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Analytical Methods

Determination of total selenium in food samples by d-CPE and HG-AFS

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ABSTRACT

A dual-cloud point extraction (d-CPE) procedure was developed for the simultaneous preconcentration and determination of trace level Se in food samples by hydride generation-atomic fluorescence spectrometry (HG-AFS). The Se(IV) was complexed with ammonium pyrrolidinedithiocarbamate (APDC) in a Triton X-114 surfactant-rich phase, which was then treated with a mixture of 16% (*v*/*v*) HCl and 20% (*v*/*v*) H_2O_2. This converted the Se(IV)-APDC into free Se(IV), which was back extracted into an aqueous phase at the second cloud point extraction stage. This aqueous phase was analyzed directly by HG-AFS. Optimization of the experimental conditions gave a limit of detection of 0.023 μg L^{-1} with an enhancement factor of 11.8 when 50 mL of sample solution was preconcentrated to 3 mL. The relative standard deviation was 4.04% (c = 6.0 μg L^{-1}, n = 10). The proposed method was applied to determine the Se contents in twelve food samples with satisfactory recoveries of 95.6–105.2%.

1. Introduction

Selenium (Se) is an essential trace element with multiple biological functions in many organisms, including humans. It is found in at least 25 human selenoproteins in the form of selenocysteine, a rare amino acid implicated in redox reactions when present at the active site of enzymes, such as glutathione peroxidases and thioredoxin reductases (Legrain, Touat-Hamici, & Chavatte, 2014; Pedrero & Madrid, 2009). However, the concentration range at which Se is beneficial to human health is narrow (50–200 μg day^{-1}) (Pyrzyn′ska, 2001).

Supplemental Se may be acquired through food, but the bioavailability and toxicity of Se depends on its concentration and chemical form in food (Fairweather-Tait, 1997; Finley, 2006). Because of the small difference between required and toxic levels, reliable and sensitive quantification methods for Se in food are very important. Although the sensitivities of modern analytical techniques are constantly increasing, preconcentration and separation technologies are required because of the low concentration of Se and complexity of the matrix.

Cloud point extraction (CPE) using a nonionic surfactant has become an alternative to conventional solvent extraction in analytical chemistry because it is simple, inexpensive, has high preconcentration factors, and the solvents are less toxic to the environment (Pytlakowska, Kozik, & Dabioch, 2013). This technique is based on the ability of nonionic surfactants to form micelles in an aqueous solution heated to a temperature known as cloud point temperature (CPT). Above the CPT, the micellar solution separates into two phases: a small volume surfactant-rich phase that contains the analyte, and an aqueous phase containing a low concentration of surfactant. To date, CPE has been widely applied to the extraction of trace metal ions from various matrices through the formation and pre-concentration of chelates (Labrecque, Whitty-Leveille, & Lariviere, 2013; Silva, Roldan Pdos, & Gine, 2009; Ulusoy, Akcay, Ulusoy, & Gurkan, 2011). There are several reports of CPE as a pre-concentration step for the determination of Se by different spectrometric techniques, including spectrophotometry (Soruraddin, Heydari, Puladvand, & Zahedi, 2011; Wen, Zhang, Li, Fang, & Zhang, 2014), spectrofluorimetry (Güler et al., 2011), graphite furnace atomic absorption spectrometry (GF-AAS) (Sun, Liu, & Wu, 2013), electrothermal atomic absorption (ET-AAS) (Ghambarian, Yamini, Saleh, Shariati, & Yazdanfar, 2009; Li, Hu, He, & Xiang, 2008; Sounderajan, Kumar, & Udas, 2010) and electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICPMS) (Chen, Hu, & He, 2006; Li et al., 2008). However, CPE coupled with hydride generation atomic fluorescence spectrometry (HG-AFS) has yet to be investigated. HG-AFS is a sensitive analytical method for the determination of trace levels of Se (Lopes Dos Santos et al., 2014; Zhao, Chen, Belzile, & Wang, 2010). The surfactant-rich phase obtained from CPE cannot be analyzed directly by HG-AFS because it is highly likely that the surfactant would produce large quantities of foam during hydride generation, which would destabilize the signal and could overflow

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from the system into the atomizer. Moreover, complexed Se cannot completely react with hydrogen to form H_2Se in the hydride generation system, although the influence of surfactants can be overcome to a great extent through the addition of antifoaming agents.

Fortunately, with a dual-cloud point extraction (d-CPE) technique, the above drawbacks of traditional CPE can be circumvented. Yin (2007) first proposed d-CPE for capillary electrophoresis speciation analysis of mercury. In d-CPE, the CPE procedure is performed twice during a single sample pretreatment process, followed by back extraction of the analyte into an aqueous phase at the second CPE step. Zhao, Zhong, Fang, Qian, and Chen (2012) reported a d-CPE procedure for the determination of several metals (Cd^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and Cu^{2+} ions) in water by inductively coupled plasma optic emission spectrometry (ICP-OES). Arain, Kazi, Arain, et al. (2014) used d-CPE for pre-concentration of As, Cd, Ni and Pb in an artificial saliva extract. Recently, this method has been applied to the extraction and pre-concentration of Cu^{2+} in serum samples from viral hepatitis patients before quantification by flame atomic absorption spectrometry (Arain, Kazi, Afridi, et al. (2014). Apart from the above-mentioned publications, d-CPE has not been used for trace metal detection. Furthermore, in all studies except that involving determination of Cu^{2+} by flame atomic absorption spectrometry, the experimental parameters of d-CPE have been optimized using a traditional univariate methodology. This optimization method is time consuming because many experiments are required, and possible interactions among variables could be missed. Multivariate techniques are faster, and more economical and effective because they allow for simultaneous optimization of more than one variable. Response surface methodology (RSM) is an effective statistical tool for determining the optimal conditions in a multivariable system with a minimum number of experiments, and its use in the field of analytical chemistry is increasing.

The purpose of this study was to develop a new method using d-CPE in combination with HG-AFS for the determination of trace level Se in food samples. Ammonium pyrrolidinedithiocarbamate (APDC) was used to form a hydrophobic complex with Se(IV), which was then encapsulated by the nonionic surfactant Triton X-114 and back extracted with a mixture of 16% (*v*/*v*) HCl and 20% (*v*/*v*) H_2O_2. This final step could minimize the adverse effects of the surfactant and convert Se-APDC complexes into Se(IV) before its reduction to SeH_2 by KBH_4 for subsequent HG-AFS detection. The experimental variables (APDC and Triton X-114 concentrations, pH and equilibration temperature) for the d-CPE were optimized using a Box-Behnken design with three variables.

2. Materials and methods

2.1. Apparatus

A model AFS-920 double-channel non-dispersive atomic fluorescence spectrometer (Beijing Jitan Instruments Co. Ltd., Beijing, China) was employed for all experiments. A selenium hollow cathode lamp (Beijing Institute of Vacuum Electronics, Beijing, China) was used as the radiation source. The instrumental parameters were adjusted according to the manufacturer's recommendations, which are shown in Table 1. A DK-600 thermostated bath (Shanghai Precision Experimental Equipment Co. Ltd., Shanghai, China) was used to maintain the desired temperature. A centrifuge (Anke KA-1000, Shanghai, China) was used to enhance phase separation. The pH values were measured with a model PHS-3C pH-meter (Shanghai Hongyi Instrument Instrument Co., Ltd., Shanghai, China).

Table 1
Operating parameters of the HG-AFS instrument.

Parameters	Conditions
PTM voltage (V)	260
Atomizer temperature (°C)	200
Atomizer height (mm)	9
Lamp current (mA)	30
Flow rate of carrier gas (Ar) (mL min^{-1})	400
Flow rate of shield gas (Ar) (mL min^{-1})	800
Read mode	Peak area
Measure method	Std. curve
Read time(s)	10
Delay time(s)	0.5
Read repeat (times)	1
Injection volume (mL)	1.0

2.2. Chemicals and reagents

All chemicals and reagents used in this work were of analytical grade or higher purity. Ultrapure water was purified with a SZ-97 automatic triple water distiller (Shanghai Yarong Biochemical Instrument Factory, Shanghai, China). Working solutions of Se(IV) were prepared by serial dilution with ultrapure water from 1000 mg L^{-1} stock solutions (Beijing Nuclear Industry Institute of Chemical Metallurgy, Beijing, China). A 1 g L^{-1} APDC (Shanghai Richjoint Chemical Reagent Co., Ltd., Shanghai, China) solution was prepared in ultrapure water. The nonionic surfactant, Triton X-114, was obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. A buffer solution was used. A mixture of 16% (*v*/*v*) HCl and 20% (*v*/*v*) H_2O_2 (Guangzhou Chemical Reagent Factory, Guangzhou, China) was used for back extractions. All vessels and pipettes used for Se(IV) analysis were treated with 10% HNO_3 for at least 24 h and washed four times with ultrapure water.

2.3. d-CPE procedure

The d-CPE procedure was based on a conventional CPE process. An aliquot (50 mL) of either the sample or Se(IV) standard with a concentration between 0.5 and 6.0 μg L^{-1} was mixed with 0.8 to 1.2 mL of 5% (*m*/*v*) Triton X-114 and 0.6 to 1.4 mL of 1 g L^{-1} APDC. The mixture was kept in a thermostatic bath at 40 to 70 °C for 15 min. The pH was buffered at 1.0 to 5.0. The turbid solution was centrifuged for 10 min at 2925*g*, and then cooled in an ice water bath for 10 min to increase the viscosity of the surfactant-rich phase, which was retained in the bottom layer. The aqueous phase was removed with a pipette. Instead of adding a diluent or proceeding with the analysis, a second CPE was performed. In the second CPE, the surfactant-rich phase, which contained the complexes, was treated with 3 mL of a solution containing 16% (*v*/*v*) HCl and 20% (*v*/*v*) H_2O_2 and then heated in a thermostatic bath at 40–70 °C for 15 min. This step was followed by centrifugation for 10 min at 2925×*g*. Finally, the supernatant was introduced into the HG-AFS system for analysis. Blank samples were also prepared in a similar way.

2.4. Sample preparation

Twelve food samples were purchased in Guangzhou, China. The samples were ground to reduce the particle size, and stored at 4 °C. Each sample (0.5–1 g) was placed in a glass beaker, and 15 mL of digestion mixture (HNO_3/$HClO_4$, 4/1, *v*/*v*) was added. The samples were gently heated on a hot plate at 120 °C to near dryness. After cooling to room temperature, 5 mL of 6 mol L^{-1} HCl was added to the mixture and the solution was heated until the volume reduced to approximately 1 mL. This reduced Se(VI) to Se(IV). Finally, the

residue was transferred to a 50-mL volumetric flask, diluted with ultra-pure water, and extracted using the procedure in Section 2.3.

2.5. Optimization procedure

Based on the results of single-factor experiments, four variables that greatly affected the recovery of Se(IV) were optimized using RSM. A four-factor, three-level Box-Behnken design with 27 runs was employed. The following four variables were selected: X_1, pH; X_2, concentration of APDC; X_3, concentration of Triton X-114; and X_4, equilibration temperature. Each variable was assessed at three different levels (Table 2). Second-order polynomial coefficients were calculated and analyzed using SAS 9.1 (SAS Institute, Cary, NC). Statistical analysis of the regression model and the significance of parameter estimates were determined by analysis of variance (ANOVA).

3. Results and discussion

3.1. Optimization by RSM

3.1.1. Statistical analysis and model fitting

A Box-Behnken design was used to investigate mutual interactions between significant factors and to determine values for these factors that would maximize the extraction efficiency. The design matrix and the experimental data are shown in Table 3. Multiple regression analysis was used to analyze the data, and a quadratic model for the Se(IV) fluorescence intensity (F) was developed:

$$F = 5689 + 829X_1 + 685X_2 + 337X_3 - 228X_4 - 923X_1^2 + 295X_1X_3 - 1198X_2^2 - 953X_3^2 - 300X_3X_4 - 715X_4^2,$$

where X_1, X_2, X_3, and X_4 represent the pH, APDC concentration, Triton X-114 concentration, and equilibration temperature, respectively. The adequacy of the regression model was evaluated using ANOVA. The model F-value (40.28) and very low p-value (<0.0001) suggested that the model was highly significant, while the lack of fit was not significant ($p = 0.1002 > 0.05$). The results of the analysis also suggested that the obtained experimental data were in good agreement with the model. The coefficient of determination (R^2) of the model was 0.9792, indicating that the model explained 97.92% of the total variation with a residual of 2.08% unexplained by the model. Meanwhile, the low value of the coefficient of variation (5.48%) demonstrated that the experiment was highly precise and reliable. According to the results of ANOVA, the linear terms X_1, X_2, X_3 and X_4, and the quadratic terms X2 1, X2 2, X2 3and X2 4 significantly affected the Se(IV) fluorescence intensity ($p < 0.05$). The interaction terms of X_1X_3 and X_3X_4 ($p < 0.05$) were also significant for the fluorescence intensity. The other interaction terms were not significant ($p > 0.05$) (see Table 4).

Table 4
ANOVA results of factors and model in the response surface experiment.

Source	DF	SS	MS	F-values	p-values
X_1	1	8256179	8256179	171.26	<0.0001
X_2	1	5632495	5632495	116.83	<0.0001
X_3	1	1366909	1366909	28.35	0.0002
X_4	1	624593	624593	12.96	0.0037
X2 1	1	4547926	4547926	94.33	<0.0001
X_1 X_2	1	15901	15901	0.33	0.5764
X_1 X_3	1	349376	349376	7.25	0.0196
X_1 X_4	1	1025	1025	0.02	0.8865
X2 2	1	7657371	7657371	158.83	<0.0001
X_2 X_3	1	62263	62263	1.29	0.2780
X_2 X_4	1	2908	2908	0.06	0.8101
X2 3	1	4839826	4839826	100.39	<0.0001
X_3 X_4	1	360901	360901	7.49	0.01806
X2 4	1	2729677	2729677	56.62	<0.0001
Model	14	27185741	1941839	40.28	<0.0001
Lack of fit	10	566425	56642	9.37	0.1002

3.1.2. Analysis of response surface

Three-dimensional response surface plots (Fig. 1) and two-dimensional counter plots (Fig. 2) were obtained using SAS 9.1. Each figure displays the effect of two independent variables while keeping the other variables at the center point. Visible peaks in the response surface plots signify that the optimum condition was inside the design range well. All four variables (pH, concentrations of APDC and Triton X-114, and equilibration temperature) affected the fluorescence intensity (Fig. 1). This finding was in agreement with the results of ANOVA. As shown in the response surface plots, the fluorescence intensity increased as two variables approached their optimum conditions, and then decreased as the variables moved away from their optimized values. However, among the interaction terms, only those between the Triton X-114 concentration (X_3) and the other two factors (pH, X_1 and equilibration temperature, X_4) were significant The fluorescence intensity rapidly increased with increases in pH, Triton X-114 concentration, and equilibration temperature (Fig. 1b and f). There were clear maxima when the Triton X-114 concentration was zero, the pH was high, and the equilibration temperature was low. These results indicated that pH influenced formation of the hydrophobic complex, and that higher equilibration temperatures led to decomposition of the hydrophobic complex. Meanwhile, the elliptical nature of contour plots (Fig. 2b and f) suggested that there were considerable interactions among them.

According to canonical analysis, the optimum extraction conditions were as follows: pH 4.0, 2.25×10^{-2} g/L APDC, 0.11% (m/v) Triton X-114 and an equilibration temperature of 51.4 °C. Under these conditions the predicted value for the fluorescence intensity was 6091, which agreed with the mean experimental fluorescence intensity of 6087. Therefore, the developed model was accurate and reliable in predicting the fluorescence intensity of extracted Se(IV).

3.2. Effect of the concentration of the back extraction agent

For the second CPE, the choice of back extraction agent is essential to successful coupling of d-CPE preconcentration to HG-AFS. The hydrophobic complex of Se(IV)-APDC should be converted to water-soluble Se(IV) before its back extraction into an aqueous phase. Dilute HCl was considered a favorable medium for hydride generation and was examined for its suitability as the back extraction agent. The HCl concentration was optimized in the 5%–25% (v/v) range, and 16% HCl was the optimal volume fraction. However, the experimental fluorescence intensity of Se(IV) was weak, suggesting that the efficiency of back extraction with this HCl concentration was rather low.

To improve the efficiency of back extraction, H_2O_2, a convenient and effective oxidizing agent, was included in the 16% (v/v) HCl solution, which allowed for rapid and complete oxidative digestion of the Se(IV)-APDC complex. H_2O_2 can oxidize the sulfhydryl group to -SOH, -SO_2H, or -SO_3H (Howard, Jurbergs, & Holcombe, 1998). APDC contains a sulfhydryl group, and addition of H_2O_2 to Se (IV)-APDC resulted in release of free Se(IV). The fluorescence intensity increased sharply when the H_2O_2 volume fraction was increased from 5% to 15%, and leveled off in the volume fraction range 15%–25%. Therefore, a solution containing 16% (v/v) HCl and 20% (v/v) H_2O_2 was employed to extract Se(IV) ions from the hydrophobic complexes for extraction back into an aqueous phase.

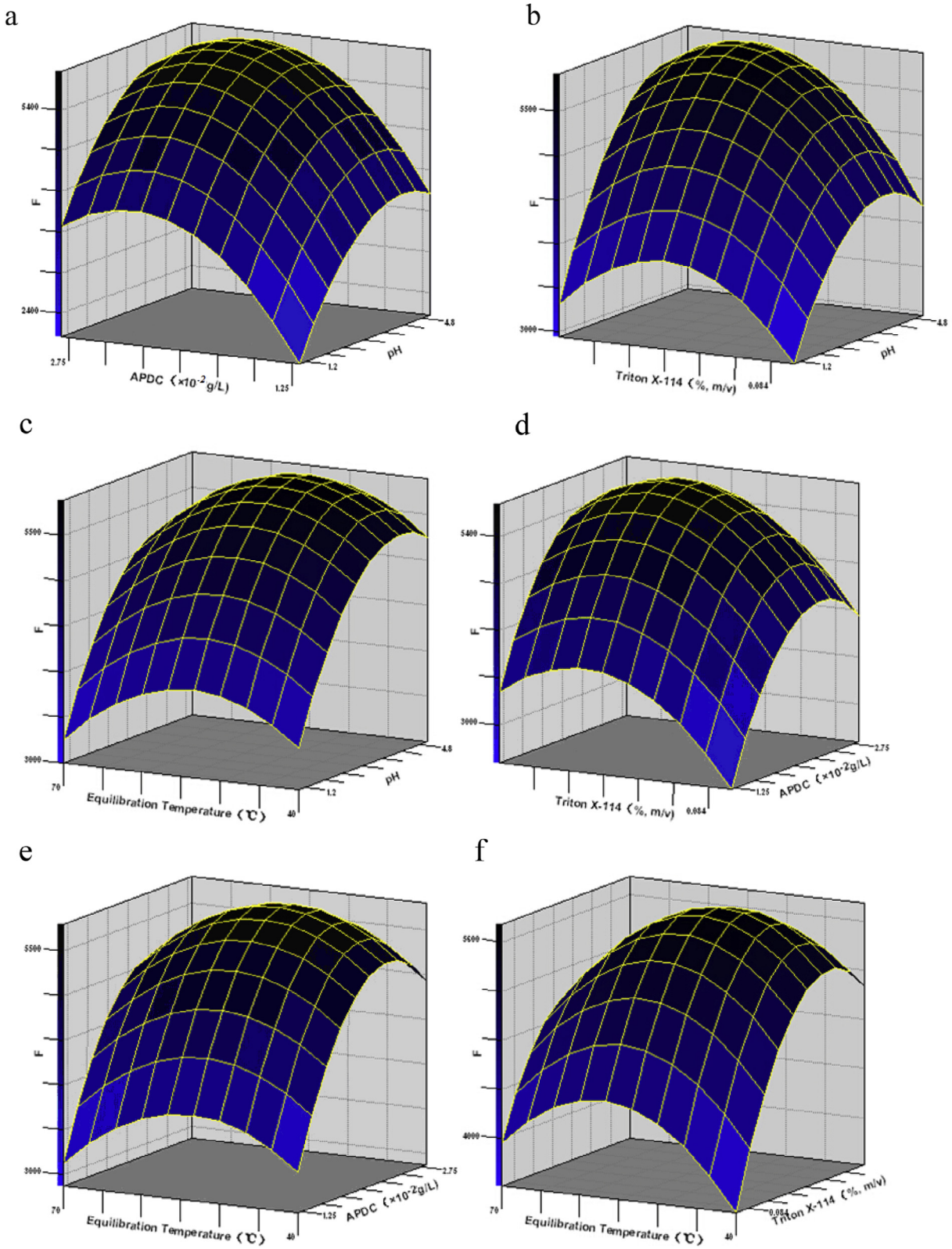

Fig. 1. Response surface for fluorescence intensity of Se(IV) extracted by the d-CPE method.

3.3. Interference

The selectivity of the proposed d-CPE towards Se(IV) was examined by investigating the effects of foreign ions on the determination of 6.0 μg L^{-1} Se(IV). The influence of each ion was considered significant when its presence altered the recovery of Se(IV) by more than ±5%. The tolerable mass ratios of foreign ions to Se(IV) were found to be greater than 5000 for K^+ and Na^+, 2000 for Mg^{2+} and Ca^{2+}, 1000 for Al^{3+}, 400 for Fe^{3+}, 100 for Pb^{2+}, 20 for Cd^{2+}, and 5 for Cu^{2+}. Most of the foreign ions investigated had no

Table 5
Analytical characteristics of the proposed method.

Parameters	d-CPE method	CPE method
Regression equation	F = 979.28 C (μg/L) +211.96	F = 146.33 C (μg/L) −54.824
Regression coefficient (r^2)	0.9980	0.9625
Linear range (μg L^{-1})	0.5–6.0	0.5–6.0
LOD (μg L^{-1})[a]	0.023	0.141
RSD% (n = 10)	4.04	12.6
Enhancement factor[b]	11.8	1.77

[a] Limit of detection (LOD) is defined as the ratio of three times the standard deviation of 10 measurements of the blank signals and the slope of the calibration curve.
[b] Enhancement factor (EF) is defined as the ratio of the slope of preconcentrated samples (50 ml) to that obtained without preconcentration.

considerable influence on the determination of Se(IV) because of the selectivity of APDC at the working pH. However, the tolerable mass ratios of Cd^{2+} and Cu^{2+} were much lower, probably because they could react with APDC in competition with Se(IV). Therefore, a high concentration of APDC was used to ensure complete chelation of Se(IV).

3.4. Analytical figures of merit

The calibration curve of Se(IV) was obtained by preconcentration of a 50-mL sample under the optimized conditions. Solutions of Se(IV) with concentrations between 0.5 and 6.0 μg L^{-1} were analyzed by conventional CPE for comparison with the d-CPE method. Table 5 lists the calibration parameters, relative standard deviations for 10 replicate determinations at the 6.0 μg L^{-1} level, and limits of detection (LOD). The calibration parameters, RSD, and LOD obtained by d-CPE were much better than those obtained by conventional CPE.

Although an antifoam solution can be added to quench surfactant foaming during hydride generation, the fluorescence signal obtained using conventional CPE was weak and the regression coefficient (r^2) was only 0.9625. This could occur if the Se(IV) is not completely released from the Se(IV)-APDC complex, leading to low efficiency of hydride generation. In d-CPE, a mixture of 16% (*v*/*v*) HCl and 20% (*v*/*v*) H_2O_2 converted the Se(IV)-APDC into free Se(IV), which was rapidly and completely reduced to H_2Se and directly determined by HG-AFS. The developed d-CPE had better sensitivity and precision with a wider dynamic linear range than CPE.

Finally, the newly developed d-CPE method was compared with other published methods for the extraction and determination of Se(IV) (Table 6). Despite the relatively low enhancement factor, which was attributed to a large extract volume (3 mL), the LOD of the proposed method was comparable to reported methods. Compared with solvent extraction, the instrumentation for CPE is simple and inexpensive, and the method does not use toxic organic solvents. The hydrophobic species, which is extracted into the surfactant-rich phase, is separated from the complicated sample matrix with a high-enrichment factor because of the small volume of surfactant-rich phase. CPE is a well-established method for separation and preconcentration of hydrophobic species. In addition to the above advantages of traditional CPE, d-CPE eliminates the effect of surfactant foaming and has a wide application range for sample pretreatment.

3.5. Analysis of food samples

The developed procedure was applied to the determination of Se(IV) in different food samples. Recovery experiments were also carried out by spiking food samples with different amounts of the Se(IV) standard before pretreatment. Reasonable recoveries (95.6–105.2%) were obtained (Table 7). To further validate the reliability of the proposed method, the certified reference materials GBW 10045 (rice), GBW 10023 (laver), and GBW 10025 (spirulina) were analyzed. The total selenium concentrations were 0.038 ± 0.010, 0.119 ± 0.012, and 0.228 ± 0.06 μg L^{-1} (n = 6) for rice, laver and spirulina respectively. These results were in good agreement with the certified values of 0.04 ± 0.013 (rice), 0.124 ± 0.014 (laver), and 0.24 ± 0.05 μg L^{-1} (spirulina). These results showed that there was no matrix interference.

Table 7
Determination of samples and recoveries (n = 3).

Sample	Se(IV) added (μg/g)	Se(IV) measured (μg/g)	Recovery (%)
Spirulina	–	0.0858	–
	0.091	0.1731	96.0
Seaweed	–	0.0711	–
	0.073	0.1440	100.3
Wakame	–	0.0992	–
	0.100	0.1970	97.8
Laver	–	0.1820	–
	0.180	0.3541	95.6
Tea leaf	–	0.0353	–
	0.036	0.0716	100.0
Mushroom	–	0.0512	–
	0.050	0.1102	97.1
Black fungus	–	0.0470	–
	0.050	0.0996	105.2
Tremella	–	0.0786	–
	0.078	0.1552	98.2
Rice	–	0.0493	–
	0.050	0.1010	103.5
Wheat	–	0.0631	–
	0.065	0.1271	98.5
Soya	–	0.0572	–
	0.050	0.1083	102.2
Corn	–	0.0356	–
	0.040	0.0772	104.0

4. Conclusions

A novel d-CPE method was developed for the preconcentration and determination of trace Se(IV) by HG-AFS. Compared with traditional CPE, the developed d-CPE technique eliminated deleterious effects of the surfactant during hydride generation and converted the Se(IV) complex into Se(IV) to enhance the fluorescence signal. The extract obtained by d-CPE could be measured directly by HG-AFS. Additionally, RSM was useful for optimization of the experimental factors, and was used to evaluate interactions between multiple variables. The proposed method was successfully applied to the determination of selenium content in twelve food samples.

The LOD of the proposed method was similar to the values reported for more complex and expensive instrumentation, despite a relatively low enrichment factor. Further improvement in the detection limit and enrichment factor could be achieved by reducing the extract volume.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.11.096.

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