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Development of a new highly sensitive and selective spectrophotometric method for the determination of selenium at nano-trace levels in various complex matrices using salicylaldehyde-orthoaminophenol

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ABSTRACT

A new spectrophotometric reagent, salicylaldehyde-orthoaminophenol (Sal-OAP) has been synthesized and characterized for the determination of selenium through novel reaction techniques. Also, a new highly selective, and sensitive spectrophotometric method for the nano-trace determination of selenium using salicylaldehyde-orthoaminophenol (Sal-OAP) has been developed. Sal-OAP undergoes reaction in a slightly acidic solution (0.0001-0.0002 M H_2SO_4) with selenium (IV) to give an orange-red chelate, which has an absorption maximum at 379 nm. The reaction is instantaneous and absorbance remains stable for over 24 h. The average molar absorption co-efficient and Sandell's sensitivity were found to be 6.4×10^5 L/mol.cm and 1.0 ng/cm^2 of, respectively. Linear calibration graphs were obtained for 0.001-40.000 mg/L of Se having detection limit of 0.1 µg/L and RSD 0-2 %. The stoichiometric composition of the chelate is 1:2 (Se:Sal-OAP). 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 developed method was successfully used in the determination of selenium in several Certified Reference Materials (Alloys, steels, human urine, bovine liver, drinking water, tea, milk, soil, and sediments) as well as in some environmental waters (Potable and polluted), biological fluids (Human blood, urine, hair, and milk), soil samples, food samples (Vegetables, rice, and wheat) and pharmaceutical samples (Tablet and syrup) and solutions containing both selenium (IV) and selenium (VI) as well as complex synthetic mixtures. The results of the proposed method for assessing biological, food and vegetables and soil samples were comparable with ICP-OES and AAS were found to be in excellent agreement. The method has high precision and accuracy (s = ±0.01 for 0.5 mg/L).

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1. Introduction

Selenium is an essential element for humans at trace level, but it is toxic at higher concentration. A maximum acceptable concentration of selenium in drinking water is 10 ng/mL [1]. Food is the main source for intake of selenium for individuals who are not occupationally exposed; thus, toxic effects have most often been associated with food intake. A safe and adequate range of selenium intake of 50-200 g per person per day has been recommended for adults, with correspondingly lower ranges for infants and children. Selenium occurs in natural waters in trace amounts as a result of geochemical processes, such as weathering of rocks and erosion of soils, and is usually present in water as selenate or selenite. Excess of selenium causes toxic effects in living organism, and toxicity depends on many factors such as chemical form, pH, presence of other ions, *etc*. [2].

It rarely occurs in its elemental state or as pure ore compounds in the Earth's crust. selenium is found in metal sulfide ores, where it partially replaces the sulfur. Commercially, selenium is produced as a byproduct in the refining of sulfide ores, most often during production. Minerals that are pure selenide or selenate compounds are known but rare. The chief commercial uses for selenium today are glass making and pigments. Selenium is a semiconductor and is used in photocells. Applications in electronics, once important, have been mostly replaced with silicon semiconductor devices. Selenium is still used in a few types of DC power surge protectors and one type of fluorescent quantum dot. Although trace amounts of selenium are necessary for cellular function in many animals, including humans, both elemental selenium and especially selenium salts are toxic in even small doses, causing selenosis. Selenium is listed as an ingredient in many multivitamins and other dietary supplements, as well as in infant formula, and is a component of the antioxidant enzymes glutathione peroxidase and thioredoxin reeducates (which indirectly reduce certain oxidized molecules in animals and some plants) as well as in three deiodinase enzymes.

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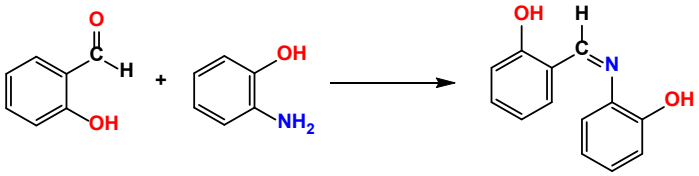

Scheme 1. Synthesis of salicylaldehyde-ortho-aminophenol (Sal-OAP).

Selenium requirements in plants differ by species, with some plants requiring relatively large amounts and others apparently requiring none [3].

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. The aim of this study was to develop a simpler direct spectrophotometric method for nano-trace determination of selenium. In the search for a more sensitive reagent, in this work a new Schiff's base reagent salicylaldehyde-ortho-aminophenol (Sal-OAP) was synthesized according to the method of Sacconi [4] and Uddin *et al.* [5] and a color reaction of Sal-OAP with Se(IV). Sal-OAP has not previously been used for the spectrophotometric determination of any metal.

This paper reports its use in a very sensitive, highly specific spectrophotometric method of ultra-trace determination of selenium. The method possesses distinct advantages over existing methods [6-53] 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 as shown in Table 1, it reveals that methods [16-52] 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 selenium in very selective and sensitive ways. The method is based on the reaction of non-absorbent Sal-OAP in a slightly acidic (0.0001-0.0002 M H_2SO_4) solution with selenium to produce a highly absorbent bright orange-red 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.

2. Experimental

2.1. 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 equipped with microcomputer-controlled air-acetylene flame and A Shimadzu (Japan) (Model: 9800) Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), (λ = 418 nm, plasma gas flow rate (L/min) = 15, LOD: below 1 µg/L of Se, RF Power (W) = 1400, Nebulizer gas flow rate (L/min) = 1-10) were used for comparison of the results. The elemental Analyzer (Exeter Analytical Inc., Model CE 440) equipped with supersensitive thermal conductivity detector for simultaneous determination of CHN was used (The National Center of Excellence in Analytical Chemistry, University of Sindh, Pakistan). Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1} (Department of Chemistry, University of Chittagong) and model: JEOL 500SS, magnetic field strength: 500 MHz solvent used: DMSO-d_6, standard: TMS, four channel NMR spectrometer with signal-to-noise ratio of ~500:1 for proton were used for characterization of the ligand (Jahangirnagar University, Savar, Dhaka).

2.2. 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 our University gave consent. University of Chittagong, Bangladesh, is committed to the protection and safety of human subjects involved in research.

2.3. Synthesis and characterization of the reagent

The reagent was synthesized in the laboratory according to the method recommended by Sacconi [4] and Uddin *et al.* [5]. The reagent salicylaldehyde-ortho-aminophenol (Sal-OAP) was synthesized by following steps (Scheme 1). A mixture of salicylaldehyde and ortho-aminophenol (25 mmol each) in ethanol (70 mL) was stirred at 30 °C and refluxed for one hour when an orange red crystalline solid appeared. This was filtered off, washed with and recrystallized from ethanol.

Salicylaldehyde-orthoaminophenol (2-((2-hydroxybenzylidene)amino)phenol) (Sal-OAP): Color: Orange red. Yield: 84%. M.P.: 182-184 °C. FT-IR (KBr, ν, cm^{-1}): 3600-3300 (OH), 1631 (C=N), 1535 (C=C), 1362 (C-N), 1313 (C-O). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 8.89 (s, 1H, CH=N), 4.52-6.91 (m, 8H, Ar-H). Anal. calcd. for $C_{13}H_{11}NO_2$: C, 73.23; H, 5.20; N, 6.57. Found: C, 73.15; H, 5.15; N, 6.57%. UV/Vis (C_2H_5OH, λ_{max}, nm, (0.45)): 326. HPLC (Isocratic: CH_3CH_2OH: H_2O = 80:20, 120 min; 0.1%): r.t. = 7.5 min., purity = 99.5%. Λ_m(S.m^2.mol^{-1}): 25. $^nD^{25}$ = 1.281. $[\alpha]_D^{25}$: -68.5 (*c* 0.5, CH_3CH_2 OH).

2.4. Reagents and solutions

All the chemicals used were of analytical reagent grade of the highest purity available. High-purity absolute ethanol and high-purity de-ionized water 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. 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 1 mL concentrated HNO_3. More rigorous contamination control was used when the selenium levels in the specimens were low.

2.4.1. Sal-OAP solution

This solution (1.17×10^{-3} M) was prepared by dissolving the requisite amount of salicylaldehyde-orthoaminophenol in a known volume solution of distilled absolute ethanol. More dilute solution of the reagent was prepared as required.

2.4.2. Selenium (IV) standard solution

A 100 mL amount of stock solution (1.72×10^{-2} M) (1 mg/mL) of tetravalent selenium was prepared by dissolving 297.45 mg of sodium selenite (Na_2SeO_3), (Merck pro-analysis grade, purity 99.6%) in doubly distilled de-ionized water and it was subsequently standardized iodometrically with standard sodium thiosulphate [53]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required. A freshly standardized solution was always used.

2.4.3. Selenium (VI) standard solution

A 100 mL amount of stock solution (1.72×10^{-2} M) (1 mg/mL) of hexavalent selenium was prepared by dissolving 325.97 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 [53]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required. A freshly standardized solution was always used.

2.4.4. Potassium dichromate solution

A 100 mL amount of stock solution (0.1 N) was prepared by dissolving 490.3 mg of finely powdered $K_2Cr_2O_7$ (E. Merck) in 100 mL deionized water.

2.4.5. Sodium azide solution

Sodium azide solution (2.5%, w/v) (A.C.S grade 99.5% pure) was freshly prepared by dissolving 2.5 g in 100 mL of deionized water

2.4.6. 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% pure) potassium sodium tartrate tetrahydrate in 100 mL de-ionized water.

2.4.7. 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.

2.4.8. EDTA solution

A 100 mL stock solution of EDTA (0.01%, w/v) was prepared by dissolving 10 mg A.C.S.-grade (≥99% pure) ethylenediaminetetraacetic acid as disodium salt dehydrate in 100 mL deionized water.

2.4.9. 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 Mukherji [54]. In the case of insoluble substances, special dissolution methods were adopted [55].

2.5. General procedure

A volume of 0.1-1.0 mL of neutral aqueous solution containing 0.01-400 µg of selenium in a 10 mL volumetric flask was mixed with a 1:80 to 1:600-fold molar excess (preferably 2 mL of 1.17×10^{-3} M) of salicylaldehyde-ortho-aminophenol (Sal-OAP) reagent solution followed by the addition of 0.8-1.6 mL (preferably 1 mL) of 0.0001 M sulfuric acid. The solution was mixed well. After 1 minute 2.0 mL of ethanol was added. The mixture was diluted up to the mark with deionized water. The absorbance was measured at 379 nm against a corresponding reagent blank. The selenium content in an unknown sample was determined using a concurrently prepared calibration graph.

2.6. 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/L) was added as preservative.

Blood, urine and milk: Blood and urine samples were collected in polypropylene bottles from effected persons of Chittagong Medical College Hospital, Bangladesh. Milk sample was collected from a Bangladeshi lactating mother. Immediately after collection they were stored in a salt-ice mixture and later, 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, fruits, and vegetables) were collected from local market of Chittagong. After collection the samples (fruits and vegetables) were stored in refrigerator for preservation. Samples (Rice and wheat) were used as dry condition and homogenized with a mortar.

Pharmaceutical samples: Pharmaceutical samples tablets of different companies were collected from local Pharmacy of Chittagong. Samples (Tablet) were homogenized with a mortar.

3. Results and discussion

3.1. Characterization of the reagent

The reagent was characterized by taking melting point, elemental analysis, FTIR, ^{1}H NMR spectrums and thermogravimetric analysis. The melting point of the reagent was 182-184 °C (Lit. 182 °C) [4]. The results of elemental analysis of the reagent was in good coincidence with the calculated values [5]. The presence of FTIR peak at 1631 cm^{-1} was due to the characteristic C=N double bond (νC=N, 1590-1631 cm^{-1}) of the Sal-OAP. The presence of ^{1}H NMR peak at δ 8.89 ppm assigned for C*H*=N and aromatic protons of Sal-OAP are observed between δ 4.52 and 6.91 ppm. Both FTIR and ^{1}H NMR spectrums and also elemental analysis data indicated the formation of the reagent. The steadiness of the thermogravimetric curve obtained for about 1 g of the reagent at 80-90 °C indicated that the reagent didn't contain any moisture.

3.2. Factors affecting the absorbance

3.2.1. Absorption spectra

The absorption spectra of a Se(IV)-Sal-OAP system in aqueous medium in presence of 1 mL 0.0001 M sulfuric acid solution, was recorded using the spectrophotometer. The absorption spectrum of Se(IV)-Sal-OAP is a symmetric curve with maximum absorbance at 379 nm and an average molar absorptivity of 6.4×10^5 L/mol.cm (Figure 1). The reagent blank exhibited negligible absorbance despite having wavelength at 379 nm. The reaction mechanism of the present method is as reported earlier [56].

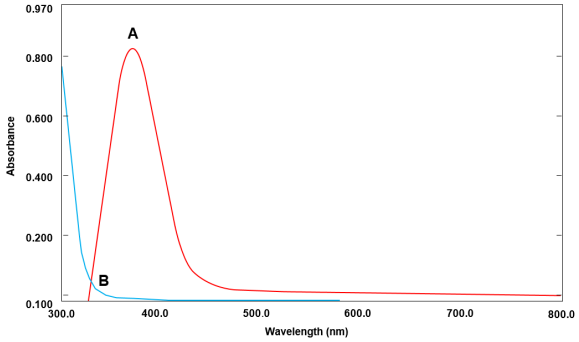

Figure 1. A and B absorbance spectra of Se(IV)-Sal-OAP (λ_{max} = 379 nm) and the reagent blank in aqueous solutions, respectively.

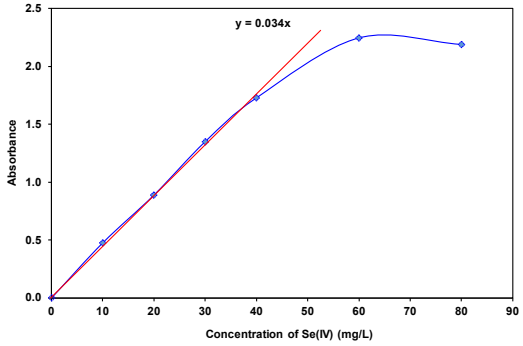

Figure 2. Calibration graph of 10-40 mg/L of selenium (IV).

3.2.2. Optimization of some parameters on the absorbance

3.2.2.1. Effect of solvent

As Sal-OAP 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, *N*,*N*-dimethylformamide, methanol, and 1,4-dioxane) studied, ethanol was found to be the best solvent for the system. Different volumes (0-5 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 20±2% (v:v) ethanol/ water medium, hence, a 20% ethanol solution was used in the determination procedure. It was observed that 10-70% (1-7 mL) ethanol produced a constant absorbance of the Se-chelate. For all subsequent measurements, 20.0 % (2 mL) of ethanol was added.

3.2.2.2. Effect of acidity

Among the various acids (Nitric, sulfuric, hydrochloric, and phosphoric acids) studied, sulfuric acid was found to be the best acid for the system. The variation of the absorbance was noted after the addition of 0.1-2.5 mL of 0.0001 M sulfuric acid to every 10 mL of test solution. The maximum and constant absorbance was obtained in the presence of 0.8-1.6 mL of 0.0001 M sulfuric acid at room temperature 25±5 °C. Outside this range of acidity, the absorbance decreased. For all subsequent measurements 1.0 mL of 0.0001 M sulfuric acid was added.

3.2.2.3. 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; a longer period of time was not studied.

3.2.2.4. Effect of temperature

The Se-Sal-OAP system attained maximum and constant absorbance at room temperature 25±5 °C. Outside this range of temperature, the absorbance decreased.

3.2.2.5. Effect of reagent concentration

Different molar excesses of Sal-OAP were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that selenium metal, the reagent molar ratio of 1:80 to 1:600 produced a constant and maximum absorbance of Se-chelate. For different (0.5 and 1 mg/L) selenium concentrations an identical effect of varying the reagent concentration was noticed. Further concentration of the reagent was not studied. For all subsequent measurements, 2 mL of 1.17×10^{-3} M Sal-OAP reagent was added.

3.3. 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. The effect of the metal concentration was studied over 0.001-100 mg/L distributed in five different sets (0.001-0.01, 0.01-0.1, 0.1-1.0, 1.0-10 and 10-100 mg/L) for convenience of the measurement. Of the five calibration graphs, one showing the limit of the linearity is given in Figure 2. The other four were straight-line graphs passing through the origin (R^2 = 0.9996). The molar absorption co-efficient and the Sandell's sensitivity [57] were found to be 6.4×10^5 L/mol.cm and 1.0 ng/cm^2 of selenium (IV), respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1 and 2.

Table 1. Summary of reviews on the existing spectrophotometric methods for the determination of selenium.

Reagent	Solvent	Medium aqueous/ surfactant/ organic	Acidity/ pH	λ_{max}, Molar absorb. coef.	Beer's law	Detection limit	RSD (%)	Interference	Remarks
Hydrazine Dihydrochloride [16]	Chloroform	Acidic	3.0	507 532×10^4	0.05-0.12	14	6	Many	i) Less sensitive ii) pH dependent iii) Less selective due to much interference iv) Solvent extractive
Dithizone [34]	CCl_4	Solvent extractive	3.0	620 5.8×10^3	0.2-1.0	70	6	Many	i) Less sensitive ii) pH dependent iii) Time consuming iv) Solvent extractive
Thionine [44]	Chloroform	Acidic	5.8	598 3.59×10^4	0.5-1.5	50	11	Many	i) Solvent extractive ii) Less sensitive iii) Less selective due to much interference
Gallocyanine by sodium sulfide [46]	Chloroform	Acidic	1.5	620 5.8×10^3	0.06-5.00	20	25	Many	i) Solvent extractive ii) Less sensitive iii) Less selective due to much interference
4,5-Diamino-6-thiopyrimidine [50]	Chloroform	Solvent extractive	3.0	600 7.33×10^4	0.1-5.0	100	4	Many	i) Lengthy and time consuming ii) Less sensitive iii) Less selective due to much interference. iv) Solvent Extractive.
1-Naphthylo amine-7-sulfonic acid (Cleve's acid) [52]	H_2SO_4	Acidic	2.0	350 8.9×10^3	0.04-3.00	20	3.0	Many	i) Less sensitive ii) Time consuming iii) Less selective due to much interference iv) Solvent extractive
Salicylaldehyde-orthoaminophenol (Sal-OAP) (Proposed method)	Ethanol	Aqueous	Slightly Acidic, 0.0001 - 0.0002 M H_2SO_4	379 6.4×10^5	0.001-40.000	0.1	0-2		i) Ultra-sensitive ii) Highly selective iii) Aqueous reaction medium. iv) Simple and rapid. v) Color stable more than 24 h at room temperature. vi) Non-extractive. vi) Application in various real, environmental, biological, soil, food and pharmaceutical samples

* Units: λ_{max} (nm), Beer's Law (mg/L), Molar absorption co-efficient, ε (L/mol.cm), and Detection limit (ng/mL).

Table 2. Summary of selected analytical parameters obtained with optimization experiments.

Parameters	Studied value	Selected value
Wavelength, λ_{max} (nm)	200 - 800	379
Solvent, mL (Ethanol)	0 - 8	1 - 8 (Preferably 2.0)
Acidity, M H_2SO_4	0.00001 - 0.01	0.0001 - 0.0002 (Preferably 0.0001)
pH	2.1 - 6.5	3.5 - 5.8 (Preferably 3.5)
Time	0 - 24 h	1 min-24 h (Preferably 2 min)
Temperature, °C	10 - 80	25±5
Reagent (fold molar excess, M:R)	1:1 - 1:700	1:80 - 1:600 (Preferably 1:100)
Linear range, mg/L	0.0001 - 100	0.001 - 40
Molar absorptivity, L/mol.cm	5.6×10^5 - 7.2×10^5	6.4×10^5
Limit of quantification, µg/L	0.1 - 10	1.0
Detection limit, µg/L	0 - 100	0.1
Sandell's sensitivity, ng/cm^2	0 - 100	1.0
Reproducibility (% RSD)	0 - 10	0 - 2
Regression coefficient, R^2	0.9993 - 0.9999	0.9996

3.4. Effect of foreign ions

The effect of over 60 anions, cations and complexing agents on the determination of only 1 mg/L of selenium (IV) was studied. The criterion for an interference [58] was an absorbance value varying by more than 5% from the expected value for selenium alone. The results are summarized in Table 3. As can be seen, a large number of ions have no significant effect on the determination of selenium. The interferences were from Fe(III) and Cr(VI) ions. Interference from these ions is probably due to complex formation with Sal-OAP. The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate interference of Fe(III) and Cr(VI), EDTA and SCN^- are used as masking agents, 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 3.

3.5. Composition of the absorbent complex

Job's method [59] of continuous variation method was applied to ascertain the stoichiometric composition of the complex under the optimum conditions (Table 2). A Se(IV)-Sal-OAP (1:2) complex was indicated by this method. The stoichiometry was found to be 1:2 (Metal: Ligand). The molar-ratio method [60] was also applied to ascertain the stoichiometric composition of the complex. A Se(IV)-Sal-OAP complex was indicated by both methods and the stoichiometry was also found to be 1:2 (Metal: Ligand).

Table 3. Tolerance limits of foreign ions, tolerance ratio [Species (x)/V (w/w)] [a].

Species x	Tolerance ratio (x/Se) (w/w)	Species x	Tolerance ratio (x/Se) (w/w)
Aluminum	20	Lead(II)	50
Arsenic(III)	20	Magnesium	100
Arsenic(V)	100	Mercury(II)	100
Antimony	100	Molybdenum(VI)	100
Azide	100	Manganese(II)	50
Ammonium	50	Manganese(VII)	20
Bismuth(III)	100	Nickel(II)	50
Bromide	100	Oxalate	100
Barium	100	Phosphate	100
Cadmium	50	Potassium	100
Calcium	50	Cobalt (II & III)	50
Carbonate	100	Selenium(VI)	50 [b]
Citrate	50	Silver	50 [c]
Chromium(III)	100 [b]	Strontium	50 [b]
Chromium(VI)	20 [b]	Sulphate	100
Cesium	100	Sodium	50
Copper(II)	50	Tartrate	1000
Cerium(III)	100	Tin(II & IV)	20 [c]
Chloride	50	Tellurium(IV)	100
Dimethylglyoxime	100	Titanium(IV)	100
EDTA	1000	Thallium(I &III)	50
Fluoride	100	Thiocyanate	100
Iron(II)	50 [b]	Tungsten(VI)	20
Iron(III)	20 [b]	Thiosulphate	100
Iodide	100	Uranium(VI)	20 [b]
Lithium	50	Zinc	50

[a] Tolerance limit was defined as ratio that causes less than ±5 percent interference.
[b] with 10 mg/L EDTA.
[c] with 10 mg/L Tartrate.

Table 4. Determination of selenium levels in a variety of synthetic mixtures.

Sample	Composition of mixtures (mg/L)	Se(IV) (mg/L)		
		Added	Found [a] (n=5)	Recovery± SD [b] (%)
A	Se(IV)	0.50	0.50	100±0.0
		1.00	0.99	99±0.8
B	As in A + Te^{IV} (25) + Pb^{2+} (25) + Zn (25) + Ba (25) + Cu^{2+} (25) + EDTA (50)	0.50	0.49	98±0.8
		1.00	1.00	100±0.0
C	As in B + K(25) + V^{V} (25) + Na (25) + Ni^{2+} (25) + Mn^{2+} (25)	0.50	0.51	102±0.9
		1.00	0.99	99±1.0
D	As in C + As^{III} (25) + As^{V} (25) + Li (25) + W^{VI} (25) + Se^{IV} (25) + Tl^{III} (25)	0.50	0.52	104±1.5
		1.00	1.03	103±1.6
E	As in D + Hg^{2+} (25) + Fe^{III} (25) + Sr (25) + Ag (25) + Cr^{VI} (25) + Tartrate (50)	0.50	0.53	106±1.6
		1.00	1.05	105±1.8
F	As in E + Ce^{III} (25) + Mo^{VI} (25) + Sn^{2+} (25) + Mn^{VII} (25) + Bi^{III} (25) + U^{VI} (25)	0.50	0.54	108±1.8
		1.00	1.08	108±2.0

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

3.6. 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 0-2.0 % for 0.01-400 µg of selenium in 10 mL, indicating that this method is highly precise and reproducible. The detection limit 3s/S (s = Standard deviation of the blank, S = Slope of calibration graph) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for selenium were found to be 0.1 µg /L and 1.0 ng/cm^2, respectively. The method was also tested by analyzing several synthetic mixtures containing selenium(IV) and diverse ions (Table 4). The results for total selenium were in good agreement with certified values (Table 5). The reliability of our Se-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 6. The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS and ICP-OES (Table 7). The results of soil analyses by the spectrophotometric method were excellent agreement with those obtained by AAS (Table 8). The results of food and vegetables analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AAS and ICP-OES (Table 9). The results of pharmaceutical samples by the spectrophotometric method were in excellent agreement with those obtained by claimed values and by ICP-OES (Table 10). The results of speciation of selenium (IV) and selenium(VI) in mixtures were highly reproducible (Table 11). Hence, the precision and accuracy of the method were excellent. With suitable masking, the reaction can be made highly selective.

4. Applications

The proposed method was successfully applied to the determination of selenium (IV) in a series of synthetic mixtures of various compositions (Table 4) and also in a number of real samples e.g. several Certified Reference Materials (CRMs) (Table 5). The method was also extended to the determination of selenium in a number of environmental, biological, food, soil 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 good agreement (Table 6). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS and ICP-OES (Table 7). The results of soil samples analyses by the spectrophotometric method were found to be excellent agreement with those of obtained by AAS (Table 8).

Table 5. Determination of selenium in some certified reference materials.

Sample	Certified reference materials (Composition, mg/kg)	Selenium, mg/kg		
		Certified value	Found [a] (n=5)	RSD [b] (%)
1	GBW 01620 * (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	15.88	1.5
2	GBW 01622 * (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	42.98	2.0
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	11.88	1.8
4	CRM-TMDW-A-100: Water Standards **: (Sb = 55, As = 55, Be = 15, Cd = 10, Se = 11, Ag = 2, Tl = 10, Mn = 40, Ni = 60, Zn = 75)	11.00 [c]	10.95±0.8	2.0
5	CEC®-CRM®-277, Estuarine Sediment ***	2.04	1.95	1.5
6	NIST®-SRM®-2711: Soil ***	4.95	4.93	1.8
7	NIST®SRM®1577b: Bovine liver ***	1.68	1.67±0.14	1.5
8	NRC®-CRM®-C85-05, Tea ***	0.041	0.043	0.5
9	NIST®SRM®-2670a-Toxic Elements in Freeze-Dried Urine ***	17.8 [c]	17.5 [c]	2.0
10	BCR®-CRM®-150: Skim Milk Powder ***	0.127	0.128	1.0

* The CRMs were obtained from the NCS Analytical Instruments Co. Ltd. Beijing, China.
** This CRM was obtained from High Purity Standards (HPS), Amazon, North Charleston, USA.
*** These CRMs were obtained from the National Research Council, Govt. of Canada.
[a] Average of five analyses of each samples
[b] The measure of precision is the relative standard deviation (RSD)
[c] Values in µg/L.

The results of some vegetable and food samples by the spectrophotometric method were found to be excellent agreement with those obtained by AAS and ICP-OES (Table 9). The results of some pharmaceutical samples by the spectrophotometric method were found to be excellent agreement with those obtained by claimed values and by ICP-OES (Table 10). The results of speciation of selenium(IV) and selenium(VI) in mixtures were highly reproducible (Table 11). The precision and accuracy of the method were excellent.

4.1. Determination of selenium in some synthetic mixtures

Several synthetic mixtures of varying compositions containing selenium (IV) 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 4. Accurate recoveries were achieved in all solutions in the range 98±0.5 to 100±0.01%. The reliability of our Selenium-Sal-OAP procedure was approved by quantitative recovery of selenium (IV) spiked in several synthetic mixture containing selenium(IV) and diverse ions. The method has high precision and accuracy (s = ±0.01 for 0.5 µg/L).

4.2. Determination of selenium in some certified reference materials

A 0.1-g amount of an alloy or steel sample containing 0.411-50.16 % of selenium was weighed accurately and placed in a 50-mL Erlenmeyer flask in presence of excess reducing agent to reduce selenium(VI) to selenium(IV) following a method recommended by Mitra [61]. To it, 10 mL of 20% (w/v) sulfuric acid was added and 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 residual carbides were decomposed. Then a further 2 mL of 1+1 H_2SO_4 and 2 mL 2.5% (w/v) freshly prepared sodium azide solution were added and the solution was evaporated carefully to dense white fumes of excess azide, 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 dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was filtered if necessary, through a Whatman No. 40 filter paper into a 100-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 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 (IV) 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 single element CRM of Se, estuarine sediment (CRM®-MESS®-3), Soil (CRM®029), human serum (CRM®-ASTMRCVD®-74231), Bovine liver (NIST® SRM®-1577c), These CRMs obtained from the National Research Council, Govt. of Canada, using tartrate or EDTA as masking agents, following a method recommended by Sun *et al*. [62]. Based on five replicate analyses, average selenium concentration determined by the spectrophotometric method was in an excellent agreement with the certified values. The results are given in Table 5.

4.3. Determination of selenium in environmental water samples

Each filtered (with Whatman No. 40) environmental sample (25 mL) contained in a 50 mL Pyrex beaker were added 1 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3 in the presence of excess freshly prepared (2.5% (w/v) sodium azide solution in a fume cupboard to reduce selenium(VI) to selenium(IV) and the mixture was heated on a hot plate until white fumes of azide to remove completely, following a method recommended by AWWA [63]. The solution was cooled and neutralized with dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. Resulting solution was then filtered through a Whatman No. 40 filter paper 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 water sample was pipetted into a 10 mL calibrated flask and the selenium content was determined as described under the general procedure using tartrate or EDTA as masking agent. To test the validity of our method, we have analyzed different types of portable and polluted waters in spike and un-spike conditions. The reliability of our spectrophotometric method was tested by recovery studies. The average percentage recovery obtained for the addition of a selenium (IV) spike to some environmental water samples was quantitative. The results of analyses of environmental water samples from various sources for selenium are shown in Table 6.

Most spectrophotometric methods for determination of selenium in natural and sea-water require preconcentration or standard addition of selenium [63].

Table 6. Determination of selenium in some environmental water samples.

Samples		Selenium (µg/L)		Recovery±s (%)	s_r [b] (%)
		Added	Found [a]		
Tap Water		0	13.0	-	-
		100	112.0	100±0.0	0.00
		500	515.0	100.5±0.6	0.22
Mineral Water		0	25.0	-	-
		100	125.0	100±0.0	0.00
		500	530.0	100.9±0.6	0.21
Well Water		0	15.0	-	-
		100	112.0	97.4±0.5	0.31
		500	525.0	101.9±0.6	0.40
Rain Water		0	10.0	-	-
		100	112.0	101.8±0.8	0.29
		500	520.0	102.0±0.5	0.23
River Water	Karnaphuli River				
	Kanaphuli (upper)	0	45.0	-	-
		100	150.0	103.4±0.6	0.25
		500	540.0	99.1±0.4	0.21
	Karnaphuli (lower)	0	50.0	-	-
		100	150.0	100±0.0	0.00
		500	560.0	101.8±1.0	0.30
	Halda (upper)	0	31.0	-	-
		100	126.0	96.2±0.5	0.22
		500	536.0	100.9±0.8	0.28
	Halda (lower)	0	36.0	-	-
		100	136.0	100±0.0	0.00
		500	540.0	100.9±0.6	0.24
Lake Water	Kaptai Lake	0	55.0	-	-
	Rangamati	100	156.0	100.6±0.8	0.21
		500	560.0	100.3±0.5	0.35
	Fayez Lake	0	26.0	-	-
	Bhatiary, Chittagong	100	126.0	100±0.0	0.00
		500	530.0	100.9±0.8	0.34
Sea Water	Potenga Sea Beach				
	Bay of Bengal (upper)	0	45.0	-	-
		100	145.0	100±0.0	0.00
		500	548.0	100.5±0.6	0.45
	Bay of Bengal (lower)	0	50.0	-	-
		100	152.0	101.3±0.8	0.39
		500	550.0	100.0±0.0	0.00
	Laboni Beach, Cox's Bazaar	0	45.0	-	-
		100	145.0	100±0.0	0.00
		500	550.0	100.9±0.7	0.29
	Kolatoli Beach, Cox's Bazaar	0	48.0	-	-
		100	148.0	100±0.0	0.00
		500	552.0	100.7±0.6	0.21
Drain Water	Eastern Cables[c]	0	140.0	-	-
		100	245.0	102±1.0	0.58
		500	650.0	101.6±0.8	0.35
	Eastern Refinery[d]	0	225.0	-	-
		100	330.0	100.6±1.0	0.49
		500	735.0	101.4±0.8	0.37
	Elite Paint[e]	0	175.0	-	-
		100	280.0	101.8±0.6	0.28
		500	670.0	99.3±0.5	0.21
	PHP Glass[f]	0	125.0	-	-
		100	230.0	102±0.8	0.29
		500	635.0	01.6±0.7	0.27

[a] Average of five replicate determinations of each sample.
[b] The measure precision is the relative standard deviation(s_r).
[c] Eastern Cables Ltd., North Patenga, Chittagong.
[d] Eastern Refinery Ltd., North Potenga, Chittagong
[e] Elite Paint and Chemical Industries Ltd., Baized Bostami Road, Chittagong.
[f] PHP Glass, Kumara, Chittagong.

The concentration of selenium in natural and sea water is a few µg/L in developed countries. The mean concentration of selenium found in US drinking water is greater than 10 µg/L [64].

4.4. Determination of selenium in some biological samples

The biological samples were digested accordingly following a method reported by Khayatian *et al.* [65]. The samples were initially dried in an oven at 120 °C for 24 h. Blood serum samples were further dried in an oven at 20°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 5 mL concentrated nitric acid and 2mL of freshly prepared 2.5% sodium azide solution. The mixture was heated to just below boiling until complete reduction of selenium(VI) to selenium(IV). 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 of an excess reducing agent according to the method recommended by Stahr [66] 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.

Table 7. Determination of selenium in human fluids.

Serial	Sample	Selenium (µg/L)						Sample source [a]
		AAS (n=5)		Proposed method (n=5)		ICP-OES (n=5)		
		Found [b]	RSD (%)	Found [b]	RSD (%)	Found [b]	RSD (%)	
1	Blood	101.5	1.7	103.5	1.5	102.5	1.6	Normal adult
	Urine	25.9	1.0	29.5	1.0	28.8	1.1	(Male)
2	Blood	414.8	3.0	415.8	2.0	416.5	3.0	Hair loss, nail discoloration patient
	Urine	121.5	2.0	120.5	1.5	122.0	1.8	(Female)
3	Blood	315.5	2.5	319.8	2.0	320.0	2.5	Neurological disorder
	Urine	81.5	2.0	87.5	1.8	88.0	1.8	(Male)
4	Blood	274.8	2.0	276.5	2.0	277.6	2.5	Dental caries patient
	Urine	71.5	1.9	77.5	1.8	79.0	2.0	(Female)
5	Blood	287.5	3.0	288.8	2.5	290.2	3.0	Pulmonary edema
	Urine	72.8	2.5	75.5	2.0	76.8	2.5	(Female)
6	Blood	298.5	3.5	300.8	3.0	302.8	3.5	Conjunctivitis patient
	Urine	77.8	2.8	78.9	2.0	80.5	2.5	(Female)
7	Blood	226.8	3.0	228.5	2.8	229.5	3.0	Heart disease patient
	Urine	65.8	2.5	66.6	2.5	68.0	2.5	(Female)
8	Blood	245.0	3.2	248.5	2.5	250.0	3.2	Kidney Dialysis patient
	Urine	62.5	2.8	65.6	2.0	67.8	2.5	(Male)
9	Blood	200.8	2.0	202.5	1.8	203.0	1.8	Hypothyroidism
	Urine	53.5	1.8	56.8	1.5	58.5	1.5	(Female)
10	Hair [c]	1.58 [c]	2.0	1.65	1.5	1.63	2.0	Normal human hair (Female)
11	Nail [c]	0.85 [c]	1.5	0.88	1.5	0.89	1.8	Normal human nails (Female)

[a] Samples were collected from Chittagong Medical College Hospital, Chittagong.
[b] The measure of precision is the relative standard deviation (RSD).
[c] Values in mg/kg.

Table 8. Determination of selenium in some soil samples.

Serial	Selenium (µg/g)				Sample sources [c]
	Proposed method (n=5)		AAS (n=5)		
	Found [a] (n=5)	RSD [b] (%)	Found [a] (n=5)	RSD [b] (%)	
S_1	2.8	1.0	3.0	1.3	Road-side soil (Dhaka-Chittagong)
S_2	0.68	1.0	0.71	1.0	Agricultural soil (Chittagong University Campus)
S_3	26.9	1.5	27.8	1.8	Industrial soil (Eastern Cables Ltd.)
S_4	58.5	2.5	59.6	2.8	Industrial soil (Eastern Refinery Ltd.)
S_5	65.6	2.8	68.0	3.0	Industrial soil (PHP Glass Ltd., Kumara, Chittagong)
S_6	25.5	2.0	26.5	2.5	Madina Tannery soil (Jalalabad, Chittagong)
S_7	75.8	2.5	78.5	3.0	Paint soil (Elite Paint & Chemical Industries Ltd., Baized, Chittagong)
S_8	7.5	1.5	8.6	1.5	Karnafuli River Bank soil (Chittagong)
S_9	25.8	2.5	26.5	2.8	T.S.P complex soil (Patenga, Chittagong)
S_{10}	18.9	2.0	19.5	2.2	Estuarine soil (Karnafuli River)
S_{11}	23.5	1.8	24.2	2.0	Industrial soil (Delhi Aluminum Factory Ltd.)
S_{12}	0.35	0.8	0.36	1.0	Normal Soil of Science Faculty (Chittagong University)

[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, Zn, Mn, Co, NO_3^-, SO_4^- *etc.*

A suitable aliquot (1-2 mL) of the final solution was pipetted into a 10 mL calibrated flask and the selenium(IV) 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 AAS and ICP-OES. The results are shown in Table 7.

The abnormally high values for the hair loss and nail discoloration and neurological disorder patient are probably due to the involvement of high selenium concentration with As and Zn. The occurrence of such high selenium contents is also reported in hair loss and neurological disorder patients from some developed countries [67].

4.5. Determination of selenium in some surface soil samples

An air-dried homogenized soil sample (10 g) was accurately weighed and placed in a 100 mL micro-Kjeldahl flask. The sample was digested in the presence of an excess reducing agent (2 mL of 2.5% freshly prepared sodium azide solution) to reduce selenium(VI) to selenium(IV) following method recommended by Jackson [68]. As the heating process continued 1-mL of H_2SO_4 is added and heated for about 5 minutes to dense white fumes to remove excess azide. The solution was then cooled at room temperature and neutralized with dilute NH_4OH solution in presence of 1-2 mL of 0.01% (w/v) EDTA solution. The content of the flask was then filtered through a Whatman No. 40 filter paper and quantitatively transferred into a 25 mL calibrated flask and made up to the mark with de-ionized water.

Table 9. Determination of selenium in some food, fruit and vegetable samples

Serial	Sample	Selenium (µg/kg or µg/L)						Sample Source
		Proposed method (n=5)		AAS (n=5)		ICP-OES (n=5)		
		Found [a]	RSD [b] (%)	Found [a]	RSD [b] (%)	Found [a]	RSD [b] (%)	
1	Chicken meat *(Gallus cibum)*	22.0	2.0	21.8	2.0	21.98	2.2	Local Market, Chittagong
2	Chicken liver *(Gallus jecur)*	25.0	2.0	25.5	2.3	25.8	2.5	Local Market, Chittagong
3	Egg *(Gallus domesticus)*	14.5	1.8	14.8	2.0	15.0	1.8	Local Market, Chittagong
4	Radish *(Raphanus sativus)*	12.5	1.5	12.8	1.8	13.3	2.0	Local Market, Chittagong
5	Carrot *(Daucus carota)*	15.9	1.8	16.5	2.0	16.8	2.0	Local Market, Chittagong
6	Tomato (*Lycopersicon esculentum)*	31.5	2.5	31.8	2.5	32.1	2.8	Local Market, Chittagong
7	Rice (Oryza sativa)	20.5	2.0	21.2	2.2	21.8	2.5	Local Market, Chittagong
8	Wheat (*Triticum aestivum*)	25.8	2.5	26.5	2.5	27.0	2.8	Local Market, Chittagong
9	Spinach *(Spinacia oleracea)*	8.5	1.5	9.0	1.6	8.9	1.8	Local Market, Chittagong
10	Mushrooms (*Agaricus bisporus*)	21.8	2.1	22.0	2.5	22.5	2.8	Local Market, Chittagong
11	Dried Loitta Fish *(Harpadon nehereus)*	28.5	2.5	29.8	3.0	30.0	3.0	Local Market, Chittagong
12	Prawn (*pandulus jordani)*	35.8	2.8	35.5	3.0	35.8	3.5	Local Market, Chittagong
13	Tea *(Camellia sinensis)*	22.5	2.0	23.0	2.5	23.5	2.8	Local Market, Chittagong
14	Banana *(Musa)*	2.5	1.5	2.8	1.8	2.6	1.8	Local Market, Chittagong
15	Milk (Diary Milk)	4.8	1.8	5.1	2.0	5.2	2.5	Local Market, Chittagong

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

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 the general procedure using tartrate or EDTA as masking agent. The selenium content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results of soil analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AAS. The average value of selenium in the Chittagong region surface soil was found to be 43.29 mg/kg [69]. The results are shown in Table 8.

4.6. Determination of selenium in some vegetable, food and fruit samples

The vegetable and fruit 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 (10 g) were ground in a mortar and taken in a 100 mL micro-Kjeldahl flask in presence of excess reducing agent and digested following a method recommended by Stahr [66] 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 1 mL of concentrated HF, and heating was continued for at least 30 min and then cooled. In the resulting solution 2 mL of 2.5% (w/v) of freshly prepared sodium azide solution was added. The mixture of each foodstuff was heated below the boiling point for 5-10 min to reduce selenium(VI) to selenium(IV). Excess azide was removed by further heating. The solutions were then cooled and neutralized with dilute NH_4OH in presence of 1-2 mL of 0.01% (w:v) EDTA solution. The resulting solution was filtered through a Whatman No. 40 filter paper and quantitatively transferred into a 25 mL calibrated flask and mixed well and made up to the mark with de-ionized water.

The food samples used were rice and wheat and these were used under dry conditions. Each sample was first ground in a mortar. Fruit samples (2 g) or rice and wheat samples (1 g) were weighed accurately and placed in a porcelain crucible and charred in an electric furnace; the sample was ashen at 555 °C in a muffle furnace in presence of excess oxidizing agent following a method recommended by Mitra [61]. To it, 2.0 mL of HCl and 10 mL of water were added to the ash. The mixture of each foodstuff was heated with 2 mL of 2.5% (w/v) freshly prepared sodium azide solution was added below the boiling point for 5-10 min to complete reduction from Se(VI) to Se (IV). Then the solution was heated for another 5 min to remove excess azide. The solutions were cooled and neutralized with dilute NH_4OH in presence of 1-2 mL of 0.01% (w/v) EDTA solution and filtered. The resulting solution was quantitatively transferred into a 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 digested solution 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 food and vegetables analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AAS and ICP-OES. The results are shown in Table 9.

4.7. Determination of selenium in pharmaceutical samples

Finished pharmaceutical samples (each Se containing tablet or 10 mL insulin or required weight) were quantitatively taken in a beaker and digested following a method recommended by Ahmed *et al.* [70]. 10 mL of concentrated nitric acid was added and heated to dryness and then added 10 mL of 20% (v/v) of H_2SO_4.

Table 10. Determination of selenium in some pharmaceutical samples.

Sample	Sample type [a]	Brand name	Trade name	Selenium (mg/kg or µg/kg)				
				Reported value / Claimed value	Proposed method (n=5)		ICP-OES (n=5)	
					Found [a] (n =5)	RSD [b] (%)	Found [a] (n =5)	RSD [b] (%)
1	Tablet	Square Pharmaceuticals Ltd. (Selenoprotine)	Mulvit plus (Multivitamin-mineral)/20 mg	20 mg	20.8	2.0	21.0	2.0
2	Tablet	Beximco Pharmaceuticals Ltd. (Selenoprotine)	Bextram Gold (A to Z) (Multivitamin-mineral)/20 mg	20 mg	19.8	1.8	20.0	2.0
3	Tablet	Opsonin pharma (Selenoprotine)	Zovia Gold (A to Z) (Multivitamin-mineral)/20 mg	20 mg	19.85	1.6	19.92	1.8
4	Tablet	Drug Int. Ltd. (Selenoprotine)	Supravit-G (Multivitamin-mineral)/20 mg	20 mg	20.5	1.8	21.0	2.0
5	Tablet	Holland and Barrett (Selenoprotine)	Selenium 50 µg	50 µg	49.6	2.0	49.8	2.0
6	Tablet	Incepta Pharmaceuticals Ltd.	Evagren 70 µg	70 µg	69.8	2.5	71.0	2.5

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

Table 11. Determination of selenium(IV) and selenium(VI) speciation in mixtures.

Serial	Se(IV): Se(VI)	Se taken (mg/L)		Se, found (mg/L)		Error (mg/L)	
		Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
1	1: 1	1.00	1.00	0.98	0.99	0.02	0.01
2	1: 1	1.00	1.00	1.00	1.02	0.00	0.02
3	1: 1	1.00	1.00	0.97	0.99	0.03	0.01
Mean error: Se(IV) = ±0.016 Se(VI) = ±0.013							
Standard deviation: Se(IV)= ± 0.015 Se(VI) = ±0.011							
1	1: 5	1.00	5.00	0.98	4.98	0.02	0.02
2	1: 5	1.00	5.00	0.99	4.99	0.01	0.01
3	1: 5	1.00	5.00	0.98	4.98	0.02	0.02
Mean error: Se(IV) = ±0.016 Se(VI) = ±0.016							
Standard deviation: Se(IV) = ± 0.0058 Se(VI) = ±0.0058							
1	1:10	1.00	10.00	0.99	9.99	0.01	0.01
2	1:10	1.00	10.00	0.98	9.98	0.02	0.02
3	1:10	1.00	10.00	0.98	9.98	0.02	0.02
Mean error: Se(IV) = ±0. 016 Se(VI) = ±0.016							
Standard deviation: Se(IV) = ± 0.015 Se(VI) = ±0.015							

The mixture was heated with 2 mL of 2.5 % (w/v) freshly prepared sodium azide solution was added below the boiling point for 5-10 min to complete reduction from Se(VI) to Se (IV). Excess azide was removed by heating and the volume was reduced to 2.5 mL and then cooled to room temperature. The solution was then 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 tartrate as a masking agent. The results of some pharmaceutical analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by reported values and ICP-OES. The analyses of pharmaceutical samples from several Pharmaceutical Companies for selenium are given in Table 10.

4.8. Determination of selenium (iv) and selenium (vi) speciation in mixtures

Suitable aliquots (1-2 mL) of selenium (IV+VI) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 250 mL Pyrex conical flask. A few drops (3–5 drops) of 4M H_2SO_4, and 5–10-mL of 2.5 % (w:v) freshly prepared sodium azide were added to reduce hexavalent selenium to tetravalent selenium and the mixture was heated gently with further addition of 10-mL water, if necessary, for 5 minutes to drive off the excess azide, then the mixture was cooled to room temperature 25±5 °C following the method recommended by Abrarin *et al.* [71]. The reaction mixture was then cooled and neutralized with dilute NH_4OH in presence of 1-2 mL of 0.01% (w:v) EDTA solution. The solution was transferred quantitatively into a 25-mL volumetric flask and 2.0 mL of 1.17×10^{-3} M Sal-OAP reagent solution was added followed by the addition of 1.0 mL of 0.0001 M H_2SO_4. It was made up to the mark with de-ionized water. The absorbance was measured then being cooled at room temperature, 25±5 °C, at 379 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 250 mL Pyrex conical flask. The solution was neutralized with dilute NH_4OH in presence of 1-2 mL of 0.01% (w:v) EDTA solution. After, the content of the beaker was transferred quantitatively into a 25 mL volumetric flask, 2.0 mL of 1.17×10^{-3} M Sal-OAP reagent solution was added, followed by the addition of 1.0 mL of 0.0001 M H_2SO_4. It was made up to the mark with de-ionized water. After 5 min the absorbance was measured following the general procedure at 379 nm against a reagent blank, as before. The selenium concentration was calculated in mg/L or µg/L 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 the assessment of speciation of Se(IV) and Se(VI) were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of selenium [72]. The results of a set of determination are given in Table 11.

5. Conclusions

A new simple, sensitive, selective and inexpensive method with the Selenium-Sal-OAP complex was developed for the

ultra-trace determination of selenium in some real, environmental, biological, soil, food and pharmaceutical samples, for continuous monitoring to establish the trace levels of selenium in different sample matrices. Compared with other methods in the literature Table 1, the proposed method has several remarkable analytical characteristics:

i) The proposed method is highly sensitive with molar absorptivity of the complex of 6.4×10^5 L/mol.cm. Thus, amount of ng/g of selenium can be determined without preconcentration.
ii) The proposed method is very simple, rapid and stable. The reaction of selenium (IV) with Sal-OAP is completed rapidly in 1 min at room temperature so it does not involve any stringent reaction conditions and offer the advantage of high complex stability (24 h).
iii) 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 Sal-OAP in aqueous solutions not only is one of the most sensitive methods for the ultra –trace determination of selenium but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the routine monitoring of trace and ultra-trace amounts of selenium in real, environmental, biological, soil, food and pharmaceutical samples. It is a new method needs neither heating nor extraction to organic phase, works satisfactorily and could be an alternative method for the rapid determination of selenium in a wide variety of sample solutions and found superior to spectrophotometric methods described in different literature [6-52,73-82].

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CRediT authorship contribution statement

Conceptualization: Muhammad Jamaluddin Ahmed, Muhammad Jihan Uddin; Methodology: Muhammad Jamaluddin Ahmed; Software: Muhammad Emdadul Hoque; Validation: Muhammad Jihan Uddin; Formal Analysis: Muhammad Jihan Uddin, Muhammad Emdadul Hoque; Investigation: Muhammad Emdadul Hoque, Muhammad Jihan Uddin; Resources: Muhammad Jamaluddin Ahmed, Muhammad Jihan Uddin; Data Curation: Muhammad Jihan Uddin, Muhammad Emdadul Hoque; Writing - Original Draft: Muhammad Jamaluddin Ahmed; Writing - Review and Editing: Muhammad Emdadul Hoque, Muhammad Jihan Uddin; Visualization: Muhammad Emdadul Hoque; Validation: Muhammad Jihan Uddin; Funding acquisition: Muhammad Jamaluddin Ahmed; Supervision: Muhammad Jamaluddin Ahmed; Project Administration: Muhammad Jamaluddin Ahmed, Muhammad Jihan Uddin.

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