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Taguchi Method Approach on Preconcentration and Speciation of Trace Amounts of Inorganic and Organic Selenium in Mushroom Samples with Cloud Point Extraction and Uv-Vis Spectrophotomety

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ABSTRACT

A micelle-mediate extraction method was developed for the speciation of selenium in mushroom samples with cloud point extraction (CPE) as a separation and preconcentration method, and determination by UV-visible spectrophotometry. The CPE is based on the complex of Se(IV) with 2,3-diaminonaphthalene (DAN) and then entrapped in non-ionic surfactant, Triton X-114.Taguchi method was used to optimize the main factors affecting CPE efficiency, such as pH of sample solution, concentration of Triton X-114 and DAN, equilibration temperature and time. Under the optimal conditions, the detection limit of this method for Se(IV) is 9 $ng\ mL^{-1}$, and relative standard deviation is 3.8% at determination of 50 $ng\ mL^{-1}$ Se(IV). The proposed method has been applied for determination of trace amounts of inorganic and organic selenium in mushroom samples with satisfactory results.

Keywords: Selenium, Edible mushroom, Cloud point extraction, Taguchi method, UV-vis spectrophotometry

INTRODUCTION

Selenium is an essential trace element for both humans and animals. It has an important role to produce some of enzymes like glutathione peroxidase (GPX) (Rotruck et al., 1973; Arthur et al., 1990). The beneficial effects of selenium in human health are strongly dependent on its concentration. The concentration range in which, selenium is considered toxic or essential is very constricted. It has been estimated that the ingestion of food stuffs with selenium content above 1 mg Se Kg^{-1} can induce toxicity; meanwhile a concentration below 0.1 mg Se Kg^{-1}leads to deficient status (O.Wada et al., 1993).The deficiency of selenium in diets would cause some disease such as cancer (Combs, 1986), heart disease (Neve, 1996) and muscle disorders (Behne et al., 1996). As well as this element was found to have a role in immune function and viral suppression in ADIS (Beck, 2001; Baum et al., 2001). However, the bioavailability, toxic and chemoprotective activity of Se are species dependent. Each species is absorbed differently by the human body and has a different tendency to bioaccumulate in organisms (Zheng et al., 2003). Selenium exists in different chemical forms: inorganic species (selenite,SeO_3^{2-}; selenate, SeO_4^{2-}) and organic species (selenoamino acids; selenomethionine and very complex selenoproteins). Several studies have shown organic selenium is more bioavailable and less toxic than inorganic forms of selenium such as Se(IV) and Se(VI), on the other hand Se(VI) is more toxic than Se(IV) (Hymer and Caruso, 2000; Pedrero and Madrid, 2009). Among the organic species, selenomethionine is one of the most effectively accumulated selenium species in different organs (Waschulewski and Sunde, 1988).

Vegetables are a recognized source of selenium into the diet. In between, edible mushrooms are known to be selenium accumulators, especially selenomethionine (Ogra et al., 2004; Michelot et al., 1997; Kalac and Svoboda, 2000), and also they are consumed for enjoyment as well as their health benefits such as containing relatively few calories and relatively high amounts of vegetable proteins. Their fruiting bodies, on a dry weight basis, contain about 39.9% carbohydrate, 17.5% protein and 2.9% fats, with the rest consisting of minerals (Latiff et al., 1996). Cultivated mushrooms are a good source of vitamin B2, niacin, and folates. As compared with other plants, mushrooms also have proven to be good sources of many mineral elements such as K, P, Zn and Cu (Mattila et al., 2001). Nowadays analytical speciation studies helps to find the different forms of selenium in mushrooms.

Elemental speciation usually requires coupling of two techniques: a technique to separate the element's chemical forms of interest and a sensitive detection method to provide determination of the analyte at low levels (B'Hymer et al., 2000). Recently cloud point extraction (CPE) using non-ionic surfactants have attracted considerable attention as an alternative to the tradition methods (Bellato et al., 2005). CPE offers many advantages over traditional liquid-liquid extraction, being simple, inexpensive, using less organic solvents with high capacity to concentrate a wide variety of analyts with high recoveries and high concentration factors (Bellato et al., 2005).

For typical CPE procedures of Se, a variety of chelating reagents have been used for the formation of extractable hydrophobic complex, such as dithizone and o-phenylenediamine (Campbell and Yahaya, 1980), 3,3-diaminobenzidine (Porcella et al., 1991), 2,3-diaminonaphthalene (Lavale and Dave, 1989), 8-hydroxyquinoline (Bhat and Gupta, 1982). Between them 2,3-diaminonaphthalene (DAN) has more selectivity, low toxicity and specially reacts with Se(IV) yielding the complex 4,5-benzopiazselenol (Se–DAN complex) which allows the determination of selenium in different matrices (Huang et al., 1996; Rodr´ıguez et al., 1994; Wang et al., 1994). Therefore Se(VI) must be reduce to Se(IV) before complex reaction.

In order to the determination of selenium, various techniques have been used such as cathodic stripping voltammetry (Mattsson et al., 1995; Papoff et al., 1998), atomic absorption spectrometry (AAS) (Stripeikis et al., 2000; Gallignami et al., 2000), inductively coupled plasma mass spectrometry (ICP-MS) (Chassaigne et al., 2002; Brindle and Lugowska, 1997), hydride generation coupled with ICP-MS or AAS (Ipolyi and Fodor, 2000; Raessler et al., 2000).

Since these methods are expensive and need to the expert operator, so the spectrophotometric techniques are the best alternative for them.

The aim of the present work was to apply CPE as a separation and preconcentration step combined with UV-vis spectrophotometry for the speciation of selenium in mushroom sample after reaction of Se(IV) with 2,3-diaminonaphthalene (DAN) and preconcentration of the produced Se-DAN complex using octyl phenoxypolyethoxy ethanol (Triton X-114) as a non-ionic surfactant. The experimental parameters affecting the CPE efficiency were optimized with Taguchi method to obtain the best results with minimum experiments.

EXPERIMENTAL

Materials and Methods

A UV-visible spectrophotometer (T90 double beam) equipped with Deuterium and Tungsten lamps, was used for all determinations. The pH measurements were carried out with a pH meter (EDT-GP353, UK). An ultrasonic bath (Bandelin Sonorex Digitec) was used in the experiments. A microwave oven (Bandelin model F115) was applied for the reduction. A centrifuge was used to accelerate the phase separation process (H-11n model, Kokusan, Japan).

All chemicals were of analytical reagent grade. De-ionised water was used in all experiments. All of the glassware and pipettes were cleaned by soaking for 24h in 10% HNO_3. After cleaning, all the containers were rinsed three times with de-ionised water and twice with acetone before the use. Stock solutions of 1000 $mg\ L^{-1}$ Se(IV) and Se(VI) were prepared by dissolving Na_2SeO_3 and Na_2SeO_4 (Sigma) in de-ionised water. The chelating agent, 2,3-diaminonaphthalene (DAN) was prepared daily by appropriate amount of reagent (Sigma) in 10.0 mL of 0.1 $mol\ L^{-1}$ HCl and 0.5 g hydroxylamine hydrochloride $NH_2OH.HCL$ (Sigma), after heating at 50℃ for 30 min in a bath water. The non-ionic surfactant, Triton X-114 (Sigma) was used without further purification. The pH of the solutions was adjusted by drop wise addition of hydrochloric acid and sodium hydroxide (1 $mol\ L^{-1}$).

The CPE Procedure

In order to the CPE procedure, aliquots of solution containing sample or standard solution of Se(IV) were prepared and placed in a graduated tube. After pH adjustment with 1 $mol\ L^{-1}$ HCl and 1 $mol\ L^{-1}$ NaOH (pH: 3), 0.5 mL chelating agent DAN ($3 * 10^{-3}\ mol\ L^{-1}$) was added and the Se-DAN complex was obtained by heating the solution in a water bath at 80℃ for 40 min. Then, 1.0 mL of 2% (v/v) Triton X-114 was added and the solution completed to 10 mL and kept in a water bath at 50℃ for 15 min. In order to separate the phases, the turbid solution was centrifuged for 10 min at 4500 rpm, and then cooled down in an ice bath for 10 min. The aqueous phase was removed and 1.0 mL of methanol was added to decrease of the viscosity of the surfactant-rich phase. Finally absorbance of the solution was measured by UV-vis spectrophotometer.

Preparation of Real Sample

For the determination of total selenium (inorganic + organic) of samples, the experiments were carried out on 1.0 g of the mushrooms. Since all of the species of selenium for reaction with DAN, should be convert to Se(IV), so at first organic selenium (selenomethionine) oxidate to Se(VI) by adding 10.0 mL of concentrated HNO_3 and heating the sample on a hot plate for 2.0 h. Then, the solution made up to 100.0 mL using de-ionised water and 10.0 mL of resulting solution was heated to remove nitric acid (2 mL). Afterwards, the reduction of Se(VI) to Se(IV) and CPE steps were applied .

Both sample digestion and selective reduction of Se(VI) to Se(IV) were always performed in a microwave oven, by using specific heating program (Ferri et al., 2007). Due to find the total inorganic selenium (Se(IV) + Se(VI)), the reduction of Se(VI) to Se(IV) was performed on 1.0 g of mushrooms by adding 10.0 mL of 12 M HCl and putting the solution in an ultrasonic bath for 20 min. Then the mixture was heated in the microwave oven with the following instrumental program: 15 min in 100℃ and 15 min in 150℃. Finally the CPE procedure was applied.

In order to achieve the concentration of Se(IV), 10.0 mL of de-ionised water was added to the 1.0 g mushroom. Then, the mixture was placed in an ultrasonic bath for 20 min and after that the CPE procedure was applied.

RESULTS AND DISCUSSION

Taguchi Design

Taguchi ̓s optimization technique is a powerful and unique discipline that allows optimization with minimum numbers of experiments (Yang, 2001).This method is capable to establish an optimal design configuration, even when significant interaction exists between and among the control variables (Casab et al., 2003; Tadayon et al., 2012). In this study, the effect of five important factors including pH of the solution, ligand concentration, surfactant concentration, equilibration temperature and time, were selected and each factor at four levels on the CPE was examined using Taguchi's method. The used level setting values of the main factors (A-E) and the $OA_{16}(4^5)$ matrix employed to assign the considered factors are shown in the table 1.

Table 1. Factors and levels of Taguchi method (A-E are the respective codes for each factor).

Levels	*Factors*				
	A(pH)	*B(ligand concentration*$(mol\ L^{-1})$*)*	*C(surfactant concentration(v/v%)*	*D(temperature ℃)*	*E(equilibration time (min))*
1	1	$2 * 10^{-3}$	1	60	20
2	2	$3 * 10^{-3}$	2	70	30
3	3	$4 * 10^{-3}$	3	80	40
4	4	$5 * 10^{-3}$	4	90	50

The optimum responses calculated using the following expression:

$$\mathrm{Y_{0pt}} = \frac{\mathrm{T}}{\mathrm{N}} + \left(\bar{A} - \frac{T}{N}\right) + \left(\bar{B} - \frac{T}{N}\right) + \left(\bar{C} - \frac{T}{N}\right) + \left(\bar{D} - \frac{T}{N}\right) + \left(\bar{E} - \frac{T}{N}\right)$$

Where T is the grand total of all results, N is the total number of results; Y_{opt} is the response under the optimum conditions, A, B, C, D and E are the mean response of the pH, ligand concentration, surfactant concentration, equilibration temperature and time at optimum levels, respectively. The results showed that pH and ligand concentration offered the main contribution to the extraction efficiency and the other parameters offered the secondary contribution. In Figure 1 the mean values of the four levels of each factor shows how the extraction efficiency changes when the level of that factor changes.

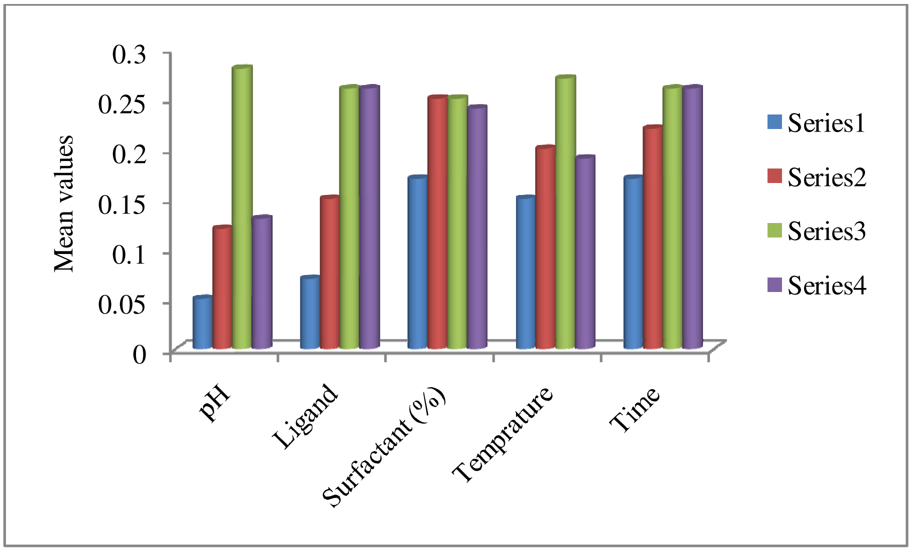

Figure 1. The response graph explaining the variation of the mean absorbance values plotted against various extraction parameters. pH [level 1=1, level 2=2, level 3=3, level 4=4]. Ligand concentration (M) [level 1=$\mathbf{2 * 10^{-3}}$, level 2=$\mathbf{3 * 10^{-3}}$, level 3=$\mathbf{4 * 10^{-3}}$, level 4=$\mathbf{5 * 10^{-3}}$]. Surfactant concentration ((v/v)%) [level 1=1, level 2=2, level 3=3, level 4=4]. Temperature (℃) [level 1=60, level 2=70, level 3=80, level 4=90]. Time (min) [level 1=20, level 2=30, level 3=40, level 4=50].

The Effect of pH

The pH of the solution is an important factor in CPE of Se(IV) using DAN, because this parameter is directly related to the formation of Se-DAN complex. The effect of pH on the CPE efficiency of Se(IV) was studied in the pH range 1.0 to 4.0 and the results are shown in Fig 1. As can be seen, maximum absorbance was obtained in pH 3.0 and by increasing the pH, the efficiency decreases, because Se(IV) species is just stable in acidic medium. Also, the lower extraction efficiency in pH: 1, 2 must be because of instability of DAN solution in acidic media (Rodriguez et al., 1994).

The Effect of DAN Concentration

The effect of DAN concentration on the analytical performance was studied with Se(IV) solution and various concentration of the reagent in the range of $2 * 10^{-3}$ to $5 * 10^{-3}\ mol\ L^{-1}$. The results showed that the signal of Se(IV) was increased with increasing of DAN concentration up to $3 * 10^{-3}\ mol\ L^{-1}$, and then remained constant. As shown in Figure 1, a final concentration of $3 * 10^{-3}\ mol\ L^{-1}$ was sufficient for optimum results.

The Effect of Surfactant Concentration

The variation of extraction efficiency upon the surfactant concentration was examined within range 1% to 4% (v/v). The results in Figure 1 showed that, the absorbance of the solution increased by increasing the Triton X-114 concentration up to 2% (v/v) and remained nearly constant between 2% to 4% (v/v). Therefore 2% (v/v) Triton X-114 was used in the proposed method.

Equilibration Temperature and Time

The dependence of extraction efficiency on equilibration temperature and time were investigated in the range of 60-90℃ and 20-50 min, respectively. The results showed that, equilibration temperature of 80℃ and equilibration time of 40 min are suitable to obtain quantitative extraction.

Analytical Figure of Merit

A calibration curve was constructed by preconcentration of 10 mL of sample standard solutions with TritonX-114. From measurements made under the optimum conditions described above, the calibration graph was linear in the range of 5-400 $ng\ mL^{-1}$ (at 294 nm) for Se(IV) with a correlation coefficient better than 0.999 (n = 5). The limit of detection (LOD), defined as $C_L = 3S_B/m$ (whereC_L,S_B and m are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively) (Hayati and Derya, 2012). The LOD value was obtained 9 $g\ mL^{-1}$. The relative standard deviations (RSD) resulting from the analysis of six replicates of 10.0 mL solution containing 50 $ng\ mL^{-1}$ Se(IV) was calculated 3.8%. The preconcentration factor as the ratio of the initial solution volume to the volume of surfactant rich phase (0.5 mL) was 20.

Analysis of Real Sample

The proposed method was applied for the speciation of Se(IV), Se(VI) and selenomethionine in edible mushroom sample cultivated in Mallard zoon of Iran. All species of Se must be convert to Se(IV), because just this species can react with DAN and determined by UV-vis spectrophotometer. However, the total selenium content (inorganic and organic) was detected after using HNO_3, reduction of Se(VI) to Se(IV) and CPE steps. The procedure for reduction of Se(VI) to Se(IV) gives the total inorganic selenium(Se(IV) and Se(VI)) content. Se(IV) procedure provides Se(IV) concentration, so the concentration of Se(VI) and organic Se are found by taking appropriate differences (Guler et al., 2011). As can be seen in Table 2, the results showed the amount of organic Se (selenomethionine) is more than inorganic Se, and the amount of Se(IV) is more than Se(VI). According to effect of selenium species on health body, Iranian mushrooms are the good sources of Se, especially selenomethionine.

Table 2. Determination ofSe(IV), Se(VI) and selenomethionine in real mushroom sample

Real mushroom sample(Iran)	*Amount of Selenium $\mu g/g$, n=3*		
	Se(IV)	Se(VI)	Selenomethionine
Mallard	0.23	0.12	2.46

Comparison of the Proposed Method with Other Methods

A comparison between the developed CPE-spectrophotometry method and CPE-spectrofluorometry as a standard method was applied. The results indicate, this proposed method provides a good low detection limit and wide linear range for determination of trace quantities of Se(IV) in real samples. The statistical F-test (the ration of two variances, $F = S_1^2/S_2^2$) was used to compare the precision of two methods. In this case, the F-test for 5 degree of freedom was calculated 1.45, that is less than the critical $F_{(5,5)}$value 5.050 for 95% confidence level. Therefore, the precision of both methods is equal and there is 95% confident that any difference in the sample standard deviations is due to the random error. So, this method is a good alternative for other expensive and complex methods.

CONCLUSION

The possibility of selenium speciation in mushroom samples has been demonstrated based on cloud point extraction of Se(IV) with DAN in the presence of non-ionic surfactant Triton X-114 and determination by UV-vis spectrophotometry. Taguchi method was used to optimization of affecting parameters on CPE. The developed method is definitely high sensitive, inexpensive, environmentally friendly, simple and reproducible, according to the distinct and advantageous of CPE. The proposed method has been successfully applied to the speciation of selenium in edible mushroom cultivated in Mallard zoon of Iran, and also can be used in various matrixes other than mushroom.

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