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Cloud point extraction and electrothermal atomic absorption spectrometry of Se (IV)—3,3′-Diaminobenzidine for the estimation of trace amounts of Se (IV) and Se (VI) in environmental water samples and total selenium in animal blood and fish tissue samples

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ABSTRACT

This paper presents a method based on the cloud point extraction for the separation and preconcentration of Se (IV) and Se (VI) in environmental water samples as well as total selenium in animal blood and tissue samples. 3,3′-Diaminobenzidine (DAB) is a selective and sensitive reagent and is known to form an intense yellow compound piazselenol with selenium (IV). When a system consisting of sample, DAB and surfactant Triton X-114 is warmed above the cloud point of the surfactant, it was seen that the DAB–Se (IV) complex gets extracted into the surfactant rich phase while the Se (VI) remains in the aqueous phase. Se (VI) in the sample was reduced to Se (IV) by microwave heating of solution in 4 mol L^{-1} HCl and total Se was estimated by carrying out the CPE. The quantification of selenium was carried out using ETAAS. The analytical parameters for the quantitative cloud point extraction of the Se–DAB complex were investigated and optimized. The proposed procedure was validated by applying it to the determination of the content of Se in Certified Reference Material BND 701-02. (NPL, India). The detection limit of selenium in environmental water samples was 0.0025 μg L^{-1} with an enrichment factor of 100. The relative standard deviation (RSD) for ten replicate measurements of 5 μg L^{-1} was 3.6%. The proposed method was successfully applied to the determination of selenium (IV), (VI) in environmental water samples and determination of total selenium in human blood, SRM-IAEA-A-13 animal blood and SRM-IAEA-407 fish tissue.

1. Introduction

Selenium is a naturally occurring element and is found throughout the environment. It is both a toxic and an essential element and the narrow concentration range between the two contrary effects makes accurate quantification of selenium at low levels increasingly important and challenging.

Our body uses selenium to produce glutathione peroxidase enzyme that regulates free radical formation, which protects cell membranes from damage hence prevents aging and disease. Selenium is present in many chemical forms. It has been reported that the inorganic forms are more toxic than the organic form and the order of toxicity is (selenite Se (IV) < selenate Se (VI) < hydrogen selenide (HSe) [1–3]. The selenite (IV) and selenate (VI) forms are predominant in environmental matrices and the plant uptake, mobility in soil and bioavailability also depends upon the oxidation state [4]. The EPA permissible level of Se (total) in water is 10 μg L^{-1} as a consequence the concentration of individual species is much lower. Therefore effective separation and low-level quantification techniques are important for differential characterization of Se (VI) and (IV).

A number of analytical techniques including ultra violet spectrometry (UV-Spec.), atomic absorption spectrometry (AAS), hydride generation AAS, inductively coupled plasma, voltammetry, X-ray fluorescence (XRF), high performance liquid chromatography atomic fluorescence spectroscopy (HPLC-AFS), gas chromatography (GC), etc. have been employed for the estimation of selenium. However some of them cannot directly differentiate between the oxidation states. Also the low levels of selenium in real samples warrant using a preconcentration procedure for the species to be detectable by these techniques. Hence a combination of effective and efficient separation chemistry with sensitive detection technique is a prerequisite for characterization of Se (IV) and Se (VI).

The separation is affected exploiting the chemistry of Se (IV) and Se (VI) using techniques like solid-phase extraction (SPE) [5–12], coprecipitation [13–15], single drop micro extraction (SDME) [16],

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dispersive liquid–liquid micro extraction (DLLME) [17,18], cloud point extraction (CPE) [19], HPLC [20], and [21,22].

Quantification of selenium has been carried out employing ETASS [5,6,10,15,17,18,22], ET vaporization-ICPMS [16,19], energy dispersive XRF [8], hydride generation ETAAS [9,23], HG AFS [14], cathodic stripping voltammetry [13,20], ICP OES [11], HPLC ICPMS [20], and ICPMS [12].

Cloud point extraction (CPE) is a liquid–liquid extraction technique; it is based on the formation of turbidity and the subsequent separation of hydrophilic and hydrophobic components of non-ionic surfactants when they are heated above their cloud point temperature (CPT). When metal complexes are present in the micellar solution they get preconcentrated in the low volume, micelle rich, hydrophobic component which is used for analysis. The high density of the surfactant rich phase facilitates easy separation. This was first reported by Watanabe and Tanaka who used it to preconcentrate trace levels of Zn [24] and since then it has been exploited for the preconcentration of a number of inorganic ions, organic and biological molecules. CPE is attracting attention as a green analytical technique because it circumvents the use of volatile organic solvents which have a negative impact on the environment and human health.

Diaminobenzidine (DAB) is known to form a yellow precipitate with Se (IV), and not with Se (VI). This is a 2:1 Se (IV) DAB complex which is insoluble in water, but soluble in solvents like alcohol, toluene, etc. [25–27]. This has been used for spectrophotometric estimation of low levels of selenium in blood, food material, pharmaceutical applications, etc. [28], and recently for the estimation of selenium in water samples [18].

This paper describes the complex formation of DAB with Se (IV) for the preconcentration and characterization by CPE. Total selenium was estimated after reduction of Se (VI) to Se (IV) by heating in a microwave oven in 4 mol L^{-1} HCl followed by CPE. Se (VI) was quantified by the difference in the estimate of total Se and Se (IV). Triton X-114 was used as the surfactant, DAB as Se (IV) specific reagent and GFAAS using palladium as modifier to quantify trace concentration of selenium. The factors affecting the CPE have been investigated and the proposed method was applied to the quantification of Se (IV) and (VI) in ground water, and tap water samples. In samples like blood and animal tissue, Se exists complexed with various organic biological moieties which get extracted into the surfactant rich phase. CPE was applied to the estimation of total selenium in blood and validated by the estimation of total selenium in animal whole blood (SRM-IAEA-A-13) and animal tissue (SRM-IAEA-407).

2. Experimental

2.1. Instrumental

2.1.1. Instrumentation

GBC 906AA AAS unit with deuterium-arc background correction, GF 3000 electro thermal atomizer and an auto sampler PAL-3000 were used in the present investigation. Pyrolytic graphite coated furnace tubes were used in all the studies. All measurements were performed using integrated absorbance (peak area). The elemental hollow cathode lamp of Selenium λ 196 nm, (GBC, Australia) was used.

A CEM MDS-2100 microwave sample preparation system, with HDF vessels capable of withstanding pressure up to 600 psi were used for sample dissolution and reduction of selenium (VI) to (IV).

2.1.2. Reagents

0.5% (w/v) freshly prepared 3,3′-Diaminobenzidine hydrochloride (SRL, India) was prepared by dissolving 100 mg in 20 mL deionised water. Standard selenium solution was prepared by suitable dilution of certified AAS standard solution (1 g L^{-1}) from E. Merck Darmstadt. Triton X-114 (Sigma–Aldrich), 2.5 mol L^{-1} Formic acid (S.D. Fine Chem., India), 1:1 NH_3 solution was prepared from extra pure NH_3(SRL., India), Supra pure nitric acid 65% (v/v), Supra pure hydrochloric acid 35% (v/v) from E. Merck Darmstadt, Germany was used for all sample treatment. Nanopure water of 18.3 MΩ delivered from Barnstead Thermolyne water purification systems were used for all dilutions and washings.

All containers and glassware were cleaned by soaking successively in three baths of 10%, 1% and 0.1% double distilled nitric acid in nanopure water. All glassware was stored in 0.1% nitric acid baths till further use.

2.2. General procedure

2.2.1. Procedure for Se (IV) in environmental water samples

2.2.1.1. CPE procedure. A suitable aliquot of a sample solution was adjusted to pH 1.90 ± 0.10 with 2.5 mol L^{-1} formic acid and 1:1 NH_4OH. 0.1 mL of 0.1 mol L^{-1} EDTA was then added. To this solution 0.5 mL 0.5% (w/v) DAB was added and the solution kept aside for 30–45 min. The pH of the solution was raised to pH 6–7, 1 mL Triton X-114 (1.0%, w/v) was added and the solution was warmed on a water bath (50 °C) till separation of aqueous and surfactant phase was seen (15 min). The solution was centrifuged for 10 min at 3500 rpm and then kept in freezing mixture for 15 min. The heavier surfactant rich phase becomes viscous and settles to the bottom. The aqueous phase was separated from the surfactant phase by decantation. To reduce the viscosity of the surfactant phase, it was made up to 0.5 mL with 5% (v/v) HNO_3. The processed sample, blanks, processed standards and modifier were injected into the graphite furnace using an auto sampler. The required amounts were adjusted using the auto mix function of the autosampler and the selenium was quantified using the optimized graphite furnace programme.

2.2.1.2. Determination of total Se. Total Se in the sample was estimated by reducing the Se (VI) to Se (IV) and carrying out the CPE procedure. A microwave oven had been used to carry out the heating and reduction as it is faster and more convenient than conventional procedures. Time and acid concentration of acid on the efficiency of reduction was studied and were able to obtain quantitative reduction using 4 mol L^{-1} HCl and heating in the microwave oven using heavy duty (HD) vessels. The optimized microwave digestion program was heating for 2 min at 100 psi, 4 min at 150 psi and 5 min at 400 psi. The CPE procedure was carried out and total Se was estimated. The Se (VI) concentration was calculated by subtracting the Se (IV) concentration from the total Se. Sample blanks were prepared by running aliquots of deionised water through the sample procedure.

The processed sample, standards, blanks and modifier were injected into the graphite furnace using an auto sampler and the selenium was quantified using the optimized graphite furnace programme.

2.2.2. Procedure for blood, SRM-IAEA-A-13 animal blood and SRM-IAEA-407 fish tissue

0.5 g of blood, animal blood and fish homogenate were weighed and transferred to the microwave digestion HD vessels. Four millilitres of conc. HNO_3 was added to all the vessels and the sample was digested using a four-stage microwave digestion program (Table 1). Blanks were prepared in a similar manner. The vessels were cooled and contents transferred and made up to volume. Suitable aliquots were taken, transferred to beakers and warmed on water bath to near dryness. The samples were made 4 mol L^{-1} wrt HCl and heated in the microwave oven as per the program mentioned in Section

Table 1
Experimental condition for microwave-assisted pressurized wet digestion of blood and tissue samples.

Stage	(1)	(2)	(3)	(4)
% Power	60	60	60	60
psi	20	40	85	140
TIME (min)	15	15	15	15
TAP (min)	5	5	5	5

Table 2
Optimized ETAAS programme.

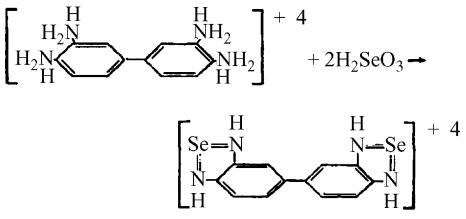
Process	Final temperature (°C)	Ramp time (s)	Hold time (s)	Gas type
Drying	90	10	5	Inert
	120	10	10	
	300	10	10	
Ashing	600	5	5	
	1000	5	5	
	1000	0	1	None
Atomisation	2100	1	2	
Tube clean	2200	1	2	Inert

2.2.1 in HD vessels wherein Se (VI) reduced to Se (IV). The sample solutions and blanks were run through the CPE procedure. The processed samples, standards, blanks and modifier were injected into the graphite furnace using an auto sampler and the selenium was quantified employing the standard addition calibration method using the optimized graphite furnace programme.

3. Results and discussion

3.1. Optimization of ETAAS: pyrolysis and atomization temperature

Optimization of furnace conditions was carried out so that effective pyrolysis and atomization temperatures were arrived at, to eliminate any matrix effect and ensure extended life of tube. Selenium compounds are known to be volatile hence palladium was used to stabilize selenium to provide a reliable analyte signal. To optimize the pyrolysis temperature the recommended atomization temperature of 1800 °C was chosen and the temperature was varied in steps of 100 °C, starting from 400 °C to 1400 °C. There was a decline in absorbance from 400 °C to 700 °C after which there was a rise which remained constant from 800 °C to 1200 °C. Pyrolysis temperature of 1000 °C was fixed for all further experiments. The atomization temperature was then varied in steps of 100 °C from 1800 °C to 2400 °C and it was observed that the analyte signal reached a well-defined plateau between 2000 °C and 2300 °C after which a decrease in signal was observed. Though the absorbance in the peak height was higher than in the peak area mode, the results in the area mode showed better precision and linear calibration. Pyrolysis temperature of 1000 °C, atomization temperature of 2100 °C and peak area mode were used in all further studies. An optimized graphite program is given in Table 2.



Acid Medium

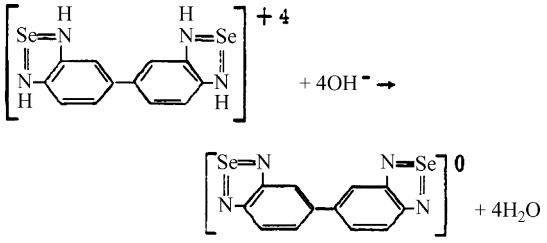
3.2. Effect of modifier

Palladium was used as matrix modifier in the present investigation to extend the pyrolysis and atomization temperatures. Studies were carried out to ascertain the range of the concentration of modifier which did not alter and affect the analyte signal. The amount of Pd (II) added was from 0 μg to 80 μg Pd per atomization cycle. It was observed that there was no change in the absorbance for 20 μL of 100 ng mL^{-1} selenium, when 10–30 μg Pd (II) was used. Less than 10 μg and more than 30 μg Pd resulted in decrease in absorbance (Fig. 1).

3.3. Effect of pH on complex formation and CPE

Complexing agents are commonly used to selectively extract metal ions by CPE from the sample solution, therefore the formation of a metal complex, kinetics of complexation and the subsequent transfer of metal chelate needs to be studied. The formation of a metal complex and its subsequent extraction into an organic solvent are known to be pH dependent.

A set of solutions containing selenium were taken and complexing agent DAB was added. The pH of the solutions was varied from 0.5 to 4.0 and selenium was estimated after extraction with CPE. It was seen that the recovery of Se (IV) was less than 10%, indicating that the complex was not quantitatively extracted into the nonionic surfactant Triton X-114. It is known that the extractability of a complex into a neutral organic medium reduces if the complex carries a net charge. Hence the extraction of the piazselenol complex was studied by varying the pH in increasing increments from pH 2 to 10. Kinetics of complex formation also affects extractability hence the solutions were rested for 10 min before extraction. It was seen that the extractability of Se (IV) increased at the higher pH and was constant from pH 5 up to pH 10 whereas Se (VI) did not show any analytical signal (Fig. 2). The hydrogen ion concentration has an important effect on the extraction, which causes the charge neutralization of the piazselenol complex resulting in a structural change which affects an increase in extractability as shown in following equation.



Neutral or Alkaline Mediun

After setting the pH of extraction, the pH of complex formation was studied. The pH of the solutions were adjusted from 0.5 to 4.0, the solutions were allowed to rest for 15 min and then the pH was raised to 6 and CPE procedure was applied. It was seen that maximum absorbance was observed at pH 2.0 ± 0.2. Therefore for subsequent experiments complexation was done at pH 2.0 ± 0.2 and CPE was carried out at pH 6 ± 1. The extraction efficiency (63.8%) was substantially lower than 100%, because this experiment was not conducted under the optimized conditions (Fig. 3).

3.4. Optimization of time for complex formation

Quantitative and reproducible recovery of analyte depends on complete complex formation and hence the kinetics of complex formation needs to be investigated. The solution containing constant concentration of Se and DAB was kept for 10 min, 20 min,

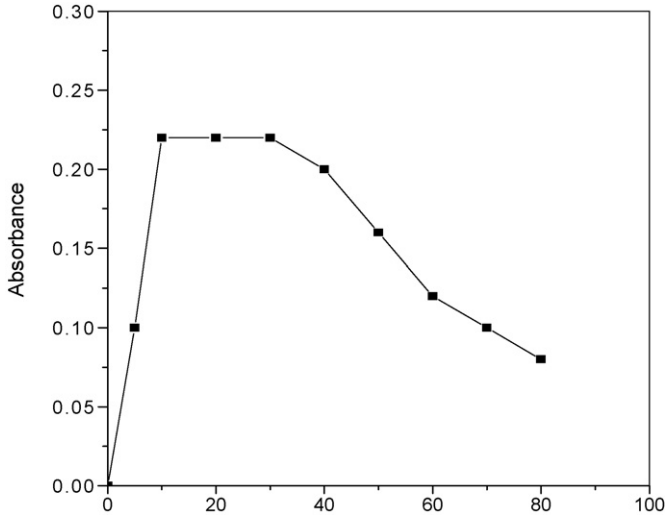


Fig. 1. Relation between concentration of palladium modifier and absorbance of selenium. Concentration of selenium: 0.1 μg mL^{-1}; instrumental parameters as given in Table 1.

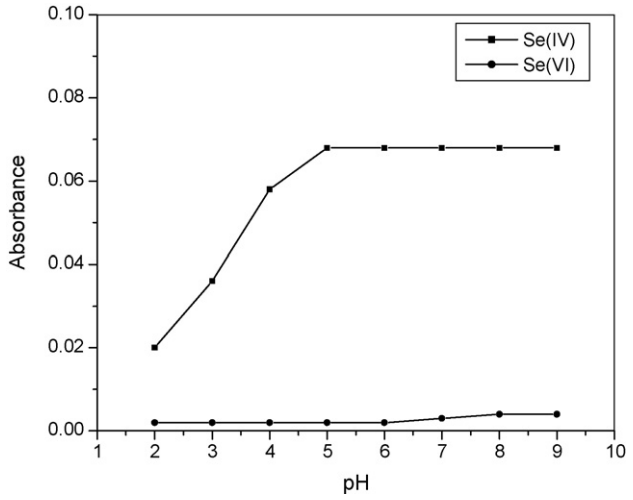


Fig. 2. Effect of pH on extraction of piazselenol with triton X-114.5 μg L^{-1} Se (IV); 5 μg L^{-1} Se (VI); 5 g L^{-1} DAB; 0.5 g $^{-1}$L triton X-114; at 50 °C; instrumental parameters as in Table 1.

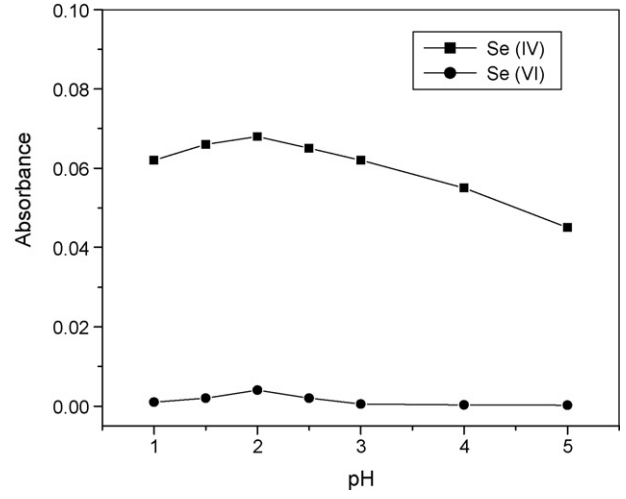


Fig. 3. Effect of pH on the complex formation of DAB and Se (IV, VI) 5 μg L^{-1} Se (IV); 5 μg L^{-1} Se (VI); 5 g L^{-1} DAB; 0.5 g L^{-1} triton X-114; at 50 °C; instrumental parameters as in Table 1.

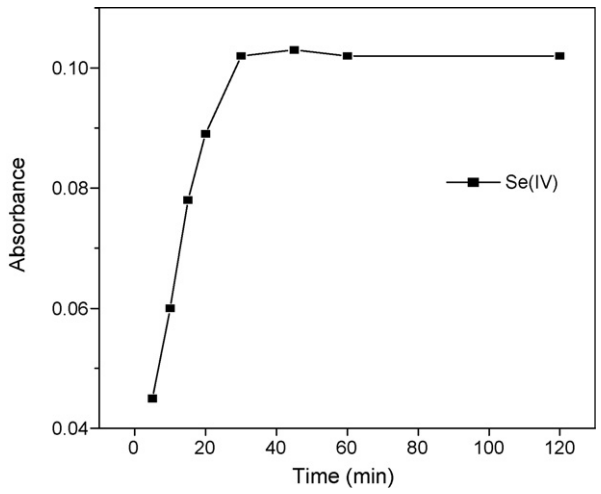


Fig. 4. Optimization of time for Se (IV)-DAB complex formation 5 μg L^{-1} Se (IV); 5 g L^{-1} DAB; 0.5 g L^{-1} triton X-114; at 50 °C; instrumental parameters as in Table 1.

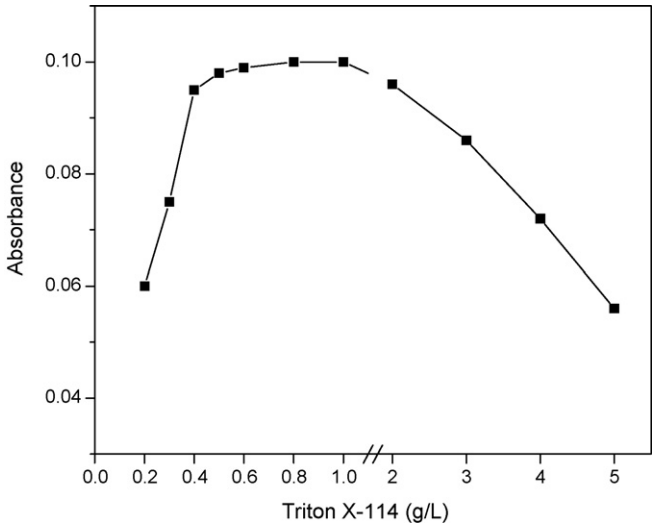


Fig. 5. Effect of surfactant concentration on recovery of Se (IV), 5 μg L^{-1} Se (IV); 5 g L^{-1} DAB; pH 6, at 50 °C; instrumental parameters as in Table 1.

30 min, 40 min, 50 min, and 60 min after which the solutions were processed by the recommended CPE procedure. As shown in Fig. 4 the optimum absorbance value was obtained after 30 min. Hence for all further experiments the experimental solution was kept for 30–45 min at pH 1.90 ± 0.10 at room temperature to ensure complex formation.

3.5. Effect of surfactant concentration

Triton X-114 is an octylphenol ethoxylate surfactant and has a CPT (25 °C) lower than other surfactants, which is favorable for laboratory experiments and hence was selected for our work. The amount of Triton X-114 affects the extraction efficiency and the volume of the surfactant rich phase. The effect of the concentration of Triton X-114 on the quantitative recovery of Se (IV) by the present method was investigated by varying the surfactant concentration from 0.1 g L^{-1} to 10 g L^{-1}. As shown in Fig. 5, it was observed that the optimum absorbance was obtained for concentration of Triton X-114 starting from 0.4 g L^{-1} and remained constant up to 1 g L^{-1}. A concentration of 0.5 g L^{-1} was chosen for subsequent experiments.

Table 3
Tolerance limits of common foreign ions for the CPE of Se (IV) 5 μg L^{-1} (n = 5).

Ions	(mg L^{-1})	Recovery (%)
Na^+	1000	101
K^+	10	102
Ca^{2+}	1000	104
Mg^{2+}	1000	101
Ions	(μg L^{-1})	Recovery (%)
Cu^{2+}	200	101
Mn^{2+}	200	98
Zn^{2+}	200	99
Al^{3+}	200	103
Fe^{3+}	200	102
V^{5+}	200	99
Ions	(mg L^{-1})	Recovery (%)
Cl^-	1000	101.
NO_3^-	1000	103
SO_4^{2-}	1000	96
	(μg L^{-1})	
PO_4^{3-}	250	97

3.6. Effect of equilibration temperature and time

It is desirable to employ the shortest equilibration time and lowest temperature and at the same time obtain effective phase separation; hence it is necessary to optimize these parameters. The equilibration temperature was varied from 20 °C to 80 °C. The maximum absorbance was observed at 30 °C, which remained constant upto 80 °C. The extraction efficiency was constant from 30 °C to 80 °C; hence a temperature of 50 °C was employed for subsequent experiments. Keeping the temperature at 50 °C the equilibration (extraction) time was varied from 5 min to 30 min. The absorbance was constant between 15 min and 30 min, therefore an equilibration time of 15 min was employed for all subsequent experiments.

3.7. Effect of sample volume

The low concentration of selenium in natural water samples makes it necessary to achieve high enrichment ratios to enable quantification of the analyte. Hence the effect of sample volume on the recovery of selenium was studied. Sample solutions of 10–50 mL containing 5 ng Se (IV) were taken and Se was estimated after carrying out CPE under optimized conditions. It was seen that quantitative recovery was obtained in solutions of 10–50 mL, therefore suitable sample volumes were taken for analysis.

3.8. Effect of foreign ions

AAS is an element specific quantification technique with little serious spectral interference. DAB is known to react with Vanadium (V), copper (II) and iron (III). Real samples inherently have a range of other cations which may affect the analytical signal and quantitative recovery of Se (IV). K^+, Na^+, Mg^{2+}, Al^{3+}, Cu^{2+}, Pb^{2+}, Fe^{3+}, V^{5+}, Cl^{1-}, $NO_3{}^{1-}$· $PO_4{}^{3-}$, and $SO_4{}^{2-}$ are commonly present in environmental samples and biological samples. Their effect was studied by adding, interfering ions in concentrations reported in literature to diluted test solution (CRM BND 701-02. NPL, India) containing 5 μg L^{-1} Se. The solution was run through the recommended CPE procedure. It was seen that the recovery of Se (IV) in the test solution was between 95% and 107%. (Table 3) It was therefore concluded that the proposed method is free from interference from diverse ions commonly found as there were no adverse effects on analytical signal of Se (IV).

Table 4
Comparison of detection limits (ng L^{-1}) in the published methods for Se speciation with the proposed method.

Analytical technique	Se (IV)	Se (VI)	Ref.
HPLC-HG-AFS	26	33	[23]
SPE-FI-ETAAS	10	10	[22]
SPE-ICPMS	7	7	[12]
SDME-ETV-ICPMS	2.7	3.0	[16]
CPE-ETV-ICPMS	8	8	[19]
CPE-ETASS	2.5	2.5	This work

3.9. Calibration, precision and figure of merit

3.9.1. Water samples

Standard selenium (IV) solution was used to obtain the calibration curve. Aliquots containing 10 ng, 20 ng, 40 ng, 100 ng, 200 ng Se (IV), were taken and processed according to the recommended CPE procedure. The processed solutions and aqueous standard solutions were injected into the graphite furnace using an auto sampler. The required amounts of each were adjusted using the auto mix function of the auto sampler. Palladium was used as modifier and optimized furnaces programme was employed for carrying out the analysis.

The calibration curve obtained using processed standards and aqueous standards showed that there was no significant difference in their absorbance values. Therefore aqueous standards were used for all estimations. The slopes for processed and aqueous standard were 0.00189 and 0.00187 and the regression was 0.9995 and 0.9980, and standard deviation was 0.0054 and 0.0083 respectively.

The limit of detection defined as $C_L = 3 \cdot S_B/m$ (where C_L is the limit of detection, S_B is the standard deviation for 10 replicate measurements of the blank and m is the slope of the processed calibration curve respectively) was 0.0025 μg L^{-1} for Se (IV). The relative standard deviation (RSD) for 10 replicate measurements of 5 ng mL^{-1} was 3.6%. The concentration factor (CF) of present method is 100. The detection limit obtained by the present method compares favorably with those reported in literature. (Table 4).

3.9.2. Blood and tissue samples

Triton X-114 is used for the preconcentration of a number of inorganic ions, organic and biological molecules and since blood and tissue samples are predominantly rich in such molecules the CPE component showed a matrix effect i.e. the instrumental response, absorbance of the analyte was affected by the matrix. Therefore a standard addition calibration was employed for the estimation of selenium in blood and tissue samples. The slopes, regression, and standard deviation were 0.00102, 0.9998 and 0.0029 respectively. The limit of detection defined as $C_L = 3 \cdot S_B/m$ (where C_L is the limit of detection, S_B is the standard deviation for 10 replicate measurements of the blank and m is the slope of the processed calibration curve respectively) was 0.025 μg g^{-1} for Se (IV). The relative standard deviation (RSD) for 10 replicate measurements of 25 ng g^{-1} was 5.4%. The detection limit by the standard addition calibration as compared to the linear calibration is reduced as the matrix causes attenuation of signal.

3.10. Validation of proposed method and application for analysis of water samples

To validate the proposed method, the procedure has been applied to the determination of the content of total selenium in water certified reference material BND 701-02.(NPL, India) The determined value 1.03 ± 0.03 mg L^{-1} (n = 7) was in good agreement with the certified value of 1.00 ± 0.02 mg L^{-1}. The t test at 95%

Table 5
Analytical results for standard addition studies for Se (IV) and Se (VI) in reference material, ground water and tap water samples. ($n = 7$).

Sample	Added ($\mu g\,L^{-1}$)		Result ($\mu g\,L^{-1}$)			Recovery (%)	
	Se (IV)	Se (VI)	Se (IV)	Se (VI)	Total	Se (IV)	Se (VI)
BND-701-02 (NPL, India)	–	–	25.3 ± 0.2	ND	25.3 ± 0.2	101.2	–
BND-701-02 (NPL, India)	20	20	45.2 ± 0.1	19.6	64.8 ± 0.3	99.5	98.0
Groundwater-1	–	–	5.1 ± 0.3	12.4	17.5 ± 0.2	–	–
Groundwater-1	2	5	7.3 ± 0.2	17.0	24.3 ± 0.3	110	92.0
Groundwater-2	–	–	4.2 ± 0.2	9.6	13.8 ± 0.3	–	–
Groundwater-2	5	2	9.1 ± 0.1	11.4	20.5 ± 0.3	98.0	98.3
Tap Water-1	–	–	nd	nd	nd	–	–
Tap Water-1	2	5	2.2 ± 0.3	5.4	7.6 ± 0.4	110	108
Tap Water-2			nd	nd	nd	–	–
Tap Water-2	5	2	4.9 ± 0.5	2.1	7.0 ± 0.3	98.0	105

n is no. of replicates.

Table 6
Analytical results for total selenium in standard reference material and blood samples. ($n = 5$).

Sample $n = 5$	Concentrations ($\mu g\,g^{-1}$)	RSD (%)	Certified value ($\mu g\,g^{-1}$)	% Recovery
SRM-IAEA-A-13 Animal Blood	0.23 ± 0.01	4.3	0.24	104
SRM-IAEA-407 Fish tissue	2.85 ± 0.04	1.4	2.83	99.3
Blood 1	0.084 ± 0.006	7.1	–	–
Blood 2	0.068 ± 0.005	7.4	–	–
Blood 3	0.073 ± 0.004	5.5	–	–

n is no. of replicates.

confidence gave a t value 2.10. The critical value of $t_{0.05}$ is 2.45 validating that the estimated value and the certified value are the same.

This protocol was applied to ground water samples received by our laboratory and to a set of tap water samples collected from the municipal domestic supply system. The samples were filtered through a 0.45 μm membrane filter; known amounts Se (IV), (VI) were spiked in these samples. All the samples were run through the CPE procedure and selenium was quantified by GFAAS. The results indicated that the recoveries of spiked Se (IV) and (VI) obtained were from 92% to 110%. (Table 5).

3.11. Validation of proposed method and application for analysis of blood and tissue samples

To validate the proposed method, the procedure has been applied to the determination of the content of total selenium in SRM-IAEA-A-13 Animal Blood and SRM-IAEA-407 Fish tissue.

This protocol was also applied to blood samples received by our laboratory. These samples were run through the CPE procedure and selenium was quantified by GFAAS. The t test at 95% confidence gave a t value 2.60. The critical value of $t_{0.05}$ is 2.78 ($n = 5$) validating that the estimated value by proposed method and the certified value are the same. (Table 6).

4. Conclusion

We have presented here a simple, sensitive and accurate method based Cloud Point Extraction of Se–DAB complex using Triton X-114 to characterize trace amounts of selenium (IV), (VI). The quantification of selenium was carried out using GFAAS. The detection limit of selenium in environmental water samples was 0.0025 $\mu g\,L^{-1}$ by GFAAS by the present technique which has an enrichment factor of 100. The detection limit of selenium in blood and tissue samples 0.025 $\mu g\,g^{-1}$.

The proposed method was successfully applied to the determination of Se (IV), (VI) in environmental water samples and total selenium in SRM-IAEA-A-13 Animal Blood and SRM-IAEA-407 Fish tissue. It is applicable for the quantification of the maximum concentration limit (MCL) of 10 $\mu g\,Se\,L^{-1}$ established by EPA.

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