

## PAPER

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# Simple and useful method for determination of inorganic selenium species in real samples based on UV-VIS spectroscopy in a micellar medium

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A preconcentration and determination methodology utilizing the cloud point phenomenon was developed for the determination of inorganic selenium species using UV-VIS spectrophotometry. The cloud point extraction method was based on the selective formation of a complex between selenium(IV) ions and 4,5-diamino-6-hydroxy-2-mercapto pyrimidine (DAHMP). Then, the formed stable complex was extracted to a nonionic surfactant phase of Triton X-114. After phase separation was carried out via decantation, the surfactant-rich phase was diluted with 20% ethanol solution and the absorbance signal was measured at 458 nm using an UV-VIS spectrophotometer. Under the optimized conditions, linear calibration curves were obtained over a range of 20–1500 ng mL<sup>-1</sup> with detection and quantification limits of 6.06 ng mL<sup>-1</sup> and 19.89 ng mL<sup>-1</sup>, respectively. The relative standard deviation was 2.80% for 5 replicate measurements at 250 µg L<sup>-1</sup> concentration level. The proposed method is very versatile and economic as only a conventional spectrophotometer is used for the measurements. The method was validated by the analysis of two certified reference materials and a recovery test. Finally, the method was applied to the determination of the total Se amount in real samples including water samples and hen eggs containing selenium.

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## 1. Introduction

Trace elements occur at very low concentrations in environmental and biological samples. The determination and speciation of these elements are always known to be hard and tedious jobs. Especially, if a sample contains two or more species of the same element, speciation studies should be made more carefully. Speciation becomes more serious and critical when the analyses of natural samples are carried out. Thousands of compounds as impurities along with metal ions are present in the unknown samples. The concentrations of selenium species in real samples are generally lower than detection limits of common instrumental techniques such as spectrophotometry and atomic absorption spectrometry.<sup>1</sup>

Selenium is one of the essential dietary constituents in mammals.<sup>2</sup> Selenium protects from several heart diseases, prevents heavy metal toxic effects and helps support anti-carcinogenic activity.<sup>3</sup> Selenium has been shown to be both essential for life and toxic at levels a little above those required for health. Indeed, dietary levels of the desired amount of Se are in a very narrow range: consumption of food containing less than 0.1 mg kg<sup>-1</sup> of this element will result in its deficiency whereas dietary levels above 1 mg kg<sup>-1</sup> will lead to toxicity.

Selenium exists in biological systems in the form of inorganic species such as Se(IV) (SeO<sub>3</sub><sup>2-</sup>; selenite), Se(VI) (SeO<sub>4</sub><sup>2-</sup>; selenate) or selenides (e.g. HgSe), or as organic species with a range of molecular masses and charges, starting from the simplest MeSeH and ending at complex selenoproteins.<sup>4–6</sup>

There are a lot of studies on selenium speciation and each of them requires a dedicated analytical approach, especially in terms of the separation technique preceding the element- or molecule-specific detection. The selenium species with different physicochemical properties, present in biological systems, represent a challenge to the analyst. Because the toxic or essential properties of selenium are dependent on its species, this has stimulated the development of analytical procedures for the determination of Se compounds in biological and environmental samples. The absorption of selenium has been observed to be higher in organic compounds, hence, the knowledge of Se speciation in food is important for a better understanding of the detection of this metalloid. Many of the problems in selenium speciation analysis are associated with the low concentration of each species to be determined and the complexity of the matrix.<sup>7,8</sup>

The influences of the matrix components in real samples are an important problem in trace analysis. Atomic and molecular absorption methods are commonly used for the determination of selenium. However, these techniques are not sensitive enough for trace selenium species without any pre-treatment. In order to determine trace selenium species in real samples,

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traditional pre-concentration and separation methods have been used in the literature such as liquid–liquid micro extraction,<sup>9</sup> solid phase extraction,<sup>10</sup> ion-exchange<sup>11</sup> and co-precipitation.<sup>12</sup> These pre-concentration methods combined with instrumental analysis have frequently been used for the determination of selenium species in real samples.

As is known, hydride generation methods combined with liquid chromatography systems and a mass detector are the best approach for the correct and selective determination of selenium species, and some electroanalytical approaches can be used to carry out analysis of selenium species in certain matrices. However, if we consider the costs of these expensive instruments and the difficulties in some matrices, we need more simple methods including pre-treatment approaches. Spectrophotometric methods are popular because of their simplicity and costs for each analysis.

Cloud point extraction (CPE) has recently attracted 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. The combination of CPE with spectrophotometric methods, which are simple, selective, inexpensive and available in almost every research laboratory, has been presented as a useful analytical tool as an alternative to expensive and complicated techniques.<sup>13–18</sup>

The main purpose of this study was to develop a simple, sensitive and accurate method for the speciation analysis of inorganic selenium species in real samples. A new method for the direct determination of inorganic selenium species using UV-VIS spectrophotometry was developed in combination with CPE. The proposed method was successfully applied to determine the total amounts of selenium and Se(IV) in real samples.

## 2. Experimental

### 2.1. Instrumentation

Spectrophotometric measurements were performed using a UV-VIS spectrophotometer (Shimadzu, UV-Visible 1800, Japan) equipped with a 1 cm quartz cell. This spectrophotometer has a wavelength accuracy of  $\pm 0.2$  nm and a bandwidth of 2 nm in the wavelength range 190–1100 nm. A pH meter with a glass-calomel electrode (Selecta, Spain) was used to measure the pH values. A thermostatic water bath (Microtest, Turkey) was used to keep a constant temperature. A centrifuge (Hettich, Universal 120, England) was used for complete phase separation. A microwave digestion system (CEM Mars X6, USA) was used to dissolve and prepare the samples for analysis.

### 2.2. Reagents and standard solutions

All reagents used were of analytical grade. Ultra-pure water with a resistivity of 18.2 M $\Omega$  cm was used in all experiments provided by an ELGA (Flex III, U.K.) water purification system. All containers (glassware, PTFE bottles) were treated with diluted HCl solution and finally rinsed with deionized water prior to experiments. Stock solutions of Se(IV) and Se(VI) ions (1000  $\mu$ g mL<sup>-1</sup>) were prepared by dissolving appropriate amounts of sodium selenite and selenate (Merck) in water. A 0.02 mol L<sup>-1</sup> 4,

5-diamino-6-hydroxy-2-mercapto pyrimidine, DAHMP, (Sigma, St. Louis, MO, USA) solution was prepared by dissolving DAHMP in 0.001 mol L<sup>-1</sup> NaOH (Merck, Darmstadt, Germany). A citric acid solution of 0.1 mol L<sup>-1</sup> was prepared using reagent pure citric acid (Carlo Erba). The solutions of all surfactants [TritonX-114, PONPE 7.5, sodium dodecyl sulfate (SDS), cetyl pyridinium chloride (CPC)] (Sigma, St. Louis, MO, USA) were used without further purification. The solutions of 5% (w/v) nonionic surfactants were prepared by dissolving 5.0 g of surfactant in 100 mL of deionized water.

### 2.3. Pretreatment of samples

Sampling is the first and most important step for speciation studies and its design and implementation have a decisive influence on the final analysis results. Due to the low concentrations of the analyte species in environmental and biological samples, sampling should be performed very carefully. The sample preparation should be focused on obtaining all inorganic selenium species as Se(IV) owing to the selectivity of the developed method for Se(IV). A similar procedure in our previous study was used for sample preparation.<sup>19</sup> A microwave digestion system was used for sample preparation and Se(VI) was reduced to Se(IV) by adopting suitable experimental conditions. 1 g solid or 10 mL liquid of the sample was put into the microwave system and 10 mL of 3 mol L<sup>-1</sup> HCl was added to the tubes. The digestion program in Table 1 was applied to all samples. After digestion, the samples were neutralized using a few drops of ammonia until the acidity was eliminated and filtered using 0.45  $\mu$ m of filter paper. Finally, the proposed method was applied under optimized conditions.

### 2.4. The CPE procedure

In a typical CPE procedure, 10 mL of sample contained inorganic selenium ions in the range of 20 to 1500 ng mL<sup>-1</sup>, 3.0 mL of 0.1 M citric acid, and 1.25 mL of 0.02 mol L<sup>-1</sup> DAHMP. The samples were allowed to stand for 10 minutes in order to complete the reaction. Then, 1.5 mL of  $3 \times 10^{-3}$  mol L<sup>-1</sup> SDS, 0.8 mL of 5% (w/v) Triton X-114, and 2.50 mL of 20% (w/v) NaCl solution were added to a Falcon tube and diluted to the mark (50 mL) with ultra-pure water. The final solution was kept in a thermostatically controlled water bath at 45 °C for 15 min. Separation of the two phases was achieved *via* centrifugation for 5 min at 4000 rpm. The surfactant-rich phase (SRP) became viscous after the solutions were kept in a refrigerator for 15 min. Then, the aqueous phase was separated by inverting the tubes or using a Pasteur pipette if necessary. 1 mL of 20% ethanol

Table 1 Microwave parameters for digestion and reduction of samples

Amount of sample	1 g solid or 10 mL liquid	
Digestion reagent	10 mL of 3 mol L <sup>-1</sup> HCl	
Time and program	0–10 min	80 °C
	10–15 min	100 °C
	15–25 min	150 °C

solution was later added before the determination step to the surfactant-rich phase in order to reduce its viscosity and facilitate determination. The analytical signal (absorbance) of this solution was monitored at 458 nm in a micro quartz cell *versus* a blank.

### 3. Results and discussion

Fig. 1 shows the absorption spectra of the formed complex in the surfactant-rich phases against a blank for six different Se(IV) levels. As can be seen in Fig. 1, the absorbance of the complex at 458 nm gradually increases with increasing selenium concentration.

After all parameters (pH, concentrations of surfactants, ionic strength, incubation conditions, *etc.*) were optimized as explained in the following sections, the amount of selenium ions in samples can be measured using the calibration equation obtained from the experimental studies. The optimization parameters and studied ranges can be seen in Table 2.

#### 3.1. Effect of pH and buffer volume

The proposed pre-concentration procedure is based on a selective chemical reaction between Se(IV) ions and DAHMP. The course and efficiency of this reaction are fairly connected with the physical and chemical properties of the medium (pH, concentration of reagents, ionic strength, and temperature). In the CPE method, the pH plays a unique role first for the main reaction but also for the subsequent extraction procedure. The effect of pH on the CPE efficiency of Se(IV) was studied in the pH range 1.0–10.0 using different buffer systems. Based on literature studies, more acidic conditions were also tested such as 0.1 mol L<sup>-1</sup> solutions of HCl, acetic acid, sulfuric acid, citric acid and phosphoric acid. The experimental studies showed that the extraction yield is very low at high pH values due to negative conditions for the main reaction. Therefore, the experiments were focused especially on the acidic range. 0.1 mol L<sup>-1</sup> of hydrochloric acid, acetic acid, sulfuric acid, citric acid,

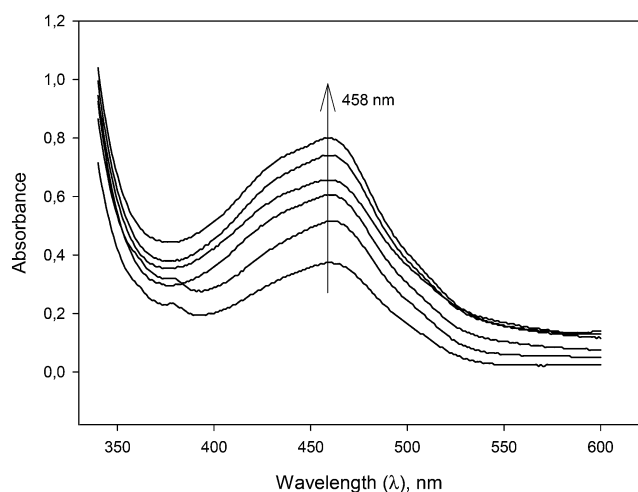


Fig. 1 Absorption spectra of Se–DAHMP complex at different concentrations of Se(IV).

Table 2 Optimization of cloud point extraction variables

Variable	Range tested	Selected value
Citric acid concentration (mol L <sup>-1</sup> )	0.001–0.015	0.010
DAHMP (mol L <sup>-1</sup> )	0.0000–0.0014	0.0006
Nonionic surfactant (% w/v)	0.000–0.100	0.080
SDS (mol L <sup>-1</sup> )	0.00000–0.00019	0.00009
NaCl (% w/v)	0.0–2.0	1.0
Incubation temperature (°C)	25–65	45
Incubation time (min)	0–60	10
Centrifugation time (min)	0–60	5

phosphoric acid, and some phosphate buffers were tried in order to supply the most appropriate medium for the reaction between Se(IV) and DAHMP. The results are illustrated in Fig. 2. As can be seen in the figure, the signal obtained with citric acid is higher than the others. The obtained signals with HCl and phosphoric acid are also acceptable, but the signal of citric acid is better than the others. In addition, the possible capacity of citric acid for complex formation with metallic species presents some advantages, such as citric acid can keep the pH constant like a buffer and also eliminates some interfering metal ions as chelating agents. The concentration of citric acid in the final solution was also studied and optimized. The results are shown in Fig. 3. As can be seen, the highest analytical signal for Se(IV) was obtained using 2.0 mL of 0.1 M citric acid in 50 mL of the final solution.

#### 3.2. Effect of DAHMP concentration on CPE

One of the main parameters in the CPE experiments is the concentration of the chelating agent. These reagents either act as complex forming reagents (mostly in metal determination), or they directly react with the target species before CPE as redox

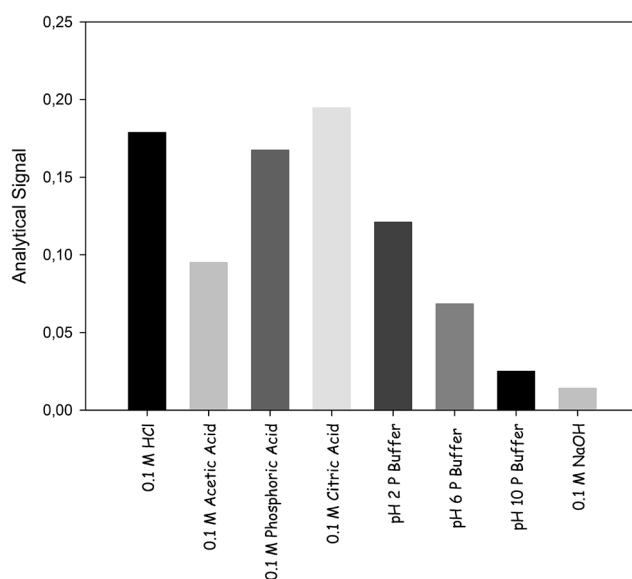


Fig. 2 The effect of different solutions on the reaction between Se(IV) and DAHMP.

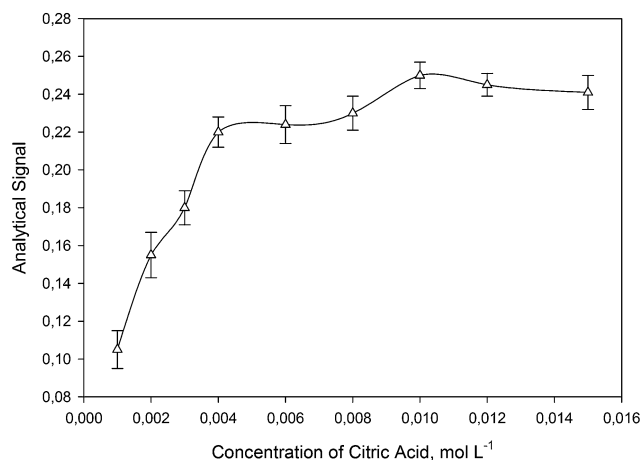


Fig. 3 The effect of citric acid concentration on the analytical signal.

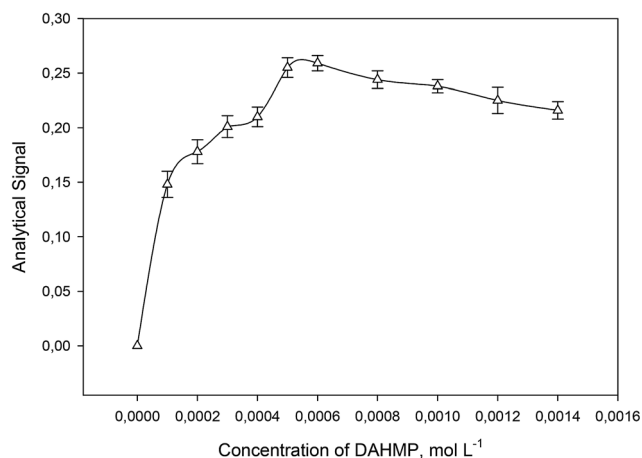


Fig. 5 The effect of DAHMP concentration on the analytical signal.

active compounds or condensation compounds (mostly in non-metallic or organic species determination).

As a result of a comprehensive literature search, we noticed that suitable and selective reagents for selenium species are generally aromatic compounds including  $-\text{NH}_2$  groups. In addition, the amino groups attached to the cyclic structure should neighbor each other (vicinal position) for a selective reaction. These compounds can be used for selective determination of  $\text{Se(IV)}$  ions due to a characteristic reaction. As a result of our pre-experiments, we obtained significant signals with DAHMP based on the selective reaction with  $\text{Se(IV)}$  in acidic media.

In the CPE experiments, if the formed complex is hydrophobic, it can be transferred very easily to the surfactant-rich phase (SRP). So, non-ionic complexes were targeted or aimed for in the CPE for a high extraction efficiency. Sometimes, ionic surfactants or secondary reagents can be added to the reaction medium in order to increase this effect.

Harrison *et al.* used a diamino compound called diamino naphthol (DAN) for selective  $\text{Se(IV)}$  determination.<sup>20,21</sup> They investigated the structure of the  $\text{Se(IV)}$ –DAN complex using several instrumental techniques. By considering the similarities between the structures of DAN and our compound (DAHMP), we can suggest a similar mechanism for the complex formation, as given in Fig. 4.

The effect of the DAHMP concentration in the final solution on the analytical signal was investigated in the range of 0–0.0014 M and the results are illustrated in Fig. 5. As can be seen, the signal intensity of  $\text{Se(IV)}$  strongly depends on the amount of DAHMP. With the increase in concentration of DAHMP, the

signal intensity initially increased, and the maximum signal intensity was achieved in the range of 0.0004–0.0008 M. Due to maximum sensitivity, a concentration of 0.006 M was selected as the optimal value for further studies.

### 3.3. Effect of nonionic surfactant concentration

Non-ionic surfactants (mainly polyoxyethylenated alkylphenols, from the Triton and PONPE series) are most widely used in CPE experiments. They are commercially available in high purity, stable, non-volatile, non-toxic and environmentally friendly. Surfactants, which have a cloud point temperature too high or too low, are not suitable for separation/pre-concentration. A successful CPE would be to maximize the enrichment factor by minimizing the volume of the surfactant-rich phase.<sup>22</sup>

PONPE 7.5 and Triton X-114 were preferred as the non-ionic surfactants due to their commercial availability and suitable structures. Therefore, the effect of the concentration of PONPE 7.5 and Triton X-114 on the analytical signal was investigated in the range of 0.00–0.10% (v/v). The results are shown in Fig. 6. As

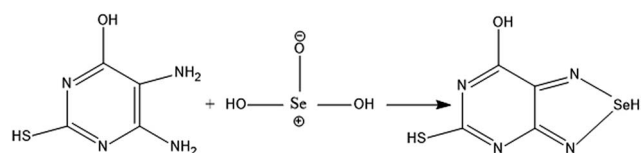


Fig. 4 Possible reaction mechanism of proposed method.

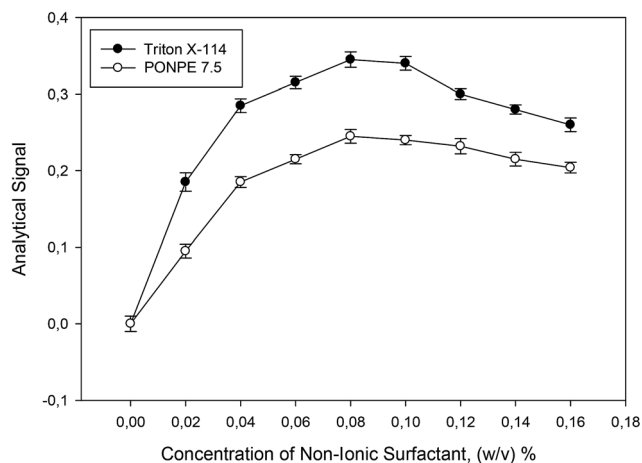


Fig. 6 The effect of concentration of non-ionic surfactants on the analytical signal.

can be seen from Fig. 6, the signals obtained for Triton X-114 are higher than those with PONPE 7.5 and the maximum absorbance value was achieved at a concentration of 0.08% (w/v). Therefore, this concentration was selected as suitable for the nonionic surfactant in subsequent studies.

### 3.4. Effect of ionic surfactant concentration

The ionic surfactants play an important role in the CPE experiments as described in the previous section. They keep the ionic equilibrium in solution using opposite charges according to the target compounds. In addition, they can increase the extraction efficiency by forming mixed surfactant systems with non-ionic surfactants.

The variation in the analytical signal as a function of the concentration of two ionic surfactants (CPC and SDS), which were chosen as auxiliary ligands, is presented in Fig. 7. One of the selected surfactants is cationic (CPC) and the other one is anionic (SDS). According to the structure and charge of the formed complex, one of them should be able to increase the signal by means of re-arrangement of the species in solution. The dependence of CPE on the ionic surfactant concentration was examined in the range of  $0\text{--}1.9 \times 10^{-4}$  M for each surfactant. As can be seen in Fig. 7, the analytical signal increased in the presence of SDS while it decreased with CPC. The extraction of Se(IV) increases up to an ionic surfactant concentration of  $9.0 \times 10^{-5}$  M, and gradually decreases at higher concentrations. Therefore, an ionic surfactant concentration of  $9.0 \times 10^{-5}$  M was selected as the optimum condition for subsequent studies.

### 3.5. Effect of equilibration temperature and incubation time

Optimal equilibration temperature and incubation time are necessary to complete the reactions and achieve an easy and efficient phase separation. The shortest equilibration time and the lowest possible equilibration temperature are always wanted for quantitative extraction and efficient phase separation. In other words, the formed complex can decompose at high temperatures and the stability of the complex can change with

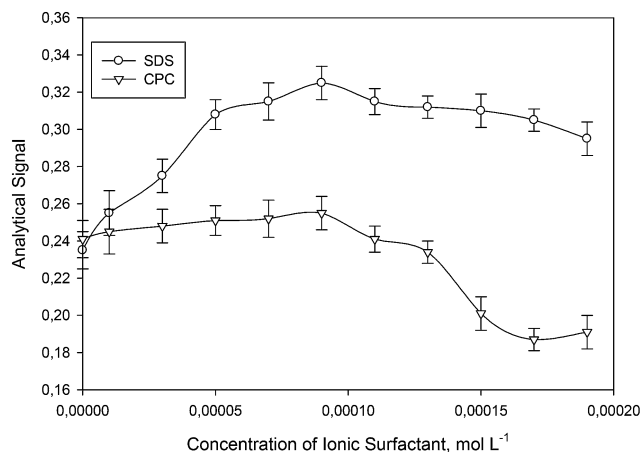


Fig. 7 The effect of concentration of ionic surfactants on the analytical signal.

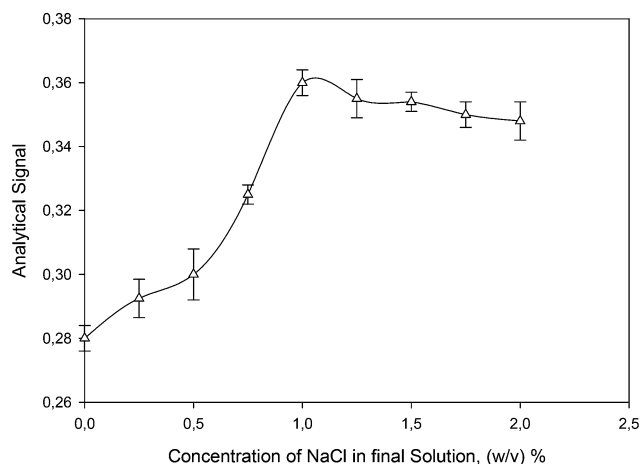


Fig. 8 The effect of concentration of ionic salt (NaCl) on the analytical signal.

time. The dependency of the extraction efficiency on equilibration temperature and time was studied in the ranges 25–65 °C and 0–60 min, respectively. The results showed that an equilibration temperature of 45 °C and a time of 10 min were adequate and enough for quantitative extraction.

### 3.6. Selection of the diluting agent for the surfactant rich phase

The surfactant rich phase (SRP) is very viscous to be applied to the detection system. In the CPE experiments, the SRP should be diluted with a suitable solvent according to the detection equipment and analyte. UV-VIS spectrophotometry may be considered as compatible equipment for CPE experiments. We have a lot of options to select a solvent for the dilution of the SRP in UV-VIS spectrophotometry. An ideal solvent should easily solve the SRP and not decompose the complex. Different solvents such as methanol, ethanol, acetonitrile, THF and acidic solutions of methanol and ethanol were tried in order to obtain the maximum signal after CPE. As a result of the

Table 3 Tolerance limits of possible interfering ions during determination of  $250 \mu\text{g L}^{-1}$  Se(IV) in model solutions

Interfering ion(s)	Tolerance ratio	Recovery (%)
<b>Anions</b>		
$\text{PO}_4^{3-}$ , $\text{Cl}^-$ , $\text{Br}^-$ , $\text{SO}_4^{2-}$ , $\text{NO}_2^-$	1000	97.5–101.0
$\text{S}^{2-}$ , $\text{C}_2\text{O}_4^{2-}$ , $\text{I}^-$ , $\text{SeO}_4^{2-}$	500	96.5–100.2
$\text{SCN}^-$ , $\text{NO}_2^-$	100	97.5–102.8
<b>Cations</b>		
$\text{Na}^+$ , $\text{K}^+$ , $\text{NH}_4^+$	1000	97.4–100.4
$\text{Mg}^{2+}$ , $\text{Ca}^{2+}$	800	95.8–104.5
$\text{Cr}^{3+}$ , $\text{Al}^{3+}$	350	98.3–104.2
$\text{Cd}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Hg}_2^{2+}$	200	96.3–101.4
$\text{Ni}^{2+}$ , $\text{Pb}^{2+}$	100	97.0–99.5
$\text{Hg}^{2+}$ , $\text{Cu}^{2+}$	50	95.0–104.2



**Table 4** Analytical characteristics of the proposed method

Parameters	The values
Linear range (ng mL <sup>-1</sup> )	20–1500
Slope	0.00067
Intercept	−0.0057
Correlation coefficient ( $r^2$ )	0.9905
Recovery (%) ( $N$ : 5)	97.5–102.6
RSD (%) (50 and 250 $\mu$ g L <sup>-1</sup> , $N$ : 5)	3.50 and 2.80
LOD ( $\mu$ g L <sup>-1</sup> )	6.06
LOQ ( $\mu$ g L <sup>-1</sup> )	19.89
Preconcentration factor <sup>a</sup>	50

<sup>a</sup> The preconcentration factor is defined as the ratio of the initial solution volume to the volume of the surfactant rich phase.

experimental studies, we saw that the signal obtained with ethanol was higher than those of other solvents. After that, a series of different concentrations was prepared in the range of 0–100% for ethanol–water, and the experiments were repeated in order to determine the ideal ethanol–water ratio. As a result of these experiments, the maximum analytical signal was obtained with 20% ethanol solution. Therefore, this solution was used to dilute the SRP after CPE in further studies.

### 3.7. Effect of ionic strength

The presence of inorganic electrolytes decreases the cloud point temperature due to dehydration of the poly(oxyethylene) chains. At low temperatures, this effect generally assists the procedure, as the experiments can be completed in a short amount of time and also decomposition of the biological samples is blocked at low temperatures.

The concentration of ionic salt was studied in the range of 0–1.2% (w/v). As can be seen in Fig. 8, the analytical signal increased with increasing NaCl concentration up to 1.0% (w/v) and then gradually decreased. Therefore, a maximum value was obtained at a concentration of 1.0% (w/v) NaCl and this amount was used in further studies.

### 3.8. Effect of interfering ions

In view of the high selectivity provided by our selective reagent for Se(IV) ions, interferences related to the extraction step, *i.e.*

co-existing metal ions and cations that may react with the Se(IV) ions, as well as cations and anions that may react with the metal binder and redox active DAHMP in the reaction medium, were investigated. Under the optimized conditions, interference studies were carried out by individually spiking gradually increased amounts of foreign interfering ions into the standard solution containing Se(IV) at a level of 250 ng mL<sup>-1</sup> before CPE, and a deviation greater than  $\pm 5.0\%$ , determined from the signals observed in absence of any foreign ions, was used as the criterion of occurrent interference. The effect of interfering ions on the proposed method is depicted in Table 3. The interference effects of Cu(II) and Hg(II) ions are probably related to their oxidant properties. As predicted, these ions can oxidize the Se(IV) ions to Se(VI) in the reaction medium. However, the amount of these ions is very low in many real samples. So, it can be considered that the method is not affected by their interference. In addition, this negative situation can be decreased by increasing citrate (buffer) concentration due to the chelating effect of citrate ions.

### 3.9. Performance properties of the proposed method

Analytical properties of the proposed method for Se(IV) are given in Table 4. The RSD values as a measure of precision for 5 replicate determinations of 50 and 250 ng mL<sup>-1</sup> of Se(IV) were 3.50 and 2.80%, respectively. The preconcentration factor, which is defined as the ratio of volume between the initial and final solutions, was calculated as about 50. The calibration graph was obtained *via* preconcentration of 50 mL of sample under the optimized conditions. The linear range was from 20 to 1500 ng mL<sup>-1</sup> for Se(IV). The detection and quantification limits, calculated according to  $3S_{\text{blank}}/s$  and  $10S_{\text{blank}}/s$  ( $3\sigma$ ), where  $S_{\text{blank}}$  was obtained from the standard deviation of 10 replicate measurements of a blank solution and  $s$  is the slope of the calibration graph, were 6.06 and 19.89 ng mL<sup>-1</sup>, respectively.

### 3.10. Speciation of inorganic selenium species

As mentioned in the previous sections, the developed method is selective for Se(IV) ions. No other analytical signal has been observed in the presence of Se(VI) ions. We used the same approach for the speciation of inorganic selenium species in

**Table 5** The speciation results in binary mixtures containing Se(IV) and Se(VI) at known concentration ratios

Added Se species			Found Se ions after CPE				
Se(IV) (ng mL <sup>-1</sup> )	Ratio	Se(VI) (ng mL <sup>-1</sup> )	Total <sup>a</sup> (ng mL <sup>-1</sup> )	Found <sup>b</sup> Se(VI) (ng mL <sup>-1</sup> )	Calculated values for Se(VI) ions		
					RE (%)	RSD (%)	Recovery (%)
100.0	×1	100.0	195.2 ± 5.6	95.2 ± 3.8	−4.80	3.99	95.2
	×2	200.0	292.1 ± 8.5	192.0 ± 4.5	−4.00	2.34	96.0
	×3	300.0	409.4 ± 10.8	305.4 ± 9.7	+1.80	3.17	101.8
	×10	1000.0	1095.2 ± 15.8	987.5 ± 14.5	−1.25	1.46	98.8

<sup>a</sup> The results indicate the total selenium concentration after reduction process based on microwave irradiation in acidic medium. <sup>b</sup> The results indicate the amount of Se(VI) ions by subtracting the Se(IV) concentration from the total Se concentration.

Table 6 Determination of inorganic selenium species as total Se concentration in real samples and SRM

Sample	Added Se(IV) (ng mL <sup>-1</sup> )	Found		
		Total Se <sup>a</sup> (ng mL <sup>-1</sup> )	Recovery (%)	RSD (%)
River water <sup>b</sup>	—	70.84 ± 4.42	—	6.23
	100	165.28 ± 5.45	94.2	3.29
	300	359.93 ± 9.52	96.4	2.64
Tap water <sup>c</sup>	—	—	—	4.97
	100	104.81 ± 3.41	104.8	3.25
	300	307.44 ± 8.50	102.5	2.76
Drinking water <sup>d</sup>	—	—	—	4.97
	100	103.21 ± 2.35	103.2	2.27
	300	294.87 ± 9.51	98.3	3.23
Egg with selenium <sup>e</sup>	—	42.71 ± 1.54	—	3.60
	100	149.51 ± 3.50	106.8	2.34
	300	345.12 ± 6.44	100.8	1.86
Rice flour, NIST-SRM 1568a (certified value: 380 ± 50)	—	375.45 ± 10.20	98.8	2.71
	100	469.12 ± 11.85	93.7	2.52
	300	684.04 ± 15.50	102.8	2.26
TMDA-53.3 (certified value: 22.70 ± 1.85)	—	23.08 ± 1.51	101.67	6.54
	100	125.01 ± 3.70	101.80	2.95
	300	327.14 ± 7.11	101.37	2.17

<sup>a</sup> Mean value ± standard deviation for 5 replicate experiments. <sup>b</sup> River water sample was taken from Kızılırmak River in Sivas. <sup>c</sup> Tap water sample was obtained from tap in our laboratory. <sup>d</sup> Drinking water was bought from a local market. <sup>e</sup> Hen eggs were bought from a local market.

our previous study.<sup>19</sup> Reduction of Se(VI) to Se(IV) was performed using HCl in a microwave digestion system. After reduction with HCl, total Se analysis was also carried out using the proposed method based on CPE under optimized conditions. Binary mixtures of Se(IV) and Se(VI) ions were prepared in order to test the response of the method. Then, reduction of Se(VI) to Se(IV) was performed in the binary mixtures using 3 mol L<sup>-1</sup> of HCl solution based on the microwave program as explained in Section 2.3.

The concentration of Se(VI) was calculated by means of the difference between the total Se and Se(IV) concentrations. The results are given in Table 5. Quantitative recovery values for Se(IV) and total Se were obtained from these mixtures.

### 3.11. Analytical applications

A newly developed method can be suggested after validation using appropriate approaches. Recovery tests, blank analyses,

analysis of certified reference materials, and parallel method analysis are known as important validation experiments. A new method should be supported using a few of these approaches at least. In our studies, we followed two of them: analysis of certified reference materials and recovery tests using different samples.

Several water samples and a hen egg with selenium were analyzed in order to check accuracy and repeatability of the proposed method. Preparation of the samples was performed following the procedure mentioned in Section 2.3. The used microwave digestion procedure both digested the samples and reduced all Se(VI) ions to Se(IV) in the medium. In addition, the method has been applied to determine the total Se concentration using two certified reference materials. The accuracy of the method was also checked with recovery tests at two levels (100 and 300 ng mL<sup>-1</sup>) of Se(IV). The results can be seen in Table 6. As can be seen from the table, the obtained results quantitatively showed sufficient recoveries with RSD values changing in the

Table 7 Comparison of the proposed CPE-spectrophotometric methods with other analytical methods in the literature<sup>a</sup>

Determination method	Chelating agent	Linear range (ng mL <sup>-1</sup> )	Detection limit (μg L <sup>-1</sup> )	RSD (%)	Media	Reference
Spectrophotometry	Dithizone	5–100	4.4	2.18	HCl, Triton X-100, (CPE)	23
Spectrophotometry	Dithizone	1–100	0.3	3.20	pH: 1.0, Triton X-114, (UCPE)	24
Spectrophotometry	3,3'-Diaminobenzidine (DAB)	5–600	1.6	2.10	pH 2.0, Undecanol, (LLME)	25
Spectrophotometry	I <sub>3</sub> <sup>-</sup> CTA <sup>+</sup>	40–1000	16.0	2.10	HCl, CTAB, Undecanol, (DLLME-SFOD)	26
Spectrofluorometry	2,3-Diaminonaphthalene (DAN)	20–700	2.10	<5.00	HCl, Triton X-114	27
Spectrophotometry	Diamino-6-hydroxy-2-mercaptopyrimidine (DAHMP)	20–1500	6.06	2.80	Citric acid, Triton X-114, (CPE)	This study

<sup>a</sup> CPE: cloud point extraction; UCPE: ultrasonic assisted cloud point extraction; LLME: liquid–liquid micro extraction; DLLME-SFOD: dispersive liquid–liquid microextraction–solidified floating organic drop; CTAB: cethyl trimethylammonium bromide; I<sub>3</sub><sup>-</sup>CTA<sup>+</sup>: triiodide anion and cethyl trimethylammonium cation.

range of 1.86–6.23% for total Se. The results indicated that the recoveries are quantitative to a reasonable level for trace selenium analysis: in the range of 91.0–101.2% in food samples and in the range of 94.0–101.8% in beverage samples. The relative standard deviations for food samples and beverages were in the range of 3.06–6.21 and 3.66–7.04%, respectively.

## 4. Conclusion

UV-VIS spectrophotometry is a comparatively inexpensive, robust and easy-to-operate analytical technique that is readily available in most laboratories. If the applied method is based on a selective reaction and the final product is stable for enough time, this tool can be used for the fast and practical analysis of a lot of species. In addition, the proposed method is combined with a separation and pre-concentration step. So, the determination of inorganic selenium species in real samples can be performed at low concentrations without any interference. The method can be considered an alternative to expensive and tedious analytical techniques such as ICP-MS, HG-AAS, and HPLC-MS.

Table 7 compares analytical parameters of the proposed system with published methods based on similar detection systems. As can be seen in Table 7, the proposed method is comparable with the published ones and better than some of those. Especially, properties of the method such as linear range, selectivity, simplicity and being environmentally friendly are very important for the application to real samples.

Generally, most methods in the literature for the determination of Se need very expensive equipment and hybrid instruments. As for the presented method, it exclusively uses conventional UV-VIS spectrophotometry. The recommended procedure could be successfully applied to the pre-concentration and determination of inorganic selenium.

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