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Determination of inorganic selenium species in water and garlic samples with on-line ionic liquid dispersive microextraction and electrothermal atomic absorption spectrometry

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ABSTRACT

A non-chromatographic separation and preconcentration method for Se species determination based on the use of an on-line ionic liquid (IL) dispersive microextraction system coupled to electrothermal atomic absorption spectrometry (ETAAS) is proposed. Retention and separation of the IL phase was achieved with a Florisil®-packed microcolumn after dispersive liquid–liquid microextraction (DLLME) with tetradecyl(trihexyl)phosphonium chloride IL (CYPHOS® IL 101). Selenite [Se(IV)] species was selectively separated by forming Se–ammonium pyrrolidine dithiocarbamate (Se–APDC) complex followed by extraction with CYPHOS® IL 101. The methodology was highly selective towards Se(IV), while selenate [Se(VI)] was reduced and then indirectly determined. Several factors influencing the efficiency of the preconcentration technique, such as APDC concentration, sample volume, extractant phase volume, type of eluent, elution flow rate, etc., have been investigated in detail. The limit of detection (LOD) was 15 ng L^{-1} and the relative standard deviation (RSD) for 10 replicates at 0.5 μg L^{-1} Se concentration was 5.1%, calculated with peak heights. The calibration graph was linear and a correlation coefficient of 0.9993 was achieved. The method was successfully employed for Se speciation studies in garlic extracts and water samples.

1. Introduction

The application of state-of-the-art solvents such as ionic liquids (ILs), in combination with microextraction techniques has attracted considerable attention in the last years in the field of analytical chemistry [1,2]. Ionic liquids are liquid salts with melting points close or below room temperature. They are generally considered to be environmentally friendlier than common organic solvents and have unique characteristics (e.g. no effective vapor pressure, adjustable viscosity and miscibility in aqueous phases) [3]. Moreover, they are also considered as highly efficient extractant phases turning them into important tools for analytical methods involving a preconcentration step [1,3–5]. Liquid–liquid microextraction (LLME) is a relatively recent concept whose main advantages are very low consumption of solvents and low cost [6]. The use of ILs in LLME is based principally on their high extraction efficiency and low volatility [5]. Furthermore, IL-LLME can be developed in different modes. Modern microextraction techniques include single-drop microextraction (SDME) [7,8], hollow fiber liquid-phase microextraction (HF-LPME) [9], ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME) [10], cold induced aggregation microextraction (CIAME) [11,12] and dispersive liquid–liquid microextraction (DLLME) [13,14]. Among these techniques, DLLME has demonstrated to be an efficient alternative to obtain excellent extraction efficiency while keeping minimal the volume of solvent required for analysis [5,15,16]. One of the main advantages of DLLME compared with others LPME techniques is that a shorter microextraction time is required. This observation can be easily explained since equilibrium conditions are not commonly achieved, as a result of the infinitely large surface area formed between extractant and aqueous phase in DLLME. However, up to date, DLLME has been performed mostly in a batch mode [5]. Consequently, the risk of contamination is very high and the operation is usually time-consuming.

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Determination of Se at trace levels has become of increasing importance because of its dual role as an essential element at low concentration levels and a toxic substance at high levels as well as its role in cancer prevention [17–19]. This broad range between the nutritional requirements and toxic effects makes important the study of a wide variety of samples such as garlic and different water samples. Despite the relevance of total content of Se, the chemical form in which Se is present is also important due to the differences in bioavailability and toxicity of the different forms [20,21]. The biochemical activity of Se depends on its oxidation state and, in general, inorganic forms of Se are more toxic than the organic forms [22]. Thus, the toxicity of Se increases in the following order: selenite [Se(IV)] < selenate [Se(VI)] < hydrogen selenide (H_2Se). Moreover, Se(IV) and Se(VI) species are the most common inorganic forms of Se [23]. In the majority of environmental matrices, Se is usually present as Se(IV) and Se(VI), as these oxidation states are the most environmentally mobile and geochemically important forms of this element. However, the concentration of Se in environmental samples is in the order of a few $\mu g\,L^{-1}$ [24]. On the other hand, the influence of matrix components occurring in common real samples is another problem in these determinations. In order to solve these drawbacks, preconcentration and separation steps are usually required. Recently, Bidari et al. have proposed the use of common organic solvents in DLLME for preconcentration and determination of an inorganic selenite [Se (IV)] derivative with gas chromatography–electron-capture detection [15]. Ionic liquids have been used in liquid chromatography as mobile phase additives for Se species separation with ICP-MS detection [25]. However, the application of ILs for Se extraction and preconcentration based on DLLME technique has not been explored so far.

In this work, inorganic Se species separation and preconcentration were mediated by chelation with ammonium pyrrolidine dithiocarbamate (APDC) reagent followed by an on-line DLLME approach using CYPHOS® IL 101. Retention and separation of dispersed IL phase containing the analyte was achieved with a Florisil®-packed minicolumn implemented in a flow injection analysis (FIA) system. The proposed methodology was designed to differentiate between inorganic Se(IV) and Se(VI) thanks to selective chelation of Se(IV) species with APDC [26]. A H_2SO_4 acid solution was used for selective extraction of inorganic Se species from garlic samples. Total inorganic Se concentration was evaluated after reduction of Se(VI) to Se(IV) with hydrochloric acid. The on-line DLLME method was successfully coupled to electrothermal atomic absorption spectrometry (ETAAS) for Se determination in both garlic extracts and water samples.

2. Experimental

2.1. Instrumentation

Elemental detection was performed using a PerkinElmer 5100ZL atomic absorption spectrometer (PerkinElmer, Norwalk, CT, USA) equipped with a pyrolytic graphite tube (PerkinElmer) and a transversely heated graphite atomizer Zeeman-effect background correction system. A Se electrodeless discharge lamp (EDL) (PerkinElmer) operated at a current of 210 mA (modulated operation) and a wavelength of 196.0 nm with a spectral band pass of 2.0 nm was used. All measurements were made based on absorbance signals with an integration time of 5 s. Instrumental parameters are listed in Table 1.

The flow injection system is shown in Fig. 1. Gilson (Villiers Le-Bell, France) Minipuls 3 peristaltic pumps equipped with Tygon-type pump tubes (Gilson) were employed to propel the sample, reagent and eluent. The sample injection was achieved using six-way rotary valves from Upchurch Scientific (Oak Harbor, WA, USA). A microbore glass column (12 mm effective bed length; 4 mm internal diameter), filled with Florisil® and porous 25 μm glass frits was used for on-line retention of the IL phase.

2.2. Reagents

All the reagents were of analytical grade and the presence of Se was not detected within the working range. CYPHOS® IL 101 was donated by Prof. Ullastiina Hakala (University of Helsinki, Finland) and supplied by CYTEC (Canada); C.A.S. number: 258864-54-9. A 1000 $\mu g\,mL^{-1}$ Se(IV) stock solution was prepared from SeO_2 (Merck, Darmstadt, Germany) in HCl 0.1 $mol\,L^{-1}$ (Ultrex® II Mallinckrodt Baker, Phillipsburg, NJ, USA). Lower concentrations were prepared by diluting the stock solution with HCl 0.1 $mol\,L^{-1}$. A 1000 $\mu g\,mL^{-1}$ Se(VI) stock solution was prepared from Na_2SeO_4 (Merck). A 1000 $mg\,L^{-1}$ palladium solution used as chemical modifier was prepared from $Pd(NO_3)_2{\cdot}2H_2O$ (Fluka, Buchs, Switzerland). A 150 $mg\,L^{-1}$ $Mg(NO_3)_2$ (Merck) stock solution was tested as chemical modifier. These solutions were prepared in 0.1% (v/v) HNO_3 (Ultrex® II Mallinckrodt Baker). A 2% (w/v) ammonium pyrrolidine dithiocarbamate (APDC) solution was prepared in ethanol (Merck). Methanol (Merck) was used as dispersant agent. H_2SO_4 (Merck) solution was used for selective extraction of inorganic Se species from garlic samples. A $NaClO_4{\cdot}H_2O$ (Merck) solution 24% (w/v) was used in order to adjust ionic strength. A surfactant solution containing 5% (w/v) Triton X-114 (Merck) was employed to avoid IL phase sticking onto the Tygon tube walls. Florisil® (100 Å pore size, 70–230 mesh particle size, Aldrich) was used to fill in the microcolumn. Ultrapure water (18 MΩ cm) was obtained from a Millipore Continental Water System (Bedford, MA, USA).

All bottles used for storing samples and standard solutions and the glassware were washed in 10% (v/v) HNO_3 for 24 h and later rinsed with ultrapure water.

2.3. Sample collection and conditioning

Garlic samples were collected from local markets of Mendoza province, in Argentina. Garlic was peeled by hand, avoiding losses of Se due to enzymes activation, taking place during a surface cut [17]. Peeled garlic samples were washed with demineralized water to remove all possible residues from soil. The bulbs were freezed, cut into small pieces and lyophilized. All samples were ground to a very fine powder using an electric coffee grinder (PEABODY, PE-MC9103, China). In order to minimize frictional heating of the sample during grinding, the process was stopped every 10 s and the sample was allowed to cool to room temperature before proceeding with the grinding. The samples were kept in a desiccator until analysis.

For collecting tap water samples, domestic water was allowed to run for 20 min and approximately a volume of 100 mL was collected in a beaker. Tap water samples were analyzed immediately after sampling. River and lake water samples were collected in cleaned bottles rinsed three times with water sample prior to collection. A sample volume of 1000 mL was collected at a depth of 5 cm below the surface. The river samples were filtered through 0.45 μm pore size PTFE membrane filters (Millipore Corporation, Bedford, MA, USA) immediately after sampling.

2.4. Extraction and separation of inorganic Se species

Ultrasound-assisted extraction of inorganic Se species was performed following the procedure described by Matos Reyes et al. [27]. Briefly, 1 g of freeze-dried garlic sample was accurately weighed inside of a 15 mL polyethylene tube and 10 mL of 1 $mol\,L^{-1}$ H_2SO_4 were added to the tube. The slurry was sonicated for 10 min, and the sulfuric extract separated by centrifugation at 3500 rpm ($2054.3 \times g$) for 10 min. The sulfuric extract was filtered. The solid

Table 1
Instrumental and experimental conditions for Se determination.

Instrumental conditions				
Wavelength (nm)				196.0
Spectral band width (nm)				2.0
EDL lamp current (mA)				210
Modifier volume (μL)				25
Modifier amount (μg)				12.5 Pd [as $Pd(NO_3)_2$]
Graphite furnace temperature program				
Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (mL min^{-1})
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Pyrolysis 1	400	90	30	250
Pyrolysis 2	1300	10	20	250
Atomization	1900	0	5	0
Cleaning	2400	1	2	250
Extraction conditions				
Sample volume				4 mL
APDC concentration				7.9×10^{-5} mol L^{-1}
HCl concentration				0.5 mol L^{-1}
Surfactant concentration (Triton X-114)				0.05% (w/v)
$NaClO_4$ concentration				1.5% (w/v)
IL amount				50 mg
Disperser solvent				Methanol (100 μL)
Eluent				Methanol (10% (v/v) HNO_3)
Eluent volume				200 μL
Loading flow rate				0.5 mL min^{-1}
Elution flow rate				0.5 mL min^{-1}

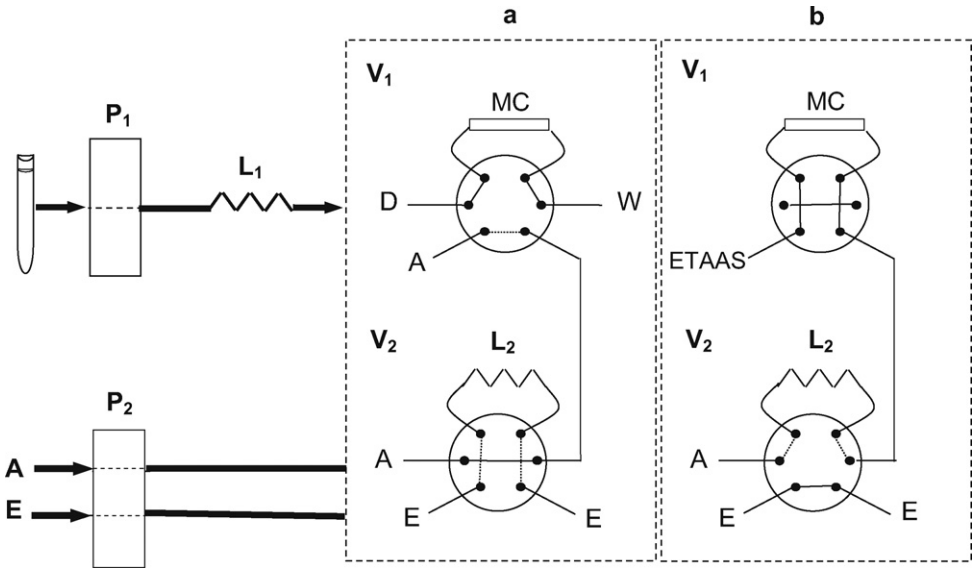

Fig. 1. Schematic diagram of the on-line IL-DLLME set-up. MC, microcolumn; D, dispersion; E, eluent; W, waste; A, air; L_1 and L_2, loops; P_1 and P_2, peristaltic pumps; V_1 and V_2, valves. Valve positions: (a) V_1 dispersion loading and V_2 eluent loading; (b) V_1 enriched phase elution and V_2, eluent delivery.

residue was washed with 10 mL of water; this suspension was centrifuged for additional 10 min and the filtered supernatant was mixed with the previous extract. A volume of 1 mL of concentrated HCl was added to a 5 mL extract aliquot and heated in a hot plate at 100 °C for at least 20 min to reduce Se(VI) to Se(IV) and then total inorganic Se was determined. Another aliquot was acidified to a final HCl concentration of 0.5 mol L^{-1} and submitted to the preconcentration procedure to determine Se(IV). On the other hand, speciation analysis of Se in water samples was performed as described for garlic extracts.

2.5. On-line separation and preconcentration procedure

A schematic diagram of the preconcentration system is shown in Fig. 1. The column was conditioned for preconcentration at the correct pH with 0.5 mol L^{-1} HCl solution. In the preconcentration step (Fig. 1), 4 mL of sample solution, 30 μL of 10^{-2} mol L^{-1} APDC solution, 40 μL of 5% (w/v) Triton X-114 and 250 μL of 24% (w/v) $NaClO_4$ were placed in a centrifuge tube. An amount of 50 mg of CYPHOS® IL solubilized in 100 μL of methanol was injected into the sample. The resultant system was shaken for about 3 s with a vortex before loading the dispersion into the column at a flow rate of 0.5 mL min^{-1}. The IL phase containing the Se–APDC complex was separated and retained by the filling material of the column. After loading, further washing with 0.1 mol L^{-1} HCl-0.025% (w/v) Triton X-114 solution served to remove any sample still present in the lines and in the column. In the elution step (Fig. 1), valves V_2 and V_3 were set on injection position and the retained IL rich phase was eluted with 200 μL of methanol acidified to 10% (v/v) HNO_3. Eluent was delivered by pump P_2 with an air stream at a flow rate

of 0.5 mL min^{-1} in countercurrent mode. A volume of 40 μL of the enriched phase was injected into the graphite furnace of ETAAS for Se determination under the conditions shown in Table 1. Calibration was performed against aqueous standards submitted to the same preconcentration procedure. Likewise, blank solutions were analyzed in the same manner as standard and sample solutions.

3. Results and discussion

3.1. Study of possible matrix effects of CYPHOS® IL 101 on Se determination by ETAAS

It was very important to select an appropriate pyrolysis temperature for removing organic matter resulting from the IL phase while preventing the pyrolysis loss of Se before the atomization step. Since the thermal behavior of ILs shows that the onset weight loss for CYPHOS® IL 101 occurs at 350 and 290 °C under nitrogen and air, respectively [28], the application of higher pyrolysis temperatures would be desirable. Two pyrolysis steps applying diverse temperature ramps during pyrolysis step were assayed as they allowed gradual elimination of organic matrix avoiding Se losses. The influence of pyrolysis temperature on the absorbance was studied within a range of 400–1400 °C. Working pyrolysis temperatures were 400 and 1300 °C. Once selected these pyrolysis conditions, the effect of the atomization temperature on Se signal was studied within the range of 1600–2100 °C. The maximum signal was obtained at about 1800 °C and remained constant up to 2000 °C. An atomization time and temperature of 5 s and 1900 °C were selected, respectively. In order to reduce interferences and increase accuracy, the use of a chemical modifier or a modifier mixture has become indispensable in ETAAS measurements. Two modifiers, $Pd(NO_3)_2$ and $Mg(NO_3)_2$, were investigated for Se determination. Sharp and well-defined absorption peaks with a reduced background were obtained in the presence of $Pd(NO_3)_2$. Therefore, 12.5 μg of $Pd(NO_3)_2$ by 25 μL injection was used for each measurement. Thus, the resulting phase was successfully analyzed by ETAAS under the conditions shown in Table 1.

3.2. Column manufacturing and on-line retention of dispersed IL phase

Important considerations were made during phase separation to develop the analysis. CYPHOS® IL 101 is a cost-effective IL but its applicability on liquid–liquid based microextraction and preconcentration methods is limited. CYPHOS® IL 101 is less dense than water and hence the separation and collection of micro volumes of IL phases is not a straightforward and reproducible task. On-line retention of IL phases in a microcolumn could solve this problem because manual operation is not required for IL phase separation process. Based on previous works of our research group regarding on-line retention of IL phase [29,30], a column packed with a suitable material such as Florisil® was used to perform on-line separation of the IL phase. Florisil® proved to be highly effective for IL phase retention [29]. Since column design is a critical parameter, inner diameter and length of the column were carefully optimized in this work [31]. It was observed that a column with a minimal effective bed length of 12 mm was necessary for IL phase retention. Shorter columns did not show efficient retention as IL phase was not entrapped by the filling material. On the other hand, increasing of column length did not enhance Se recovery and larger amounts of eluent were necessary for longer columns. Therefore, a 20-mm long column was chosen as optimal for IL phase retention. Another variable considered in column design was inner diameter. Thus, a reduced inner diameter was preferred in order to fully elute the IL phase retained in the column with a minimal volume of eluent, leading to obtain a high preconcentration factor for Se determination. A 4-mm inner diameter was found to be effective for both IL phase retention and later elution prior to the injection into the graphite furnace of ETAAS instrument. A high back pressure generated in the FI system limited the use of columns with lower inner diameter.

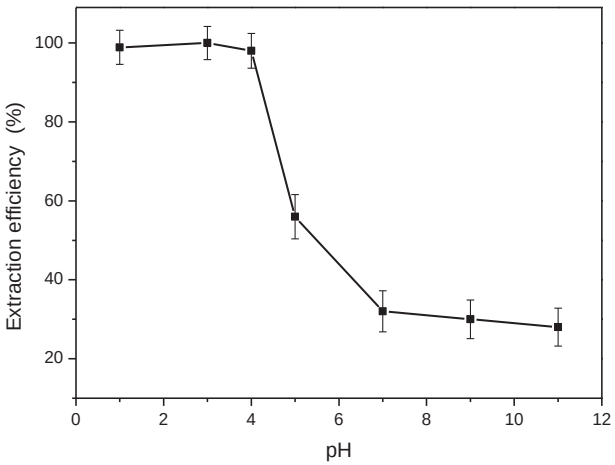

Fig. 2. Influence of pH on the extraction efficiency of Se(IV) by the on-line DLLME system. Experiments performed at a Se concentration of 0.5 μg L^{-1} and APDC concentration of 7.9×10^{-5} mol L^{-1}. Other conditions are listed in Table 1.

3.3. Optimization of the loading variables for Se retention

Several variables were studied in order to optimize Se–APDC complex formation and extraction, as well as retention of the IL phase into the column. Among them pH, surfactant and chelating agent concentration, dispersant solvent volume, IL amount and loading flow rate were studied.

Since the formation of highly stable chelates between Se(IV) species APDC is feasible [26], this reagent was used to improve affinity of Se for the IL phase. The complexation phenomenon is strongly conditioned by the pH of solutions and subsequently affects the extraction efficiency of Se–APDC complex. Therefore, the effect of pH on Se(IV) complexation and extraction with APDC was studied. The results illustrated in Fig. 2 show that the highest extraction efficiency occurs in the pH range of 0.1–4. Furthermore, different HCl concentrations were tested within a range of 0.1–4 mol L^{-1}. The final HCl concentration was adjusted to 0.5 mol L^{-1}.

Reagent concentration is a critical variable to be optimized in extraction methods based on a chelating agent such as APDC. Thus, it is highly important to establish the minimal reagent concentration that leads to total complex formation while achieving the highest extraction. A concentration of 7.9×10^{-5} mol L^{-1} APDC was the minimum concentration required to obtain the highest extraction efficiency.

The extraction process is a complex result of several parameters (e.g., partition coefficient, diffusion coefficient of solute, solubility of extraction solvent, liquid viscosity and complex hydrophobicity). Chelate compounds, in which the metal has become part of the organic structure, are only slightly soluble in water but dissolve readily in organic solvents [32]. In this case, the partitioning mechanism that transfers the analyte from aqueous into IL phase could be similar to that occurring in traditional organic solvents. Hence, it is highly important to establish the minimal volume of IL leading to total complex extraction while achieving the highest signal for a sample volume of 4 mL. The variation of Se signal upon IL amount was examined within the range 10–80 mg (Fig. 3). It was observed

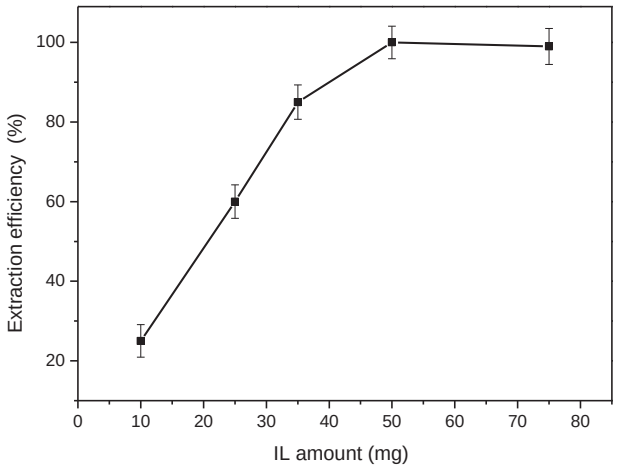

Fig. 3. Influence of CYPHOS® IL 101 amount on the Se(IV) extraction efficiency of the on-line DLLME system. Experiments performed at a Se concentration of 0.5 μg L^{-1} and APDC concentration of 7.9×10^{-5} mol L^{-1} for 4 mL of sample. Other conditions are listed in Table 1.

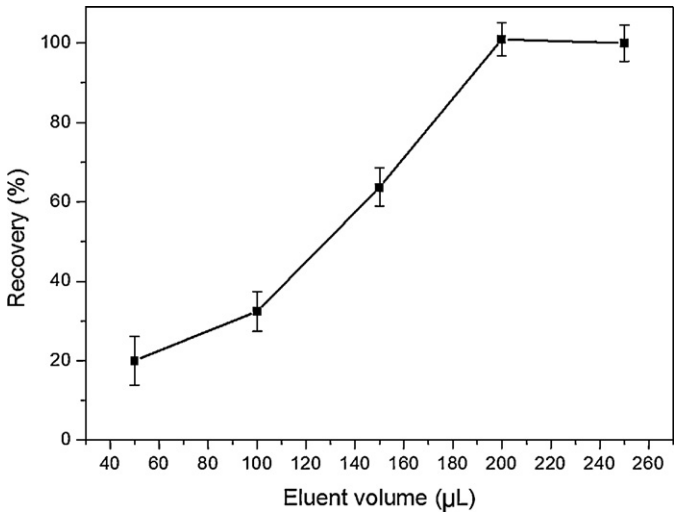

Fig. 4. Dependence of Se recovery with different volume of acidified methanol used as eluent. Experiments performed at a Se concentration of 0.5 μg L^{-1} and APDC concentration of 7.9×10^{-5} mol L^{-1}. Other experimental conditions are listed in Table 1.

that extraction efficiency of the system and Se signal were remarkably affected by IL amount. Quantitative extraction and higher signal were observed for a minimal IL amount of 50 mg. No significant changes were observed on the extraction efficiency by adding higher IL amounts. On the other hand, it was considered the effect of IL amount on the retention capacity of the column. Experiments performed with different IL amounts showed that effective retention of the IL phase was achieved up to 70 mg of the IL. A significant reduction in the IL retention was observed for higher IL amounts. Thus, in order to achieve the best enhancement factor, 50 mg IL amount was chosen as optimal. It was observed that CYPHOS® IL 101 was easily dispersed in water forming a cloudy solution. Furthermore, only 100 μL of methanol were used as dispersant solvent. Additionally, methanol served to solubilize the Se-enriched IL phase making easier and more reproducible its injection into the graphite furnace.

Accordingly to previous observations, the presence of surfactants, such as Triton X-114, in an on-line extraction system based on ILs is crucial to reduce adherence of the IL droplets on the inner walls of the tubes. Therefore, flow ability of the IL throughout the FI system is improved, forcing the sole retention of the IL dispersed phase in the column [30]. The fine droplets of IL are surrounded by Triton X-114 molecules, hence, IL interactions with the inner walls of the lines decrease and consequently, IL phase does not stick on it. Although the presence of a surfactant facilitates the flowing of the IL phase, it can negatively affect the retention of the IL phase by the filling material of the column. Therefore, the effect of Triton X-114 on Se–APDC extraction and later IL phase retention into the column was studied within a surfactant concentration range of 0.01–2.0% (w/v). A 0.05% (w/v) surfactant concentration was chosen for further work as yielded high extraction efficiency. Higher surfactant concentrations led to inefficient retention into the column and non-reproducible results. Moreover, the highest analytical sensitivity enhancement factor was reached at 0.05% (w/v) Triton X-114.

The sample flow rate through the column is an important parameter since this is one of the steps that controls the time of analysis. Moreover, retention of the IL phase into the column can be mainly explained by a filtering-like process, rather than a chemical one. However, more studies are required to fully understand the mechanisms involved in ILs retention on Florisil®. Thus, the effect of sample flow rate through the column was a critical variable to achieve high retention of the IL phase. The presence of IL dispersed phase did not allow the utilization of flow rates higher than 0.6 mL min^{-1}. A flow rate of 0.5 mL min^{-1} was chosen for further experiments.

3.4. Elution of Se-enriched IL phase from the column

In order to elute the IL phase retained inside the column, solvents miscible with CYPHOS® IL 101 were studied. Both acetone and methanol resulted to be the most effective for removing the IL phase and Se–APDC complex from the column. However, it was preferred methanol to acetone as sharper peaks were observed with methanol during Se determination by ETAAS. Furthermore, the eluent was acidified with nitric acid to induce dissociation of Se–APDC complex and further releasing of Se into solution. A nitric acid concentration of 10% (v/v) was chosen. With the aim of reducing eluent volume and minimize dispersion of analyte, air-segmentation, consisting of sandwiching the eluate by air segments, was also applied. Dependence of recovery on eluent volume is shown in Fig. 4. A volume of 200 μL of methanol acidified to 10% (v/v) nitric acid was enough to obtain quantitative elution of Se from the column, while lower volume resulted in incomplete elution of the analyte and poor sensitivity. The optimum flow rate of eluent in countercurrent mode was 0.5 mL min^{-1}.

3.5. Interferences study

Most common matrix constituents of real samples under study, such as alkali and alkaline earth elements, do not react with APDC because of its selectivity at the working pH [26]. However, the effect of other concomitant ions regularly found in environmental samples was evaluated. The study was performed by analyzing 4 mL of 0.5 μg L^{-1} Se standard solution containing concomitant ions at different concentrations and following the recommended extraction procedure. A concomitant ion was considered to interfere if it resulted in an analytical signal variation of ±5%. Thus, Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{+2} and Fe^{3+} could be tolerated up to at least 600 μg L^{-1}. Analytical signal of the blank was not modified in presence of the concomitant ions assayed.

3.6. Analytical performance and determination of Se species in real samples

The relative standard deviation (RSD) resulting from the analysis of 10 replicates of 4 mL solution containing 0.5 μg L^{-1} of Se

Table 2
Selenium species determination and recovery study in real samples (95% confidence interval; $n = 6$).

Sample	Se added as ($\mu g\,L^{-1}$) Se(IV)	Se(VI)	Se(IV) Found ($\mu g\,L^{-1}$)	Recovery (%)[a]	Se(VI) Found ($\mu g\,L^{-1}$)	Recovery (%)[a]
Garlic sample 1	–	–	0.45 ± 0.02	–	0.23 ± 0.01	–
	0.5	–	0.93 ± 0.03	96	0.23 ± 0.01	–
	–	0.5	0.45 ± 0.02	–	0.74 ± 0.03	102
	0.5	0.5	0.95 ± 0.03	100	0.75 ± 0.03	104
Garlic sample 2	–	–	0.09 ± 0.01	–	<LOD	–
	0.5	–	0.57 ± 0.02	96	<LOD	–
	–	0.5	0.09 ± 0.01	–	0.49 ± 0.02	98
	0.5	0.5	0.57 ± 0.04	96	0.50 ± 0.03	100
River water	–	–	0.15 ± 0.01	–	0.17 ± 0.01	–
	0.5	–	0.66 ± 0.03	102	0.17 ± 0.01	–
	–	0.5	0.15 ± 0.01	–	0.66 ± 0.03	98
	0.5	0.5	0.64 ± 0.03	98	0.65 ± 0.03	96
Lake water	–	–	0.22 ± 0.01	–	<LOD	–
	0.5	–	0.73 ± 0.03	102	<LOD	–
	–	0.5	0.22 ± 0.01	–	0.51 ± 0.03	102
	0.5	0.5	0.70 ± 0.03	96	0.52 ± 0.03	104
Tap water	–	–	<LOD	–	<LOD	–
	0.5	–	0.48 ± 0.03	96	<LOD	–
	–	0.5	<LOD	–	0.51 ± 0.03	102
	0.5	0.5	0.52 ± 0.03	104	0.51 ± 0.03	102

[a] $100 \times [(\text{found} - \text{base})/\text{added}]$.

Table 3
Characteristic performance data obtained by using the proposed method and other reported for Se determination in water.

Method	LOD ($ng\,L^{-1}$)	RSD (%)	Enhancement factor	Sample consumption (mL)	Reference
Au-W-coil trap HG-AAS	39	3.9	20.1	27	[33]
On-line l-methionine, controlled pore glass column ETAAS	6	3.0	20	1	[34]
On-line CTAB-modified alkyl silica sorbent ICP-OES	100	3.6	27.6	3	[35]
On-line IL DLLME ETAAS	15	5.1	20	4	Proposed method

was 5.1%. Analytical sensitivity was enhanced by a factor of 20. The enhancement factor was obtained from the ratio of the calibration curve slopes for Se(IV) with and without application of the extraction/preconcentration step. Calibration curve without preconcentration was obtained by direct injection of Se(IV) standard solutions at different concentrations into ETAAS. The calibration graph obtained with the proposed method was linear with a correlation coefficient of 0.9993 at levels near the detection limits and up to at least 12.5 $\mu g\,L^{-1}$. The limit of detection (LOD) was calculated based on the signal at intercept and three times the standard deviation about regression of the calibration curve. A LOD of 15 $ng\,L^{-1}$ Se was obtained for the proposed methodology.

In order to demonstrate the wide applicability of the proposed method, different matrix samples including river water, tap water, lake water and garlic extracts were specially considered for analysis in this work. Due to the absence of a certified reference material for inorganic Se species in water and garlic samples, the selectivity of the proposed method for Se species determination was assayed by analyzing various synthetic samples with equimolar concentrations of Se(IV) and Se(VI). It can be observed in Table 2, that both Se species were completely separated and quantitatively recovered. The method showed an acceptable accuracy under different conditions, with recovery percentages between 96% and 104% for Se(IV) and Se(VI), respectively.

A comparison of the proposed method with other reported preconcentration methods for Se determination is shown in Table 3. The proposed method shows comparable analytical performance with respect to previously reported methods. Thus, on-line IL-DLLME method shows some advantages over on-line preconcentration procedures using a retention microcolumn, such as no need of using retention materials with specific surface functionalization and construction of home-made columns. Therefore, the use of an inexpensive IL, such as CYPHOS® IL 101, minimal reagent consumption and waste generation plus high simplicity and automation of DLLME technique, turns the proposed method into a valuable alternative for widespread application in routine analytical laboratories.

4. Conclusions

The results in this work demonstrate the possibility of using APDC for selective separation and preconcentration of Se(IV) species as Se–APDC complex was effectively extracted by CYPHOS® IL 101. The on-line retention and separation of IL enriched phase increases the speed of the preconcentration and analysis process, in addition to reduced sample consumption and contamination risks generally present in batch procedures. Moreover, on-line IL-DLLME approach makes feasible the use of ILs with lower density than aqueous media, which represents a considerable drawback in regular LLME procedures. The extraction and preconcentration method fulfill the requirements of analytical selectivity and sensitivity for reliable speciation analysis of inorganic Se species in water and garlic samples.

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