

Analytical Methods

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Optimization of the preconcentration of selenium IV on palladium nanoparticles (PdNP's), using multivariate analysis for the inorganic speciation of selenium in environmental water samples

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21 **Abstract:** A simple method for the characterization and quantification of ultra trace
22 quantities of Se (IV) in environmental water samples by preconcentration of selenium (IV) on
23 PdNP was optimized employing the multivariate strategy. The 12 run Plackett Burman factorial
24 design experiments was carried out to identify and the central 2³+star orthogonal composite
25 design was used to optimize the relevant experimental factors. Sodium borohydride (NaBH₄)
26 was used to reduce Se (IV) to Se (0) and also also to generate PdNP. The PdNP were collected
27 and dissolved in minimum amount of nitric acid and selenium was quantified by ETAAS. Se
28 (VI) in the sample was reduced to Se (IV) and Se (VI) was the difference in Se (IV) and total Se
29 (VI) + Se (IV). The standard addition studies of Se (IV) and Se (VI) at the low, mid and high
30 concentration levels of the calibration curve indicated recoveries between 97-104%. The
31 detection limit and the preconcentration factor (PF) for the estimation of selenium employing
32 this methodology were 0.025 µg L⁻¹ and 100 respectively. The adsorption capacity of PdNP in
33 the present study, for Se (0) was found to be 28 mg g⁻¹. The proposed procedure was validated
34 by applying it for the determination of the content of total Se in Certified Reference Material
35 BND 701-02 (NPL, India) and applied for the determination of sub ppm to ppm levels of Se
36 (IV) and Se (VI) in ground water samples from Nawanshahar-Hoshiarpur region, Punjab, India.
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51 *Keywords:* speciation, Selenium (IV), selenium (VI), multivariate techniques, palladium nanoparticles, sodium
52 borohydride, electro thermal atomic absorption spectrometry.
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Introduction

Selenium is an essential trace element nutrient for humans and its deficiency is associated with several disease conditions. Se supports the expression of selenoproteins which include glutathione peroxidases (GPX) and thioredoxin reductases (TrxR), the former helps to protect the body against free radical damage and cancer while the latter is required for proper functioning of the thyroid gland. However the permissible concentration range for Se is very narrow and, if the intake level is exceeded, it is known to causes acute toxicity leading to even death (Nuttall, 2006). Food and drinking water are the usually sources of Se and the permissible average intake per day is 30-55 μg . (WHO, FAO, 2004). According to water quality guidelines for Se the maximum contamination level (MCL) of Se in water for drinking purposes is 10 $\mu\text{g L}^{-1}$ (EPA 2009, WHO, 2011). and the maximum permissible level (MPL) for water used for irrigation is 20 $\mu\text{g L}^{-1}$ (NAS-NAE, 1973).

The availability of Se in ground water is determined by its soil type. In regions of extensive irrigation and where the soil is known to have seleniferous deposits, the ground water has been found to be associated with high levels of Se (Dhillon and Dhillon, 2003). Several such areas have been identified in different states of India (Dhillon and Dhillon, 1991; Dhillon and Dhillon, 1997), where Se concentrations upto 65.5 $\mu\text{g L}^{-1}$ in ground water and 29-185 $\mu\text{g g}^{-1}$ in locally produced cereal grains (Bansal, et al., 2012) have been reported. Toxicity of Se is dependent on the chemical form of the species and the inorganic forms of selenium found in drinking and environmental waters are mainly selenite Se (IV) and selenate Se (VI). The former is thermodynamically favored and is 40 times more toxic than the organic forms (Vinceti, et al., 2013). Therefore there is a need for efficient separation and preconcentration protocols for the differential characterization of Se (IV) and Se (VI) species in ground water.

Literature survey indicates that there are numerous reports on strategies for the determination of selenium species in food and water samples at sub ppb levels. These include co precipitation with $\text{La}(\text{OH})_3$ (Tang, et al., 2005), $\text{Mg}(\text{OH})_2$ (Tuzen, et al., 2007), dispersive liquid-liquid micro extraction (DLLME) (Martinis, et al., 2011; Bidari, et al., 2008; Zhang, et al., 2013; Liu et al., 2015), hollow fiber liquid phase micro extraction (HF-LPME) (Ghasemi, et

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4 al., 2010), cloud point extraction (CPE) (Suvarna, et al., 2010), solid phase extraction (SPE)
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6 (Xiong, et al., 2008; Saygi, et al., 2008; Gezer, et al., 2011; Fu, et al., 2012; Deng, et al., 2012;
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8 Kocot, et al., 2015)
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11 Multivariate techniques have been employed for the optimization of analytical
12 methodology (Ferreira, et al., 2007; Baig, et al., 2009; Ghambarian, et al., 2009) as they allow
13 many significant variables to be identified and optimized simultaneously (Montgomery, 1997;
14 Deming and Morgan, 1987). Among the different types of designs, Plackett-Burman Design
15 (PBD) which use only a few experiments have been used as a screening method to select the
16 variables that have influence on a system [Massart, et al., 1988]. To obtain the optimum values
17 for each significant variable involved in a certain system, central composite designs (CCD) are
18 frequently used for second-order response surface modeling within k factor experiments (Reddy,
19 et al., 2008).
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30 Palladium is known to be a versatile catalyst and sodium borohydride (NaBH_4)
31 (Bonnemann, et al., 2001) is used to produce PdNP and these generated in situ have been used as
32 a carrier for the separation and preconcentration of trace elements from a variety of matrices
33 (Zhuang, et al., 1996) and for the differential characterization of Cr (VI), Cr (III) (Omole, et al.,
34 2007) As (III) , As (V) (Sounderajan, et al., 2009) in soil and ground water respectively.
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41 In this paper we report the use of multivariate techniques for the optimization of in situ
42 reduction and adsorption of selenium (IV) on palladium nanoparticles (PdNP) for the
43 characterization and quantification of Se (IV) and Se (VI) in environmental water samples. Here,
44 sodium borohydride has been used for the reduction of Pd (II) to Pd (0) to obtain PdNP and also
45 Se (IV) to Se (0). The reduced elemental selenium was adsorbed on the surface of PdNP and thus
46 separated from Se (VI). Se (VI) in the sample was reduced to Se (IV) and Se (VI) was quantified
47 by the difference in estimate of total Se (IV+VI) and Se (IV). The significant analytical factors
48 influencing the quantitative recovery of Se on PdNP were identified employing 12 run Plackett-
49 Burman Design (PBD), while the optimum value of each significant variable was obtained by
50 central composite design (CCD).
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The method has been validated by applying it for the determination of total selenium in Certified Reference Material BND 701-02 (National Physical Laboratory, India). The standard addition studies of Se (IV) and Se (VI) at the low, mid and high concentration levels of the calibration curve indicated recoveries between 96-104%. The proposed method was successfully applied for the determination of sub ppm to ppm levels of Se (IV) and Se (VI) in ground water samples collected from Nawanshahar-Hoshiarpur region, Punjab, India.

2. Experimental

2.1. Instrumentation

GBC906AA 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 (GBC part no. 99-0059-00) were used in all the studies. All measurements were performed using integrated absorbance (peak area). The elemental hollow cathode lamp of Se λ 196.0 nm GBC, Australia was used in the estimation of Se and Pd (NO_3)₂ was used as a matrix modifier. The pyrolysis and atomization temperatures were optimized and used in the determination of Se.

2.2. Reagents

Supra pure nitric acid 65%, Supra pure hydrochloric acid 35% from E. Merck Darmstadt, Germany were used for all sample treatments. Nanopure water of 18.3M Ω delivered from Barnstead Thermolyne Water Purification System was used for all dilutions and washings. Standard solutions of Se (IV) were prepared by suitable dilution of certified AAS standard solution (1mg mL⁻¹) from E. Merck Darmstadt. Se (VI) solution was prepared by dissolving sodium selenate in 1% nitric acid. Palladium solution was prepared by dissolving weighed quantities of palladium metal (99.999%) in 10mL conc. nitric acid and making up to volume. 10% sodium borohydride (Riedel Haen, AG 96%) was prepared by dissolving weighed quantities and making up to volume.

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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.

2.3. Analytical procedure

Optimization of the preconcentration of Se (IV)

2.3.1. The fractional factorial design

For the evaluation of the significant experimental factors among the five factors for the estimation of Se (IV), two levels Plackett–Burman designs with 12 experiments were described instead of the $2^5 = 32$, required for full factorial designs. Experimental parameters such as palladium concentration (Pd), sodium borohydride concentration (NaBH_4), pH (p), time (t) and volume of the sample (V), were identified as factors which may affect the recovery of Se (IV) in the insitu reduction/adsorption of Se (IV)/Se (0) on palladium nanoparticles (PdNP). The Plackett–Burman matrix where the low (–) and high (+) levels are those as specified in Table 1.

(Table 1) —→

Procedure used in the factorial design

Three replicates of each set of solutions from 25 -100 mL with known concentration of Se (IV) + Se(VI) were taken, $\text{Pd}(\text{NO}_3)_2$, 0.3mg to 1.0 mg was added to the solutions and the pH was adjusted in the range of 2 and 6. Then, 30mg to 100mg of NaBH_4 was added to the beakers which were stirred vigorously and then allowed to rest for a period of 2 to 4 hours. After the prescribed times the solutions were centrifuged at 3000 rpm and the PdNP collected. The supernatant was decanted and the PdNP dissolved in 5% HNO_3 . The blanks were processed in a similar manner. The resulting recovery values of the experiments (1–12) were of three replicates of each set as indicated in Table 2. The experimental data was evaluated with the help of STATISTICA computer program Package 7.

(Table 2) —→

2.4.2. Optimization plan

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A central 2^3 +star orthogonal composite design involving 20 experiments with 6 center points were carried out for three factors at five levels with three replicates in order to optimize the levels of effective parameters for improving the sensitivity of Se (IV) recovery. The total number of experiments needed (N=20) is determined by the following equation.

$$N = 2^f + 2f + C_p \quad \text{Eq. (1)}$$

where, f is the number of variables and C_p is the number of center points.

In the present work the statistically significant variables [Pd], [NaBH₄] and [V] were regarded as factors for the optimization experiment where time and pH were taken at fixed values (i.e., p=3 and time= 2 h). Three replicates sets of solution were taken and processed as per the matrix design where low (-1), high (+1), 0, $-\alpha$ and $+\alpha$ values of each variable were specified in Table 3. The design matrix with two blocks, including the factors, their coded levels and the results from each test, is shown in Table 4. Design and analysis of the central composite experiment were carried out with STATISTICA Release 7.0 statistical package.

(Table 3) —————>

(Table 4) —————>

2.5 Determination of Se (VI)

Se (VI) in the water sample was reduced to Se (IV) by microwave heating (Table 5) and the total Se (VI+IV) in the sample was determined by carrying out the optimized procedure. Sample blanks were prepared by running aliquots of de ionized water through the sample procedure. The concentration of Se (VI) was calculated as the difference of Se (IV) + Se (VI) and Se (IV).

(Table 5) —————>

3. Results and Discussion:

3.1. Optimization of graphite furnace atomic absorption spectrometer

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5 Optimization of ashing and atomization furnace conditions were carried out so that
6 effective temperatures were arrived at, to eliminate any matrix or memory effect and ensure
7 extended life of the tube. Se compounds are volatile hence Pd was used to stabilize selenium to
8 provide a reliable analyte signal. The processed solutions contained Pd however it did not affect
9 the absorbance of Se, as it remain unaltered up to 10–30 μg Pd (II) per injection of 100 ng Se.
10 (Suvarna, et al., 2010).
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12 To optimize the pyrolysis temperature the recommended atomization temperature of 2300 °C
13 was chosen and the pyrolysis temperature was varied in steps of 100°C, starting from 600°C to
14 1600°C. An optimum and constant absorbance was observed between 1100°C to 1400°C. A
15 pyrolysis temperature of 1100°C was selected and the atomization temperature was then varied
16 in steps of 100°C from 2000°C to 2500°C. The analyte signal reached a well-defined plateau
17 between 2200°C and 2400°C after which a decrease in absorbance was seen. Pyrolysis
18 temperature of 1100°C and atomization temperature of 2300°C and peak area mode was used in
19 all further studies. The absorbance was measured in the peak area mode, as the results showed
20 better precision.
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22 (Table 6) \longrightarrow
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32 33 34 35 36 **3.2. PdNP**

37 The particles obtained on reduction of palladium by NaBH_4 were examined in secondary
38 electron mode at 30 kV beam acceleration indicated the presence of agglomerated particles. The
39 PdNP had an average size of 50–100nm.(Suvarna, et al., 2009)
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47 **3.3. Optimization of experimental variables**

48 The results obtained from PBD (12 experiments) with five experimental parameters such
49 as palladium concentration (Pd), sodium borohydride concentration (NaBH_4), pH (p), time (t)
50 and volume of the sample (V) (Table 3) were evaluated by determining standardized effect for
51 each factor at a 95% confidence level. These standardized effects were visualized in Pareto
52 chart (Fig.1) and presents the results obtained from this evaluation. The length of the bars in the
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Pareto chart is proportional to the absolute value of the standardized effects and indicated the threshold for this test at a level $p= 0.05$ (for $t_{crit}=2.2$). All factors, whose absolute values of the standardized effects are above critical t-value, were considered to be statistically significant.

(Fig. 1) 

3.3.1. Estimated effects of variables for estimation of Se (IV)

The results as shown in Pareto chart (Fig. 1) indicate that the levels of pH and time used in the experiment were insignificant. The PBD indicates only the estimate effect of the main factors and not of the effects of the interactions of the factors. It was seen that the coefficient estimate of volume was the largest and was negative while that of Pd was higher than NaBH₄ and both of which were positive. This indicated that higher concentrations of Pd and NaBH₄ and lower volumes of sample were favorable for quantitative extraction of Se (IV) on PdNP. The % recovery of Se (IV) in experiment 2 and 5 (Table 3) was found to be 102 % and 100% respectively. Here V was at (-) level in experiment 2 and (+) level in experiment 5 while Pd and NaBH₄ were at (+) optimum level. In experiment 8 and 9 where V was (+) while Pd and NaBH₄ were (+) and (-) alternately the % recovery was 41.5 % and 54.4%, thus indicating the negative effect of volume on recovery of Se IV. Pd was the next critical variable evaluated for its effect on the SPE of Se IV. In experiments 2 and 7, the Pd concentration was (+) high and (-) low respectively while the NaBH₄ was (+) and Volume was (-) low in both. The % recovery in experiments 2 and 7 was about 102.1% and 74.9% respectively, thus illustrating that altering the Pd concentration greatly affected the recovery of Se IV. The NaBH₄ concentration in experiment 5 was at the (+) level while that in experiment 10 was at the (-) level while Pd and V were both at (+) level in both experiments. The recoveries were 100.4% and 47.4% respectively thus establishing the positive effect of NaBH₄. In the experiments 11 and 12, t and pH were varied while the levels of Pd, V and NaBH₄ were the constant in both. The recoveries in both experiments were 57.4 % and 73.6% substantiating the finding that both pH (p), time (t) did not affect the recovery of Se (IV). Time (t) and pH (p) did not affect the recovery of Se because the PdNP were stable and formed instantly and also the process was physical adsorption and not complex formation.

3.3.2. Optimization of variable by CCD

Having screened out the variables that did not have significant effect on the recovery of Se (IV)/Se(0), namely time and pH they were taken at fixed values (i.e., pH=3 and time= 2 h). The remaining three factors Pd, NaBH₄ and V were optimized by CCD to provide values for the maximum recovery of Se (IV). A central 2³+star orthogonal composite design with six central point's involving 20 experiments were performed for three factors with three replicates and average results as a response are listed in Table 5. The results were analysed by STATISTICA 7.0 software package.

The mathematical relationship of the response (Y) on the three significant independent variables [Pd],[NaBH₄] and [V] was approximated by a nonlinear second-order polynomial equation

$$Y = b_0 + b_1 \times Pd + b_2 \times NaBH_4 + b_3 \times V + b_{11} \times Pd^2 + b_{22} \times NaBH_4^2 + b_{33} \times V^2 + b_{12} \times Pd \times NaBH_4 + b_{13} \times Pd \times V + b_{23} \times NaBH_4 \times V$$

Eq. (2)

where, Y is the experimental response, b_0 is the intercept obtained by the average of three main effects, b_1, b_2 and b_3 are the main effects of the coded variables of [Pd],[NaBH₄] and [V] respectively; b_{11}, b_{22} and b_{33} are the squared effects; b_{12}, b_{13} and b_{23} are two factor interaction half-effects.

Optimum values were found, by partial differentiation of Eq. (2)

$$\left(\frac{\partial Y}{\partial X_1} \right)_{X_2, X_3} = 0 \quad \left(\frac{\partial Y}{\partial X_2} \right)_{X_1, X_3} = 0 \quad \left(\frac{\partial Y}{\partial X_3} \right)_{X_1, X_2} = 0$$

The response Y presented the maximum recovery of 99.5% at the optimal values 1.09 mg of Pd (X₁), 102.5mg of NaBH₄ (X₂) and 116.0 mL of V (X₃).

(Fig. 2 i-iii) →

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The response surfaces for each pair of variable indicated that as shown in Figs. 2 (i-iii) indicated that there was a nonlinear relation between Y and variables X_1 , X_2 and X_3 .

(Fig. 3) \longrightarrow

3.3.3. Model Validation

The experimental results and the predicted values obtained using model (Eq. 2) are given in Fig 3. As can be seen, the predicted values match the observed (experimental) values reasonably well with $R^2=0.997$.

3.3.4. Optimized analytical procedure for the estimation of Se (IV) & Se (VI)

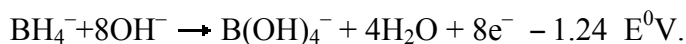
A suitable aliquot of sample solution (100 mL) was adjusted to pH 3.0 ± 0.1 using dilute ammonia and nitric acid. 1.0 mg Pd (II) was then added followed by 100 mg of NaBH_4 and the contents were vigorously stirred. The pH of this solution was around 8.0 ± 0.2 . After 2.0 hours, the contents were transferred and centrifuged for 10 minutes at 3500 rpm. The supernatant solution was discarded and the collected palladium particles were dissolved in 5% HNO_3 and made up to volume 1 mL.

In another aliquot, the Se (VI) component of the sample was reduced to Se (IV) by microwave heating as described in Table 5. The resulting solution was processed by the optimized procedure and total Se (VI) + Se (IV) was estimated.

Se was determined in the processed solutions by ETAAS using the optimized furnace programme. The processed samples, blanks, standards and modifier were injected into the graphite furnace using the auto mix mode of the auto sampler. Se (VI) was estimated as the difference of Se (VI) + Se (IV) and Se (IV).

Reaction mechanism:

NaBH_4 is a powerful reducing agent and reduce Pd and Se. The reaction mechanisms are:



3.4 Adsorption Capacity of Se (IV)/ Se (0) on PdNP

The adsorption capacity of Se (0) on PdNP is a measure of the effectiveness of the PdNP's as an extraction phase. To study this, a series of working solutions of Se (IV) were prepared in the concentration range of 0.001, 0.005, 0.01 upto 0.50 mg L⁻¹ and the experiments were carried out under the optimized experimental conditions. The experimental data was plotted using eqn 3 and 4.

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_{\max}} + \frac{1}{KQ_{\max}} \quad \text{Eq.3}$$

$$Q_e = \frac{KQ_{\max}C_e}{1 + KC_e} \quad \text{Eq. 4}$$

(Fig.4) →

Here Q_e is the amount of solute adsorbed by gram of PdNP colloid (mg/g), C_e is the equilibrium concentration ($\mu\text{g L}^{-1}$), K ($\text{L } \mu\text{g}^{-1}$) and Q_{\max} (mg/g) represent Langmuir equilibrium constant and the adsorption maximum capacity of the solute on the PdNP surface, respectively. The nonlinear fit of the experimental data Eq. (3) (correlation coefficient R^2 is 0.989) is shown in Fig.4, while the inset shows the linear fit (correlation coefficient R^2 is 0.9997) of the experimental data to Eq. (4). The adsorption constants and adsorption capacity for the adsorption of Se (IV)/Se (0) on PdNP as obtained from the non linear fit are 381 L/ μg and 27.8 mg g⁻¹ and from the linear fit are 521 L/ μg and 28.5mg g⁻¹, respectively. The results indicated that the interaction between Se (IV)/ (0) and PdNP's confirms to the Langmuir adsorption isotherm and is a mono layer mono molecular physical adsorption.

3.5 Effect of foreign ions

AAS is a technique with little serious spectral interference however real samples inherently have a range of cations and anions which may affect the analytical signal and

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quantitative recovery of Se (IV). The effect of these ions was studied by adding them in concentrations reported in literature to diluted test solution (CRM BND 701-02. NPL, India) containing $5\mu\text{gL}^{-1}$ Se. An ion was considered as interferent, when it causes a variation greater than $\pm 5\%$ in the recovery of Se in the samples. The results presented in Table 7 demonstrated that common cations and anions do not interfere on the determination of trace quantities of Se by the present procedure.

(Table 7) \longrightarrow

3.6 Method Validation

This procedure was applied for the determination of the content of total Se in certified reference material (CRM) BND 701-02 (NPL, India). The calculated t-value for measured value was 1.63 which was lower than the critical t-value at 95% C.L ($t_{0.05,6} = 2.45$) indicating that there is no significant difference between estimated value and the certified value and they are in good agreement.

This method was applied to ground water samples collected from Nawanshahar-Hoshiarpur region, Punjab, India. Before applying this procedure samples were filtered through a $0.45\mu\text{m}$ membrane filter. Known amounts of Se (IV) and Se (VI) were spiked in these samples at three concentration values namely, the low, mid and high levels of the calibration curve. Selenium was quantified by GFAAS. The results indicated that the recoveries of spiked Se (IV) and Se (VI) were in the range of 97% to 102%. Results of ground water samples and spiked standards addition recoveries are given in Table 8. It is seen that the dominant species present in water samples from Nawanshahar-Hoshiarpur was Se (VI) selenate, as leaching of selenium from soil was due to the increased oxidizing nature of the groundwater. This has important implications on the health of people consuming groundwater from seleniferous areas which exceeded the maximum permissible level for irrigation water.

(Table 8) \longrightarrow

3.7 Calibration and figure of merit

The calibration curve obtained using processed standards and aqueous standards from 10 $\mu\text{g L}^{-1}$ to 200 $\mu\text{g L}^{-1}$ of Se was linear. The slopes for aqueous standard and processed standard were 0.00189 and 0.00187, respectively and their regression and standard deviation were 0.999 and 0.998, and 0.0054 and 0.0083, respectively. Since the properties of the calibration curve are similar, aqueous standards were used for quantification of selenium in all the experiments. The limit of detection defined as $DL = 3 \times S_B / m$ (where DL is the limit of detection, S_B is the standard deviation for 10 replicate measurement of the blank and 'm' is the slope of the calibration curve) was 0.025 $\mu\text{g L}^{-1}$ for Se (IV).

The uncertainty of measurement of Se (IV) in ground water samples by the proposed procedure was estimated following the guidelines of EURACHEM CITAC (Guide 2012). The contributions considered, were those from the studies of the repeatability of the analytical values, recovery of the standard addition studies and calibration linearity. A summary of the individual uncertainty combined uncertainty and expanded uncertainty of Se concentration in CRM BND701-02. NPL, India, ground water sample and the spiked samples is summarized in Table 9.

(Table 9). \longrightarrow

The detection limit 0.025 $\mu\text{g L}^{-1}$, preconcentration factor (100) and adsorption capacity (28.2 mg g⁻¹ g⁻¹) of Se (IV) on PdNP when compared with the published results of Saygi (2007), Xiong (2008), Deng (2012) and Kocot (2015) as reported in the literature are found to be favorable. (Table 10)

(Table 10) \longrightarrow

4. Conclusion

A methodology based on the quantitative adsorption of reduced Se (IV)/Se (0) on PdNP generated in situ by NaBH_4 has been described to quantify Se (IV) and Se (VI) in environmental

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water samples. The quantification of selenium was carried out using ETAAS. The proposed method for the determination of Se (IV) was optimized by the multivariate technique. The Plackett-Burman Design was used to find the significant factors and the data was evaluated by determining standardized effect for each factor at (p~ 95.0%) and visualized by a Pareto chart. Pd, NaBH₄ and V emerged as the significant factors. The optimum values of three significant factors were calculated from second order polynomial regression modeling (quadratic equation) and the values are 1.09mg, 102.5mg and 116.0mL for Pd, NaBH₄ and V respectively. The experimental data were analyzed using STASTICA 7.0 software package.

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The standard addition method showed recoveries for Se (IV) and Se (VI) in the range of 97% to 102%. The detection limit of selenium in environmental water samples was 0.025 µg L⁻¹ by the present technique and has a pre concentration factor of 100. The uncertainty of estimation, for 10.0 µg L⁻¹ Se calculated for the present protocol was 1.6 µg L⁻¹. The proposed method was successfully applied for the determination of Se (IV) and Se (VI) in ground water samples collected from Nawanshahar-Hoshiarpur region, Punjab, India and is applicable for the quantification of the maximum concentration limit of 10 µg L⁻¹ of Se established by WHO.

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17 **FIGURE CAPTIONS:**
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20 Fig 1: Pareto Chart
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22 Fig.2.i: Pd vs NaBH vs recovery
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24 Fig 2.ii: Pd vs V vs recovery
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26 Fig 2.iii: NaBH vs V vs recovery
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28 Fig.3. Model validation: Experimental results and the predicted values
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30 Fig. 4: Nonlinear fit of Langmuir adsorption isotherm of Se (IV)/Se (0) on
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34 Inset: Linear fit of Langmuir adsorption isotherm of Se (IV)/Se (0) on
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Table 1. Variables and levels used in the Plackett Burman design for redn of Se (IV)

Variables	Symbol	Low (-1)	High (+1)
Palladium Concentration (mg)	<i>Pd</i>	0.3	1
NaBH ₄ Concentration (mg)	<i>NaBH</i>	30	100
Time (h)	<i>t</i>	2	4
pH	<i>p</i>	2	6
Volume (mL)	<i>V</i>	25	100

Table 2. Plackett Burman matrix design and results for % recovery of Se (0) (n=3)

Experiments	Pd	t	p	NaBH	V	Recovery (%)
1	+1	-1	+1	-1	-1	98.9±0.1
2	+1	+1	-1	+1	-1	102.1±0.3
3	-1	+1	+1	-1	+1	40.3±0.3
4	+1	-1	+1	+1	-1	98.4±0.5
5	+1	+1	-1	+1	+1	100.4±0.3
6	+1	+1	+1	-1	+1	52.3±0.5
7	-1	+1	+1	+1	-1	74.9±0.4
8	-1	-1	+1	+1	+1	41.5±0.4
9	-1	-1	-1	+1	+1	54.4±0.4
10	+1	-1	-1	-1	+1	47.4±0.4
11	-1	+1	-1	-1	-1	57.4±0.5
12	-1	-1	-1	-1	-1	73.6±0.6

Table 3. Variables and levels used in 2³+star orthogonal composite design

Variables	Symbol	- α	Low (-1)	0	High (+1)	+ α
Palladium Concentration (mg)	<i>Pd</i>	0.0327	0.3	0.65	1	1.27
NaBH ₄ Concentration (mg)	<i>NaBH</i>	3.27	30	65	100	126.7
Volume (mL)	<i>V</i>	1.73	25	62.5	100	121.7

Table 4. Central 2^3 +star orthogonal composite design and results for % recovery of Se.
(n=3)

Experiments	Blocks	Pd	NaBH	V	Recovery (%)
1	1	-1	-1	-1	82.0±0.2
2	1	-1	-1	+1	61.1±0.3
3	1	-1	+1	-1	91.9±0.4
4	1	-1	+1	+1	73.8±0.2
5	1	+1	-1	-1	98.7±0.2
6	1	+1	-1	+1	68.6±0.4
7	1	+1	+1	-1	100.6±0.6
8	1	+1	+1	+1	98.6±0.5
9	1	0	0	0	90.9±0.3
10	1	0	0	0	91.0±0.4
11	1	0	0	0	91.0±0.3
12	1	- α	0	0	15.3±0.4
13	2	+ α	0	0	100.4±0.5
14	2	0	- α	0	16.4±0.4
15	2	0	+ α	0	90.6±0.2
16	2	0	0	- α	96.0±0.4
17	2	0	0	+ α	65.8±0.7
18	2	0	0	0	91.2±0.2
19	2	0	0	0	91.2±0.2
20	2	0	0	0	91.4±0.3

Table 5. Experimental programme for microwave-assisted pressurized reduction of Se (VI) to Se (IV)

Stage	(1)	(2)	(3)
Power (W)	100	100	100
Pressure (psi)	100	150	400
Ramp Time (min.)	5	5	5
Hold Time (min.)	2	4	2

Table 6. Optimized ETAAS program

Process	Final Temperature (°C)	Ramp Time (S)	Hold Time (S)	Gas Type
Drying	90	10	5	Inert Gas
	120	10	10	
	300	10	10	
Pyrolysis	600	5	5	
	1100	5	5	
	1100	1	0	
Atomization	2300	1	2	
Tube Clean	2400	1	2	Inert Gas

Table 7. Effect of foreign ions on the recovery of Se IV: $5\mu\text{g L}^{-1}$, Se VI: $5\mu\text{g L}^{-1}$ (n=3)

Ion	Concentration (mg L ⁻¹)	Recovery (%) of ^a Se (IV)	Recovery (%) of ^b Total Se
Na ⁺	1000	99	101
K ⁺	1000	98	100
Ca ²⁺	1000	102	99
Mg ²⁺	1000	101	97
	Concentration (μg L⁻¹)		
Cu ²⁺	200	101	98
Mn ²⁺	200	98	100
Zn ²⁺	200	99	102
Al ³⁺	200	103	98
Fe ³⁺	200	102	99
	Concentration (mg L⁻¹)		
Cl ⁻	1000	101	102
NO ³⁻	1000	103	100
SO ₄ ²⁻	1000	97	98
	Concentration (μg L⁻¹)		
PO ₄ ³⁻	250	97	101

Table 8. Analytical results of Se (IV) and Se (VI) and spike addition recovery of ground water samples, ground water samples collected from Nawanshehar and Hoshiarpur district, Punjab,India and CRM. (n=6)

Sample	Added ($\mu\text{g L}^{-1}$)		Results ($\mu\text{g L}^{-1}$)		Recovery (%)	
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Se(IV)	Se(VI)
Tap Water	-	-	<0.025	<0.025	-	-
	10	10	9.9±0.2	9.8±0.2	99	98
	50	50	49.5±0.8	49.2±0.9	99	98
	100	100	97.4±1.0	97.6±1.0	97	98
GW-1	-	-	1.7±0.2	65.0±0.4	-	-
	5	50	6.9±0.3	75.2±0.4	104	102
GW-2	-	-	2.5±0.2	47.1±0.4	-	-
	5	50	7.4±0.2	98.0±0.5	98	102
GW-3	-	-	3.0±0.2	17.3±0.2	-	-
	5	20	8.1±0.2	37.5±0.3	102	101
GW-4	-	-	<0.025	112.0±4.0	-	-
	5	100	4.7±0.4	211.0±4.0	94	100
GW-5	-	-	<0.025	180.0±5.0	-	-
	5	200	4.8±0.3	382.0±5.0	96	100
GW-6	-	-	<0.025	200.0±4.0	-	-
	5	200	4.8±0.3	401.0±4.0	96	100
BND 701-02	-	-	1.02±0.03*	<0.025	-	-
	200	200	1.20±0.02*	198.5±4.0	100	99

*mg L⁻¹

Table 9. Summary of uncertainty

	BND (10ng)	Sample	Fortified I (20ng)	Fortified II (50ng)	Fortified III (100 ng)
Concentration Se ($\mu\text{g L}^{-1}$) (\bar{x})	9.9	112.0	121.8	157.6	210.6
u_{rep}	1.2	2.00	1.90	1.90	1.70
u_{rec}	5.3	5.4	5.4	5.4	5.3
u_{cal}	6.1	6.2	6.0	6.0	5.9
u_{comb} (%)	8.1	8.2	8.3	8.3	8.4
u_{comb} ($\mu\text{g L}^{-1}$)	0.81	9.2	10.1	13.0	15.8
U	1.62	18.4	20.2	26.0	31.6

Table 10 Comparison of detection limits ($\mu\text{g L}^{-1}$), adsorption capacity (mg g^{-1}) and pre concentration factor (PF) obtained in the present method for Se speciation

Separation and pre-concentration technique	Analytical Technique	Se(IV) ($\mu\text{g L}^{-1}$)	Se(VI) ($\mu\text{g L}^{-1}$)	Adsorption capacity mg g^{-1}	PF	Ref.
SPE: PdNP	ETAAS	0.025	-	22.	100	(this work)
SPE: APDC on Diaion HP-2MG	ETAAS	0.01	-	5.2	100	(18)
SPE: CTAB-modifies alkyl silica	ICP-OES	-	0.10	5.0	27.6	(19)
SPE: TiO ₂ NP	HG-AFS	0.024	0.042	27.1	24	(20)
SPE: graphene	EDXRF	0.032	-	-	1013±15	(22)

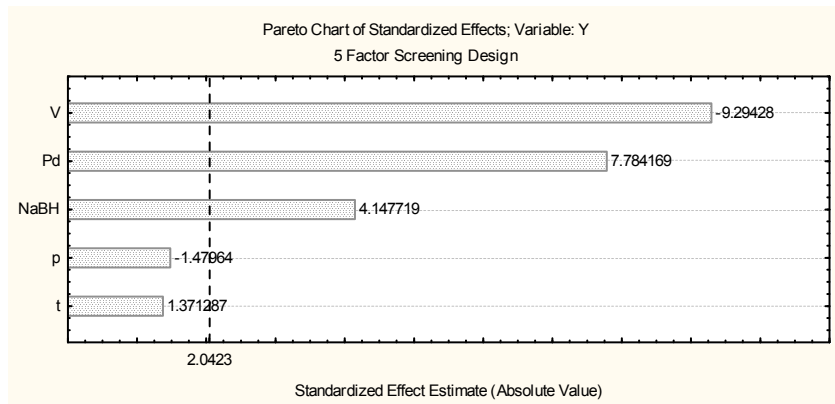


Fig. 1

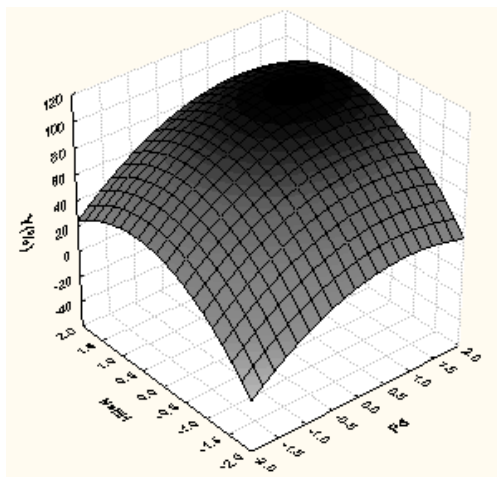


Fig.2.i

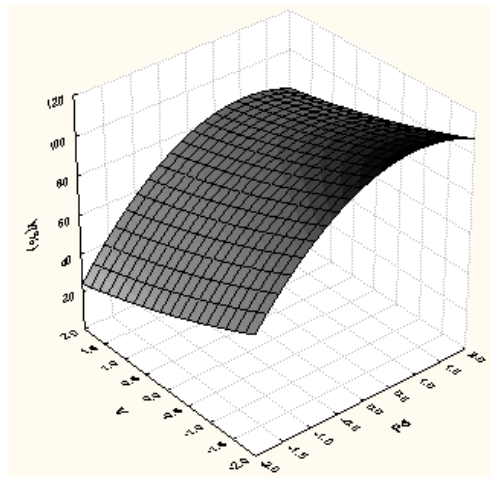


Fig. 2.ii

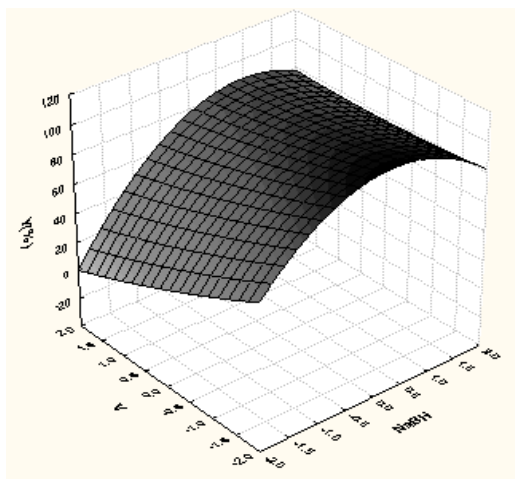


Fig. 2.iii

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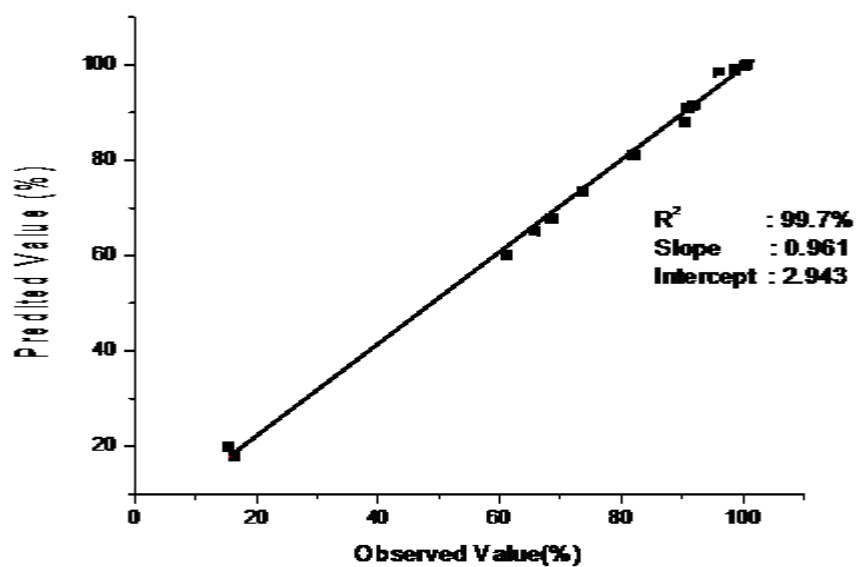


Fig. 3

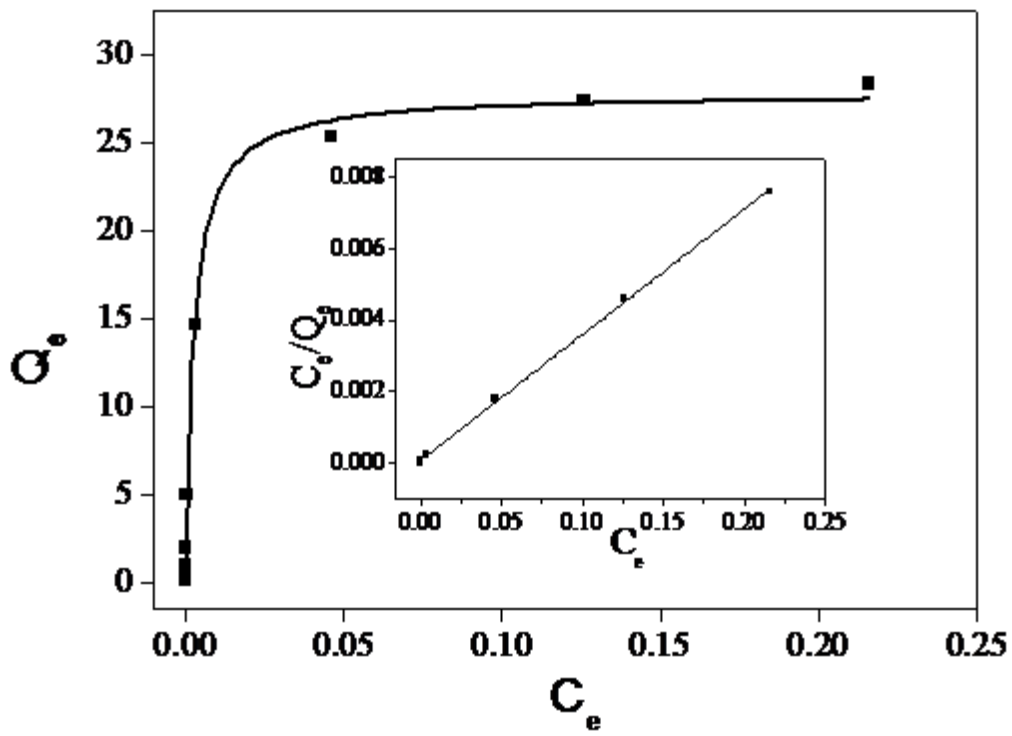
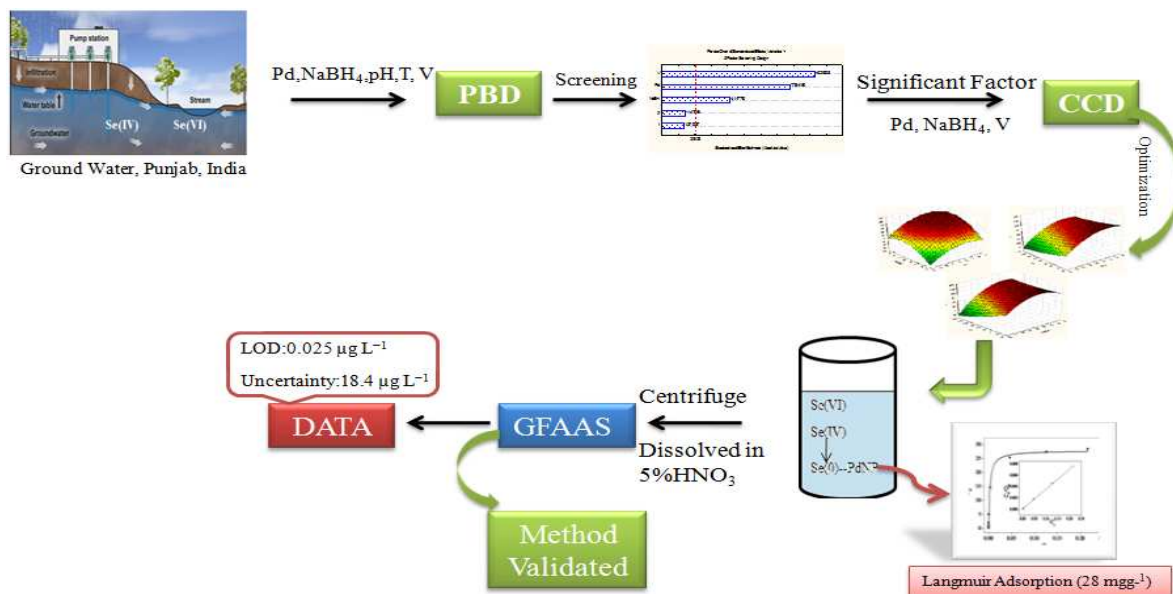


Fig. 4

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