Spectrophotometric and thermodynamic study for Separation and preconcentration of Trace Amounts of Selenium Species After CPE

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Abstract

A new prepared chelating agent namely, 2-(Benzimidazolyl azo-4- benzyl phenol) (BIABP) is used for the determination of selenium species in water, rice and vegetables samples by a combination of cloud point extraction (CPE) method and spectrophotometric techniques. The method involved a selective hydrophobic complex formation between selenium (IV) (and selenium (VI) after reduction with HCl under specified conditions) and (BIABP) at pH 5 which can efficiently be extracted in surfactant-rich phase of Triton X-114 and determined spectrophotometrically at λmax of 534 nm. All significant factors for selenium (IV) complex that impact the separation and determination steps were discussed in detail by one factor-at-a-time (OFAT) optimization. The interferences and thermodynamic studies are also considered. At optimum conditions, the enrichment factor of 58 fold was obtained for Se (IV), leading to detection limits of 2.55 ng Se (IV) mL⁻¹. The linear dynamic range, relative standard deviation (n=7 at 30 ng Se (IV), and the percent recovery were of 5-80 ng mL⁻¹, 1.07% and 97.83±1.40. The described method is sensitive, easy to apply and has a slight interferences thereby the determination of selenium species in water, rice and vegetables samples was performed

Keywords: Speciation of Selenium, 2-(Benzimidazolyl azo-4- benzyl phenol), water and foods, cloud point extraction, Visible Spectrophotometry

Introduction

Two research papers that recently published by us concerning the speciation analysis of iron[1] and vanadium [2] have paid insistence that we continue to design a new analytical eco-friendly method for the determination of ultra-trace amounts of selenium species using a new prepared chelating agent. The main reasons for the choice of selenium in this study lay in its importance as an essential element for plant,
animal, and human body, but at high concentration it can become toxic and the range between the concentration in which selenium is essential and toxic is very narrow [3]. In addition, there are so scanty works published in chemical literature concerning selenium speciation using CPE coupling with instrumental techniques. Selenium exists in the environment and biological materials as inorganic species (selenite \( \text{SeO}_3^{2-} \), selenate \( \text{SeO}_4^{2-} \)) and organic species seleno amino acids, Selenomethionine and very complex seleno proteins [4]. Most drinking water contain concentrations of selenium but the most important inorganic species of selenium in water are selenate (\( \text{SeO}_4^{2-} \)) and selenite (\( \text{SeO}_3^{2-} \)) and the levels of selenium in surface water and ground water ranged from 0.06 µg/L to about 400 µg/L [5]. Therefore, the American environmental protection agency (USEPA) specified that the permissible level of Se (total) in water is 10 µg L\(^{-1} \); as a consequence the concentration of individual species is much lower [6]. In foods the Se content is highly variable and strictly depends on the geographic where food is produced. It depends on the amount of selenium in the soil and several other factors [7]. In animal tissues and plant, selenium is found mostly bound to proteins. Therefore, the most important food sources of selenium are seafood and meats (0.3–50 mg/kg), due to their high protein contents, and cereals (0.1–10 mg/kg), because they tend to be consumed in large amounts than recommended [8]. In the light of the above information, the determination of Se species is of prime importance in order to assess the level of its bioavailability, essentiality and toxicity.

Direct determination of Se speciation at low concentration is difficult by using spectrometric techniques owing to lack of sensitivity. Thus analyte pre-concentration is a must for its detection. Many separation/ pre-concentration methods have been used for the determination of selenium species like solid-phase extraction(SPE), dispersive liquid–liquid micro extraction (DLLME), co-precipitation and hollow fiber liquid phase microextraction (HFLPME) in combination of different spectrometric techniques such as electrothermal atomic absorption spectrometry (ETAAS), indicatively coupled plasma-optical emission spectrometry (ICP-OES) and electrothermal vaporization-indicatively coupled plasma–mass spectrometry (ETV-ICP-MS [9-14]. Cloud point extraction (CPE), on the other hand, is attracting attention as a green analytical technique because it circumvents the use of volatile organic solvents which have a negative impact on human health in addition to other characteristics features of this extraction methodology. However, few studies have
appeared in the chemical literatures reflect the applications merit of CPE coupled with atomic spectrometers for selenium extraction and preconcentration from water, saline water and food matrices [3,15-24]. Most of these methods use sophisticated instrumentation and commercial chromogenic reagents which are most likely not available in all laboratories and expensive as well.

In this work, we present a simple, cheap and highly sensitive method based on combination of CPE with visible spectrophotometry using a prepared chromogenic reagent in our laboratory. The method involved a hydrophobic complex formation between Se (IV) and 2-[(Benzoimidazolyl) azo]-4-benzyl phenol (BIABP) at pH 5 which can efficiently be extracted in surfactant-rich phase of Triton X-114 and determined spectrophotometrically at λ_{max} of 534 nm. Whilst Se (VI) is determined by difference between total Se and Se (IV).

**Experimental**

**Apparatus**

Two spectrophotometer systems were used in this study namely, a PG Instrument T80+ UV/Vis spectrometer (England) and UV-7804C (China) equipped with a 10-mm quartz cell, for recording of absorption spectra of the complexes formed and absorbance measurements respectively. A double-beam Atomic Absorption Spectrophotometer novAA-400(Analytic Jena, Germany) equipped with graphite furnace and provided with Ultra fast background correction using a Deuterium lamp and vanadium hallow cathode lamp (operated at 6 mA) as the radiation source at the wavelength of 196.0 nm with 0.5 nm spectral band pass was used for determination of Se species The cloud point temperature of the surfactant was monitored via using a microprocessor-controlled water bath WB 710 model with temperature accuracy of ±0.3°C at 37 °C (OPTIMA, Japan). equipped with combined electrode was employed for solution pH during the optimization and measurement steps.

**Materials and Reagents**

The chemicals used in this work including, a hydrochloric acid, sodium hydroxide, acetic acid and sodium acetate (BDH, England), ethanol (GCC, England sodium selenate and sodium selenite (Fluka Germany), and Triton X-114 (ACROS ORGANICS, New Jersey, USA) were used as received without any further
purification. Doubly distilled and/or deionized water used throughout. The stock solutions (1000 μg mL\(^{-1}\)) of Se (IV) and Se(VI) ions were prepared by dissolving 0.109 g (Na\(_2\)SeO\(_3\)) in 50 mL distilled water and 0.119 g of (Na\(_2\)SeO\(_4\)) in 50 mL distilled water, respectively and diluted standard solutions of each metal ion were daily prepared by serial dilution of the stock solutions. A 10% (v/v) of Triton X-114 was prepared by diluting 10 mL of concentrated solution to 100 mL with water in a 100 mL volumetric flask. The stock solutions (1x 10\(^{-2}\)M) of ligand (BIABP) were prepared by dissolving appropriate amounts in ethanol. The pH buffer solutions in the range (2-7) were prepared by mixing different volumes of 0.1 mol L\(^{-1}\) of acetic acid and sodium acetate in 1L.

**General CPE Procedure**

To an aliquot of 1 mL of a solution containing known amount Se (IV) standard or sample solution, 0.5 mL of 1.0x10\(^{-2}\) mol L\(^{-1}\) (BIABP), 1.0 mL of acetate buffer solution at pH = 5.0, 0.2 mL of Triton X-114 (10%) were mixed in a 10-mL volumetric flask and diluted to mark with deionized water. The contents of the flask were transferred into a 10 mL centrifuging tube and the phase separation was induced by heating the contents in a water bath at 60 ºC for 10 min. For Se (IV) the separation of the phases was accelerated by centrifuging at 4000 rpm for 10 min. The viscosity of the surfactant-rich phase was enhanced by cooling the system in an ice-bath for 5 min. After decantation, the surfactant-rich phase that remained adhered to the tube was dissolved with a 3 mL of 0.1M nitric acid in ethanol and the concentration of the Se (IV) ions was determined spectrophotometry at \(\lambda_{\text{max}}\) of 534 nm. For Se (VI), the content was reduced to Se(IV) by adding 2 mL 4M HCl, and the solution was then heated in a water bath (80ºC) for 10 min, then the above-mentioned CPE procedure was followed. The Se (VI) content was calculated by subtracting Se (IV) from the total amount of Se. Blank experiments were carried out using the same procedure without Se added.

**Preparation of samples**

**Water** [25]: An aliquot of (1 ml) of water sample was treated with 0.5 ml of 1M NaOH and 0.5 mL of 0.2 M EDTA. The solution was mixed and centrifuged to remove any precipitate formed. The centrifuged was transferred to a 50 mL calibrated
flask and its selenium content was determined according to the general CPE procedure.

Vegetables and Rice: Three vegetables samples (potato, spinach, carrot) were collected from local markets and subjected to the procedure described by Ulusoy et al [26] with minor modification. Vegetable samples were carefully washed; the edible parts were cut and crushed, and finally frozen at 20°C. Then the samples were air-dried under uncontaminated condition. The dried samples were crumbled and pulverized with a mill. 1.0000 g of each fine powder sample was transferred into a glass beaker and digested with 3 mL of a mixture of HNO₃–H₂O₂ to volume ratio of 5:1 on a hot plate at a fairly low temperature till complete digestion. The content was cooled to room temperature and the solution was made to 50 mL with deionized water in 50 mL volumetric flask. An aliquot (1 mL) of the sample solution was taken, CPE procedure was followed and the amount of two species was determined spectrophotometrically.

1.00 g of each fine powder of rice sample was transferred into a glass beaker and treated with a mixture of HNO₃–H₂SO₄ (9:1) on a hot plate at a fairly low temperature till complete digestion. The content was cooled to room temperature and the solution was made to 50 mL with deionized water in 50 mL volumetric flask. An aliquot (1 mL) of the sample solution was taken. CPE procedure was followed and the amount of the two species was determined spectrophotometrically.

Results and Discussion

Absorption Spectra

The absorption spectra of the reagent (BIABP) solution and its complex with selenium (IV) at pH 5 in surfactant-rich phase against blank solution prepared under similar conditions were showed in Figure 1. It was observed that the absorption maximum (λ_max) of Se-(BIABP)_2 complex occurs in visible region at 534 nm, while the reagent (BIABP) solution alone displays an absorption maximum at λ_max of 422 nm. Therefore a wavelength maximum at 534 nm for the complex was used throughout this work.
Figure 1. Absorption spectra (a) Reagent (BIABP) = 5 x 10^{-4} M (b) Se (IV) - (BIABP) complex, Se (IV) = 60 ng mL^{-1}.

The reagent (BIABP) reacts with Se (IV) at pH 5 forming pink complex and the absorbance reached its maximum within 5 min and remained stable for at least 24 h. The mole ratio (Figure 2) was used to study the stoichiometry of Se (IV) - BIABP complex and confirmed by Job’s method (Figure not shown) that the composition of the pink complex was of 1:2 (M: L) at pH 5.0.

Figure 2 Mole ratio of complex between Se (IV) and (BIABP) in buffer solution at pH= 5

The stability constant \( (K_d) \) of the complex could be determined by using data from the molar ratio (Yoe-Jones plot) or continuous variation method (Job’s plot) by assume that only single complex is present. The calculation was carried out according to the procedure adopted elsewhere [27]. The average stability constant \( (K_d) \) of Se (VI)-BIABP complex in both methods was of 1.85x10^8 L^2 (mol^{-1})^2 at 534 nm. On the basis of above results, the
most probable structure of the complex formed between Se (VI) and BIABP at pH 5 is (BIABP)$_2$Se$^{IV}$ as displayed in Figure 3.

![Figure 3. The probable structure of the complex formed between Se (VI) and BIABP at pH 5.](image)

**Optimization of CPE Procedure**

In order to achieve the best sensitivity of the method and maximum extraction efficiency for the extraction of selenium species, the effect of different analytical factors which impact the CPE procedure for the two species, such as, solution pH, reagent concentration, non-ionic surfactant amount, temperature and time were investigated and optimized using a classical OFAT optimization.

**Effect of pH**

The pH of solution is one of the most important factors affecting the formation of complexes and their subsequent extraction, in order to evaluate the effect of pH on absorbance of complex formation of Se (IV) with (BIABP) to ensure that the reaction between metal ions and chelating molecule has sufficient hydrophobicity and subsequent extraction into the small volume surfactant-rich phase. The influence of pH on absorbance signals at (534) nm for the formation of the Se(IV)- BIABP complex in Triton X-114 medium was studied by varying the pH from 2 and 8. The results are shown in Figure 4. It was observed that the absorbance increased with increasing pH and reached a maximum at pH =5 for on the formation of Se (VI) -BIABP complex. Thereafter, it suddenly decreases at high pH which may result in dissociation of complex and in incomplete extraction in micelle due to the shifting in the formation reaction toward left. Consequently pH of 5 selected as the optimal for complete formation of Se (VI) -BIABP complex.
Figure 4. Effect of pH on the formation of Se (VI) -BIABP complex by CPE.

Effect of the (BIABP) Concentration

In separation and preconcentration by CPE, ligand selected with requirement that formed quickly, derived complex with sufficient hydrophobicity and quantitatively with the least possible excess. The effect of the (BIABP) concentration was investigated by measuring the absorbance signal according to the general CPE procedure of solution containing 40 ng mL\(^{-1}\) Se(IV) and varying volume from 0.1 to 1.0 mL of 1 x 10\(^{-2}\) mol L\(^{-1}\) (BIABP). The analytical responses increase rapidly as the volume of (BIABP) increases and reach maximum up to 0.5 mL and decreases thereafter with further increase in the chelating agent indicating that any excessive amount of chelating reagent was not necessary (Figure 5). Consequently, 0.5 mL of 1 x10\(^{-2}\) mol L\(^{-1}\) of (BIABP) was chosen as optimum for Se (IV) species.

Effect of Triton X-114 Amount

The effect of the surfactant amount on CPE of 10 ml containing 40 ng Se (IV), 0.5 ml (1x10\(^{-2}\)) BIABP and varying amount of TX-114 from 0.1-0.5 mL was studied and the results showed in Figure 6. It was shown that the absorbance signal increases and reach maximum at 0.2 mL 10% Triton X-114 and decreases suddenly thereafter.
Figure 5. Effect of (BIABP) concentration on the determination of Se (IV) by CPE.

Figure 6. Effect of Triton X-114 amount on the CPE of Se (IV) complex by CPE.

At low amount of surfactant the absorbance for ion complex is low may be related to the inadequacy of the volume of the surfactant rich phase to entrap the (Se-BIABP) complex quantitatively, while at higher amount of surfactant the extraction efficiency is low probably due to the increase in surfactant rich phase volume at which the analyte become more diluted. As a result, 0.2 mL of 10% (v/v) Triton X-114 was used as the optimum concentration for selenium for obtaining high sensitivity for ion complex.
Effect of the Equilibrium Temperature and Time

Since extraction by CPE is performed under isothermal condition and to ensure phase separation and preconcentration of an analyte efficiently. The effect of the equilibrium temperature was examined by taking aqueous phase containing 40 ng mL\(^{-1}\) Se (IV) and keeping other parameters at optimum conditions. The temperature was varied from (30-80) °C at 10 min. It was observed from (Figure 7) that the highest absorbance signals were obtained when temperature at 60 °C. Thus, an equilibration temperature of 60 °C for maximum extraction of Se (IV) was chosen as optimal. On the other hand, it was noticed that the incubation time of 10 min was sufficient for the maximum absorbance of Se (IV) ion as showed in Figure 8.

![Figure 7. Effect of temperature on the extraction Se (IV) complex by CPE.](image)

![Figure 8. Effect of time on the CPE of Se (IV) complex by CPE.](image)
The effect of centrifugation rate and time was also taken into account on extraction efficiency. A centrifuging time of 10 min at 4000 rpm was selected for the entire CPE procedure as being optimum and beyond this time no confirmation was observed for improving extraction efficiency.

Thermodynamic study

Thermodynamic parameters for the extraction of Se (VI) complex during the cloud point using Triton X-114 as a mediated extracting agent at different temperatures were determined. The equilibrium constants (K_{ex}) at the selected temperature were calculated from equation below and the results are shown from Table 1.

\[
K_{ex} = \frac{D}{[M^{n+}]_{aq}[R]_{org}^n} \quad (1)
\]

where \(D\), \([M^{n+}]_{aq}\) and \([R]_{org}^n\) are distribution ratio, metal ion concentration in aqueous phase and reagent concentration in organic phase respectively.

| T °C | 30  | 40  | 50  | 60  |
|------|-----|-----|-----|-----|
| T °K | 333 | 323 | 313 | 303 |
| 1/T  | 3.300×10^{-3} | 3.194×10^{-3} | 3.095×10^{-3} | 3.300×10^{-3} |
| K_{ex} | 0.6×10^{9} | 0.8×10^{9} | 1.3×10^{9} | 2.1×10^{9} |

These thermodynamic equilibrium constants (K_{ex}) are actually represents all equilibrium constants that affect the separation process [28]. From the results in Tables 1, log K_{ex} gave straight line relation against \(1/T \cdot K\) (Figures 9), and from which the slope of line, the enthalpy of extraction (\(\Delta H_{ex}\)), Gibbs free energy (\(\Delta G_{ex}\)) and entropy (\(\Delta S_{ex}\)) were calculated for CPE method from the relationships shown in equations 2 (Van’t Hoff equation [29]), 3 and 4.

\[
\frac{\Delta \log K_{ex}}{\Delta 1/T} = -\frac{\Delta H_{ex}}{2.303 R} \quad (2)
\]

\[
\Delta G = -2.303RT \log K_{ex} \quad (3)
\]

\[
\Delta G = \Delta H - T\Delta S \quad (4)
\]
where R is the gas constant, T is the absolute temperature. The activity coefficients for other components are assumed to be constant under the experimental conditions [30]. The slopes equal to $-\Delta H / 2.303R$.

![Figure 9. Effect of the equilibrium extraction temperature on Se (IV) complex formation by CPE](image)

On the basis of Eq (2) and the slope of Figure 9, $\Delta H$’s are calculated to be (35.91) KJ mol$^{-1}$ for Se (IV) complex extraction (Table 2), indicating that the extraction reactions by CPE process are endothermic. This parameter reflects a high efficiency of the extraction process of the complexes was achieved thermodynamically into the surfactant-rich phase. The values of Gibb's free energy ($\Delta G_{ex}$) and entropy change ($\Delta S_{ex}$) were obtained at different temperatures for all extraction reaction from equations 2 and 3 as presented in Table 2.

| $^\circ$T (ºK) | $\Delta H_{ex}$ (KJ mole$^{-1}$) | $\Delta G_{ex}$ (KJ mole$^{-1}$) | $\Delta S_{ex}$ (J mole$^{-1}$K$^{-1}$) |
|----------------|-------------------------------|---------------------------------|---------------------------------|
| 303            | 35.91                         | - 50.759                        | 286.036                         |
| 313            |                               | - 53.391                        | 285.309                         |
| 323            |                               | - 56.400                        | 285.789                         |
| 333            |                               | - 58.990                        | 283.213                         |

$\Delta G_{ex}$ was found to be negative in all cases and its value increased approximately with temperature, indicating the extraction process is a spontaneous phenomenon because the complex transportation and surfactant phase formation are synchronized processes.
occur at the same time. Thus the more negative value of $\Delta G_{ex}$, the large spontaneous process is. Similarly, $\Delta S_{ex}$ of the complex calculated showing the positive values of $\Delta S_{ex}$ which proved that the solubilized complex molecules are organized in more random fashion during extraction process. Consequently, at optimum temperatures, the micelles are quantitatively agglomerated with distinct lose of water so that they accumulate in surfactant-rich phase and the separations of phases occur due to the influence of internal energy of the micelles[30].

**Calibration curve and Statistical Treatments**

Under the optimized conditions, a series of standard Se (IV) solution ranging from 5-80 ng mL$^{-1}$ was taken and subjected to general CPE procedures in order to test the linearity of the method. The analytical figures of merit are summarized in Table 3. The statistical evaluation for calibration graph has shown that a strong correlation between absorbance and Se (IV) concentration may exist ($R^2$=99.91). On the other hand, the analysis of variance (ANOVA) also proved the linear regression equations $[y= (0.0071 \pm 0.000188)x+0.0374 \pm 0.00899]$ was statistically valid (Table 4). This because of the ratio ($MS_{reg}/MS_{err}$) for 1and 7 DOF, larger than critical values (F17 = 5.987 at 95% CI), indicating that the prediction based on the regression is satisfactory. The limit of detection (LOD=2.55 ng mL$^{-1}$) and limit of quantitation (LOQ=8.49 ng mL$^{-1}$) obtained for Se (IV) with the prepared ligand (BIABP) by developed CPE-Spectrophotometry were based on the standard deviation of the response and the slope of the calibration curve using the following equations; LOD = 3 $\sigma_B/s$; LOQ = 10 $\sigma_B/s$, where ($\sigma_B$) is the standard deviation of the calibration plot and ($s$) its slope. The ratio of the slope of calibration curves obtained with and without CPE, giving enrichment factor of 58 for Se (IV). This revealed that the prepared ligand beside CPE-spectrophotometry gave satisfactory analytical figures of merit which is more comparable with those obtained by previous studies using commercial ligands and sophisticated techniques [9-14].

Concerning the limit of detection for Se(VI) ,our finding was of 8-fold, 4-fold, and 2.5-fold better than that obtained by Güler et al[20] , Tadayon and Mehrandoost[15],Oktariza et al[16] and Ulusoy [23] respectively, whom used CPE-UV-Vis spectrophotometric technique for the determination of Se species in various samples. It was approximately in harmony with that obtained by Wen et al [19] who used CPE-ICP-MS for the detection se (VI) in tea. However, our LOD observed that
Table 3 Method validation of the determination of Se (IV) - (BIABP) using the proposed method

| Parameter                                      | Se (IV)                          |
|------------------------------------------------|----------------------------------|
| Regression equation                            | y=0.0071x + 0.0374               |
| Correlation coefficient(r)                     | 0.9995                           |
| Correlation of Determination (R²%)             | 99.91                            |
| Std. dev. of regression line (s_y/x)           | 0.005921                         |
| C.L. for the slope (b± ts_b) at 95%            | 0.0071 ± 0.000188                |
| C.L. for the intercept (a± ts_a) at 95%        | 0.0374 ± 0.00899                 |
| Concentration range (ng mL⁻¹)                  | 5-80                             |
| Limit of Detection ( ng mL⁻¹)                  | 2.55                             |
| Limit of Quantitation (ng mL⁻¹)                | 8.49                             |
| Sandell’s sensitivity (μg cm⁻²)                | 1.399 x10⁴                       |
| Molar absorptivity(L.mol⁻¹ .cm⁻¹)              | 1.23 x10⁸                       |
| Composition of complex (M:L)*                  | 1 : 2                           |
| RSD(n=7)% at 30 ng mL⁻¹ Se(IV)                 | 1.07                            |
| Recovery% at 95% C.I                           | 97.83±1.40                      |
| Enrichment factor(PF)*                         | 70                              |
| Enrichment factor(EF)**                        | 58                              |

*Calculated by dividing the volume of original solution to the volume of surfactant-rich phase obtained, ** Calculated as the ratio of slope of calibration curve obtained by CPE to that obtained without pre-concentration

Table 4 Analysis of Variance table of regression line for Se(IV).

| Source                  | dof | SS    | MS    | F     | P    |
|-------------------------|-----|-------|-------|-------|------|
| Regression              | 1   | 0.2871| 0.2871| 337.764| 0.00 |
| Residual Error          | 7   | 0.0060| 0.00085|       |      |
| Total                   | 8   | 0.2931|       |       |      |

it was not good as with those obtained by dos Santos Depoi and Pozebon [21], and Yingjie et al [22] whom used CPE with more sophisticated instrumental techniques such as HG-ICP-OES and ETA-ICP-MS respectively, for the determination of this metal species in various water samples. However, by considering the detection limit of Se (VI) ions is 2.55 μg/L in aqueous solution and 1 g of sample in 50 mL, the method detection limit is 0.128 μg /g. This finding has encouraged the application of
the proposed method successfully in the estimation of Se ion species in water, rice and vegetable samples to test its applicability and reliability.

**Precision and Accuracy**

Because the commercial certified reference material (RCM) sample that contains known selenium was unavailable and in order to investigate if the proposed method is subjected to systematic error, the accuracy in the term of recovery percent was studied by spiking of 20, 30, 40 ng mL\(^{-1}\) Se (IV) and 15, 30, 40 ng mL\(^{-1}\) Se(VI) to an appropriate amount of rice sample solution prepared previously and then the same steps were followed by the general CPE procedure. The results were tabulated in Table 5, indicating that there is no highly significant systematic error in case of the presence other constituents in rice sample.

**Table 5. Accuracy of the proposed method**

| Amount metal ion taken (ng mL\(^{-1}\)) | Amount metal ion found (ng mL\(^{-1}\)) | Rec. (%) | E\(_{rel}\) (%) | Mean Rec % | x±t.s /√n |
|----------------------------------------|----------------------------------------|----------|----------------|------------|----------------|
| 20                                     | 19.7                                   | 98.5     | -1.5           | 97.83      | 97.3±1.40      |
| 30                                     | 29.3                                   | 97.6     | -2.4           |            |                |
| 50                                     | 48.7                                   | 97.4     | -2.6           |            |                |

| Amount metal ion taken (ng mL\(^{-1}\)) | Amount metal ion found (ng mL\(^{-1}\)) | Rec. (%) | E\(_{rel}\) (%) | Mean Rec % | x±t.s /√n |
|----------------------------------------|----------------------------------------|----------|----------------|------------|----------------|
| 15                                     | 14.5                                   | 96.7     | -3.3           | 96.7       | 97.2±1.2       |
| 30                                     | 29.3                                   | 97.7     | -2.3           |            |                |
| 40                                     | 38.9                                   | 97.2     | -2.8           |            |                |

**Interference Study**

The effect of most diverse ions expected in the samples matrix on the determination of 40 ng mL\(^{-1}\) Se (IV) solution was also investigated following the general CPE procedure. The results indicated that some of metal ions like K(I), Na(I), Ca(II), Mg(II), Ni(II), Cd(II), Zn(II), Hg(II), and Fe(II) have no appreciable effect on the Se response, while the other metal ions such as Co(II), Cu(II), V(IV) and Cr(III) have exceeded the allowable limits of interferences for Se(IV) as shown in Table 6.
Table 6. Effect of interfering ions on the absorption signal of Se (IV) [40 ng mL\(^{-1}\), Abs= 0.320] by proposed method

| Interferent ion | A unit | ΔA | E\(_{rel}\)% | Interferent ion | A unit | ΔA | E\(_{rel}\)% |
|-----------------|-------|----|-------------|-----------------|-------|----|-------------|
| Na(I)           | 0.323 | 0.003 | 0.9        | Cd(II)          | 0.335 | 0.150 | 4.6        |
| K(I)            | 0.325 | 0.005 | 1.5        | Zn(II)          | 0.312 | -0.008 | -2.5      |
| Ca(II)          | 0.329 | 0.009 | 2.8        | Hg(II)          | 0.325 | 0.005 | 1.5        |
| Mg(II)          | 0.326 | 0.006 | 1.8        | Fe(II)          | 0.334 | 0.014 | 4.3        |
| Co(II)          | 0.350 | 0.030 | 9.3        | V(IV)           | 0.342 | 0.022 | 6.8        |
| Ni(II)          | 0.331 | 0.011 | 3.4        | Cr(II)          | 0.347 | 0.027 | 8.4        |
| Cu(II)          | 0.349 | 0.029 | 9.0        |                 |       |       |            |

Experiments have also been carried out in an attempt to eliminate the Interference effects on Se (IV) ions by using several masking agents such as oxalic acid, citric acid, tartaric acid and sodium fluoride. The experiments have shown that the interference effect of the above ions on Se (IV) absorbance signal was held efficiently by adding 1mL of 0.01M tartaric acid for masking Se (IV) from Co (II), Cu (II), Cr (III) and V (IV) ions.

**Determination of selenium species in real samples**

For further checking of the applicability and reliability of CPE- spectrophotometric method, the paired t-test was applied for comparison of means between the proposed method and sensitive ETAAS method for selected samples. The statistical analysis of result shown in Tables 7 to 9 proved that there was no significant difference at 95% confidence level for the determination of Se (IV) and Se (VI) by both methods.

**Conclusions**

An eco-friendly method was proposed for speciation analysis of selenium in various matrices. The synthesized organic reagent (BIABP) proved to be selective for Se (VI) species in mild acidic medium which enhanced the analytical capability of the combined CPE with visible spectrophotometry in terms of sensitivity, detection limit and reliability. The proposed method was simple and cheap compared to the
sophisticated methods that reported in chemical literature. The drawback of the established method couldn’t determine the two selenium species simultaneously in the

### Table 7. Spectrophotometric determination of vanadium species in water samples

| Sample    | Proposed method | ETAAS method | Paired t- test* |
|-----------|-----------------|--------------|-----------------|
| Tap water |                 |              |                 |
| 1         | 3.12±0.03       | 2.26±0.15    |                 |
| 2         | 1.78±0.11       | 1.72±0.09    | $\bar{x}_d=0.218$ |
| 3         | 2.11±0.35       | 1.95±0.12    | $S_d=0.316$     |
| 4         | 2.35±0.14       | 2.31±0.23    | $t_{cal}=1.68$  |
| 5         | 2.05±0.08       | 1.98±0.51    | $t_{crit}$ at 95% |
|           | 2.24±0.13       | 2.12±0.22    | $df_5=2.57$     |

### Table 8. Spectrophotometric determination of selenium species in rice samples

| Sample    | Proposed method | ETAAS method | Paired t- test* |
|-----------|-----------------|--------------|-----------------|
| Rice      |                 |              |                 |
| Iraqi 1   | 0.28±0.04       | 0.16±0.07    | $\bar{x}_d=0.047$ |
| Iraqi 2   | 0.23±0.10       | 0.21±0.04    | $S_d=0.049$     |
| Indian    | 0.31±0.09       | 0.27±0.10    | $t_{cal}=1.8$   |
| Vietnamese| 0.25±0.02       | 0.24±0.03    | $t_{crit}$ at 95% |
|           |                 |              | $df_3=3.18$     |

| Rice      |                 |              |                 |
| Iraqi 1   | 0.24±0.12       | 0.14±0.02    | $\bar{x}_d=0.0425$ |
| Iraqi 2   | 0.28±0.03       | 0.26±0.07    | $S_d=0.038$     |
| Indian    | 0.19±0.09       | 0.17±0.04    | $t_{cal}=2.2$   |
| Vietnamese| 0.25±0.01       | 0.25±0.02    | $t_{crit}$ at 95% |
|           |                 |              | $df_3=3.18$     |
Table 9. Specrophotometric determination of selenium species in vegetables

| Sample | Proposed method | ETAAS method | Paired t-test* |
|--------|----------------|--------------|---------------|
| Carrot | 0.21±0.13      | 0.19±0.09    | \( \bar{x}_d = 0.049 \) |
| Spinach| 0.84±0.28      | 0.79±0.31    | \( S_d = 0.02 \) |
|        |                |              | \( t_{cal} = 3.03 \) |
|        |                |              | \( t_{crit} at 95\% \) |
|        |                |              | \( df = 4.3 \) |
|        | 0.32±0.05      | 0.32±0.05    | \( 0.09\pm0.31 \) |
|        | 0.21±0.13      | 0.21±0.13    | \( 0.84\pm0.28 \) |
|        | 0.84±0.28      | 0.84±0.28    | \( 0.40\pm0.03 \) |
|        | 0.40±0.03      | 0.40±0.03    | \( 0.21±0.13 \) |
|        | 0.84±0.28      | 0.84±0.28    | \( 0.19±0.09 \) |
|        | 0.32±0.05      | 0.32±0.05    | \( 0.32±0.05 \) |

*The mean value and its standard deviation for three replicate measurements at 95% confidence level \( (\bar{x} \pm s/\sqrt{n}) \) **paired t-test = \( \bar{x}_d - \bar{x}_t \sqrt{n}/s_d \)

same solution because the prepared chelating agent was selective only to Se (VI). Therefore, the concentration of Se (VI) was computed by the difference between total Se and Se (VI) ions.

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