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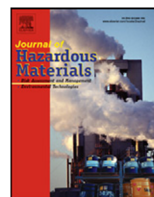

Development of an on-line temperature-assisted ionic liquid dispersive microextraction system for sensitive determination of vanadium in environmental and biological samples

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ABSTRACT

An original flow injection (FI) system was developed for on-line microextraction of Vanadium (V) based on room temperature ionic liquid (RTIL). Vanadium was complexed with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) at pH 4.0. A 40 μL-volume of 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4mim][PF_6]$) RTIL was mixed with 5 mL of sample solution containing the V-5-Br-PADAP complex. Then, a fully on-line temperature-assisted dispersion procedure was developed, followed by, analyte microextraction; and final on-line separation of the RTIL phase with a florisil-containing microcolumn. Vanadium was removed from the microcolumn with a 10% (v/v) nitric acid (in acetone) solution, and finally measured by electrothermal atomic absorption spectrometry (ETAAS). The detection limit achieved after preconcentration of 5 mL of sample solution, was 4.8 ng L^{-1}. The relative standard deviation (RSD) for 10 replicate determinations at 5 μg L^{-1} of vanadium level was 4.1%, calculated from the obtained peak heights. The calibration graph was linear, with a correlation coefficient of 0.9982 at levels from the detection limits up to 15 μg L^{-1}. The method was successfully applied for the determination of vanadium in environmental and biological samples.

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1. Introduction

The effects of trace elements in the environment and therefore on man's health, have fostered the development of analytical techniques and instrumentation, capable of measuring concentrations no greater than parts per million level and more frequently, parts per billion or less [1,2]. Despite electrothermal atomic absorption spectrometry (ETAAS) is one of the most used analytical techniques for ultra trace determinations [3], in order to achieve accurate, reliable, and sensitive results, it is necessary to perform separation and preconcentration procedures for trace element detection. However, sample preparation, which involves digestion, extraction and preparation of the analytes before the analysis, is the time limiting step, and is responsible for about 30% of the total error. Nowadays, the goals to be reached are: to obtain the best results, in the least time, with minimal contamination, low reagent consumption and generation of minimal residue or waste [4].

Conventional liquid–liquid extraction (LLE), is widely employed for sample preparation, due to its simplicity and flexibility [5]. This procedure can effectively decrease the detection limit while eliminating matrix interferences. However, since generally LLE is performed in batch, in order to reach low detection limits, some drawbacks like the use of large volumes of toxic organic solvents and sample makes LLE expensive; time-consuming, laborious and environmentally unfriendly [6]. Contamination risks and a not always straightforward application in routine analysis, should also be mentioned as well [7]. On the other hand, flow injection (FI) systems provide advantages for reducing contamination, because are usually closed systems and made of inert materials [8]. Moreover, high sample throughout, reduced sample and reagent consumption, minimal waste production, and effective means for matrix modification are among its several advantages [9–11].

Extractions of metal ions using RTILs combined with suitable complexing agents have been recently developed [12], specially employing hydrophobic RTILs, to extract low polar compounds from aqueous solution [13]. Since one of the most remarkable properties of RTILs is reduced or non-detected volatility, these solvents have been proposed as alternatives to classical organic solvents, therefore diminishing environmental and safety concerns related with high solvent consumption during classical LLEs [14,15]. Likewise, miniaturization of sample pretreatment protocols by diminishing the extractant phase volume, is especially important, when expensive samples and reagents are employed or only very

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limited amounts of these are available [3]. Thus, RTILs based on 1-alkyl-3-methylimidazolium hexafluorophosphates ($[C_n mim][PF_6]$, n = 4, 6, 8) have been used in single drop microextraction (SDME) technique in both direct (SDME) and headspace (HSME) modes [5]. However, both methods are time-consuming; have limited reproducibility and present some practical drawbacks, such as, the fact that the drop is broken up and air bubbles are formed when increasing agitation rate [14]. Both, ionic liquid dispersive liquid–liquid microextraction (IL-DLLME) based on regular molecular organic solvents as dispersive agents [16], and temperature-controlled IL dispersive liquid-phase microextraction (TILDLME) [14,17], have been developed as novel homogeneous L–L microextraction techniques based on ILs. However, these methods were developed in batch-mode, carrying similar drawbacks as regular LLE. Therefore, there is an urgent need for developing RTIL-based microextraction techniques in fully on-line mode. Recently, we reported initial developments of an on-line L–L phase separation system, for RTIL extraction and determination of Cd by flame atomic absorption spectrometry (FAAS) [18]. However, the extraction of the analyte by the RTIL phase was not performed in on-line mode. Therefore, a fully on-line microextraction procedure involving; formation of RTIL dispersion, extraction of analytes, and final RTIL phase separation has not been so far reported in literature.

In the present work, a novel FI system for on-line TILDLME development is proposed. Vanadium was chosen to be determined, due to its major importance at trace levels for biological and environmental studies [19,20]. The method was developed with initial chelation of vanadium with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) reagent followed by TILDLME. Formation and separation of the RTIL phase, as well as analyte extraction, were fully performed in an on-line system and coupled to ETAAS for vanadium determination in biological and environmental samples.

Table 1
Instrumental and experimental conditions for vanadium determination.

Instrumental conditions	
Wavelength	318.4 nm
Spectral band width	0.7 nm
Lamp current	30 mA
Injecting volume	200 μL
Modifier volume	10 μL
Modifier mass	5 μg Pd

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
	30	10	20	250
Drying 2	150	99	10	250
Pyrolysis 1	600	10	10	250
Pyrolysis 2	800	5	10	250
Pyrolysis 3	1800	20	20	250
Atomization	2500	0	3	–
Cleaning	2600	1	2	250

Extraction conditions	
Working pH	4.0
Sample volume	5 mL
5-Br-PADAP concentration	10^{-4} mol L^{-1}
Ethanol concentration	5% (v/v)
Buffer concentration	5×10^{-3} mol L^{-1}
Surfactant concentration	0.025% (w/v)
Volume of RTIL	40 μL
Heating temperature	45 °C
Heating time	5 min
Eluent	Acetone (in 10% HNO_3)
Eluent volume	200 μL (2 × 100 μL)
Loading flow rate	1 mL min^{-1}
Elution flow rate	0.25 mL min^{-1}

2. Experimental

2.1. Instrumentation

Experiments were performed using a PerkinElmer (Shelton, CT, USA) Model 5100PC atomic absorption spectrometer equipped with a graphite furnace module (HGA 500), a pyrolytic graphite tube (PerkinElmer) and a transversely heated graphite atomizer Zeeman-effect background correction system. A vanadium hollow cathode lamp (PerkinElmer) operated at a current of 30 mA and a wavelength of 318.4 nm with a spectral bandwidth of 0.7 nm was used. All measurements were made by using integrated absorbance with an integration time of 5 s. The temperature and time programmes for the atomizer are shown in Table 1.

The FI on-line microextraction system is shown schematically in Fig. 1. It consisted of; two Gilson (Villiers Le-Bell, France) Minipuls 3 peristaltic pumps, two six-port two-position injection valves (Oak Harbor, WA, USA) and a home-made microcolumn (23 mm length; 2 mm internal diameter) filled with the retention material of the RTIL dispersed phase and fitted with porous 25 μm glass frits. Tygon-type pump tubes (Gilson) were employed for all aqueous streams delivery. Solvent-resistant pump tubes (Gilson) were employed for delivery of the acidified-acetone stream.

2.2. Reagents

All the reagents were of analytical grade and the presence of vanadium was not detected within the working range. A 1000 mg L^{-1} vanadium (V) stock solution was prepared by dissolving 2.2966 g of ammonium metavanadate (99.99%) (Merck, Darmstadt, Germany) in 1000 mL of 0.1 mol L^{-1} nitric acid (Merck). Lower concentrations were prepared by diluting the stock solution with 0.1 mol L^{-1} nitric acid. A 10^{-2} mol L^{-1} 5-Br-PADAP solution was prepared by dissolution of 349.24 mg 5-Br-PADAP (Aldrich, Milwaukee, WI, USA) in ethanol (Merck). Lower concentrations were prepared by serial dilution with ethanol. A 2.0 mol L^{-1} acetic acid–acetate solution (Merck) adjusted to pH 4.0 by dissolution of sodium hydroxide (Merck) was employed as the buffer solution. A surfactant solution containing 5% (w/v) Triton X-100 (Merck) was employed to avoid RTIL phase sticking within the Tygon tube walls. Florisil (100–200 mesh particle size, 289 $m^2\,g^{-1}$ surface area) (Aldrich) was used as the selected material to fill the microcolumn. For chemical modification, a 1000 mg L^{-1} palladium solution was prepared, by dissolving 62.73 mg $Pd(NO_3)_2 \cdot 2H_2O$ (Fluka) in 25 mL 0.1% (v/v) HNO_3.

The RTILs $[C_4mim][PF_6]$ and $[C_6mim][PF_6]$ were synthesized according to a method proposed by Huddleston et al. [21] and stored in contact with ultrapure water to equilibrate the water content in the RTIL phase [22]. Qualitative analysis of synthesized IL was performed by comparison of infrared spectra, with commercially available $[C_4mim][PF_6]$ and $[C_6mim][PF_6]$ (Solvent Innovation GmbH, Köln, Germany).

Ultrapure water (18 MΩ cm) was obtained from a Millipore Continental Water System (Bedford, MA, USA). All glassware was washed with 0.1 mol L^{-1} HNO_3 for at least 24 h and thoroughly rinsed five times with ultrapure water before use.

2.3. Sampling collection and conditioning

2.3.1. Saliva samples

Saliva samples were collected from men and women volunteers, aged between 25 and 35 years living in Mendoza, Argentina. In order to minimize the possibility of contamination with food

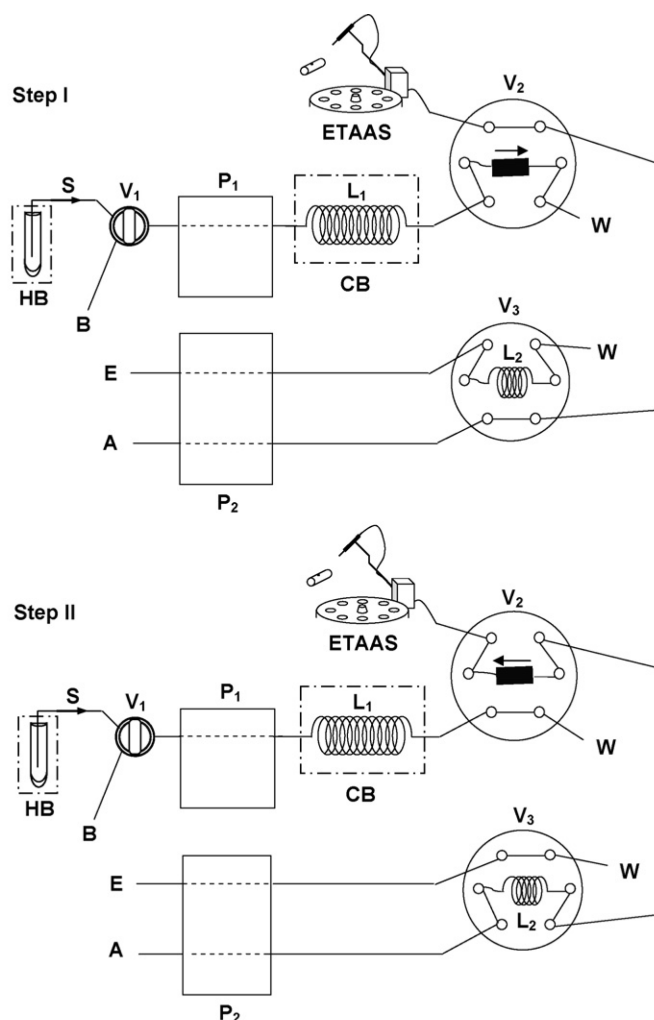

Fig. 1. Flow injection device and its operation sequence for on-line microextraction and determination of vanadium. ETAAS: electrothermal atomic absorption spectrometer; P_1 and P_2: peristaltic pumps; V_1, V_2, and V_3: injection valves; HB: hot bath; CB: cold bath; L_1: reaction loop; L_2: solvent loop; S: sample; B: buffer; E: eluent; A: air; W: waste.

debris or cigarette and airborne particles, the subjects were asked to thoroughly rinse their mouths three times with bidistilled deionised water. Human saliva samples were collected between 8 and 10 h to reduce possible circadian contributions into vanadium-free polystyrene test tube [23]. Sample volume was 3 mL for all cases. The samples were then placed in a graduated centrifuge tube, and centrifuged for 15 min at 1500 rpm (377.2 × g). Two and a half millilitres of the supernant was diluted to 5 mL with bidistilled water, and the vanadium content was determined by the on-line procedure proposed. Dilution prior to analysis is practical, because collecting larger volumes may be tedious and uncomfortable to the donor. Blanks were prepared with the same reagents, without the samples, undergoing an identical process.

2.3.2. *Water samples*

For tap water samples collection, domestic water was allowed to run for 20 min and approximately a volume of 1000 mL was collected in a beaker. Tap water samples were analyzed immediately after sampling. River 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 membrane filters (Millipore Corporation, Bedford, MA, USA) immediately after sampling. All the instruments used were previously washed with a 10% (v/v) HNO_3 water solution and then with ultrapure water.

2.4. *On-line microextraction and preconcentration procedure*

A volume of 40 μL [C_4mim][PF_6] was added to a 10 mL glass marked conical tube with 5 mL of the pre-treated sample; 250 μL ethanol, 500 μL of 10^{-3} mol L^{-1} 5-Br-PADAP solution, 12.5 μL of acetate/acetic acid buffer (2 mol L^{-1}, pH 4.0) and 25 μL of 5% (w/v) Triton X-100. For optimizing the preconcentration and determination system, 5 mL of 5 μg L^{-1} vanadium (V) standard solution was used instead of the sample. The mixture was heated in a thermostated bath at 45 °C for 5 min. The tube was then shaken with a vortex stirrer in order to fully dissolve the IL phase.

A schematic diagram of the on-line system is shown in Fig. 1. Length and function of each step are summarized in Table 2. Before loading, the column was conditioned for preconcentration at correct pH with a buffer-diluted solution, valve V_1 in position B (buffer load), which was propelled by the peristaltic pump P_1. After this procedure, V_1 was switched to S position (sample load). The warm mixture was placed in a hot bath at 45 °C and propelled (Step I) by pump P_1 at a flow rate of 1 mL min^{-1}. The dispersed RTIL phase was formed on-line by reducing the temperature of the solution in a reaction loop (L_1), which was immersed in an ice bath (CB). Hence, the V-5-Br-PADAP complex was extracted on-line into the dispersed RTIL phase, and finally, retained in the Florisil-packed microcolumn. Valve V_2 was set in load position during this operation (Table 2). Simultaneously, pump P_2 delivered 10% (v/v) nitric acid acidified-acetone to the solvent loop (L_2) of valve V_3 until it was filled. Then, pump P_2 was turned off. After that, the arm of ETAAS autosampler was manually moved into the dosing hole of the graphite tube.

In Step II, valves V_2 and V_3 were set on injection position, and the retained RTIL phase containing V-5-Br-PADAP complex, was eluted from the microcolumn with 200 μL of 10% (v/v) nitric acid acidified-acetone in countercurrent mode. Eluent was delivered by pump P_2 with an air stream at a flow rate of 0.25 mL min^{-1} directly inside the graphite tube for vanadium determination. As the total volume of eluent (200 μL) exceeded the capacity of the graphite furnace, two successive 100 μL-aliquots were injected, introducing a drying step of 10 s between each injection. First elution was performed

Table 2
Operating parameters and sequence of on-line FI automated liquid-phase microextraction system. Experimental conditions were as mentioned in Table 1.

Step[a]	Duration	Flow rate (mL min^{-1})	Active pump	Valve positions[b]			Purpose
				V_1	V_2	V_3	
I	30 s	1	P_1	B	L	L	Column conditioning
	2 min	1	P_1 and P_2	S	L	L	Sample and Solvent loading
	30 s	1	P_1	B	L	L	Lines washing
II	1 min	0.25	P_2	B	I	I	Analyte elution into the graphite tube

[a] Refer to Fig. 1.
[b] Valve positions: B: buffer load position; L: load position; S: sample load position; I: injection position.

with a stopped-flow procedure filling the microcolumn with the eluent solution and keeping it for 1 min before injection into ETAAS. After the two aliquots of eluent were run through the microcolumn, the autosampler arm was then retracted to the washing position. The concentration of vanadium was determined by ETAAS using peak heights, and under the instrumental conditions mentioned in Table 1.

Calibration was performed against aqueous standards submitted to the same preconcentration procedure. Blank solutions were analyzed in the same manner as standard and sample solutions.

3. Results and discussion

Since typical variables affecting complex formation and stability such as; pH, acetic/acetate buffer solution concentration, ethanol concentration, 5-Br-PADAP concentration, and the salting out effect were already studied in a previous work [17], these variables were not re-evaluated in the present study. On the other hand, this current work was focused on critical variables involved in the on-line RTIL phase formation, and extraction of the analyte with the proposed FI system.

3.1. Volume and choice of ionic liquid

Physical and chemical properties of imidazolium-containing ILs, such as; density, viscosity, and solubility, are defined by the alkyl group of the imidazolium cation and might affect the extraction efficiency of target analytes. Therefore, the influence of alkyl group of $[C_n\text{mim}][PF_6]$ ILs on extraction of V-5-Br-PADAP complex was investigated in this work. $[C_4\text{mim}][PF_6]$ and $[C_6\text{mim}][PF_6]$ ILs were tested for V-5-Br-PADAP complex extraction (Fig. 2). Significant differences on the extraction capacity of 1-alkyl-3-methylimidazolium ILs were observed. Even though it was expected that extraction would be higher for longer chain of 1-alkyl group due to the affinity of the complex for more hydrophobic ILs, better extraction was observed when $[C_4\text{mim}]^+$ was employed. The extraction process is a complex result of various parameters including; partition coefficient, diffusion coefficient of solute, solubility of extraction solvent, liquid viscosity, and complex hydrophobicity [21]. Therefore, further studies should be undertaken in order to fully explain these results. For the analytical interests of this work, $[C_4\text{mim}][PF_6]$ was chosen as the extracting IL phase. Extraction efficiency of the system and the signal can be remarkably affected by the RTIL amount. Therefore, it is highly important to establish the minimal volume of RTIL that leads to total complex extraction, while achieving the best analytical sensitivity. Recovery of vanadium upon the RTIL volume was examined within the range of 0–150 μL. Results revealed that the highest extraction efficiency was achieved with a minimal RTIL volume of 40 μL. A significant reduction in column retention was observed for higher RTIL volumes, hence 40 μL RTIL was selected as optimum for further experiments. Higher volumes of RTIL exceeded the column retention capacity.

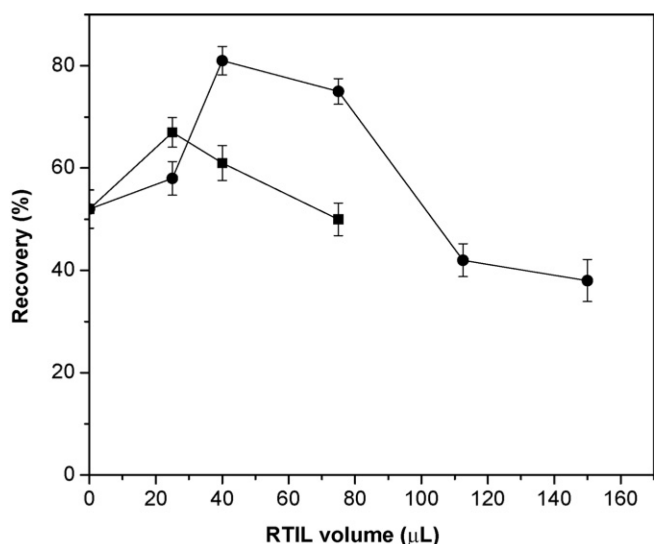

Fig. 2. Effect of type and amount of RTIL on vanadium extraction. $[C_4\text{mim}][PF_6]$ (●); $[C_6\text{mim}][PF_6]$ (■). Other conditions were as indicated in Table 1.

3.2. Column manufacturing and on-line RTIL phase separation

From the beginning of this work, it was supposed that on-line retention of the RTIL phase could be performed due to the high viscosity of the RTIL $[C_4\text{mim}][PF_6]$ (352.2 mPa s) at room or low temperature. Therefore, the appropriate combination of; loading flow rate, cooling loop length, type of filling material, and dimensions of the microcolumn would determinate the efficient retention of the RTIL phase by the FI system [24].

Home-made columns packed with potentially-suitable filtering materials such as; cotton, polyurethane foam, silica gel, XAD-16 resin or Florisil, were tested for on-line retention and separation of the dispersed RTIL phase (cloudy solution). When soft filling materials, such as cotton and polyurethane foam were employed, the RTIL droplets were not completely retained in the column. This effect could probably be due to RTIL draining through the cotton fibers or holes in the foam, causing retention difficulty. On the other hand, XAD-16 resin, silica gel and Florisil were successful for retention because the RTIL phase was placed in a more confined volume inside the microcolumn. This effect would allow achieving a more efficient condensation of the RTIL microdrops, as well as analyte elution with low dispersion. Florisil showed a higher analyte recovery for the experiment conditions than silica gel or XAD-16 resin, and therefore this material was chosen to fill in the column. The smaller particle size of Florisil (100–200 mesh particle size) in comparison with silica gel (70–230 mesh particle size, Aldrich) and XAD-16 resin (20–50 mesh particle size, Fluka) could explain this phenomenon. Smaller particles could form a more compact filtering media, hence improving RTIL phase condensation and separation.

The length of the microcolumn was an important variable to be considered in this work. It was observed that a minimal length of 20 mm was needed for total RTIL phase retention. Shorter columns did not lead to good retention as the RTIL droplets were not completely retained by the filling material. On the other hand, increasing the column length did not enhance vanadium recovery, and a high back pressure was generated within the FI system. Moreover, larger amounts of eluent were necessary for longer columns. Therefore, a 20-mm-long column with a 2 mm inner diameter was chosen as optimal for RTIL phase retention.

The sample flow rate is one of the most important variables which control the analysis time, and determine the linear velocity of the RTIL microdroplets through the column. This study was developed for loading flow rates ranging between 0.25 and 2.0 mL min^{-1}. No major changes on the analytical response were observed in the range of 0.25–1 mL min^{-1}. The response decreased at flow rate values higher than 1 mL min^{-1}. A filtering-like retention process of the RTIL phase into the column, rather than a chemical one, could explain this phenomenon. Lower sample flow rates, mean longer times into the cooling loop, thus allowing a better condensation of the IL phase. A flow rate of 1 mL min^{-1} was therefore selected.

Both, the complete solubility, and dispersion of $[C_4\text{mim}][PF_6]$ into the aqueous solution depend on temperature [25]. This variable played a critical role in obtaining a homogenous solution that contains the RTIL and V-Br-PADAP complex. Therefore, the RTIL-

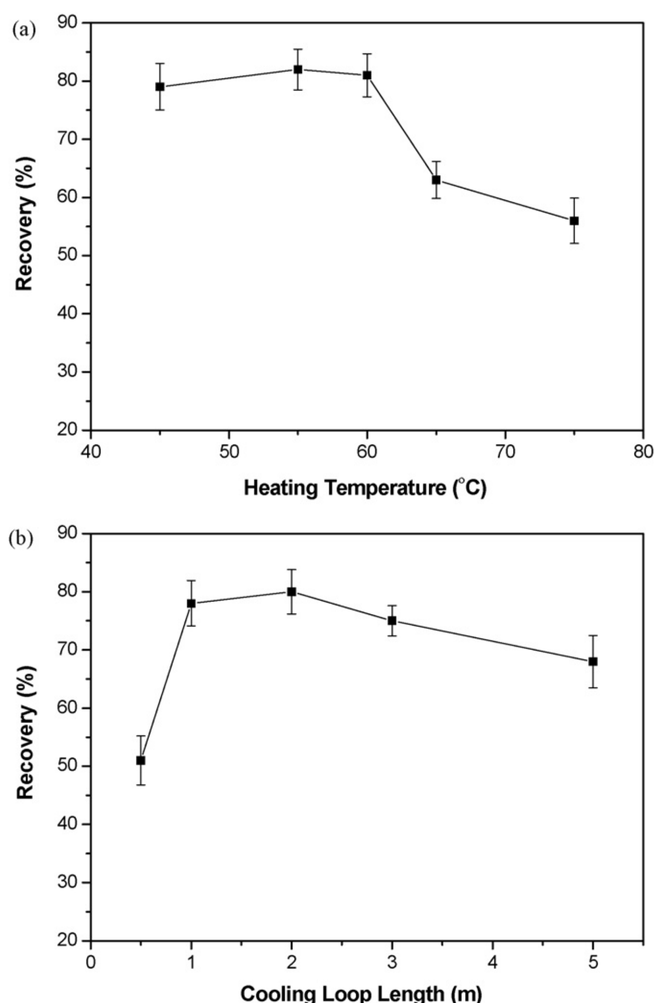

Fig. 3. (a) Effect of heating temperature and (b) length of cooling loop on vanadium extraction. Other conditions were as indicated in Table 1.

containing solution was heated in the range of 45–75 °C. As it can be observed in Fig. 3(a), the extraction efficiency decreased when increasing temperature. This effect could be explained as a hotter solution needs a longer cooling time for increasing RTIL viscosity and subsequent retention in the microcolumn. Hence, a minimal temperature of 45 °C was chosen for heating step development. Effect of heating time on vanadium extraction was also studied. Vanadium extraction efficiency increased when the initial homogenous solution was placed for about 1–5 min in a hot bath. No major changes on vanadium recovery were observed when longer times were assayed. A 5 min-heating time was chosen for further experiments.

The extraction time was considered, from the moment the RTIL-aqueous phase homogenous solution started flowing in the cooling loop, until it arrived to the microcolumn for RTIL microdrops retention and separation from the aqueous solution. A turbidity phenomenon is generated when temperature of the RTIL-aqueous solution is low enough for decreasing RTIL solubility, thus forming a dispersed RTIL phase which is subsequently condensed and retained in the microcolumn. The minimal extraction time required for RTIL phase formation was obtained by varying the cooling loop length. As shown in Fig. 3(b), the best analytical recovery was obtained when a 2-m-loop was employed. On the other hand, lower signals were observed with shorter cooling loops. This phenomenon could be explained due to insufficient time for properly cooling the RTIL-containing solution, thus limiting the formation

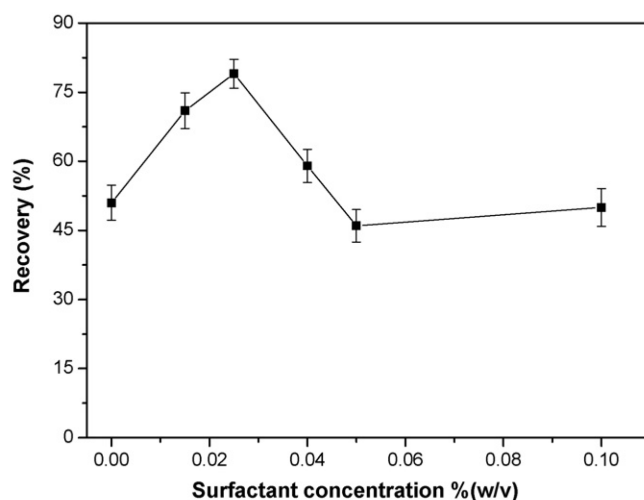

Fig. 4. Effect of Triton X-100 concentration on vanadium extraction and recovery. Other conditions were as indicated in Table 1.

of the two-phase dispersion system. Furthermore, lower signals were observed when solutions were cooled for longer times. Therefore, a 2-m-cooling loop was used for further experiments. It is worth pointing out, that lower times for RTIL phase separation were needed with the on-line system, as compared to the same procedure developed in batch-mode [17]. Moreover, only in batch-mode a centrifugation step was required for phase separation, increasing the temperature of the solution with respect to that obtained in the cooling bath. This rise in temperature led to partial dissolution of the RTIL phase, and therefore, loss of extraction efficiency [25]. On the other hand, with the proposed on-line system, separation time was significantly reduced as there was no need of a centrifugation step. Moreover, the temperature of the whole process could be controlled, minimizing RTIL solubilization into aqueous media. This last effect allowed the use of a reduced volume of RTIL to perform the extraction of the analyte.

A common surfactant, Triton X-100, was added to the sample solution in order to avoid the adherence of the RTIL on the inner walls of the tubes, thus, forcing its sole retention into the microcolumn, while reducing analyte dispersion on the whole FI system. Triton X-100 molecules surround the RTIL fine droplets, decreasing RTIL interactions with the inner walls of the lines and avoiding RTIL phase sticking [14]. However, it was supposed that the presence of large amounts of surfactant could negatively affect the retention of the RTIL phase in the microcolumn. The effect of Triton X-100 on V-5-Br-PADAP extraction and later RTIL phase retention into the microcolumn was studied within a concentration range of 0.025–0.1% (w/v). As shown in Fig. 4, 0.025% (w/v) Triton X-100 concentration was chosen for further work as it yielded high extraction efficiency while allowing the free running of the RTIL droplets in the lines. Higher surfactant concentrations led to insufficient retention into the column, and hence non-reproducible results.

3.3. Analyte and RTIL removal from the microcolumn

In order to fully elute vanadium retained in the microcolumn, different types and amounts of organic eluents miscible with $[C_4mim][PF_6]$ were evaluated. The selection of solvents was made, based on the solubility that $[C_4mim][PF_6]$ shows in these media [13]. Thus; ethanol, methanol, acetone, 10% (v/v) nitric acid, methanol acidified to 10% (v/v) nitric acid and acetone acidified to 10% (v/v) nitric acid in volumes ranging from 80 to 250 μL were assayed for vanadium elution. Among these eluents, the best results were obtained when acidified-acetone was employed. As shown in

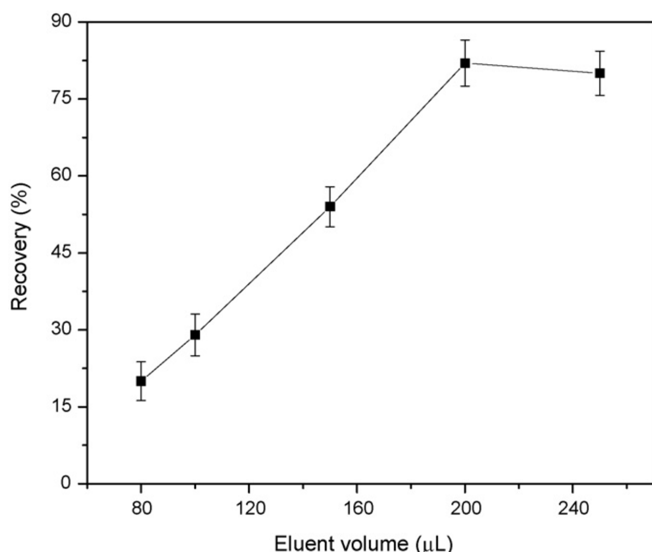

Fig. 5. Evaluation of the minimal eluent volume required for complete analyte elution from the microcolumn loaded with the RTIL phase. Conditions were as indicated in Table 1.

Fig. 5, a volume of 200 μL of acetone acidified to 10% (v/v) nitric acid was sufficed to obtain quantitative elution of the RTIL phase and V-5-Br-PADAP complex removal from the column. A lower volume resulted in an incomplete elution of the analyte and a reduction of sensitivity, whereas a larger volume was not possible to be assayed as it exceeded the graphite furnace sample capacity. Acetone was acidified with nitric acid in order to induce dissociation of V-Br-PADAP complex and further releasing of vanadium into solution. Additionally, best results were obtained when the elution of the analyte was developed in countercurrent through the microcolumn, which was especially favourable in order to obtain a more efficient elution by minimizing the analyte dispersion [26].

With the aim of reducing the eluent volume and minimizing analyte dispersion when the eluate is delivered to the graphite furnace electrothermal atomizer, the use of air-segmentation, which consists of sandwiching the eluate by air segments and transporting it into the graphite tube, was also applied [27]. As the total eluent volume (200 μL) exceeded the sample capacity of the graphite furnace, two successive 100 μL-aliquots were sequentially injected into the atomizer, introducing a drying step of 10 s between each injection (Table 1), while the loop of valve V_3 was refilled for the second elution. First elution was performed by a stopped-flow procedure, filling the microcolumn with the eluent solution, and holding it for 1 min before injection into ETAAS. The furnace autosampler arm was inserted manually inside the dosing hole of the graphite tube, and held in place during eluate delivery, which was performed by using an air stream at 0.25 mL min^{-1}. As a complete study on vanadium measurement by ETAAS in the presence of a RTIL matrix was developed in a previous work [17], the same chemical modifier and graphite furnace program (Table 1) were applied in this study.

3.4. Interferences study

The effects of common coexisting ions were investigated. The tests were made at the concentration levels at which they may occur in the studied samples. Five millilitres of 5 μg L^{-1} of vanadium solution, containing concomitant ions regularly found in these samples at different concentrations, was evaluated following the recommended extraction procedure. Thus, Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and Fe^{3+} could be tolerated up to at least 2000 μg L^{-1}. Alkali and alkaline earth elements, commonly encountered concomitant ions, do not form stable complexes with 5-Br-PADAP at working pH. Contribution to the ionic strength of the system was insignificant and did not affect the extraction efficiency. Analytical blank signal was not modified in the presence of the concomitant ions assayed. As expected, humic substances do not produce interference effects on analyte extraction with imidazolium hexafluorophosphate-type RTILs [28].

3.5. Analytical performance

At optimum experimental conditions, an 80% extraction efficiency of vanadium in water samples was achieved (Table 1). The relative standard deviation (RSD) resulting from the analysis of 10 replicates of 5 mL solution containing 5 μg L^{-1} of vanadium was 4.1%. The calibration graph was linear with a correlation coefficient of 0.9982 at levels near the detection limits and up to at least 15 μg L^{-1}. The limit of detection (LOD), calculated based on the signal at intercept and three times the standard deviation about regression of the calibration curve [29], was 4.8 ng L^{-1} for the proposed methodology. The frequency of analysis was 6 samples per hour.

The proposed method presents a detection limit that is comparable to, or better than, other methodologies developed for total vanadium determination (Table 3), with good calibration range using a reduced amount of sample.

3.6. Determination of vanadium in environmental and biological samples

Saliva could be a good biomarker for studying metal exposure and metabolism, since a good correlation between saliva and plasma levels for health investigation parameters, makes saliva, an attractive health diagnostic tool for systemic diseases [30]. However, a major obstacle for detection of chemical contaminants in saliva, is that concentrations are often 1 or 2 orders of magnitude lower than in blood [31]. Because trace amounts of vanadium have yet unclear biological functions, vanadium levels in water must be strictly controlled [32]. As water is one of the main sources of vanadium for biological systems [33], containing normally less than 3 μg L^{-1} [20], commonly, a suitable preconcentration procedure has to be applied, before elemental detection, in order to make vanadium determination possible at trace levels.

Since water quality is considered the main factor controlling health, and the state of disease in both man and animals [34], the proposed method was applied to the determination of solu-

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

Method	LOD (ng L^{-1})	RSD (%)	Sample consumption (mL)	Calibration range (μg L^{-1})	Ref.
SPE-FI-ICP-OES	60	3.4	10	100	[36]
SPE-FI-ICP-MS	25	4.4	2	0.5–100	[37]
SPE-FI-ICP-AES	90	n.r.[a]	5	n.r.[a]	[26]
SPE-ICP-MS	4	n.r.[a]	7	0.1–5	[38]
FI-TILDLME-ETAAS	4.8	4.1	5	15	Proposed method

[a] Not reported.

Table 4
Vanadium concentration in water and saliva samples (95% confidence interval; $n = 6$). Experimental conditions were as shown in Table 1.

	Sample	Added (μg L^{-1})	Found (μg L^{-1})	Recovery (%)[a]	Sample	Added (μg L^{-1})	Found (μg L^{-1})	Recovery (%)[a]
River water	1	0	0.60 ± 0.05	–	3	0	0.98 ± 0.05	–
		2.00	2.60 ± 0.07	100		2.00	3.03 ± 0.14	102
	2	0	0.81 ± 0.04	–	4	0	1.12 ± 0.06	–
		2.00	2.74 ± 0.08	96.5		2.00	3.10 ± 0.15	99.0
Tap water	1	0	0.19 ± 0.02	–	3	0	0.48 ± 0.05	–
		2.00	2.16 ± 0.07	98.5		2.00	2.52 ± 0.12	102
	2	0	0.23 ± 0.01	–	4	0	0.39 ± 0.04	
		2.00	2.29 ± 0.09	103		2.00	2.34 ± 0.13	97.5
Saliva	1	0	n.d.[b]	–	3	0	n.d.[b]	–
		2.00	1.99 ± 0.07	99.5		2.00	1.96 ± 0.12	98.0
	2	0	n.d.[b]	–	4	0	n.d.[b]	–
		2.00	2.02 ± 0.07	101		2.00	2.03 ± 0.10	102

[a] [(Found − Base)/Added] × 100.
[b] Not detected.

ble vanadium in tap and river water samples. Results are shown in Table 4. Recovery of vanadium was between 96.5 and 103.0%. Vanadium concentrations in river water samples were in the range of 0.60–1.12 μg L^{-1} and in tap water were in the range of 0.18–0.48 μg L^{-1}. Results were not significantly different to those reported previously in river and tap water samples [26]. Additionally, the accuracy of the proposed methodology was evaluated by analyzing a certified reference material (CRM) of natural water NIST SRM 1643e, with a vanadium content of 37.86 ± 0.59 μg L^{-1}. Using the method developed in this work, the vanadium content found in the CRM was 34.93 ± 1.01 μg L^{-1} (95% confidence interval; $n = 6$).

To our knowledge, there have been no reports demonstrating the viability of performing a RTIL-based microextraction technique in human saliva. When no standard reference materials with a certified content of vanadium are available, a recovery study could be considered as an alternative for validation studies [35]. The proposed analytical method was applied to three portions of sample and the mean vanadium concentration of each sample was taken as a base value. Then, increasing quantities of 2 μg L^{-1} of vanadium in saliva aliquots were added to the other aliquots and the same procedure was followed. Recovery values were between 98.0 and 101.6% for vanadium (Table 4).

4. Conclusions

In this work, an original FI system for on-line RTIL phase formation and separation coupled with ETAAS detection for vanadium determination in environmental and biological samples is proposed. The implementation of RTILs in on-line procedures opens up an attractive alternative for automated separation and preconcentration methodologies. The on-line retention of the dispersed RTIL phase in a Florisil-packed-microcolumn, significantly simplifies the microextraction technique, by reducing manual operation and contamination risks. Moreover, the on-line procedure required lower amounts of RTIL than a similar technique proposed in batch. A fundamental advantage of using this on-line system is its suitability to work with RTILs with higher density than aqueous media, which is a major limitation in regular L–L extraction-based techniques. In this work, the different steps of the process, including; formation of a dispersed RTIL phase, analyte extraction, and final separation of the RTIL droplets completely took place in an on-line FI system.

Finally, the method allowed the reliable and accurate determination of vanadium in environmental and biological samples, showing the possibility of using RTIL-based microextraction techniques for analysis of real or complex samples. We therefore suggest that, the method could be of key interest especially for routine analytical laboratories.

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