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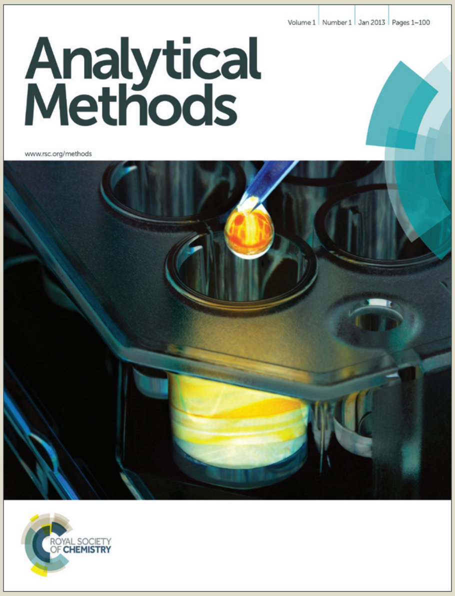

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Simple and Useful Method for Determination of Inorganic Selenium Species in Real Samples Based on UV-VIS Spectroscopy in Micellar Medium

Halil İbrahim ULUSOY

Cumhuriyet University, Faculty of Pharmacy, Department of Analytical Chemistry,

58140, SIVAS, TURKEY

***Corresponding Author**

Department of Analytical Chemistry, Faculty of Pharmacy

Cumhuriyet University, Sivas, Turkey

E-mail: hiulusoy@yahoo.com

Tel. : +90 346 219 1010 -3905

Fax : +90 346 219 11 86

Abstract

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

Keywords: Cloud Point Extraction, Inorganic Selenium Species, Spectrophotometry, Diamino-6-hydroxy-2-mercaptopyrimidine (DAHMP), Water Samples

1. Introduction

Trace elements occur at very low concentrations in environmental and biological samples. Determination and speciation of these elements are always known hard and tedious job. 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 to be carried out. Thousands of compounds as impurities are present along with metal ions in 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 [1].

Selenium is one of the essential dietary constituent in mammals [2]. Selenium protects from several heart diseases, prevents heavy metal toxic effects and helps anti-carcinogenic activity [3]. Selenium has been shown to be both essential for life and toxic at levels 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^{-1} of this element will result in its deficiency whereas dietary levels above 1 mg kg^{-1} will lead to toxic manifestations. Selenium exists in biological systems in the form of inorganic species such as Se(IV) (SeO_3^{2-} ; selenite), Se(VI) (SeO_4^{2-} ; selenate) or selenides (e.g. HgSe), or as organic species having a range of molecular masses and charges, starting from the simplest MeSeH and ending at complex seleno proteins [4-6] .

There are a lot of studies for 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 represents a challenge to the analyst. Because the toxic or essential properties of Selenium are dependent on its speciation, 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 established to be higher from organic compounds; hence the knowledge of Se speciation in foods is important for a better understanding of utilization of this metalloid. Many of problems in selenium speciation analysis are associated with the low concentration of each species to be determined and complexity of matrix [7,8].

The influences of the matrix components in real samples are an important problem at the trace analysis. Atomic and molecular absorption methods are commonly used for determination of selenium. Furthermore, these techniques are not sensitive enough for trace selenium species without any pre-treatment. In order to make determination of selenium species in real samples, the traditional pre-concentration and separation methods have been used in literature such as liquid–liquid micro extraction [9], solid phase extraction [10], ion-exchange [11] and co-precipitation [12]. These pre-concentration methods combined with instrumental analysis have frequently been used for the determination of selenium species in real samples.

As known, hydride generation methods combined with liquid chromatography systems and mass detector are the best way for correct and selective determination of selenium speciation. And, some electro analytical approaches can carry out analysis of selenium species in certain matrix. But if we consider that costs for these expensive instruments and difficulties in some matrix, 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) is recently attracting attention as a green analytical technique because it circumvents the use of volatile organic solvents which have a negative impact on the environment and human health. The combination of CPE with spectrophotometric methods which is simple, selective, inexpensive and available in almost every research laboratory was presented as a useful analytical tool being alternative to expensive and complicated techniques [13-18].

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

2. Experimental

2.1. Instrumentation

Spectrophotometric measurements were performed on a UV-VIS spectrophotometer (Shimadzu, UV-Visible 1800, Japan) equipped with a 1 cm quartz cell. This spectrophotometer has a wavelength accuracy of ±0.2 nm and a bandwidth of 2 nm in the wavelength range of 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 constant the 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 to analysis.

2.2. Reagents and standard solutions

All reagents used were of analytical grade. Ultra-pure water with a resistivity of 18.2 MΩ cm was used in all experiments provided by 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 µg mL^{-1}) were prepared by dissolving appropriate amounts of sodium selenite and selenate (Merck) in water. 0.02 mol L^{-1} of 4, 5-Diamino-6-hydroxy-2-mercapto pyrimidine, DAHMP, (Sigma, St. Louis, MO, USA) solution was prepared by dissolving in 0.001 mol L^{-1} NaOH. (Merck, Darmstadt, Germany). The citric acid solution of 0.1 mol L^{-1} was prepared by using reagent pure citric acid (Carlo Erba). The solutions of all surfactant [TritonX-114, PONPE 7.5, sodium dodecyl sulfate (SDS), cetyl pyridinium chloride (CPC)] (Sigma, St. Louis, MO, USA) were used without further purification. 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 analyte species in the environmental and biological samples, sampling should be made very carefully. The preparation of samples should be focused to obtain all inorganic selenium species as Se (IV) owing to selectivity of developed method for Se (IV). We used a resemble procedure in our previous study for sample preparation [19]. A microwave digestion system was used for preparation of samples and also reduction of Se (VI) to Se (IV) by adopting suitable experimental conditions. 1 g solid or 10 mL liquid of sample was presented to microwave system and 10 mL of 3 mol L^{-1} HCl was added to tubes. The digestion program in Table 1 was applied to all samples. After digestion, the samples were neutralized by a few drop of ammonia until its acidity was eliminated and filtrated by 0.45 µm filter paper. Finally, the proposed method was applied under the optimized conditions.

(Table 1)

2.4. The CPE procedure

In a typical CPE procedure, 10 mL of sample containing inorganic selenium ions in the range of 20 to 1500 ng mL^{-1}, 3.0 mL of 0.1 M Citric Acid, and 1.25 mL of 0.02 mol L^{-1} DAHMP. Samples were allowed to stand for 10 minutes in order to complete reaction. Then, 1.5 mL of 3×10^{-3} mol L^{-1} 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 dilute to the mark (50 mL) with ultra-pure water. The final solution was kept into a thermostatic controlled water bath at 45 ºC for 15 min. Separation of the two phases was achieved by centrifugation for 5 min at 4000 rpm. The surfactant-rich phase (SRP) became viscous after solutions were kept in 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 solution was later added before determination step to 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 blank.

3. Results and discussions

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

(Figure 1)

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

(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 succession and efficiency of this reaction is fairly connected with physical and chemical properties of medium (pH, concentration of reagents, ionic strength, and temperature). In CPE method, pH plays a unique role on first main reactions and also subsequent extraction procedures. The effect of pH on the CPE efficiency of Se(IV) was studied in range of pH 1.0-10.0 by using different buffer systems. Basis on literature studies, more acidic mediums were also tested such as 0.1 mol L^{-1} solutions of HCl, acetic acid, sulfuric acid, citric acid and phosphoric acid. The experimental studies provided that the extraction yield is very low at high pHs due to negative conditions for main reaction. So, the experiments were focused especially on acidic range. 0.1 mol L^{-1} of hydrochloric acid, acetic acid, sulfuric acid, citric acid, phosphoric acid, and some phosphate buffers were tried in order to supply the most appropriate medium for reaction between Se(IV) and DAHMP. Results were illustrated in Figure 2. As can be seen in figure, the signal obtained with citric acid better than others. The obtained signals with HCl and phosphoric acid were also acceptable but signals of citric acid were better than others. In additions, possible capacity of citric acid for complex formation with metallic species presents some advantages. Because, citric acid can keep constant the pH like a buffer and also eliminate some interfering metallic ions as a chelating agent. Then, the concentration of citric acid in final solution was also studied and optimized. The results were shown in Figure 3. As can be seen that the highest analytical signals for Se(IV) were obtained by using 2.0 mL of 0.1 M citric acid in 50 mL of final solution.

(Figure 2 and Figure 3)

3.2. Effect of DAHMP Concentration on CPE

One of the main parameters in CPE experiments is concentration of chelating agent. Sometimes, these reagents act as a complex forming reagent (mostly in metal determinations) or they directly react with target species before CPE as redox active compounds or condensation compound (mostly in non-metallic or organic species determinations).

As a result of a comprehensive scanning in literature, we noticed that suitable and selective reagents for selenium species are generally aromatic compounds including $-NH_2$ groups. In additions, amino groups in cycle should be neighbor each other (vicinal position) for a selective reaction. These compounds can be used for selective determination of Se (IV) ions due to characteristic reaction. As a result of our pre-experiments, we obtained meaningful signals with DAHMP based on selective reaction with Se (IV) ions at acidic mediums.

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

Harrison et al. used a di-amino compound called di-amino naphthol (DAN) for selective Se (IV) determination [20,21]. They investigated the structure of the Se (IV)-DAN complex by using several instrumental techniques. By considering the similarities in structures between DAN and our compound (DAHMP), we can suggest a resemble mechanism as given in Figure 4 for forming complex.

(Figure 4)

The effect of DAHMP concentration in final solution on analytical signal was investigated in range of 0-0.0014 M and the results were illustrated in Figure 5. As can be seen that the signal intensity of 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 range of 0.0004-0.0008 M. Due to be enough a concentration of 0.006 M for maximum sensitivity, this value was selected as optimal value for further studies.

(Figure 5)

3.3. Effect of nonionic surfactant amount

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

PONPE 7.5 and triton X-114 was preferred as non-ionic surfactant due to their commercial availability and suitable structures. Therefore, the concentrations of Ponpe 7.5

and Triton X-114 on the analytical signal were investigated in range of 0.00-0.10% (v/v). The results were shown in Figure 6. As can be seen from Figure 6, the signals obtained from Triton X-114 are bigger than PONPE 7.5's signals and the maximum absorbance value at concentration of 0.08 % (v/v). Therefore, this concentration was selected as suitable nonionic surfactant for subsequent studies.

(Figure 6)

3.4. Effect of ionic surfactant amount

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

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

(Figure 7)

3.5. Effect of equilibration temperature and incubation time

Optimal equilibration temperature and incubation time are necessary to complete reactions, and achieve an easy and efficient phase separation. The shortest equilibration time and the lowest possible equilibration temperature are always wanted for quantative extraction and efficient phase separation. In other words, the formed complex can be decomposed at high temperatures and stability of complex can change by time. The dependences of extraction efficiency upon equilibration temperature and time were studied with a range of 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 diluent agent for the surfactant rich phase

Surfactant rich phase (SRP) is very viscous to present for detection system. In CPE experiments, SRP should be diluted with suitable solvent according to detection equipment and analyte. UV-VIS spectrometry may be considered as compatible equipment for CPE experiments. We have a lot of options to select solvent for SRP in UV-VIS spectrometry. 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 experimental studies, we saw that the signal obtained with ethanol better than other solvents. After a series with increasing concentrations were prepared in the range of 0-100 % for ethanol-water, experiments were repeated in order to determine ideal ethanol ratio. As a result of 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. In the low temperatures, this effect generally gets easy the processes. Due to experiments can be completed in short time and also decomposition of biological samples are blocked at low temperatures.

The concentration of ionic salt was studied in the range of 0-1.2 (w/v) %. As it can be seen in Figure 8, the analytical signal increases with concentration of NaCl up to 0.5 (w/v) % and then gradually decreased. Therefore, a maximum value was obtained at a concentration of 0.5 (w/v) % NaCl and this amount was used at further studies.

3.8. Effect of interfering ions

In view of the high selectivity provided by selective reagent for Se(IV) ions, the interferences can be related with extraction step, *i.e.* the co-existing metal ions and the cations that may react with Se(IV) ions, as well as the cations and anions that may react with metal binder and redox active DAHMP in the reaction medium. 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 level of 250 ng mL^{-1} before CPE, and a deviation greater than ±5.0 % from the signals observed in absence of any foreign ions was used as the criterion of interference occurring. The effect of interfering ions on proposed method was depicted in Table 3. The interference effects of Cu(II) and Hg(II) ions are probably related with their oxidant properties. As predicted, these ions can oxidize the Se(IV) ions to Se(VI) in the reaction medium. But the amount of these ions are very low in a lot of very real samples. So it can be considered that the method is not affected by their interfering effects. In addition, this negative situation can be decreased by increasing citrate (buffer) concentration due to chelating effect of citrate ions.

(Table 3)

3.9.The performance properties of the proposed method

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

3.10. Speciation of inorganic inorganic selenium species

As it was mentioned in previous section, the developed method is selective for Se(IV) ions. Any analytical signal has been observed in the presence of Se(VI) ions. We used same approach in our previous study [19] for speciation of inorganic selenium species. Reduction of Se(VI) ions to Se(IV) was performed by using HCl in a microwave digestion system. After reduction with HCl, total Se analysis was also made by using proposed method based on CPE under optimized conditions. Binary mixtures of Se(IV) and Se(VI) ions were prepared in order to test response of method. Then reduction of Se(VI) ions to Se(IV) in the binary mixtures were performed by using 3 mol L^{-1} of HCl solution based on microwave program as explained in section 2.3.

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

(Table 5)

3.11. Analytical applications

A newly developed method can be suggested after validation by 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 by using a few of these approaches at least. In our studies, we followed two of them: analysis of certified reference materials and recovery test on 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 sample was performed the procedure mentioned in section 2.3. The used microwave digestion procedure digested the samples both and reduced all Se(VI) ions to Se(IV) in the medium. In addition, the method has been applied to the determination of total Se at two certified reference materials. The accuracy of the method was also checked by recovery test at two levels (100 and 300 ng mL^{-1}) of Se(IV). The results can be seen in Table 6. As can be seen from Table, the obtained results quantitatively showed sufficient recoveries with RSDs changing in range of 1.86–6.23% for total Se. The results indicated that the recoveries are quantitatively at reasonable levels 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 respectively were in the range of 3.06–6.21% and 3.66–7.04%.

(Table 6)

4. Conclusion

UV-VIS spectrophotometry is a comparatively inexpensive, robust and easy-to-operate analytical technique that is readily available in most laboratories. If your method is based on a selective reaction and final product is stable for enough time, this tool can be used fast and practical analysis of a lot species. In addition, the proposed method is combined with a separation and pre-concentration step. So, determination of inorganic selenium species in real samples can be performed at low concentration 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 published ones and better some ones. Especially, the properties of method such as linear range, selectivity, simplicity, environmental friendly are very important for application to real samples.

Generally, most of methods in literature for 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 preconcentration and determination of inorganic selenium.

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Table 1. Microwave parameters for digestion and reduction s ofamples

Amount of sample	1 g solid or 10 mL liquid	
Digestion reagent	10 mL of 3 mol L^{-1} HCl	
Time programme	0-10 min.	80 ºC
	10-15 min.	100 ºC
	15-25 min.	150 ºC

Table 2. Optimization of Cloud Point Extraction Variables

Variable	**Range Tested**	**Selected Value**
Citric Acid Concentration (mol L^{-1})	0.001-0.015	0.010
DAHMB (mol L^{-1})	0.0000-0.0014	0.006
Nonionic Surfactant (% w/v)	0.000-0.100	0.080
SDS (mol L^{-1})	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

Table 3. Tolerance limit of possible interfering ions at determination of 250 μg L^{-1} Se(IV) in model solutions

Interfering ion(s)	**Tolerance ratio**	**Recovery%**
	Anions	
PO_4^{3-}, Cl^-, Br^-, SO_4^{2-}, NO_2^-	1000	97.5–101.0
S^{2-}, $C_2O_4^{2-}$, I^-, SeO_4^{2-}	500	96.5–100.2
SCN^-, NO_2^-	100	97.5-102.8
	Cations	
Na^+, K^+, NH_4^+	1000	97.4–100.4
Mg^{2+}, Ca^{2+}	800	95.8–104.5
Cr^{3+}, Al^{3+}	350	98.3–104.2
Cd^{2+}, Zn^{2+}, Hg_2^{2+}	200	96.3–101.4
Ni^{2+}, Zn^{2+}, Pb^{2+}	100	97.0–99.5
Hg^{2+}, Cu^{2+}	50	95.0-104.2

Table 4. Analytical characteristics of the proposed method

Parameters	**The Values**
Linear range	20-1500 ng mL^{-1}
Slope	0.00067
Intercept	-0.0057
Correlation coefficient (r^2)	0.9905
Recovery % (N: 5)	97.5-102.6
RSD (%) (50 and 250 µg L^{-1}, N: 5)	3.50 and 2.80
LOD (µg L^{-1})	6.06
LOQ (µg L^{-1})	19.89
*Preconcentration factor	50

*Preconcentration factor is defined as the ratio of the initial solution volume to the volume of surfactant rich phase

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^{-1}	Ratio	Se(VI) ng mL^{-1}	Total[1] ng mL^{-1}	Found[2] Se(VI) ng mL^{-1}	The 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
	× 5	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

[1] The results indicate the total selenium concentration after reduction process based on microwave energy in acidic medium.

[2] The results indicate the amount of Se(VI) ions by subtracting Se(IV) concentration from total Se concentration

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

Sample	**Added Se(IV) ng mL^{-1}**	**Found**		
		Total Se[1] ng mL^{-1}	**Recovery %**	**RSD%**
River water[2]	-	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[3]	-	-	-	4.97
	100	104.81±3.41	104.8	3.25
	300	307.44±8.50	102.5	2.76
Drinking Water[4]	-	-	-	4.97
	100	103.21±2.35	103.2	2.27
	300	294.87±9.51	98.3	3.23
Egg with Selenium[5]	-	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

[1] Mean Value±Standard deviation for 5 replicate experiments

[2] River water sample was taken from Kızılırmak River in Sivas

[3] Tap water samples was obtained from tap in our laboratory

[4] Drinking water was bought from a local market

[5] Hen eggs were bought from a local market

Table 7 Comparison of the proposed CPE-spectrophotometric methods with the other analytical methods in literature

Determination Method	**Chelating Agent**[a]	**Linear rang ng mL^{-1}**	**Detection limit, µg L^{-1}**	**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	$I_3^{-}CTAA^{+}$	40-1000	16.0	2.10	HCl, CTAB, undecanol, (DLLME–SFOD)	[26]
Spectrofluorometry	2,3-diaminonaphtalene (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

CPE: Cloud Point Extraction; UCPE: Ultrasonic Assisted Cloud Point Extraction; LLME: Liquid-liquid Micro Extraction; DLLME-SFOD: Liquid–liquid microextraction–solidified floating organic drop; CTAB: Cethyl Trimethyl Ammonium Bromide; $I_3^{-}CTAA^{+}$: triiodide anion and cetyltrimethylammonium cation

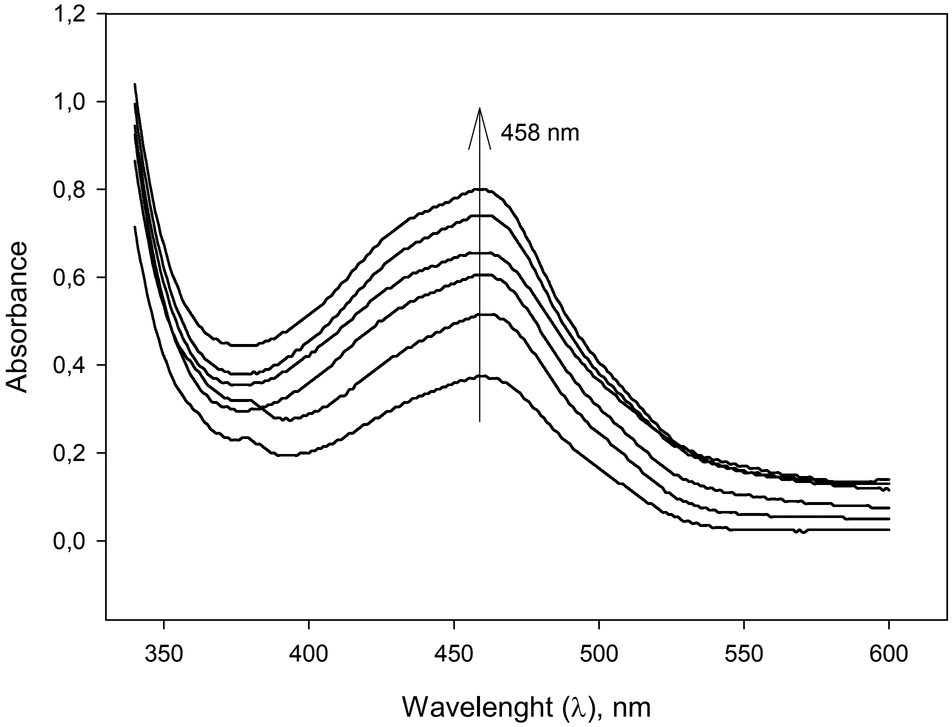


Figure 1. Absorption spectrum of Se-DAHMP complex in the presence of Se (IV) ions at different concentrations.

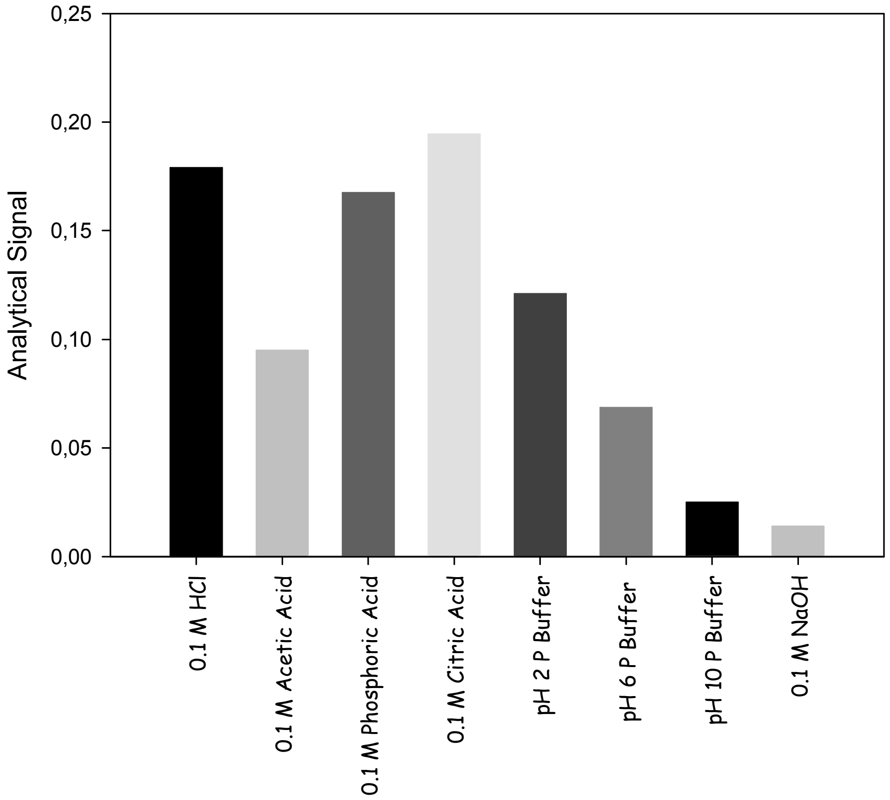


Figure 2. The effect of different solutions on reaction between Se(IV) and DAHMP

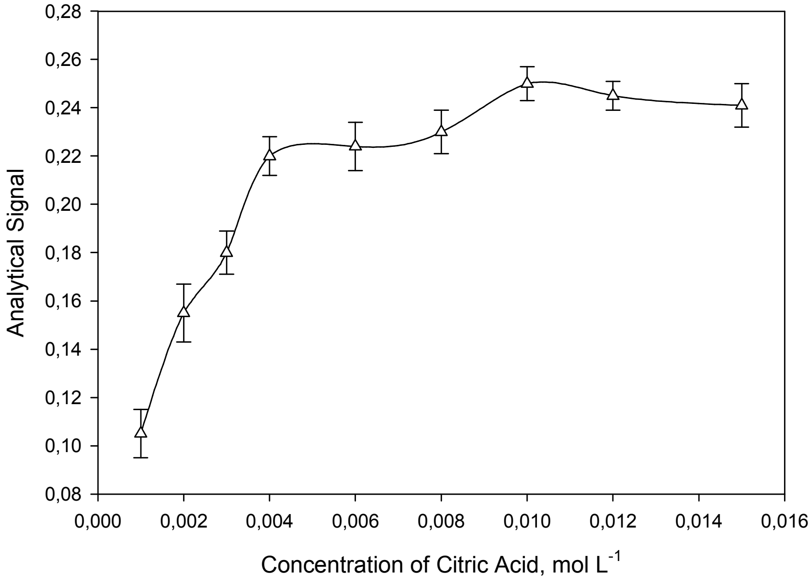


Figure 3. The Effect of Concentration of Citric Acid on Analytical Signal

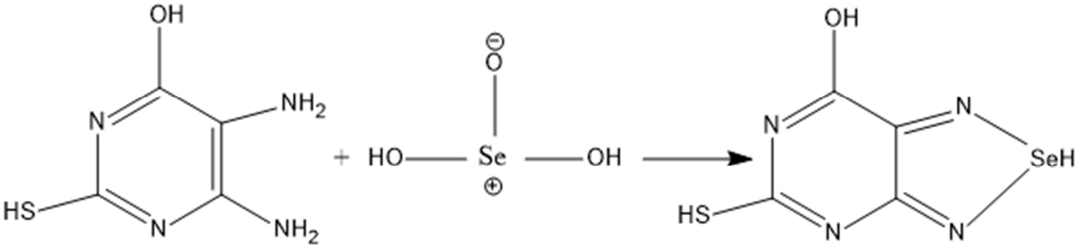


Figure 4. The possible reaction mechanism for proposed method

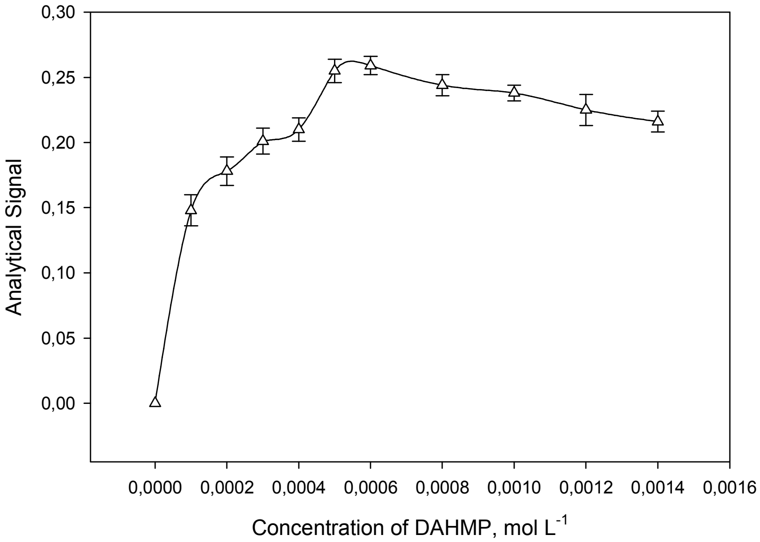


Figure 5. The Effect of Concentration of DAHMP on Analytical Signal

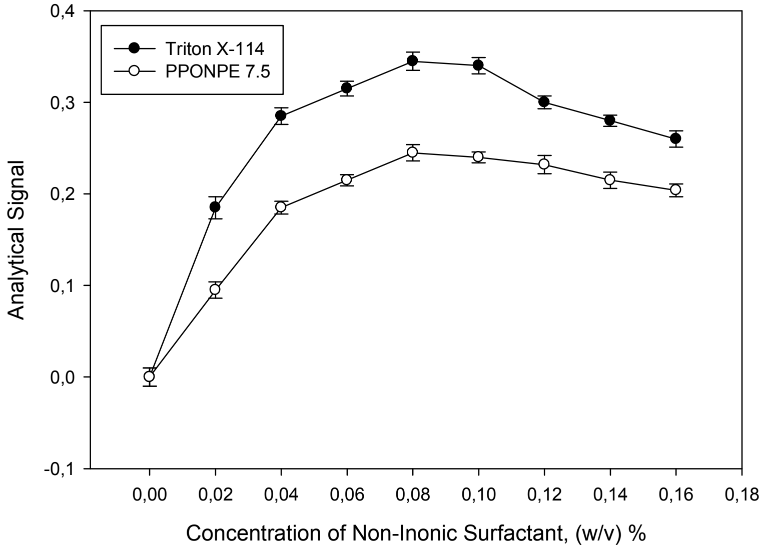


Figure 6 The Effect of Concentration of Non-ionic Surfactant on Analytical Signal

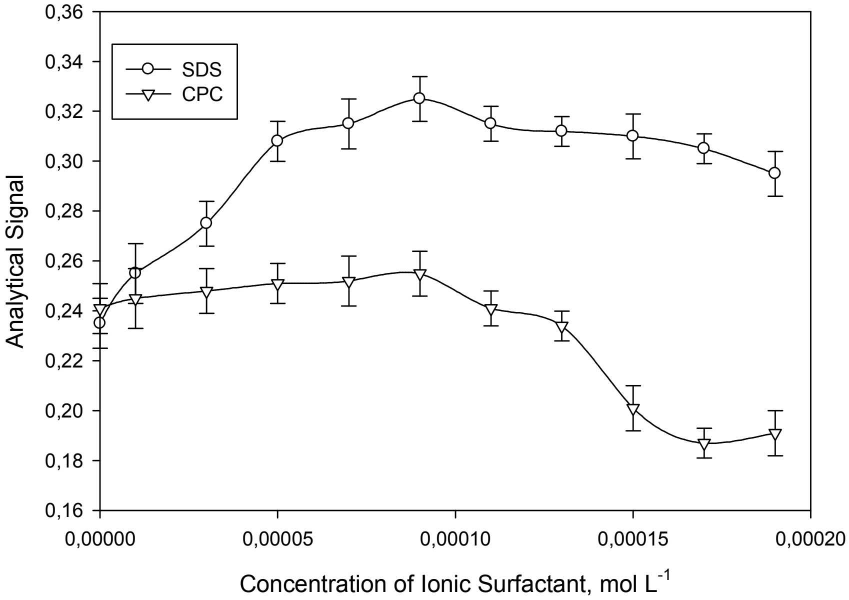


Figure7. The Effect of Concentration of Ionic Surfactant on Analytical Signal

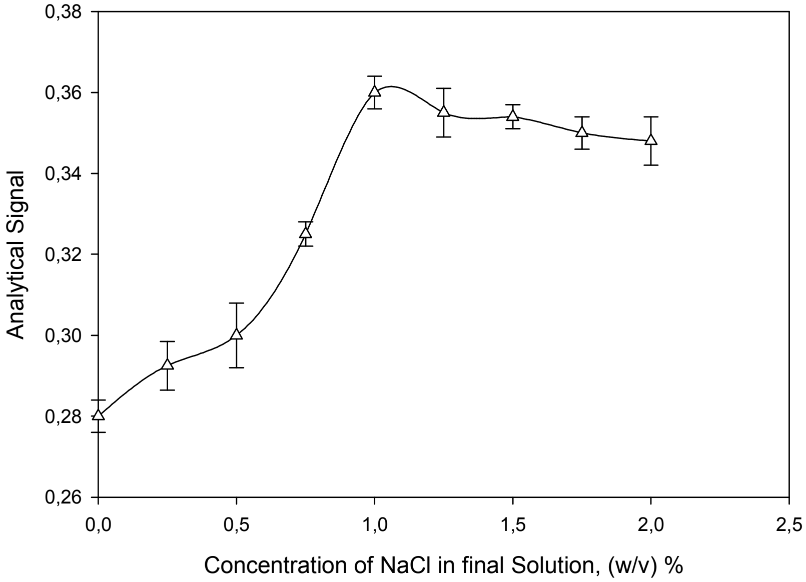
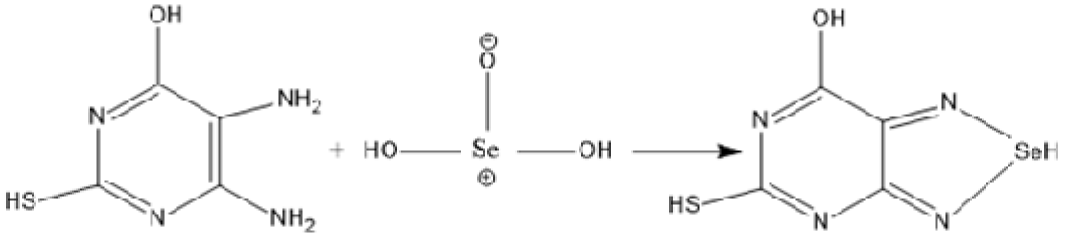
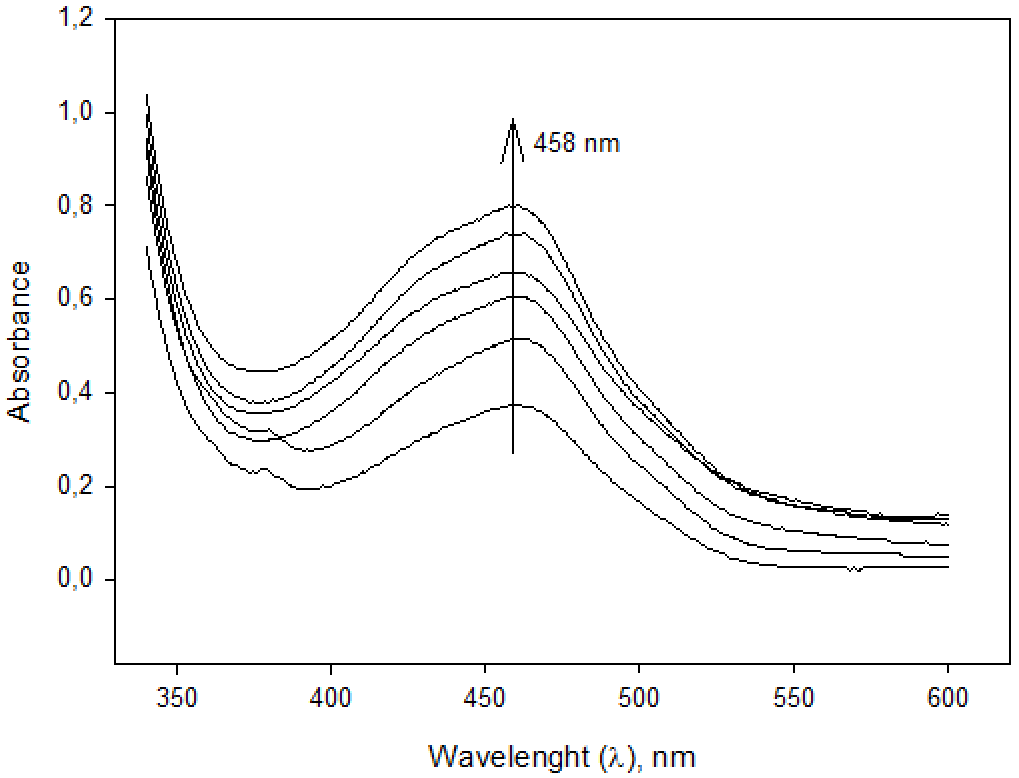


Figure 8. The Effect of Concentration of Ionic Salt (NaCl) on Analytical Signal


OH
N
NH2
HS
N
NH2
+
HO
Se
OH
O
⊖
⊕
OH
N
N
SeH
HS
N
N


458 nm
Absorbance
1,2
1,0
0,8
0,6
0,4
0,2
0,0
350
400
450
500
550
600
Wavelenght (λ), nm