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Highly selective ionic liquid-based microextraction method for sensitive trace cobalt determination in environmental and biological samples

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ABSTRACT

A simple and rapid dispersive liquid–liquid microextraction procedure based on an ionic liquid (IL-DLLME) was developed for selective determination of cobalt (Co) with electrothermal atomic absorption spectrometry (ETAAS) detection. Cobalt was initially complexed with 1-nitroso-2-naphtol (1N2N) reagent at pH 4.0. The IL-DLLME procedure was then performed by using a few microliters of the room temperature ionic liquid (RTIL) 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6mim][PF_6] as extractant while methanol was the dispersant solvent. After microextraction procedure, the Co-enriched RTIL phase was solubilized in methanol and directly injected into the graphite furnace. The effect of several variables on Co–1N2N complex formation, extraction with the dispersed RTIL phase, and analyte detection with ETAAS, was carefully studied in this work. An enrichment factor of 120 was obtained with only 6 mL of sample solution and under optimal experimental conditions. The resultant limit of detection (LOD) was 3.8 ng L^{-1}, while the relative standard deviation (RSD) was 3.4% (at 1 μg L^{-1} Co level and $n = 10$), calculated from the peak height of absorbance signals. The accuracy of the proposed methodology was tested by analysis of a certified reference material. The method was successfully applied for the determination of Co in environmental and biological samples.

1. Introduction

Cobalt (Co) is an essential trace element in nature, having an important role in many body functions, as a component of vitamin B_{12}. However, depending on its concentration, it can be either essential or toxic for many living beings, including humans [1]. Therefore, it is clear that determination of trace amounts of Co in biological and environmental samples plays an important role in the fields of environmental surveillance, medicine and toxicology [2]. Electrothermal atomic absorption spectrometry (ETAAS) is a widely used analytical technique for the determination of trace elements in biological fluids due to its reliability, sensitivity and relatively low cost of instrumentation. However, difficulties still lie on determination of trace heavy metals because of both, their low abundance levels and high complexity of biological matrices [3,4]. Hence, preliminary preconcentration and sample clean-up steps are frequently required to achieve accurate, reliable and sensitive results.

Numerous separation and preconcentration techniques for Co determination in water and biological samples have been proposed, including solid phase extraction (SPE) [2,5–7], classical liquid–liquid extraction (LLE) [8,9], cloud point extraction (CPE) [10,11], liquid–liquid microextraction (LLME) using organic solvents [12,13], and membrane filtration [14]. Conventional LLE with regular organic solvents is widely employed for sample preparation due to its simplicity and flexibility [15]. Even though this procedure can effectively decrease detection limits and eliminate matrix interference, it also requires large amounts of high purity organic solvents for the extraction, resulting in environmental and safety concern due to high volatility, toxicity and flammability [15]. On the other hand, many of the problems linked with regular organic solvents as well as loss of solvent by evaporation can be significantly avoided using ionic liquids (ILs) as alternative solvents, since they have no detectable vapor pressure and are relatively thermal stable even at elevated temperatures [16]. Extractions of metal ions using room temperature ionic liquids (RTILs) combined with suitable complexing agents have been recently developed in analytical chemistry, thus allowing extraction of low polar compounds from aqueous solution [17]. Since miniaturization of sample pretreatment protocols is of special importance when expensive samples

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and reagents are employed, or only very limited amount of these are available [18], 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 immersion (DI-SDME) and headspace (HS-SDME) modes [15]. However, both methods are time-consuming, have limited reproducibility and presents some practical drawbacks such as emulsion formation and the fact that the drop is broken up and air bubbles are formed when increasing agitation rate or when dealing with some dirty samples [16,19]. Classical dispersive liquid–liquid microextraction based on ILs as extractant phase (IL-DLLME), with organic solvents as dispersing agents [20–22], and temperature-controlled IL dispersive liquid phase microextraction (TILDLME) [19,23] have both been proposed as novel homogeneous LLME techniques for metal extraction, thus avoiding many of the problems observed in earlier methods.

The application of RTILs in LLME procedures has been already reported for Co determination, by chelation of this metal with 1-(2-pyridylazo)-2-naphthol (PAN) [24,25]. In fact, pyridylazo-type reagents in combination with ILs have been used for determination of several metal ions. However, it has to be noticed that, despite the favorable stability constants of their complexes, this class of reagents shows limited selectivity towards metal chelation. Thus, extraction procedures based on these reagents could be prone to suffer from matrix interferences occurring in real complex samples. On the other hand, 1-nitroso-2-naphthol (1N2N) forms stable complexes with numerous metal ions and it can selectively react with Co under specific conditions. Moreover, 1N2N has been employed for spectrophotometric determination of Co in the past [26], but no report has been so far published regarding its use and combination with RTILs, for development of LLME procedures.

In this work, a highly selective separation and preconcentration method for Co determination at trace levels is proposed. Cobalt was initially complexed with 1N2N reagent, followed by application of IL-DLLME technique based on the RTIL 1-hexyl-3-methylimidazolium hexafluorophosphate ([C_6mim][PF_6]). The proposed method was successfully applied for the determination of Co at trace levels in environmental and biological samples.

2. Experimental

2.1. Instrumentation

Experiments were performed using a Perkin Elmer (Shelton, CT, USA) model 5100ZL atomic absorption spectrometer equipped with a graphite furnace module, a pyrolytic graphite tube (Perkin-Elmer) and a transversely heated graphite atomizer Zeeman-effect background correction system. A Co hollow cathode lamp (SCP Science, Champlain, NY, USA) operated at a current of 15 mA and a wavelength of 240.7 nm with a spectral bandwidth of 0.2 nm was used. All measurements were performed using integrated absorbance with an integration time of 5 s. Temperature and time programs for ETAAS instrument were as shown in Table 1.

A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used to accelerate the phase separation process. A thermostated bath (Vicking, Buenos Aires, Argentina) model Masson Digital, maintained at the desired temperature, was used for heating. A vortex model Bio Vortex V1 (Boeco, Hamburg, Germany) was used for mixing the reagents. UV-photolysis of urine samples was performed with a 15W/G15T8 UV-C lamp (Philips, Holland).

2.2. Reagents

A 1000 mg L^{-1} Co(II) stock standard solution was prepared by dissolving 0.503 g of Co(II) nitrate hexahydrate (98%) (Aldrich, Milwaukee, WI, USA) in 100 mL of 0.1% (v/v) HNO_3 (Merck, Darmstadt, Germany). Lower concentrations were prepared by diluting the stock solution with 0.1 mol L^{-1} HNO_3. A 10^{-2} mol L^{-1} 1N2N solution was prepared by dissolving 0.177 g of 1N2N (98%) (Aldrich) in 100 mL of methanol (Merck). Lower concentrations were prepared by serial dilution with methanol. 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 buffer solution. Individual surfactant solutions containing 1.9×10^{-2} mol L^{-1} Triton X-114 (Merck) or 1.5×10^{-2} mol L^{-1} Triton X-100 (Merck) were evaluated as anti-sticking agents. A 50% (w/v) sodium nitrate solution was prepared by dissolving 5 g of $NaNO_3$ (Merck) in 10 mL of ultrapure water. For chemical modification, a 1000 mg L^{-1} Pd solution was prepared by dissolving 62.7 mg $Pd(NO_3)_2{\cdot}2H_2O$ (Fluka, Buchs, Switzerland) in 25 mL 0.1% (v/v) HNO_3.

[C_6mim][PF_6] was synthesized according to a method proposed by Huddleston et al. [27] and stored in contact with ultrapure water to equilibrate the water content in the RTIL phase [28]. Qualitative analysis of synthesized IL was performed by comparison of infrared spectra with commercially available [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 a 0.1 mol L^{-1} HNO_3 solution at least for 24 h and thoroughly rinsed 5 times with ultrapure water before use.

2.3. Sample collection and conditioning

2.3.1. 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. 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. Tap water samples were analyzed immediately after sampling. River water samples were filtered through 0.45 μm pore size membrane filters (Millipore Corporation, Bedford, MA, USA) immediately after sampling. All samples were acidified to pH 1 with concentrated HNO_3 and stored at 4 °C in bottles (Nalgene; Nalge, Rochester, NY, USA). The samples were analyzed as soon as possible.

2.3.2. Biological samples

Urine and saliva samples were collected from men and women volunteers, aged from 25 to 35 years, living in Mendoza (Argentina), without having eaten breakfast. In order to minimize the possibility of contamination with food debris or cigarette and airborne particles, the subjects were asked to thoroughly rinse their mouths three times with ultrapure water. Human saliva samples were collected between 8 and 9 h to reduce possible circadian contributions, into Co-free polystyrene test tubes [29]. The samples (7 mL) were acidified with HNO_3 to pH 2 and then placed in a graduated centrifuge tube and centrifuged for 20 min at 1500 rpm (377.2 g). Five milliliters of the supernatant were diluted to 25 mL with bi-distilled water and Co was determined by the proposed method. Dilution prior to analysis is practical since collection of large volumes may be tedious and uncomfortable to the donor. Blanks were prepared with the same reagents, without the samples, undergoing an identical process.

Urine samples were digested by UV-photolysis as described by Husakova et al. [3]. Briefly, 5 mL of sample was placed in a decomposition glass beaker, added with 200 μl of 30% (w/w) H_2O_2, and the mixture was then irradiated for 45 min. Then, another 200 μl-aliquot of 30% (w/w) H_2O_2 was added and irradiation process was continued for 45 min. Finally, 10 mL of H_2O was added and the irradiation process was repeated for another 120 min. After completion

Table 1
Instrumental and experimental conditions for Co determination.

Instrumental conditions	
Wavelength	240.7 nm
Spectral band width	0.2 nm
Lamp current	15 mA
Injection volume	50 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
Drying 2	250	15	30	250
Pyrolysis 1	600	90	30	250
Pyrolysis 2	900	20	20	250
Pyrolysis 3	1200	10	20	250
Atomization	2400	0	3	
Cleaning	2600	1	2	250

Extraction conditions	
Sample volume	6 mL
1N2N concentration	4×10^{-5} mol L^{-1}
pH for complex formation	4.0
pH for complex extraction	2.0
Buffer concentration (pH 1)	2×10^{-2} mol L^{-1}
HCl concentration (pH 2)	1 mol L^{-1}
Surfactant concentration (Triton X-114)	9.3×10^{-5} mol L^{-1}
$NaNO_3$ concentration	1.5% (w/v)
RTIL amount	60 mg
Dispersant and RTIL phase solvent	Methanol
Volume of dispersant solvent	500 μL
RTIL phase solvent volume	50 μL

of the irradiation procedure the volume of the digested sample was set to 25 mL.

2.4. Dispersive liquid–liquid microextraction procedure based on ionic liquid

A mixture of 6 mL of the pre-treated sample or a 1 μg L^{-1} Co(II) standard solution (for method optimization), 200 μL of 10^{-3} mol L^{-1} 1N2N solution, 50 μL of 2 mol L^{-1} (pH 4.0) acetate/acetic acid buffer, 180 μL of 50% (w/v) sodium nitrate solution and 29 μL of 1.9×10^{-2} mol L^{-1}Triton X-114, was heated in a thermostated bath at 50 °C for 15 min. After formation of Co–1N2N complex, the tube was placed in an ice bath for 10 min to diminish the temperature, and pH 2 was adjusted by adding HCl (1 mol L^{-1}). An amount of 60 mg of [C_6mim][PF_6] (extraction solvent) and 500 μL of methanol (dispersant solvent) were then added to the sample solution. A cloudy solution was immediately formed, by dispersion of the immiscible RTIL into the aqueous sample, thus greatly enlarging the contact area between the two phases. Consequently, the Co–1N2N complex was extracted into the dispersed RTIL phase. After 7 min of extraction time, centrifugation at 1500 rpm (377.2 g) for 15 min allowed the formation of two well-defined phases. The upper aqueous phase was then manually removed with a syringe and the RTIL phase dissolved with 50 μL of methanol, followed by direct injection into the graphite furnace for Co determination (Table 1). Calibration was performed against aqueous standards and blank solutions.

3. Results and discussion

3.1. ETAAS conditions for Co determination in RTIL phase

Initial studies were focused on obtaining high accuracy and precision for ETAAS measurements of Co in the presence of the RTIL matrix. Direct automatic injection of RTILs into ETAAS carries some drawbacks due to the high viscosity of the resulting phase. Therefore, in order to achieve reproducible injection of the RTIL into the graphite furnace, dissolution in an appropriated solvent was studied. Acetone and methanol were assayed in this work. Although dilution of the RTIL phase in both solvents was feasible, the best performance was achieved with methanol as diluent. Total dissolution of the RTIL phase was observed for 50 μL methanol, while lower volumes turned out into a deterioration of analytical sensitivity. Thereby, 50 μL of methanol was employed for further experiments.

In the presence of the RTIL matrix, increased background signal was observed during atomization step, due to its organic nature. Since background correction is more accurate when background absorbance is minimal as compared with atomic absorption signal, different heating ramps in the pyrolysis step, were evaluated to increase matrix elimination and then obtain minimal or none background signal in the atomization step. Although a background reduction was accomplished, it was also observed that Co signal gradually decreased by increasing IL concentration. This could be due to smoke originated during thermal decomposition of [C_6mim][PF_6] in the pyrolysis step, resulting in loss of Co during ashing stage. Therefore, different amounts of $NH_4H_2PO_4$, $Mg(NO_3)_2$, $Pd(NO_3)_2$ and a mixture of them were tested as chemical modifiers to improve Co signal. A significant background reduction (about 50%) as well as sharp and well-defined absorption peaks were obtained when 5 μg of Pd were injected into the graphite furnace. Thus, Pd was used in subsequent experiments as chemical modifier.

Finally, the effect of pyrolysis and atomization temperatures on the absorbance signal generated by a 100 μg L^{-1} Co solution with equal amount of [C_6mim][PF_6] as resulting from the extraction procedure, were studied. The optimal pyrolysis temperature was selected by considering absorption-to-background ratio and peak shape. Well defined Gaussian and sharp peaks were considered as optimal. For temperature values higher than 1200 °C, analyte loss was evidenced during pyrolysis (Fig. 1). On the other

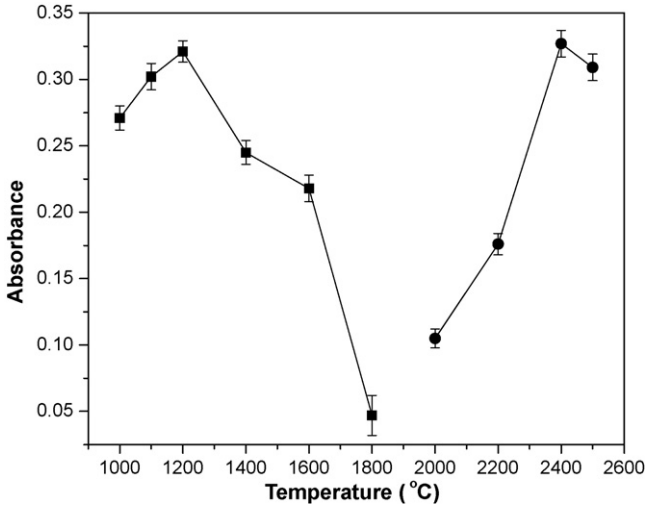

Fig. 1. Pyrolysis (■) and atomization (●) temperature curves obtained by injection of a 100 μg L^{-1} Co solution in the presence of 4×10^{-5} mol L^{-1} 1N2N in RTIL-methanol, with 5 μg of Pd as modifier. Atomization temperature for pyrolysis optimization: 2500 °C. Pyrolysis temperature for atomization optimization: 1200 °C. Additional instrumental variables were as mentioned in Table 1 (95% confidence interval; $n = 6$).

hand, no signal was observed when this temperature was assayed in the atomization step. The effect of atomization temperature on Co measurement was studied within the range of 2200–2600 °C. The maximum absorption signal was obtained at 2400 °C (Fig. 1). Therefore, pyrolysis and atomization temperatures selected for next experiments were 1200 and 2400 °C, respectively. The resulting methanol-RTIL phase was thus successfully analyzed by ETAAS under the conditions showed in Table 1.

3.2. Selection of RTIL and dispersant solvent

The selection of a suitable RTIL was performed based on specific properties, such as low solubility in water, good extraction ability, and higher density than water. Thus, we focus on hydrophobic and relatively inexpensive imidazolium-ILs containing $[PF_6]^-$ as counteranion. For the most used within that class, i.e. [C_4mim][PF_6], [C_6mim][PF_6] and [C_8mim][PF_6], the solubility in water diminishes following: 18.8, 7.5, to 2.0 g L^{-1}, respectively [19]. On the contrary, viscosity of these RTILs increases as follows: 450, 585, to 710 mPa s, respectively [19]. Both parameters have to be considered, since a lower solubility allows minimal RTIL consumption, while a high viscosity could lead to practical drawbacks during the microextraction procedure. Thus, [C_6mim][PF_6] was chosen as the extractant phase considering its relatively high hydrophobicity, lower solubility as compared with [C_4mim][PF_6] while showing an acceptable viscosity to work with the DLLME approach. Since both extraction efficiency and analyte detection in ETAAS can be remarkably affected by RTIL amount, it was critical to establish the minimal amount of RTIL yielding total Co extraction while achieving the best analytical sensitivity. Recovery of Co upon RTIL amount was examined within the range of 40–80 mg and using 0.5 mL methanol as dispersant solvent. The results revealed that 60 mg was the lowest amount of [C_6mim][PF_6] required to achieve 100% recovery. Higher amounts of the RTIL did not improve extraction efficiency, while could lead to increase background signals. Therefore, 60 mg was used for subsequent experiments in this work.

The choice of a dispersant solvent was done considering the immiscibility between IL phase and aqueous sample. Thus, acetone and methanol were particularly evaluated. Recovery efficiency was evaluated using 500 μL of each dispersant solvent and 60 mg [C_6mim][PF_6]. Methanol yielded the highest recovery for Co, and thereby this solvent was selected as the dispersant for our studies. This higher recovery can be attributed to the better dispersion obtained in methanol [30]. On the other hand, the volume of dispersant directly affects RTIL solubility in aqueous phase, significantly determining the volume of the final phase, and thus influencing the efficiency of the microextraction technique. Thus, methanol volumes ranging within 200–900 μL were assayed. It was observed that the extraction efficiency increased by increasing the methanol volume up to 500 μL. A higher volume of methanol slightly reduced the preconcentration factor. Finally, 500 μL was chosen as the optimum volume of disperser solvent.

3.3. Influence of sample volume on extraction efficiency

Since [C_6mim][PF_6] solubility has been reported to be 7.5 g L^{-1} [19], the final volume of the RTIL phase and its effect on Co recovery were evaluated upon sample volume increase. As can be observed in Fig. 2, Co recovery remained constant up to 6 mL of sample. Despite a higher volume of the RTIL sedimented phase was achieved for lower sample volumes, it was more difficult to obtain reproducible signals due to background deterioration originated from insufficient pyrolysis treatment during Co measurements. Thus, the best signal-to-background ratio was obtained when 6 mL-aliquots of sample were chosen.

3.4. Complex formation conditions and selectivity of Co extraction

The pH plays an important role, not only on metal-chelates formation but also on DLLME performance, as it defines the charge of the complex and its affinity for the RTIL phase. The effect of pH on the formation of Co–1N2N was studied in the range of 1–8 (Fig. 3(a)). In good agreement with Da Silva et al. [31], the optimum pH was observed in the interval of 3.5–5, confirming that the complex requires a weakly acidic solution for quantitative formation [31]. Therefore, samples and standards were adjusted at pH 4.00 before IL-DLLME procedure. A neutral Co–1N2N chemical form is obtained at the chosen pH, since pK_a value of reagent is 7.63 ± 0.02 [32]. In order to maintain a constant working pH that allows formation and stability of the complex, an acetic/acetate buffer solution was selected. The possible influence of buffer concentration on Co extraction efficiency was studied in the range of $0–4 \times 10^{-2}$ mol L^{-1}. It was observed that Co extraction increased by increasing the buffer concentration up to 2×10^{-2} mol L^{-1}. This

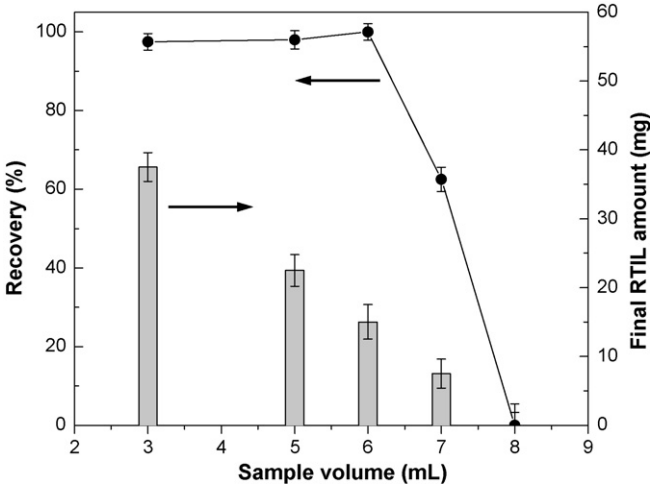

Fig. 2. (●) Evaluation of sample volume capacity of the proposed IL-DLLME approach for efficient Co recovery. (■) The effect of sample volume on IL phase solubilization is represented as final RTIL amount. Other conditions were as indicated in Table 1 (95% confidence interval; $n = 6$).

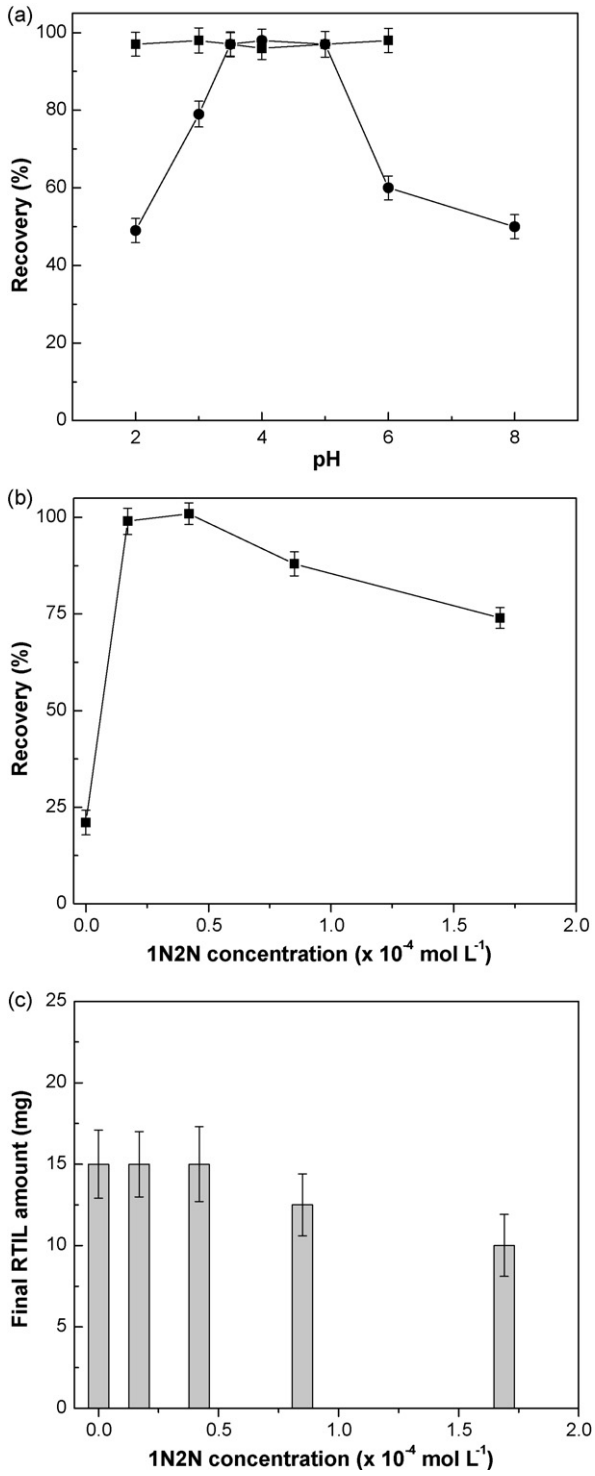

Fig. 3. Selectivity of Co complex formation and extraction. (a) Influence of pH on Co–1N2N complex formation (●) and extraction (■). (b) Effect of 1N2N concentration on Co recovery (■). (c) Effect of 1N2N concentration on final RTIL amount. Other conditions were as indicated in Table 1 (95% confidence interval; $n = 6$).

improvement on the system performance could be explained due to major stability of Co in solution at low pH when acetic acid is present [33]. A buffer concentration of 2×10^{-2} mol L^{-1} was chosen for subsequent experiments.

The high stability of the Co–1N2N complex, at different pH values after formation, has been already reported [26]. Therefore, the effect of pH on complex formation and the performance of IL-DLLME procedure could be individually study in this work. After complex formation, the effect of pH on the extraction performance was studied within the range of 2.0–6.0 by adding appropriate volumes of HCl or NaOH solution (Fig. 3(a)). No changes on the extraction efficiency were observed within this pH interval. Thus, in order to significantly increase the selectivity of Co chelation with 1N2N reagent and determination, solutions with low pH are preferred due to high instability of others metal–1N2N complexes [31]. Consequently, after the Co–1N2N complex was formed at pH 4, IL-DLLME procedure was performed at pH 2 by adding HCl (1 mol L^{-1}).

Due to the polarity of Co ions, their extraction efficiency by the sole application of $[C_6mim][PF_6]$ could be too low. In order to increase the extraction efficiency of metal ions it is necessary to improve their affinity for the RTIL phase by complexing with a suitable reagent such as 1N2N [17]. Moreover, imidazolium-based ILs present a high chemical affinity to substances with one or more aromatic rings in their structures, since they are extracted from the sample matrix through CH–π hydrogen bonds between the C_2H of the imidazolium ring and the aromatic parts of compounds [34]. The effect of 1N2N concentration on the analytical signal was evaluated (Fig. 3(b)). A maximum Co extraction was observed at 4.2×10^{-5} mol L^{-1} 1N2N, while a drop of efficiency was observed upon an increase in 1N2N concentration (Fig. 3(b)). This effect could be attributed to an increase of RTIL solubility in acidic aqueous phase when a neutral extractant, such as 1N2N, is present (Fig. 3(c)) [35]. Since the formation of the Co–1N2N complex is a slow process that can be speeded up by heating the solution [26], the effect of temperature on reaction kinetic and final Co extraction was studied. A 0–60 min time window was chosen to investigate the formation of the complex both, at room temperature and at 50 °C in a thermostated bath. It was observed that extraction recovery reached the highest value for 15 min in a thermostated bath at 50 °C before developing the IL-DLLME procedure. Furthermore, it has been demonstrated that Co–1N2N is a stable complex over a 24-h period [36].

3.5. Surfactant and salt as additives

The Co–1N2N complex precipitates in aqueous medium due to its low polarity, negatively affecting the extraction efficiency of the technique. A non-ionic surfactant not only can avoid this problem, but also reduce the adherence of the RTIL on the wall of the centrifuge tube. The effect of different concentrations ($0–5.6 \times 10^{-4}$ mol L^{-1}) of two non-ionic surfactants (Triton X-100 and Triton X-114) was studied and compared. It was observed that both the complexing agent and the metallic complex remained in solution within the range studied. For Triton X-100, it was observed that extraction efficiency decreased by increasing surfactant concentration. On the other hand, when using Triton X-114, Co extraction improved up to 9.3×10^{-5} mol L^{-1}. Thus, Triton X-114 was chosen as anti-sticking agent.

Generally, the addition of salt in traditional L–L extraction using conventional organic solvents increases the extraction performance due to salting out effect. This effect was investigated over a $NaNO_3$ concentration range of 0–6% (w/v). As shown in Fig. 4, the extraction efficiency increased as a result of salting out effect in the range of 0–2% (w/v) $NaNO_3$, while it decreased at concentrations higher than 2% (w/v) $NaNO_3$ due to solubilization of the RTIL phase into aqueous phase. Thus, a concentration of 1.5% (w/v) $NaNO_3$ was selected for subsequent experiments.

3.6. Evaluation of minimal extraction and centrifugation time

Extraction is a time-dependant process involving transferring of analytes from aqueous into RTIL phase. The extraction time, defined

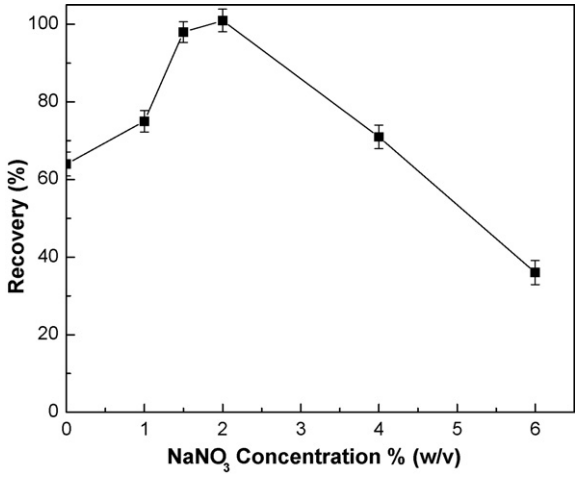

Fig. 4. Influence of salt addition on Co extraction efficiency obtained by application of the proposed IL-DLLME procedure. Experimental conditions were as mentioned in Table 1 (95% confidence interval; $n = 6$).

as the interval between addition of the mixture of methanol and RTIL and the moment the centrifugation process started, was evaluated in the range of 0–20 min. The recovery–time study showed that the highest extraction efficiency could be attained since 5 min and longer extraction times did not significantly improve Co extraction. These results show that IL-DLLME is a very fast extraction process, as right after the cloudy solution was formed; the surface area between the RTIL droplet and the aqueous phase was very large, thus improving the diffusion of Co–1N2N into the extractant. In order to achieve the highest extraction efficiency in the shortest time, extraction was performed during 7 min.

The effect of centrifugation time on Co recovery was studied in the range of 5–25 min at 1500 rpm (377.2 g). The volume of the sedimented IL phase, and consequently recoveries, increased as the centrifugation time was extended up to 15 min. The analyte recovery remained constant for longer times, indicating total definition of RTIL phase at the bottom of centrifuge tube. A centrifugation time of 15 min was then selected.

3.7. Study on potential interfering species

In view of the high selectivity achieved for Co–1N2N complex formation at pH 4, followed by extraction at pH 2, interference effects for our method could be mainly considered during the extraction/preconcentration step. Therefore, the selectivity of the proposed method was assayed by evaluating the individual effect of possible concomitant ions at the levels usually found in water and biological samples. The procedure was performed with 6 mL of 1 μg L^{-1} Co solutions individually containing different concentrations of such ions. As shown in Table 2, quantitative separation and determination of Co were obtained even when foreign ions were at higher concentrations than those normally found in the samples under study. Additionally, their contribution to the ionic strength of the system is insignificant and does not affect the extraction efficiency.

3.8. Analytical performance

In order to evaluate the performance of the proposed method, three main parameters were employed, namely: extraction recovery, enrichment factor and consumptive index. Extraction recovery (ER) was defined as the percentage of total analyte which was extracted into the IL phase:

$$\mathrm{ER} = \frac{m_{\mathrm{ILphase}}}{m_{\mathrm{aq}}} = \frac{C_{\mathrm{ILphase}} \times V_{\mathrm{ILphase}}}{C_{\mathrm{aq}} \times V_{\mathrm{aq}}} \times 100$$

where m_{ILphase} and m_{aq} are the mass of analyte in the final IL phase and the initial concentration in the sample solution, respectively. C_{ILphase} and C_{aq} are the concentration of the analyte in the IL phase and in the sample phase, respectively. V_{ILphase} and V_{aq} are the volumes of the phases involved [30]. Therefore, an extraction recovery of about 99.9% was achieved when the procedure was developed under optimal experimental conditions (Table 1).

Table 2
Effect of foreign ions on the recovery of Co[a].

Ion	Added as	Concentration (mg L^{-1})	Co recovery (%)
Ca^{2+}	$Ca(NO_3)_2$	400	103
Cd^{2+}	$Cd(NO_3)_2$	1000	101
Cu^{2+}	$Cu(NO_3)_2$	100	100
Fe^{3+}	$FeCl_3$	100	99.7
Hg^{2+}	$HgCl_2$	1000	101
K^{+}	KNO_3	1000	103
Mg^{2+}	$Mg(NO_3)_2$	100	99.0
Mn^{2+}	$MnSO_4$	1000	101
Na^{+}	$NaNO_3$	1000	102
Zn^{2+}	$Zn(NO_3)_2$	100	105
Cl^{-}	KCl	3000	100
NO_3^{-}	$NaNO_3$	2700	101
PO_4^{3-}	NaH_2PO_4	2000	100
SO_4^{2-}	$MnSO_4$	1700	98.3

[a] This study was performed using 6 mL of 1 μg L^{-1} Co standard solution.

Likewise, the enrichment factor (EF) is defined as the ratio of the calibration curve slopes for Co before and after the preconcentration step [37]. The obtained enrichment factor (EF) for a sample volume of 6 mL and a resulting RTIL phase in methanol volume of 50 μL was 120. The relative standard deviation (RSD) was 3.4% (Co concentration: 1 μg L^{-1}, $n = 10$). The calibration graph was linear between 0.038 and 3.5 μg L^{-1}, with a correlation coefficient of 0.9987. The limit of detection (LOD), calculated based on the signal at intercept and three times the standard deviation about regression of the calibration curve [35], was 3.8 ng L^{-1} for the proposed methodology. Finally, the consumptive index (CI) can be defined for practical purposes as:

$$\mathrm{CI} = \frac{V_s}{\mathrm{EF}}$$

where V_s is the volume of sample (in milliliters) consumed to achieve the EF value [38]. The CI obtained for the proposed method was 0.05. Regarding the frequency of analysis, although the whole preconcentration procedure (metal chelation, extraction into the dispersed IL phase, and centrifugation) could take about 45 min, it is possible to simultaneously treat as many samples as can be placed in the centrifugation equipment. For our work, the frequency of analysis was at least 30 samples per hour.

Finally, a comparative study on analytical performance allows us to show the strengths of our method with respect to others reported in the literature. Our method presents a linear range and a detection limit that is comparable to, or better than other methodologies developed for Co determination in biological and environmental samples (Table 3). A high enrichment factor was obtained with a reduced sample volume, yielding a low CI. Thus, CI reflects the efficiency of sample utilization, and it is useful tool for selecting a preconcentration method when sample amount is limited, such as the case of body fluid analysis [38]. All in all, the results indicate that the proposed method is a simple, fast, interference-free, selective and environment-friendly analytical approach for trace Co determination in biological and water samples. On the other hand, electrothermal vaporization coupled to inductively coupled plasma

Table 3
Characteristic performance data obtained by using the proposed method and other techniques reported for Co determination.

Method	LOD (ng L^{-1})	RSD (%)	EF	Sample consumption (mL)	Calibration range (μg L^{-1})	Analysis frequency (h^{-1})	CI (mL)	Ref.
CPE/spectrometry	7500	2.2	10	10	20–200	[b]	1	[43]
SPE/FAAS	550	1.63	300	900	[b]	>60[a]	3	[2]
DLLME-FAAS	900	5.8	16	5	3–100	[b]	0.31	[44]
SPE-ICP-MS	4	6.0	150	150	0.5–100	>120[a]	1	[6]
CIAME-FO-LADS	140	2.32	165	10	1.5–65	[b]	0.06	[24]
IL-SDME-ETV-ICP-MS	1.5	7.7	350	1.5	0.01–50	6[a]	0.004	[25]
IL-DLLME-ETAAS	3.8	3.4	120	6	0.038–3.5	30	0.05	This work

[a] On-line preconcentration procedures.
[b] Non reported.
FAAS: Flame atomic absorption spectrometry CIAME: cold-induced aggregation microextraction; FO-LADS: fiber optic-linear array detection spectrophotometry.

mass spectrometry (ETV-ICP-MS) has been exposed as a powerful analytical technique for Co determination [25]. However, the major degree of sophistication, high cost, and limited frequency of analysis originated from its combination with SDME technique, could be prohibitive for application in routine analytical laboratories. On the contrary, IL-DLLME technique combined with ETAAS detection, presents high frequency of analysis, comparable and good limit of detection, with the advantage of using low cost and widely spread instrumentation.

3.9. Determination of Co in environmental and biological samples

Cobalt is commonly used in dental cast alloys, orthodontic wires and implantable orthopedic devices, releasing it into human tissue due to corrosion [39]. Since saliva is an easy-to-collect low-cost sample which is very useful for screening large populations [40], it can be used for monitoring Co released from orthopedic devices. However, a major challenge for detection of chemical contaminants in saliva is that concentrations are often 1 or 2 orders of magnitude lower than in blood [41]. On the other hand, blood and urine are proposed as biomarker of recent exposure to soluble Co species [1]. However, urine is preferred for heavy metals monitoring due to non-invasive sampling and easier collection [40]. To best of our knowledge, there have been no reports demonstrating the viability of performing a RTIL-based microextraction technique for metal extraction from non-invasive biological samples such as saliva and urine. Only Xia et al. [25] applied an IL-LLME technique for metal extraction in human serum samples. Therefore, the results obtained after urine and saliva analysis are summarized in Table 4. Furthermore, analyte recovery in the presence of biological matrix was studied. The proposed method was applied to six portions of both saliva and urine matrices and the average concentrations of Co were taken as base values. Then, 1 μg L^{-1} Co was added to samples and the same procedure was followed. The results obtained with the proposed method were in good agreement with those previously reported for urine samples [42], while Co recoveries were highly satisfactory for all cases.

The proposed method was applied to the determination of soluble Co in tap and river water samples (Table 3). The recovery of Co was between 98.0 and 103%. The Co concentrations in river water samples were in the range of 0.45–0.57 μg L^{-1} and in tap water were in the range of 0.53–0.69 μg L^{-1}. Results were not significantly different to those previously reported in river and tap water samples [5]. Additionally, the accuracy of the proposed methodology was evaluated by analyzing a certified reference material (CRM) of natural water NIST SRM 1643e, with a Co content of 27.06 ± 0.32 μg L^{-1}. This CRM contains several ions commonly present in natural water samples. Since the certified concentration value in the CRM was higher than the upper limit of the lineal range achieved by this method, a dilution by a factor of 15 had to be implemented for analysis. Using the method developed in this work, the Co content found in the CRM was 27.26 ± 0.83 μg L^{-1} (95% confidence interval; $n = 6$).

Table 4
Determination of Co in water and biological samples (95% confidence interval; $n = 6$).

	Sample	Added (μg L^{-1})	Found (μg L^{-1})	Recovery (%)[a]
River water	1	0	0.45 ± 0.01	–
		1.00	1.48 ± 0.08	103
	2	0	0.57 ± 0.02	–
		1.00	1.59 ± 0.08	102
	3	0	0.53 ± 0.01	–
		1.00	1.51 ± 0.07	98.0
	4	0	0. 48 ± 0.01	–
		1.00	1.47 ± 0.06	99.0
Tap water	1	0	0.65 ± 0.02	–
		1.00	1.63 ± 0.07	98.0
	2	0	0.53 ± 0.01	–
		1.00	1.55 ± 0.06	102
	3	0	0.69 ± 0.02	–
		1.00	1.67 ± 0.09	98.0
	4	0	0.58 ± 0.01	–
		1.00	1.60 ± 0.08	102
Saliva	1	0	n.d.[b]	–
		1.00	0.98 ± 0.06	98.0
	2	0	0.15 ± 0.00	–
		1.00	1.12 ± 0.05	97.0
	3	0	0.07 ± 0.00	–
		1.00	1.08 ± 0.07	101
	4	0	n.d.[b]	–
		1.00	1.03 ± 0.07	103
Urine	1	0	0.60 ± 0.02	–
		1.00	1.58 ± 0.06	98.0
	2	0	0.92 ± 0.03	–
		1.00	1.94 ± 0.09	102
	3	0	0.32 ± 0.01	–
		1.00	1.29 ± 0.06	97.0
	4	0	0.35 ± 0.03	–
		1.00	1.36 ± 0.08	101

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

4. Conclusions

A highly selective and rapid microextraction method based on [C_6mim][PF_6] RTIL for Co determination was developed. The great potential that IL-based microextraction has for trace Co determination, with the help of 1N2N as a selective chelating reagent was demonstrated. The variation of pH is an effective way to eliminate possible interfering species that on other hand could form stable complexes with the organic reagent and would be co-extracted with the analyte. Thus, 1N2N showed good tolerance to possible interferences caused by other co-existing metal ions, due to the high stability of Co–1N2N complex at pH 2.

This study indicates that IL-DLLME technique using [C_6mim][PF_6] and 1N2N chelating reagent is a highly efficient

(∼100%) and green extraction technique for Co separation and preconcentration, even from complex matrices like biological ones. In fact, the preconcentration method was successfully applied for Co determination in water, urine and saliva samples, with good accuracy and good reproducibility.

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