Room Temperature Ionic Liquid-based Microextraction for Pre-concentration of Cadmium and Copper from Biological Samples and Determination by FAAS

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INTRODUCTION

The influence and toxicity of trace metals such as cadmium (Cd) and copper (Cu) on the environment and on human health are today receiving greater consideration in pollution and nutritional assessments (1-2). Cd is a non-essential element and plays no positive role for humans or animals. It is categorized as a predominantly toxic metal even at very low levels, and affects destruction of body organs, i.e., lungs, kidneys, and liver (3). The most significant anthropogenic sources of Cd are emission from industrial plants, such as steel works, zinc smelters, power stations, and incinerators (1,4). The intake of Cd by humans takes place by respiration of polluted air or through ingestion of different foods (5,6). The productions of free radicals, which deteriorate the physiological functions of the human body, are carried out by Cd through different mechanisms (7,8). On the other hand, Cu is an essential trace element, plays vital roles in many biochemical reactions of the human organisms and in the metabolism of lipids, carbohydrates, proteins, and the synthesis of nucleic acids (9-10). Even though Cu is important for humans, high levels of Cu can be harmful and cause vomiting, nausea, diarrhea, irritation of throat and nose, and form toxic-free hydroxyl radicals resulting in cancer by destroying the DNA (11-12).

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ABSTRACT

Room temperature ionic liquids (RTILs) are considered "Green Solvents" for their thermally stable and non-volatile properties. They are employed to replace traditional volatile organic solvents in solvent extraction, organic synthesis, and electrochemistry. In the present work, a simple, selective, sensitive and economical microextraction methodology was developed based on the separation and pre-concentration of RTILs for trace cadmium (Cd) and copper (Cu) determination in biological samples. The Cd and Cu pre-concentration was established using the RTIL (1-butyl-3-methylimidazolium hexafluorophosphate ([C4mim][PF6])) with dithizone complex. For the quantitative recovery of Cd and Cu, the effects of the parameters inducing the extraction efficiency and its subsequent determinations, i.e., pH, amount of complexing reagent, volume of RTILs and dispersant solvent, sample volume, extraction time, and temperature were studied. For Cd and Cu determination under the optimized conditions, the detection limits (LOD) of 0.05 and 0.08 µg/L and quantification limits (LOQ) of 0.15 and 0.25 µg/L, respectively, were obtained. An enrichment factor of 75 was achieved for both analytes and the relative standard deviation was < 3%. BCR 144R sewage sludge of domestic origin certified reference material (CRM) was used for validation of the present method. The proposed RTIL microextraction method was applied for the pre-concentration of Cd and Cu in biological samples with satisfactory results.

The low levels of these analytes present in a complex sample matrix require a procedure that does not affect the accuracy and sensitivity of the analytical method selected. Therefore, a cleanup or enrichment step is most important. The procedures proposed in the literature for the separation and pre-concentration of Cd and Cu include cloud point extraction (CPE), solid phase extraction (SPE), coprecipitation, capillary microextraction, single-drop microextraction (SDME), and solid phase microextraction (SPME) (13-21), etc. Even though liquid-liquid solvent extraction can efficiently reduce the limit of detection and remove interferences, it is tedious, time-consuming, and laborious, and requires high purity organic solvents. However, their removal after usage from the sample solution results in high risk of pollution to the environment (22-23).

Within the last few years, room temperature ionic liquids (RTILs), which are considered green solvents, were used as alternative extractants for traditional liquid-liquid extraction procedures (24-25). Various problems related to the loss of solvent by evaporation as well as regular organic solvents were avoided by using ionic liquids (ILs) as substitute solvents. RTILs have high thermal stability even at elevated temperatures, good solubility for organic and inorganic compounds, and insignificant vapor pressure (26). The main benefit of ILs is that they are environmentally friendly for the extraction of elements (27). RTILs have been

applied in SPE (28), hollow fiber membrane extraction (29), and liquid phase microextraction (30–31), and offer significantly high enrichment factors.

The aim of the present work was to establish a rapid, simple, novel, and highly efficient analytical procedure for Cd and Cu determination in biological samples using the combination of RTIL with flame atomic absorption spectrometry (FAAS). Dithizone is an extensively used complexing agent that can produce a complex with many metal ions. It acted as the ligand with methanol as the dispersant solvent for the Cd and Cu pre-concentration in biological samples. The influence of several experimental parameters on the extraction of Cd and Cu were investigated to examine the extraction behavior of ionic liquids.

EXPERIMENTAL

Instrumentation

A PerkinElmer® Model 3110 flame atomic absorption spectrometer, equipped with a 10-cm air-acetylene burner, was used for the determination of Cd and Cu (PerkinElmer, Inc., Shelton, CT, USA). At 12.5 and 15 mA, the hollow cathode lamps of Cd and Cu were operated with a 0.7 nm spectral bandwidth. The analytical wavelengths were set at 228.8 and 324.8 nm, respectively. A Sartorius Model PT-10 glass-electrode was used to establish the pH levels (Sartorius Co., Goettingen, Germany). An ALC PK Model 120 centrifuge machine and a Vortex 3000 ANA Shaker (Wiggen Hauser, Salenger, Malaysia) were used. For the purification of water, a Model RO 180 (Human Corporation, Korea) reverse osmosis water purification system was used to achieve 1 μ S/cm water conductivity. The instrumental settings are listed in Table I.

Standards and CRM

Standard solutions of Cd and Cu were prepared by dilution of the stock solution (1000 mg/L), obtained from Merck, Darmstadt, Germany. The standard solutions were prepared by stepwise dilution of the stock solution. Certified reference material (CRM) BCR 144R sewage sludge of domestic origin was provided by the Community Bureau of Reference (Geel, Belgium).

Chemicals, Reagents, and Glassware

In this study, all reagents used were analytical grade. Concentrated nitric acid (HNO_3 , purity 65%) and methanol were obtained from Merck (Germany) and Sigma Aldrich (St. Louis, USA), respectively. Ionic liquid (IL) 1-butyl-3-methylimidazolium hexafluorophosphate ($[\text{C}_4\text{mim}][\text{PF}_6]$) was obtained from Merck. A 0.1% (w/v) dithizone was obtained from Riedel-deHaen (Sleeze, Germany). The dithizone solution was prepared in methanol. A 0.1 M phosphate buffer solution was prepared from di-sodium hydrogen phosphate (Na_2HPO_4) in reverse osmosis water, and a pH range of 4–10 was prepared with (0.1 M) HNO_3/NaOH . All laboratory glassware and plastic containers used in the present experimental work were kept in 10% (v/v) HNO_3 and, prior to use, were rinsed with reverse osmosis water.

Sample Preparation

A 1-mL aliquot of sample solution was taken and Cd and Cu was instantly determined and pre-concentrated using the RTIL method.

Portions of 1 g human scalp hair and 2 mL of urine samples were placed into a PTFE beaker (10 mL). Then, 10 mL of concentrated HNO_3 was added and the solution kept standing overnight. The solution was evaporated and 10 mL of HNO_3 and H_2O_2 (2:1) was added, then heated to near dryness. The residue was dissolved in 0.2 M HNO_3 . Before analysis, the phosphate buffer solution was used to adjust to pH 9. The certified reference material (CRM) BCR 144R sewage sludge of domestic origin and the blank samples were prepared using the same method as for the biological samples in order to assess Cd and Cu concentrations with the reagents.

Recommended RTIL Procedure

For the enrichment of Cd and Cu, 15-mL aliquots of standard solution containing the Cd and Cu analyte in the range of 0.12–100 $\mu\text{g/L}$, three replicate samples of 10 mL of BCR 144R and triplicate samples of each real biological (scalp hair and urine) sample were taken into 50-mL graduated centrifuge tubes. Then, 3 mL of different buffers were added to adjust the pH in the range of 4–10. The pH of the solution was adjusted to the desired pH by the addition of 1 M HCl and/or NaOH . After adjusting the pH, 300–1200 μL dithizone solution (0.01%), 100 μL of each methanol and ($[\text{C}_4\text{mim}][\text{PF}_6]$) were added as disperser and extractant solvents, respectively. This step resulted in a cloudy solution (water, methanol, and ($[\text{C}_4\text{mim}][\text{PF}_6]$)) in the tube. The centrifuge tubes were shaken carefully and gradually for a few

TABLE I
Instrument Settings

Parameters			
Elements	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Cd	228.8	0.7	4
Cu	324.8	0.7	30

seconds and allowed to stand for 15 minutes. Then the cloudy solution was centrifuged for 10 minutes at 4000 rpm. This allowed the formation of two well-defined phases, and dispersive droplets of IL were sedimented at the bottom of the centrifuge tube. After enrichment, the upper aqueous phase was decanted, the IL phase dissolved in 200 μL of concentrated HNO_3 , and then introduced into the FAAS using a home-made micro-sample aspiration system. For the respective analyte determinations, the samples were introduced into the nebulizer of the FAAS by using the home-made microsample aspiration system. In this system, 100 μL of the sample was injected into a mini home-made Teflon® funnel with an Eppendorf® pipette. The Teflon funnel was connected to the nebulizer with capillary tubing (19,32).

Calibration of Cd and Cu was prepared by subjecting the aqueous standards to the same RTIL procedure. A blank submitted to the RTIL was measured similar to the calibration solution of the standards, CRM, and real biological samples. The validation and accuracy of the proposed RTIL method were checked by using the CRM BCR 144R sewage sludge of domestic origin for both analytes.

RESULTS AND DISCUSSION

The RTILs procedure in comparison to conventional dispersive liquid phase microextraction was carried out using a single solvent reagent. RTILs and dispersive liquid phase microextraction procedures substantially increase the surface area of the extraction solvent, and the contact area between extraction solvent and analytes enhances the recovery of the analytes. In addition, ILs are used as an alternative to toxic organic solvents in RTILs as compared to dispersive liquid-phase microextraction (DLLME). Some significant experimental factors that would affect the enrichment efficiency were studied in order to achieve optimum extraction recovery.

Effect of pH

The pH plays a distinctive role on metal-ligand development and affects production of the complex and its consequent extraction. The influence of pH on optimum complex production for the extraction of Cd and Cu was studied on replicate subsamples of standards or real samples by altering the pH of the aqueous solution ranging from 4–10. The results illustrated in Figure 1 show that the Cd and Cu recoveries in the presented system decrease with a pH range of 4–7,

then increases with a pH higher than 7. Optimum extraction efficiency was obtained at pH 9. To prevent hydrolyzation of the metal ion at a higher pH value, the pH of 9 was selected for both analytes. The metal chelator is in neutral form at a lower pH, and distributes or mixes in the organic phase. This leads to a very low level metal chelate complex because there is only a limited amount of deprotonated chelator present for the production of the complex. On the other hand, at a higher pH, a high level of deprotonated chelator leads to a high level of metal chelate complex, resulting in high extraction efficiency.

Effect of Dithizone Concentration

Dithizone is a strong chelating ligand for metal ion extraction because it can produce stable complexes with many metal ions under appropriate conditions. The recovery of Cd and Cu increased with an increase in 0.01% (w/v) dithizone from 400 to 1200 μL , and remained constant with a further increase in the dithizone level. At 1000 μL , optimum recovery of Cd and Cu was obtained and 0.01% dithizone was chosen for further experiments to avoid the competitive chelation of matrix ions in real biological samples (see Figure 2). Optimum extraction efficiency was dependent on the concentration of the complexing agent. When the volume of dithizone was >1000 μL , the analytical signal remained constant.

Effect of Volume of Ionic Liquid

The volume of the extractant is an important factor in conventional liquid phase extraction and significantly affects the extraction efficiency of the analytes. In the present work, $([\text{C}_4\text{mim}][\text{PF}_6])$ was chosen as the extraction solvent due to its hydrophobicity. The volume of $([\text{C}_4\text{mim}][\text{PF}_6])$ was opti-

Fig. 1. Effect of pH on the (%) recovery of Cd and Cu: 1000 μL of a dithizone (0.01%), 100 μL of ILs and methanol, centrifugation at 10 minutes at 4000 rpm.

mized in the range of 50–150 μL . The results in Figure 3 show that the Cd and Cu recoveries increased along with an increase in the volume of $([\text{C}_4\text{mim}][\text{PF}_6])$ from 50 to 100 μL , while the extraction efficiency of $([\text{C}_4\text{mim}][\text{PF}_6])$ remained constant when the volume was >100 μL . Therefore, 100 μL IL was chosen in order to achieve perfect analytical characteristics.

Disperser Solvents and Their Effects

The disperser solvent must be miscible in both extractant and aqueous phase and should have

good dispersive ability. Therefore, methanol, acetone, ethanol, and acetonitrile were used as disperser solvents. The influence of these solvents on the extraction efficiency of RTILs was determined using different volumes of each disperser solvent with 100 μL of $([\text{C}_4\text{mim}][\text{PF}_6])$ and 0.01% dithizone. The results indicated that among the four disperser solvents, methanol has the highest extraction efficiency for Cd and Cu. Thus, methanol was selected as the disperser solvent for further studies.

The effect of volume of methanol on the extraction efficiency of Cd and Cu was also studied. To attain the optimum volume of methanol, different volumes (25–150 μL) of methanol were tested. The results showed that the extraction efficiency increased with an increase in the volume of methanol up to 100 μL , then reduced with a further increase in methanol volume (see Figure 4). It can be concluded that a minimum volume of methanol results in $([\text{C}_4\text{mim}][\text{PF}_6])$ not being dispersed well and consequently decreases the extraction efficiency. On the other hand, the solubility of the metal-dithizone complex in the aqueous phase increases with a higher volume of methanol but reduces the extraction efficiency. For further studies, 100 μL of methanol was chosen as the optimum volume.

Sample Volume

The effects of sample volume with a constant amount of 100 μL of $([\text{C}_4\text{mim}][\text{PF}_6])$ and methanol on the extraction of Cd and Cu were studied from 10.0 to 50.0 mL. By increasing the sample volume to >20 mL, the extraction efficiency of the respective analytes decreased.

Fig. 2. Effect of dithizone concentration on the (%) recovery of Cd and Cu: pH 9, 100 μL of ILs and methanol, centrifugation at 10 minutes at 4000 rpm.

Fig. 3. Effect of ILs volume on the (%) recovery of Cd and Cu: 1000 μL of 0.01% of a dithizone, pH 9, 100 μL of methanol, centrifugation at 10 minutes at 4000 rpm.

Fig. 4. Effect of methanol volume on the (%) recovery of Cd and Cu: 1000 μL of 0.01% of a dithizone, pH 9, 100 μL of ILs, centrifugation at 10 minutes at 4000 rpm.

The optimal extraction recovery was found at 15 mL. Therefore, a sample volume of 15 mL was selected for subsequent work.

Effects of Centrifugation Rate and Time

The centrifugation rate and time that induced the pre-concentration of ILs from the aqueous phase was investigated from 5 to 20 minutes at the 2500–5000 rpm range, respectively. The analytical signals of Cd and Cu increased by increasing the extraction time up to 10 minutes and then keeping the time constant. Mass transfer is a time-dependent process and the maximum integrated absorbance is attained when the system is at equilibrium. However, it takes time for a system to reach complete equilibrium. The extraction time was investigated by monitoring the variation of the integrated absorbance. The integrated absorbance increases with an increase in extraction time up to 10 minutes, and after 10 minutes the increase becomes slower. Therefore, to avoid dropwise dissolution, the extraction time of 10 minutes was selected for quantitative complex formation of the analytes (3). Greater extraction efficiency was obtained at 4000 rpm, and above this centrifugation rate, there was no effect on the extraction efficiency.

TABLE II
Effect of Interfering Ions on the Recovery of Cd and Cu Using RTILs

Interferent Ions	Conc. (µg/mL)	Added Ions	% Recovery	
			Cd	Cu
Fe ³⁺	25	Fe(NO ₃) ₃ ·9H ₂ O	98.4	95.6
Zn ²⁺	25	Zn(NO ₃) ₂ ·6H ₂ O	96.9	97.4
Pb ²⁺	25	Pb(NO ₃) ₂ ·6H ₂ O	95.7	93.1
Na ⁺	2000	NaNO ₃	98.7	97.2
K ⁺	2000	KCl	97.8	96.3
Ca ²⁺	250	Ca(NO ₃) ₂ ·4H ₂ O	96.9	93.4
Mg ²⁺	250	Mg(NO ₃) ₂ ·4H ₂ O	89.3	90.4
Cl ⁻	2000	KCl	97.5	94.8
SO ₄ ²⁻	2500	Na ₂ SO ₄	91.4	91.2

Influences of Concomitants

The reliability of the proposed pre-concentration method on the recovery of Cd and Cu was studied in the presence of possible concomitant ions. The results listed in Table II demonstrated that the existence of greater amounts of interfering ions had no noticeable effect on the recovery of the respective analytes within a ± 5% error. The influence of interfering ions present up to 5000 µg/mL each was insignificant on the pre-concentration of the analytes. The results indicate that the proposed pre-concentration procedure can be useful in the analysis of biological samples without any systematic error.

Calibration, Precision, and Detection Limits

Calibration graphs were established for Cd and Cu by enrichment of the sample with 0.01 % dithizone, ionic liquid, and disperser solvent in a medium buffered at pH 9. Calibrations were achieved with a series of Cd and Cu standards after subjecting to RTIL. The calibration curve equations after the pre-concentration method for Cd and Cu, respectively, were :
(A = 0.0324 C (Cd) + 0.0024) and
(A = 0.0193 C (Cu) + 0.001),
where A is the absorbance and C is the concentration of the analyte in

the extraction solvent. The correlation coefficients of the equations were 0.993 and 0.995 for Cd and Cu, respectively, which shows that a good linear regression was obtained between absorbance and concentration.

The limits of detection (LOD) and limits of quantification (LOQ) for Cd and Cu were estimated as follows: LOD = 3 x s/m and LOD = 10 x s/m, respectively, where s is the standard deviation of 10 measurements of the blank and m is the slope of the calibration graph. The LOD and LOQ of Cd and Cu, respectively, was found to be (0.05 and 0.08) and (0.15 and 0.25) µg/L. The relative standard deviations (RSDs) of the proposed method were 2.2 and 2.9% for both analytes by measuring the standard solution (n = 5). The enrichment factors (EF) for Cd and Cu were found to be 75, respectively, designed as the slope ratio of the two calibration curves of the standard with micro-extraction and without micro-extraction.

Accuracy of Proposed Method

For verification of accuracy, validation, and precision of the proposed method, CRM BCR 144R sewage sludge of domestic origin, triplicate samples, procedural and reagent blanks were applied for Cd and Cu determination. The percentage recoveries of Cd and Cu in the CRM samples by RTILs were in the 98.4–98.7% range (see Table III). The analytical results were in good

TABLE III
Analytical Results of Cd and Cu Determination in CRM BCR 144R (Sewage Sludge of Domestic Origin) using RTILs (n = 3) in (mg/Kg)

	Certified Value x ± s ^a	Observed Value x ± s ^a	Recovery ^b (%)
Cd	1.84 ± 0.02	1.81 ± 0.04	98.4
Cu	300 ± 7.5	296 ± 8.6	98.7

^a mean ± standard deviation (n=10).

^b (%)Recovery = [Observed value] / [Certified value] × 100.

agreement with the CRMs and the presented values, and no significant differences were detected ($p > 0.05$). The mean levels for Cd and Cu differed by less than 1–2% from the certified values. These results demonstrate the validity of the proposed procedure for Cd and Cu pre-concentration.

Comparative Data of Analytical Characteristics of DLLME

A comparison of the proposed procedure with other described pre-concentration procedures for Cd and Cu pre-concentration is summarized in Table IV. The developed RTIL procedures have relatively lower LOD (0.05 and 0.08 $\mu\text{g/L}$) and higher EF (75) for Cd and Cu, respectively, than some of the other methods listed in Table IV and require only 10 mL of sample solution. This is useful for pre-concentration of both Cd and Cu at trace levels in real biological samples. As shown in Table IV, the proposed RTIL method, compared with literature data, indicates that the RSD, LOD/LOQ, and the EF of the present study are comparatively better than in previously described studies (3, 22–23, 33–40).

Applications

The RTIL method is suitable for the determination of Cd and Cu in biological samples (scalp hair and urine) as listed in Table V. The analytical applicability of the proposed methodology was verified by measuring the Cd and Cu levels in 10 mL each of CRMs and biological samples. The samples were pre-concentrated with 0.01% dithizone and 100 μL of each IL and methanol, respectively. The results show that the proposed RTILs can be successfully applied for the separation and pre-concentration of biological samples.

TABLE IV
Comparison of Analytical Characteristics of the RTILs
With Other Reported Methods for Cd and Cu Pre-concentration

System	Analysis Method	Enrichment Factor	RSD (%)	Linear Range ($\mu\text{g/L}$)	LOD ($\mu\text{g/L}$)	Reference
Cd						
On-line SPE	GFAAS	24.6	3.2	0.006–0.3	0.0028	(33)
CPE	FAAS	5	0.8–3.0	3–300	1.00	(34)
DLLME	GFAAS	122	2.9	0.002–0.021	0.0005	(23)
SDME	GFAAS	65	7.4	0.01–1	0.0007	(3)
Cu						
On-line SPE	FAAS	560	2.1	0.16–12	0.04	(35)
CPE	FAAS	64.3	1.6	0.27–100	0.27	(36)
DLLME	FAAS	42	5.1	50–2000	3	(37)
IL-SDME	UV-Vis.	33	3.4	—	0.15	(22)
RTILs						Present
Cd/Cu	FAAS	75	2.2/2.9	0.12–100	0.05/0.08	study

TABLE V
Analytical Results of Cd and Cu Determination in Standard and Spiked Biological Samples by RTILs (n=3) in $\mu\text{g/mL}$

	Spiked Sample	Found	Recovery (%)	Found	Recovery (%)
		Hair sample		Urine sample	
Cd	0	1.76 ± 0.07^a	—	0.015 ± 0.002	—
	5	6.65 ± 0.02	98	5.01 ± 0.08	99.9
	10	11.7 ± 0.36	100	10.1 ± 0.25	100.0
	20	21.4 ± 0.62	98	20.1 ± 0.42	100.1
Cu	0	20.3 ± 0.52	—	0.25 ± 0.05	—
	5	25.2 ± 0.56	99	5.13 ± 0.12	97.4
	10	30.1 ± 0.78	98	10.1 ± 0.32	98.8
	20	40.2 ± 0.86	100	20.2 ± 0.51	99.7

^a mean $\pm$ standard deviation ($x \pm s$) ($n = 4$).

CONCLUSION

An effective procedure for the pre-concentration and separation of Cd and Cu in biological samples is described using room temperature ionic liquids (RTILs) consisting of 1000 μL of dithizone, 100 μL each of IL and methanol, followed by FAAS determination. The detection limits (LODs) for Cd and Cu were

0.05 and 0.08 $\mu\text{g/L}$ and the quantification limits (LOQs) were 0.15 and 0.25 $\mu\text{g/L}$, respectively. In comparison to traditional extraction methods, the proposed method is rapid and simple, cost-effective, environmentally save, offers an enhancement factor of 75, enhanced sensitivity, and requires no toxic organic solvents. The greater toler-

ance of coexisting ions and excellent analytical performance shows that the developed method is applicable for the investigation of trace elements in biological samples.

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