

## Solidified floating organic drop microextraction for speciation of Se (IV) and Se (VI) in water samples prior to electrothermal atomic absorption spectrometric detection

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**Abstract:** A simple and fast solidified floating organic drop microextraction (SFODME) method is suggested for the speciation of Se (IV) and Se (VI) in water samples prior to electrothermal atomic absorption spectrometric (ETAAS) detection. For that purpose, 1-phenylthiosemicarbazide (PTC) was used as chelating agent and undecanol was used as extraction solvent. The pH of solution, extraction solvent volume, amount of ligand, effect of time for complex formation, and effect of possible foreign ions were also evaluated for quantitative and effective extraction of analyte. Under optimized parameters, detection limit ( $0.19 \mu\text{g L}^{-1}$ ), limit of quantification ( $0.60 \mu\text{g L}^{-1}$ ), relative standard deviation (4.6%), linear range ( $0.60\text{--}24 \mu\text{g L}^{-1}$ ), relative error (−4.3%), and enrichment factor (53) were calculated, respectively. The accuracy of the SFODME method was confirmed with analysis of reference material (LGC 6010 Hard drinking water). The presented method was applied to water samples.

**Key words:** Speciation, SFODME, selenium, microextraction, electrothermal atomic absorption spectrometry

### 1. Introduction

Selenium is known as an essential micronutrient for all living organisms. It is found in organic and inorganic forms. Se (IV) and Se (VI) are the most common inorganic species in the environment.<sup>1–5</sup> The toxicity of selenium is associated with concentration level in a sample and as well as its chemical forms. Therefore, it is essential to determine concentration levels of selenium species in environmental and food samples.<sup>6–8</sup> However, it is difficult to obtain more reliable results because of interference caused by the matrix and very low concentrations of selenium in many samples.<sup>9</sup> To obtain reliable results, separation and enrichment steps are necessary prior to the determination of selenium in samples.<sup>10–12</sup>

Nowadays the popular trend in analytical chemistry is to develop simplified and miniaturized extraction methods. Miniaturization of the preconcentration methods provides many benefits like minimizing toxic solvent consumption and being simple, unexpansive, and nontedious. Many microextraction methods coupled with ETAAS have been applied to analyze trace level and overcome matrix problems in environmental samples. These methods are single-drop microextraction (SDME), solid-phase microextraction (SPME), dispersive liquid–liquid microextraction (DLLME), stir-bar sorptive microextraction (SBSE), solidified floating organic drop microextraction (SFODME), headspace liquid-phase microextraction (HS-LPME), and hollow-fiber membrane liquid-phase microextraction (HF-LPME).<sup>13–17</sup> Among these methods, SFODME procedures have many

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advantages like simplicity, short extraction time, applicability, reduced efforts, and minimized toxic solvent.<sup>18–22</sup> In the solidification-based microextraction methods, a small volume of suitable organic extraction solvent (melting point: 10–30 °C) is stirred for a desired extraction time and then transferred into an ice bath (5–10 min) for solidification of organic solvent.<sup>23–26</sup> The solidified extraction solvent containing the analyte is separated with a spatula and after dissolving in a suitable solvent, it is injected into the instrument for analysis.

In this study, a simple and fast SFODME method in combination with ETAAS for speciation and determination of Se (IV) and Se (VI) in water samples was developed. Factors for quantitative and effective extraction such as the pH of solution, extraction solvent volume, amount of ligand, effect of time for complex formation, and effect of possible foreign ions were evaluated and optimized.

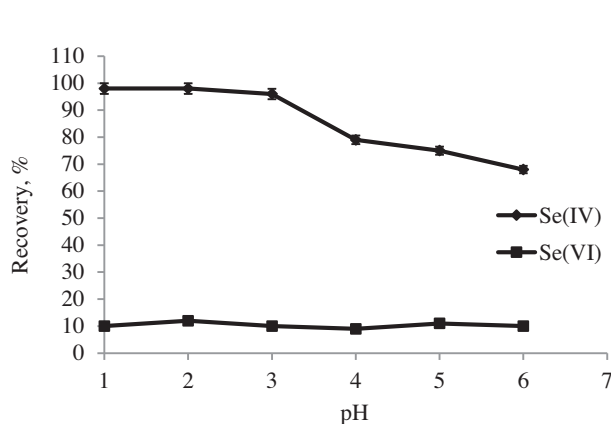
## 2. Results and discussion

### 2.1. Effect of pH

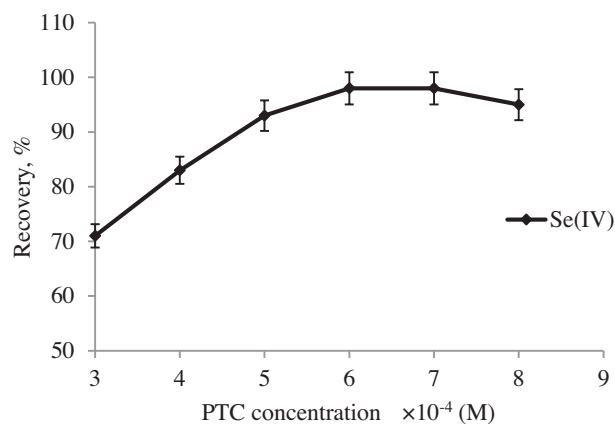
The pH of sample solution plays an important role in all speciation works<sup>27</sup> because it affects the interactions between the analyte and chelating agent. For quantitative and selective speciation of selenium 6–7 mL of solution containing 1.0  $\mu\text{g L}^{-1}$  Se (IV) and Se (VI) ions was studied in the range of pH 1.0–6.0 by using 0.1 M HCl and NaOH. As it can be seen from Figure 1, Se (IV) recoveries were quantitative in the range of pH 1–3 but Se (VI) recoveries were less than 10% for all pH values. Therefore, pH 1.5 was chosen as the optimum pH.

### 2.2. Optimization of ligand amount and undecanol volume

Formation of complexes is an important factor and it depends on the amount of chelating agent.<sup>28,29</sup> The effect of PTC amount for the excellent extraction ability of Se(IV) on SFODME procedure was evaluated from  $3.0 \times 10^{-4}$  to  $8.0 \times 10^{-4}$  M solution (see Figure 2). It was seen that sample solution including  $6 \times 10^{-4}$  M of PTC was of sufficient concentration for quantitative extraction of Se (IV).



**Figure 1.** Effect of pH on recoveries of Se (IV) and Se (VI) (N = 3).



**Figure 2.** Effect of PTC concentration on SFODME (N = 3).

In order to optimize extraction solvent volume, different volumes of undecanol (50, 60, 70, 80, 90, and 100  $\mu\text{L}$ ) were treated to perform SFODME. As shown in Figure 3, the recovery of Se (IV) increased with increasing volume of undecanol until 70  $\mu\text{L}$ ; then recoveries stayed nearly stable. Quantitative extraction was also observed at this volume and 70  $\mu\text{L}$  volume of undecanol was chosen as the optimal volume for further

works. This undecanol phase was treated with 0.1 M HNO<sub>3</sub> in methanol and settled to 150 μL volume for reducing its viscosity before the determination step of Se (IV) by ETAAS.

### 2.3. Optimization of other parameters

The effect of four other conditions (shaking time, centrifugation time and rate, effect of time to complex formation, sample volume) on SFODME was studied (6–7 mL of solution containing 1.0 μg L<sup>-1</sup> Se (IV) and Se (VI), pH: 1.5, 6 × 10<sup>-4</sup> M PTC, 70 μL undecanol). In this work, a vortex was used for shaking and increasing the interaction of sample and extraction solvent. For that purpose, a vortex time in the range of 1–6 min was investigated and 4 min was found to be the optimum vortex time. Centrifugation time (1–5 min) and rate (500–3000 rpm) were also evaluated. An excellent separation of phases and quantitative extraction of selenium were achieved at the centrifugation rate of 2500 rpm for 5 min.

Sometimes the formation of metal–ligand complex takes time but sometimes it ends in a short time. This time factor affects extraction recovery. Because of this reason, complex formation time was evaluated in the range of 1–20 min for the SFODME procedure. It was observed that complex formation of Se (IV)–PTC takes 15 min; yellowish complex. A complex formation time of 15 min was chosen for further studies of SFODME. For providing a high preconcentration factor and low detection limit, volume of sample was also studied in the range of 2–11 mL. As can be seen from Figure 4, the targeted analyte ion was recovered quantitatively for the whole working range of 2–8 mL and the highest sample volume for extraction was chosen as 8 mL and preconcentration factor was calculated as 53 according to ratios of sample (8 mL) volume and final diluted volume (150 μL).

### 2.4. Influence of matrix ions

PTC can react with some ions in water samples and can cause decreased recovery of Se (IV). The effects of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and PO<sub>4</sub><sup>3-</sup> were tested for selective speciation and preconcentration with the concentration 2000, 2000, 1000, 750, 3000, 3000, 1500, and 1500 mg L<sup>-1</sup>, respectively. In the evaluation of the results, tolerable limit was used as causing a relative error ±5%. Under optimized conditions (8 mL of solution containing 1.0 μg L<sup>-1</sup> Se (IV) and Se (VI), pH: 1.5, 6 × 10<sup>-4</sup> M PTC, 70 μL undecanol) interfering cations and anions generally present in water samples were added separately and altogether. These added cations and anions showed no significant effect on the SFODME method.

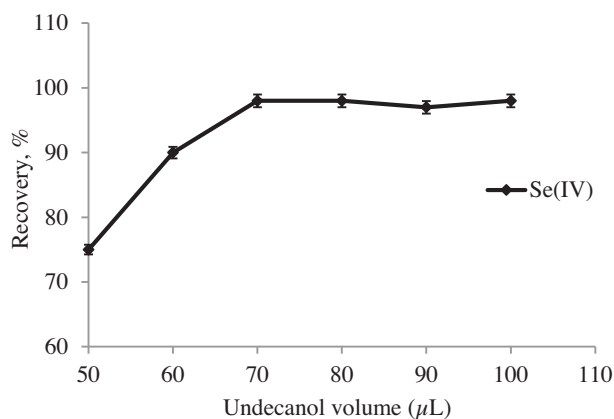


Figure 3. Effect of extraction solvent volume (N = 3).

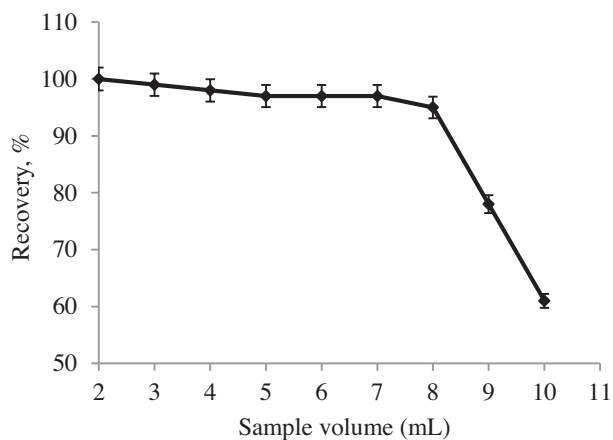


Figure 4. Effect of sample volume (N = 3).

## 2.5. Analytical performance of SFODME

Under optimized parameters, limit of detection (LOD), limit of quantification (LOQ), analytical range, relative standard deviation (RSD), and relative error were calculated. The LOD ( $3S_b$ ,  $S_b$  is the standard deviation of eleven replicates of the blank measurement) and LOQ ( $10S_b$ ) were  $0.19 \mu\text{g L}^{-1}$  and  $0.60 \mu\text{g L}^{-1}$ , respectively. The linear range was calculated as  $0.60\text{--}24 \mu\text{g L}^{-1}$ . The RSD (4.6%) was calculated from seven replicates determination of  $1.0 \mu\text{g L}^{-1}$  Se (VI). LGC 6010 Hard drinking water was used to establish the validity of the developed methodology. The determined ( $8.9 \pm 0.3 \mu\text{g L}^{-1}$ ) value and certified value ( $9.3 \mu\text{g L}^{-1}$ ) of Se (IV) showed good agreement. Relative error was found as  $-4.3\%$ .

## 2.6. Analyses of water samples

Yeşilirmak river water, Kelkit river water, and Almus dam water samples were collected from the city of Tokat (Turkey) and a seawater sample was collected from the city of Mersin (Turkey). All collected water samples were filtered before application. As can be seen from Table 1, the developed SFODME (in Section 3.2) procedure can be applied to water samples for speciation of selenium without any matrix effect.

**Table 1.** Determination Se (IV) and Se (VI) in water samples (sample volume: 8 mL, final volume: 150  $\mu\text{L}$ ; N = 3).

Samples	Added ( $\mu\text{g L}^{-1}$ )		Found ( $\mu\text{g L}^{-1}$ )			Recovery (%)		
	Se (IV)	Se (VI)	Se (IV)	Se (VI)	Total Se	Se (IV)	Se (VI)	Total Se
Yeşilirmak river water	-	-	$1.13 \pm 0.08$	$0.95 \pm 0.18$	$2.08 \pm 0.17$	-	-	-
	3.0	3.0	$4.08 \pm 0.15$	$3.85 \pm 0.34$	$7.93 \pm 0.30$	$98 \pm 2^*$	$97 \pm 3$	$98 \pm 3$
	6.0	6.0	$6.98 \pm 0.38$	$6.62 \pm 0.62$	$13.6 \pm 0.5$	$98 \pm 3$	$95 \pm 4$	$96 \pm 3$
Kelkit river water	-	-	BDL	BDL	BDL	-	-	-
	3.0	3.0	$2.90 \pm 0.20$	$2.90 \pm 0.36$	$5.80 \pm 0.31$	$97 \pm 2$	$97 \pm 4$	$97 \pm 2$
	6.0	6.0	$5.83 \pm 0.28$	$5.87 \pm 0.48$	$11.7 \pm 0.4$	$97 \pm 2$	$98 \pm 3$	$98 \pm 2$
Almus dam water	-	-	$0.99 \pm 0.06$	$1.05 \pm 0.14$	$2.04 \pm 0.13$	-	-	-
	3.0	3.0	$3.84 \pm 0.11$	$3.94 \pm 0.23$	$7.78 \pm 0.21$	$95 \pm 2$	$96 \pm 3$	$96 \pm 3$
	6.0	6.0	$6.88 \pm 0.30$	$6.92 \pm 0.50$	$13.8 \pm 0.4$	$96 \pm 3$	$98 \pm 4$	$98 \pm 3$
Seawater	-	-	BDL	BDL	BDL	-	-	-
	3.0	3.0	$2.93 \pm 0.19$	$2.85 \pm 0.39$	$5.78 \pm 0.34$	$98 \pm 2$	$95 \pm 3$	$96 \pm 2$
	6.0	6.0	$5.90 \pm 0.25$	$5.93 \pm 0.47$	$11.8 \pm 0.4$	$98 \pm 3$	$99 \pm 4$	$98 \pm 3$
Tap water	-	-	BDL	BDL	BDL	-	-	-
	3.0	3.0	$2.86 \pm 0.15$	$2.97 \pm 0.30$	$5.83 \pm 0.26$	$95 \pm 2$	$99 \pm 4$	$97 \pm 3$
	6.0	6.0	$5.76 \pm 0.29$	$5.84 \pm 0.49$	$11.6 \pm 0.39$	$96 \pm 3$	$97 \pm 4$	$98 \pm 4$

\*Mean  $\pm$  standard deviations, BDL: Below detection limit.

## 2.7. Comparison with existing methods

The optimized SFODME method was compared with other selenium preconcentration works in the literature. The developed SFODME methodology has excellent precision, very low detection limit, and high preconcentration factor when compared with some studies in the literature in Table 2. Moreover, use of disperser solvent in liquid phase microextraction methods leads to an increase in the cost of the method and environmental contamination. Use of any disperser solvent in this SFODME reveals another advantage. However, long complex formation time and low pH working media could be considered disadvantages of this work.

## 2.8. Conclusions

In this study, a simple SFODME method was developed for Se (IV) and Se (VI) speciation in combination with ETAAS. The miniaturized SFODME has many advantages like simplicity, short extraction time, applicability,

reduced efforts, and minimized toxic solvent for selective speciation and determination of Se (IV) and Se (VI) in water samples. In addition, it represents a green technology for selective speciation and determination of Se (IV) and Se (VI) in water samples, due to the use of low volumes of undecanol and no disperser reagent.

**Table 2.** Comparison of proposed SFODME method with other reported methods in the literature.

Method	Techniques	LOD ( $\mu\text{g L}^{-1}$ )	PF	R.S.D. (%)	References
DLLME	ETV-ICP-MS	47	64.8	7.2	30
DLLME-SFO	UV	16	250	2.1	31
SFODME	ETV-ICP-MS	0.19	500	5.5	32
DLLME	ETV-ICP-MS	0.008	107	9.2	33
SPE	ICP-MS	0.016	10	6.2	34
DLLME	ETAAS	0.05	70	4.5	35
DLLME	GC-ECD	0.005	122	4.1	36
DLLME	TXRF	1.1	10	-	37
On-line IL-DLLME	ETAAS	0.015	20	5.1	38
USAEME; DLLME	GC-FID	0.05; 0.11	2491; 1129	5.32; 4.57	39
DLLME	ETAAS	3	140	< 5.1	40
DLLME-SFOD	UV	1.6	133	2.1	41
DLLME	HPLC	0.11	25	2.3	42
SFODME	ETAAS	0.19	53	4.6	This work

DLLME: Dispersive liquid–liquid microextraction, DLLME-SFO: Dispersive liquid–liquid microextraction-solidified floating organic drop, SFODME: Solidified floating organic drop microextraction, SPE: Solid phase extraction, ETV-ICP-MS: electrothermal vaporization inductively coupled plasma mass spectrometry. ETAAS: electrothermal atomic absorption spectrometry, GC-ECD: gas chromatography–electron-capture detection, TXRF: total reflection X-ray, USAEME: ultrasound-assisted emulsification microextraction, GC-FID: gas chromatography-flame ionization detection, HPLC: high-performance liquid chromatography.

### 3. Experimental

#### 3.1. Instruments and chemical reagents

Selenium concentrations were determined by using a PerkinElmer Analyst 700 (Norwalk, CT, USA) atomic absorption spectrometer equipped with a deuterium background correction system and HGA-800 electrothermal atomizer. A selenium electrodeless discharge lamp was used at 200 mA. The wavelength and spectral band pass were 196.0 nm and 2.0 nm, respectively. Pyrolytic-coated (platformed) graphite tubes were used during the analysis step (temperature ( $^{\circ}\text{C}$ )/ramp time (s)/hold time (s) for drying 1 100/5/20, drying 2 140/15/15, ashing 1200/10/20, atomization 2200/0/5, and cleaning 2600/1/3, argon flow rate  $250\text{ mL min}^{-1}$ ). The pH of model solutions and water samples was adjusted by Sartorius pp-15 model pH meter (Gottingen, Germany). A Nüve model NF 200 (Ankara, Turkey) centrifuge was used for centrifugation of solutions.

Analytical reagent grade undecanol, 1-phenylthiosemicarbazide (PTC), hydrochloric acid, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). A  $1 \times 10^{-3}$  M solution of 1-phenylthiosemicarbazide was prepared by dissolving in methanol. Matrix modifiers (Pd and  $\text{Mg}(\text{NO}_3)_2$ ) were obtained from Merck. Samples of 20  $\mu\text{L}$  plus 10  $\mu\text{L}$  of mixture of 0.015 mg Pd + 0.010 mg  $\text{Mg}(\text{NO}_3)_2$  as matrix modifier were injected into the graphite furnace. The reagents for selenium standard solutions were obtained from Sigma and Aldrich (St. Louis, MO, USA).

### 3.2. SFODME method

First 8 mL of solution containing Se (IV) and Se (VI) was adjusted to pH 1.5. Next PTC was added to obtain the desired working value ( $6 \times 10^{-4}$  M) and the mixture was left for 15 min for yellowish complex formation. Then 70  $\mu$ L of undecanol was added. This mixture was shaken by vortex (3.300 rpm) for 4 min then the solution was centrifuged at 2500 rpm for 5 min and transferred into an ice bath (5–10 min) for solidification of the upper undecanol phase. The solidified undecanol phase containing analyte was separated with a spatula and after dissolving with 0.1 M HNO<sub>3</sub> in methanol (settled to 150  $\mu$ L), it was injected into the instrument for determination of Se (IV).

### 3.3. Reduction process of Se (VI) to Se (IV)

Total selenium concentrations were determined after reduction of Se (VI) to Se (IV) using a reduction procedure:<sup>2</sup> 2 M HCl was added to the water samples; then the microwave program of 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min was performed. Se (VI) concentration was calculated as the difference between total selenium and Se (IV) concentrations.

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