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New sample preparation method based on task-specific ionic liquids for extraction and determination of copper in urine and wastewater

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Abstract In this study, four hydrophilic ionic liquids (ILs) containing 1-alkyl-3-methylimidazolim cation and either salicylate or chloride anions were synthetized and studied as new task-specific ionic liquids (TSILs) suitable for aqueous biphasic system (ABS) formation and selective one-step extraction of copper(II). TSILs are designed so that the anion is responsible for forming the complex with metal(II) and preventing hydrolysis of metal cations at very strong alkaline pH, whereas the cation is responsible for selective extraction of metal(II)-salicylate complexes. It was found that 1-butyl-3-methylimidazolium salicylate could be used for selective extraction of Cu(II) in the presence of Zn(II), Cd(II), and Pb(II) at very alkaline solution without metal hydroxide formation. It was assumed that formation of metal(II)–salicylate complexes prevents the hydrolysis of the metal ions in alkaline solutions. The determined stability constants for Cu(II)–salicylate complexes, where salicylate was derived from different ionic liquids, indicated that there was no significant influence of the cation of the ionic liquid on the stability of the complexes. The ABS based on 1-butyl-3-methylimidazolium salicylate has been applied as the sample preparation step prior to voltammetric determination of Cu(II). The effect of volume of aqueous sample and IL and extraction time were investigated and optimum extraction conditions were determined. The obtained detection limits were 8 ng dm^{-3}. The optimized method was applied for the determination of Cu(II) in tap water, wastewater, and urine. The study indicated that application of the ABS based on 1-butyl-3-methylimidazolium salicylate ionic liquid could be successfully applied as the sample preparation method for the determination of Cu(II) from various environmental samples.

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Abbreviations
ABS   Aqueous biphasic system
E   Extraction efficiency
EF   Enrichment factor
ILs   Ionic liquids
LLE   Liquid-liquid extraction

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P   Partition coefficient
TSIL   Task-specific ionic liquid
TLs   Tie-lines
TLL   Tie-line length

Introduction

Sample preparation is an important step in analysis of trace metals in complicated matrix such as environmental, biological, and food samples. It includes clean-up and concentrate of the targeted analytes. Various types of solid-phase and liquid-liquid extractions (LLE) have been applied as the sample preparation step [1]. Several liquid-liquid microextraction techniques such as single drop microextraction, dispersive liquid-liquid microextraction, and hollow fiber liquid-phase microextraction have been introduced as alternatives to the traditional sample preparation techniques based on LLE [2]. These techniques integrate sampling, extraction, and enrichment of analytes into one step which are usually easy to perform. Also, they are low-cost techniques with high enrichment factor but time and solvent consuming. The main drawback is the limited number of suitable extractants and at the same time high volatility and toxicity.

Recently, ionic liquids (ILs) as a new class of the compounds with negligible vapor pressure (and thus they are claimed to be non-volatile) gained attention as alternative solvents and media for LLE. These solvents have other remarkable features such as high thermal stability, variable viscosity, possibility to dissolve both organic and inorganic compounds, and tunable physicochemical properties. Usually, hydrophobic ILs have been used for IL + water biphasic system formation. Many application of hydrophobic ILs in microextraction procedures were published earlier [3, 4]. The water immiscible imidazolium-based IL was firstly applied for the biphasic metal extraction [5] and still remains the most frequently employed system for this task [6]. Extraction of metal ions based on ILs covered following approaches: the use of neutral ligand (e.g., crown ethers and calixarenes) dissolved in IL to complex metal ions [7], selective extraction of the metal ions through interaction with anions from IL [8], and task-specific ILs suitable for complexation of the metal ions either with specific functional groups attached to cation [9, 10] or with the anion [11]. However, compared to hydrophilic ILs, hydrophobic ILs are more expensive and their number is rather limited.

Polymer-polymer, polymer-salt, or salt-salt aqueous biphasic systems have been widely used for separation of organic molecules and biomolecules [12, 13]. The application of aqueous biphasic system (ABS) based on ionic liquids have been continuously increased since 2003 when Rogers et al. published this method for the first time [14]. These systems found numerous applications in green separation processes [15, 16].

Although large number of articles reported the applications of ABS based on ILs for extraction of different organic and bioactive compounds, only a few papers reported application of ABS for metal extraction such as selective extraction of Cd(II) and Cr(VI) using ABS based on tetrabutylammonium bromide ionic liquid [17, 18], extraction of Sc(III) by ABS based on carboxyl-functionalized phosphonium-based ILs [19], and selective extraction of Cr(III) applying 1-octyl-3-methylimidazolium salicylate IL [20]. The metal extraction in ABS involves the addition of either extracting agent (in view of enhancement of the extraction efficiency), mineral acids (in order to avoid hydrolysis/precipitation of the metal), or metallic salts themselves [21]. Advantages of ABS based on ILs are fast phase separation and rare formation of stable emulsion or third phase, high extraction efficiency, and mild environment. However, this method is still not widely applied in analytical chemistry especially for metal extraction.

Determination of copper(II) ions in biological samples is very important since it may be used as biochemical marker for some diseases such as Wilson disease, Menkes disease, hemochromatosis, biliary cirrhosis, thyrotoxicosis, various infections, and variety of other acute, chronic, or malignant diseases (including leukemia) [22]. Copper is an essential element for many biological functions and human life, but at the same time, it is potentially toxic due to its high chemical redox potential and ability to participate in free radical reactions. Copper homeostasis is carefully regulated through a system of protein transporters. The copper excretion is disordered in several human diseases. The biliary system which involves specific transport protein for copper is the major pathway of copper excretion. Mutations in the gene for the transporter protein cause hepatolenticular degeneration so-called Wilson disease. Also, the primary copper-bounding protein in the blood ceruloplasmin is reduced in Wilson disease. As a result of these disorders, excretion of copper in urine increases and for 24 h excretion is higher than 100 μg (normal values is lower than 40 μg/day). Low urine copper level (lower than 15 μg/day) is a characteristic for several diseases such as malnutrition, hypoproteinemia, malabsorption, and nephrotic syndrome.

On the other side, determination of Cu(II) in the wastewater from industrial manufacturers, processors, and users of copper and copper compounds before released to the receiving water streams is of outstanding relevance for environmental protection. The maximum allowable discharge concentration of Cu(II) in waste water is prescribed on European [23] and national levels.

Thus, the aim of this work was the identification and application of the most suitable task-specific ionic liquid (TSIL) for the extraction of copper ions using ABS formation method from biological and environmental samples. This is distinct from previous studies of the Cu(II) extraction since these studies generally used hydrophobic chelating agent to solubilize the metal in the IL phase [24, 25]. In this work, four differently substituted ILs have been synthesized: 1-butyl-3-methylimidazolium

salicylate, [C₄mim][Sal]; 1-(3-hydroxypropyl)-3-methylimidazolium salicylate, [HOC₃mim][Sal]; 1-(3-hydroxypropyl)-3-methylimidazolium chloride, [HOC₃mim][Cl]; and 1-(4-hydroxy-2-oxybutyl)-3-methylimidazolium salicylate, [HOC₂OC₂mim][Sal]. The phase behavior of the ternary systems {IL + K₃PO₄ + H₂O} was studied, and conditions for Cu(II) extraction were optimized. Finally, ABS based on [C₄mim][Sal] was identified as the most attractive and was applied for the quantification of Cu(II) in a wastewater obtained from copper mines and in biological samples (urine).

Experimental part

Materials and methods

Cadmium(II) nitrate, copper(II) nitrate, lead(II) nitrate, zinc(II) nitrate, potassium chloride, sodium acetate, sodium hydroxide, ammonium hydroxide, ammonium chloride, and potassium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were analytical grade reagents. Solutions were prepared using Milli-Q water (Millipore Corporation, USA).

The concentration of the metal ions in IL-rich phase after extraction was determined by polarography voltammetric system 797 VA Computrace analyzer (Metrohm, Herisau, Switzerland) applying differential pulse anodic stripping voltammetry (DPASV) at a hanging mercury drop electrode and a Metrohm's procedure for the voltammetric determination of Cd(II), Cu(II), Pb(II), and Zn(II) No. 231/2e in 0.13 mol dm⁻³ KCl and 0.045 mol dm⁻³ CH₃COONa as the electrolytes. The procedure No. 231/2e was slightly modified for the determination of the metal ions in the salt-rich phase: in this case, 1.5 mol dm⁻³ KCl and 0.5 mol dm⁻³ CH₃COONa were used as the electrolytes.

The chemical names, structures, abbreviations, provenance, and purity of the synthesized ILs are given in Table 1. ILs were synthesized by procedures published elsewhere [26, 27].

Phase diagrams and tie-lines

The binodal curves for the ABS based on the targeted IL were determined via cloud point method at room temperature of 293 ± 1 K and at atmospheric pressure of p = 0.1 MPa. The method was described in details in Refs. [14, 27]. The aqueous solution of K₃PO₄ (55%) was added drop by drop into an aqueous solution of IL (60%) until turbidity was observed. Then, pure water was added until the mixture became clear. The amounts of IL and the added solutions were measured on analytical balance (CP224S, Sartorius) with the accuracy of ± 10⁻⁴ g. The mixture was shaken on a vortex agitator (Reax Top, Heidolph, Germany) at 2500 rpm after each step. These two steps were repeated until turbidity was no longer detected.

The experimental binodal data were fitted by an empirical Merchuk correlation [28]:

$$Y = A exp\left(BX^{0.5} - CX^3\right) \tag{1}$$

where Y and X are the mass fractions of IL and salt, respectively, and A, B, and C are constants obtained by least-squares regression.

The tie-lines (TLs) were determined by a gravimetric method [29]. A mixture at the biphasic region {IL + salt + water} was gravimetrically prepared, vigorously stirred, and left for 3 h at room temperature to reach the complete separation and equilibration. The top (IL-rich) and bottom (salt-rich) phases were separated and weighted. The mass fractions of IL (Y) and salt (X) in IL-rich (Y_{IL} and X_{IL}) and salt-rich phases (Y_S and X_S) were calculated by solving following system of four equations with four unknown values applying MathCad 15.0 program:

$$Y_{IL} = A exp\left[BX_{IL}^{0.5} - CX_{IL}^3\right] \tag{2}$$

$$Y_S = A exp\left[BX_S^{0.5} - CX_S^3\right] \tag{3}$$

$$Y_{IL} = \frac{Y_M}{\alpha} - \frac{1-\alpha}{\alpha} Y_S \tag{4}$$

$$X_{IL} = \frac{X_M}{\alpha} - \frac{1-\alpha}{\alpha} Y_S \tag{5}$$

Here, subscript M denotes the mixture and α is determined mass ratio of the top phase and the mixture. Coefficients A, B, and C were taken from the fitting parameters of binodal curve. Tie-line length (TLL) was calculated using obtained values for Y_{IL}, Y_S, X_{IL}, and X_S and the following equation [28]:

$$TLL = \sqrt{\left(Y_{IL} - Y_S\right)^2 + \left(X_{IL} - X_s\right)^2} \tag{6}$$

Determination of copper stability constants

For the determination of stability constants of Cu(II) complexes with salicylate anion that originated from ILs, potentiometric titration was performed at 298.15 K in a nitrogen atmosphere with decarbonated NaOH (c = 0.09841 mol dm⁻³). The constant ionic strength was adjusted by addition of KClO₄ in investigated solutions. Also, perchloric acid was added to solution in order to completely protonate salicylate anion. The composition of the solutions with different ionic strength is given in Table 2.

From measured pH and calculated concentrations, the average coordination number, $\overline{n}$, was calculated based on equation:

$$\overline{n} = \frac{c(Sal) - [Sal]'}{c(Cu^{2+})} \tag{7}$$

Table 1 The chemical structures, provenance, and purity of the synthesized ILs

IL	Chemical structure	Purification method	Mass fraction, ω	Water content (ppm)
[C4mim][Sal]		Rotary evaporation followed by vacuum	0.96[a]	226
[HOC3mim][Sal]		Rotary evaporation followed by vacuum	0.97[a]	198
[HOC3mim][Cl]		Rotary evaporation followed by vacuum	0.97[a]	239
[HOC2OC2mim][Sal]		Rotary evaporation followed by vacuum	0.97[a]	212

aConfirmed by NMR spectroscopy after purification [25, 26]

where $c(Sal)$ is a total concentration of ionic liquid (ligand), $[Sal]'$ represents free concentration of ligand, and $c(Cu^{2+})$ is a concentration of copper ion. The free concentration of ligand can be calculated from the relation:

$$[Sal]' = [Sal^-]\alpha_H \tag{8}$$

where α_H represents:

$$\alpha_H = 1 + K_{p1}[H_3O^+] + K_{p1}K_{p2}[H_3O^+]^2 \tag{9}$$

K_{p1} and K_{p2} are protonation constants of salicylate ion taken from Ref. [30] and $[Sal^-]$ is calculated from equation:

$$[Sal^-] = \frac{c(Sal) + c(HClO_4) - c(NaOH) - [H_3O^+] + \left[OH^-\right]}{K_{p1}[H_3O^+] + K_{p1}K_{p2}[H_3O^+]^2} \tag{10}$$

where $c(HClO_4)$ is the concentration of added $HClO_4$ and $c(Sal)$ is the concentration of ionic liquid. From dependence of $\bar{n} = f(-\log[Sal^-])$, stability constants were determined.

Extraction procedure

A ternary mixture within the biphasic region was prepared containing 20% of IL, 20% of K_3PO_4, and 60% of water. The final volume of extraction mixture was 0.5 cm^3 and the concentration of each metal ion was 1.82 mmol dm^{-3}. The mixture was shaken for 2 min using a vortex agitator at 2500 rpm and left to equilibrate for at least 2 h. The top IL-rich phase was carefully separated and weighted. The concentration of the heavy metal ions was determined in both phases. The blank solution was prepared on the same way using distilled water instead of aqueous metal solution.

Optimization of extraction parameters

The following conditions for application of ABS based on [C4mim][Sal] as sample preparation step were optimized separately: amount of IL and sample volume, extraction, and centrifugation time. To investigate the effect of IL amount, the different volume of IL from (0.060 to 0.250 cm^3) was added into 0.250 cm^3 of aqueous solution of Cu(II)

Table 2 Concentration of the components in solutions for determination of stability constants

Ionic strength	$c(IL)/mol\ dm^{-3}$	$c(Cu(ClO_4)_2)/mol\ dm^{-3}$	$c(HClO_4)/mol\ dm^{-3}$	$c(KClO_4)/mol\ dm^{-3}$
0.50	0.020	0.010	0.050	0.50
0.30	0.010	$5 \cdot 10^{-2}$	0.025	0.30
0.10	$5 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$	0.010	0.10
0.05	$1 \cdot 10^{-3}$	$5 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	0.05
0.01	$2 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	$5 \cdot 10^{-3}$	0.01

(5 mg dm^{-3}) and 60% solution of K$_3$PO$_4$ was added to the final concentration of 18%. The mixture was shaken for 2 min using a vortex agitator at 2500 rpm and left to equilibrate for at least 2 h. To investigate the influence of amount of sample solution, 0.060 cm^3 of [C$_4$mim][Sal] was mixed with different volume (0.500 to 2.000 cm^3) of aqueous solution of Cu(II) (5 mg dm^{-3}) and 2 cm^3 of 55% solution K$_3$PO$_4$. The influence of extraction and centrifugation time was investigated in the range from 1 min to 4 h and 2 min to 15 min, respectively. The mixture of 1 cm^3 aqueous solution of Cu(II) (5 mg dm^{-3}), 0.060 cm^3 of IL, and 1 cm^3 of 55% solution K$_3$PO$_4$ was shaken using a vortex agitator at 2500 rpm stirring. Then, the IL-rich and salt-rich phases were separated by centrifugation (laboratory centrifuge LLG Labware uniCFUGE5) at 10000 rpm (RCF 6932×g). For investigation of longer extraction time, the samples were firstly vortex agitated for 2 min, then shaking was continued using a laboratory shaker (Promax 2020, Heidolph, Germany) at 200 rpm.

Calculations

Partition coefficients of the studied metal ions in ABS based on ILs (P_{IL}) were calculated as the ratio of the equilibrium concentration of the metal ions in the IL-rich (C_{Me}^{IL}) and in the salt-rich (C_{Me}^{s}) phases:

$$P_{IL} = \frac{C_{Me}^{IL}}{C_{Me}^{s}} \quad (11)$$

The extraction efficiency (E) was defined as the fraction of the initial amount of the heavy metal ions (n_{Me}^{in}) which was extracted into IL-rich phase and calculated as:

$$E = \frac{n_{Me}^{in} - n_{Me}^{s}}{n_{Me}^{in}} \quad (12)$$

where n_{Me}^{s} is the amount of metal ions tht remained into the salt-rich phase after separation of phases. The recovery (R) was defined as amount of metal ion extracted in the IL-rich phase (n_{Me}^{IL}) against initial amount of metal ion:

$$R = \frac{n_{Me}^{IL}}{n_{Me}^{in}} \quad (13)$$

The enrichment factor (EF) was calculated as the ratio of the metal ion concentration in the IL-rich phase and in the sample.

Sample collection and analysis

Sample of wastewater was collected within the Bor copper mines and production facilities (Bor, Republic of Serbia) at the point where the wastewater enters the river Borska. The sample of 24-h urine from a healthy male, 35 years of age, was obtained from a local medical center. Cu(II) was extracted from the sample applying previously optimized ABS based on [C$_4$mim][Sal] and then determined applying DPASV at a hanging mercury drop electrode and a Metrohm's procedure No. 231/2e. No filtration or any further treatment was applied before the extraction.

Results and discussion

Phase diagrams and tie-lines

In this paper, four hydrophilic ILs based on different 1-alkyl-3-methylimidazolim cations were synthesized and investigated for building ABS and selective extraction of copper(II) (Table 1). For the investigation of the effect of alkyl substituent of the cation, we choose butyl at one of two N atoms of the imidazole as one of the most common and the most frequently used and two oxygenated alkyl substituent 3-hydroxypropyl and 4-hydroxy-2-oxybutyl due to decreasing of the ionic liquid's toxicity [25]. Salicylate anion was selected in regard to its biodegradability and ability to form stable complexes with copper(II) with pronounced anti-inflammatory and antifungal activities [31], and chloride anion was chosen for comparison.

The phase diagrams of the aqueous biphasic systems based on the targeted IL and potassium phosphate are shown in Fig. 1. The corresponding experimental data for ternary mixtures of the selected {IL + K$_3$PO$_4$ + H$_2$O} systems which represent the minimum concentration of the constituents required for the formation of two aqueous phases at 293 K and pressure of 0.1 MPa are given in Table S1 in the Electronic Supplementary Material. The experimental binodal data were fitted by Merchuk correlation and obtained fitting parameters (A, B, and C), corresponding standard deviations (σ) and correlation coefficients (R^2) are given in Table S2 in the ESM. The regression parameters higher than 0.9991 and low values of the standard deviations confirmed that Merchuk equation are applicable to fit the experimental data. The position of biphasic region which is localized on the right side of binodal curves depends on both cation and anion of IL. The influence of cation on ability of the targeted ILs with the salicylate anion to form ABS follows the order: [C$_4$mim][Sal] > [HOC$_2$OC$_2$mim][Sal] > [HOC$_3$mim][Sal]. The introduction of oxygen groups (in the form of hydroxide and/or ether group) into the alkyl side chain of the imidazolium cation decreases the ability of IL to form ABS due to decreasing hydrophobicity. Also, Fig. 1 shows the significant effect of anion (salicylate and chloride) with the same cation [HOC$_3$mim]$^+$ on ABS formation. Different solvation of anion with water molecules directly influences on the ability of IL to form ABS [32].

The liquid-liquid equilibrium data (tie-line compositions, TLL, and slope) for {IL + K$_3$PO$_4$ + H$_2$O} systems at 293 K were calculated applying Eqs. (2–6) and reported in Table 3

Fig. 1 Ternary phase diagrams of the studied {IL + K_3PO_4 + H_2O} system at T = 293 K and atmospheric pressure p = 0.1 MPa. [C_4mim][Sal] (black square), [HOC_2OC_2mim][Sal] (red circle), [HOC_3mim][Sal] (blue up-pointing triangle), [HOC_3mim][Cl] (pink-down pointing triangle)
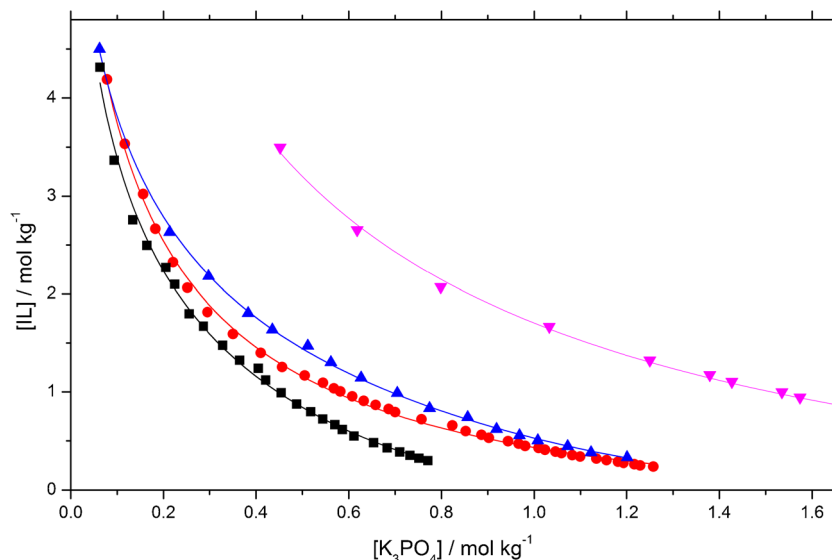
and Fig. S1 in ESM. TLL is an important parameter for the determination of extraction conditions because TLLs in specific ABS show the content of IL in IL-rich and salt-rich phases. The longest TLL has the lowest concentration of IL in the salt-rich phase and vice versa. It should be noted the lowest mass fraction of IL in salt-rich phase was found for

Table 3 Experimental tie-line data as mass fraction for the ternary system composed of {IL(Y) + K_3PO_4(X) + H_2O} at 293 K and pressure of = 0.1 MPa

| Total composition		Top phase		Bottom phase		100TLL	Slope
100X	100Y	100X	100Y	100X	100Y		
{[C_4mim][Sal] + K_3PO_4 + H_2O}							
9.09	25.36	4.85	34.97	14.76	6.25	30.38	−2.9
8.68	24.69	3.42	40.80	16.04	4.23	38.69	−2.9
9.34	26.81	2.25	46.80	17.52	2.50	46.85	−2.9
9.67	28.04	1.74	50.03	18.37	1.78	51.03	−2.9
{[HOC_3mim][Sal] + K_3PO_4 + H_2O}							
13.28	24.90	4.67	41.16	25.09	2.98	43.29	−1.87
13.58	26.21	3.48	45.07	26.51	2.06	48.79	−1.87
13.86	27.54	2.60	48.55	27.79	1.44	53.42	−1.87
14.21	28.87	1.92	51.82	29.12	0.95	57.69	−1.87
{[HOC_2OC_2mim][Sal] + K_3PO_4 + H_2O}							
15.55	24.11	3.14	47.45	27.34	1.95	51.54	−1.88
15.00	23.26	3.97	43.53	25.96	2.69	46.38	−1.88
14.57	22.59	4.42	41.69	24.73	3.52	43.24	−1.88
15.86	25.43	2.62	50.27	28.61	1.41	55.34	−1.88
{[HOC_3mim][Cl] + K_3PO_4 + H_2O}							
28.92	13.43	14.86	30.69	32.58	8.94	28.06	−1.23
29.34	13.99	12.56	34.59	35.05	6.96	35.63	−1.23
29.80	14.72	10.70	38.07	37.27	5.43	42.09	−1.23
30.27	15.68	8.88	41.89	39.72	4.03	48.83	−1.23

ABS based on [C_4mim][Sal] due to better ability of this IL to form ABS.

Extraction of copper(II)

In order to find the most suitable TSIL for one-step extraction of Cu(II), ABSs based on the synthesized ionic liquids were applied for investigation of Cu(II) extraction in the presence of three other divalent metals (Zn, Cd, Pb). The ternary mixture within the biphasic region was prepared containing 20% of IL, 20% of K_3PO_4, and 60% of water, except for [HOC_3mim][Cl] where the concentration of phosphate was 27% due to a lower possibility to form ABS. The final volume of extraction mixture was 0.5 cm³ and the initial concentration of each metal ion was 1.82 mmol dm⁻³. The mixture was shaken for 2 min using a vortex agitator at 2500 rpm and left to equilibrate for 2 h. The top IL-rich phase was carefully separated and weighted. pH values of the phases measured after their separation, in all cases, were strong alkaline (pH = 13 ± 0.3). Metal ions in the ABS based on [HOC_3mim][Cl] were precipitated due to the hydrolysis and this IL was excluded from further research. From this observation, it might be concluded that anion is the key parameter responsible for extraction efficiency and hydrolytic stability. Thus, one of the most important advantages of aqueous biphasic systems is the possibility to perform extraction in very alkaline solutions in the pH range where the ligand is completely deprotonated and metal hydroxide formation is in the same time omitted.

Figure 2 shows voltammograms of IL-rich (top) phase and salt-rich (bottom) phase after extraction and separation of the phases. The concentration of the metal ions was determined in both phases. It is clear from Fig. 2 that Cu(II) was extracted completely into IL-rich phase in all investigated ABS and should be emphasized that Cu(II) was selectively extracted in the ABS based on [C_4mim][Sal], whereas Zn(II), Cd(II), and Pb(II) were not partitioning in this system. Zn(II), Cd(II),

Fig. 2 Voltammograms of IL-rich (solid red line) and salt-rich (dash black line) phases after extraction of the selected metals in ABS based on **a** [C$_4$mim][Sal], **b** [HOC$_3$mim][Sal], and **c** [HOC$_2$OC$_2$mim][Sal]. Experimental conditions: 20% IL, 20% K$_3$PO$_4$, and 60% water. Concentration of each metal ion was 1.82 mmol dm^{-3}
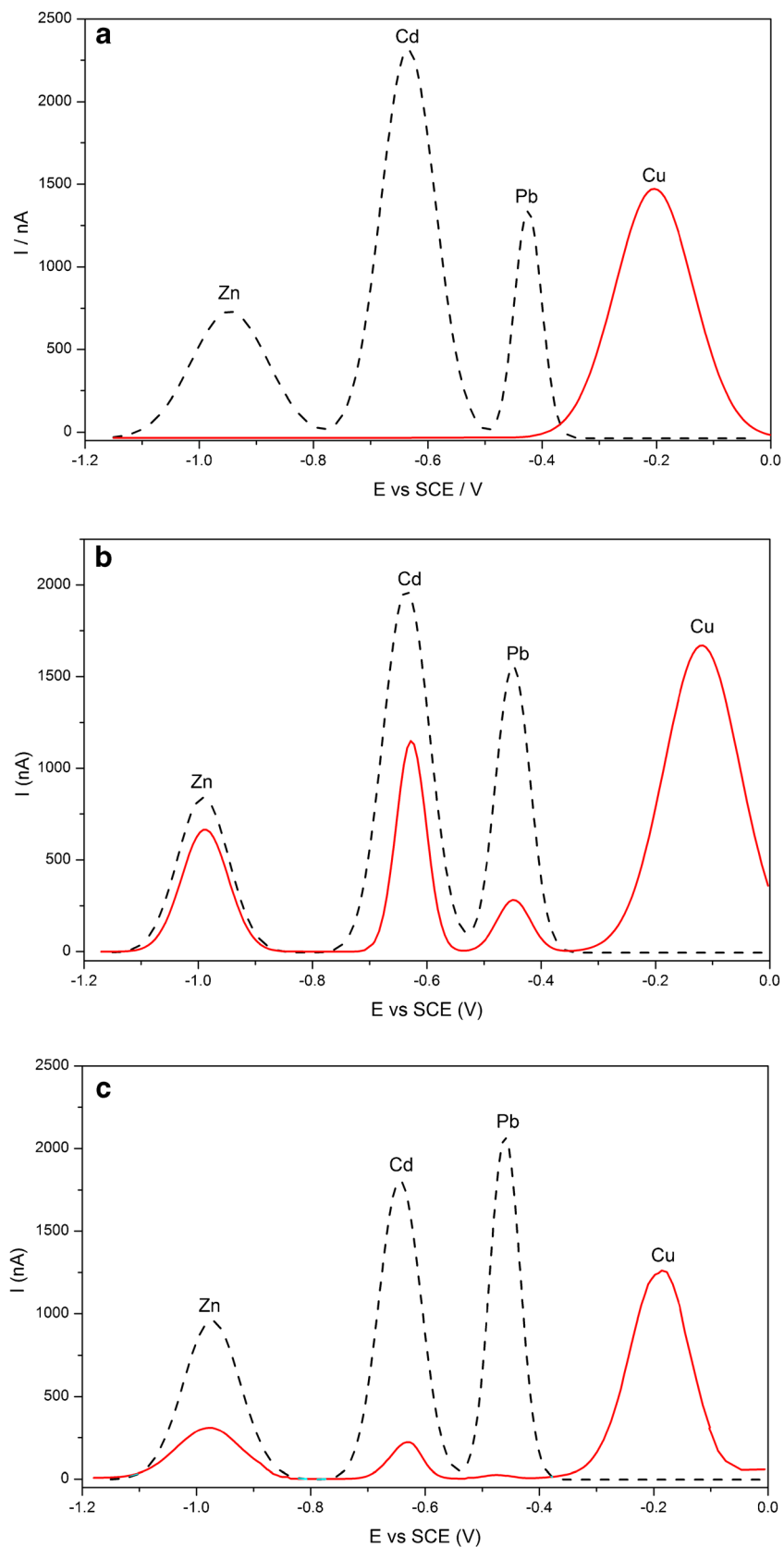
Table 4 Partition coefficients (P_{IL}) and extraction efficiencies (E) of the studied heavy metals in the targeted ABS based on the synthetized IL

| Metal	[C$_4$mim][Sal]		[HOC$_3$mim][Sal]		[HOC$_2$OC$_2$mim][Sal]	
	P_{IL}	E, %	P_{IL}	E, %	P_{IL}	E, %
Cu(II)	∞	100	∞	100	∞	100
Zn(II)	–	0	0.72	41.75	0.35	25.82
Cd(II)	–	0	0.37	27.14	0.09	7.97
Pb(II)	–	0	0.18	14.97	0.03	2.90

and Pb(II) were extracted in ABS based on [HOC$_3$mim][Sal]. Zn(II) and Cd(II) were also extracted in ABS based on the third applied ionic liquid [HOC$_2$OC$_2$mim][Sal].

It should be emphasized that hydrolysis of the investigated divalent metal ions did not occur in the ABSs based on ILs with salicylate anion despite very strong alkaline condition in both phases. This result suggests that Cu(II) form complex with

salicylate anion from IL, then the complex is extracted in IL-reach phase. In the case of Zn(II), Cd(II), and Pb(II) which are not extracted in the ABS based on [C$_4$mim][Sal] or partially extracted in the ABS based on [HOC$_3$mim][Sal] [HOC$_2$OC$_2$mim][Sal], we also assume formation of metal(II)–salicylate complexes that prevent hydrolysis of these metal ions, but these complexes are partially or non-extracted in IL-rich phase. This implies that IL cation has a decisive effect on the extraction of metal(II)–salicylate complexes.

The partition coefficients (P_{IL}) and extraction efficiency (E) of the studied metal ions in the investigated ABS are listed in Table 4. It may be noted that P_{IL} equals ∞ denotes that the complete extraction was obtained, i.e., no detection of Cu(II) in the salt-rich phase was observed [33] and that extraction efficiency in the case of Cu(II) was 100%. The calculated parameters implied that Cu(II) was completely extracted in all investigated ABSs, whereas Zn(II), Cd(II), and Pb(II) were not separated in ABS based on [C$_4$mim][Sal] and their P_{IL} and

Fig. 3 Values of stability constants **a** K_1^a and **b** K_2^a for Cu(II)–salicylate complexes at various ionic strengths for different ionic liquid solutions: [C$_4$mim][Sal] (black square), [HOC$_2$OC$_2$mim][Sal] (red circle), [HOC$_3$mim][Sal] (blue up-pointing triangle)
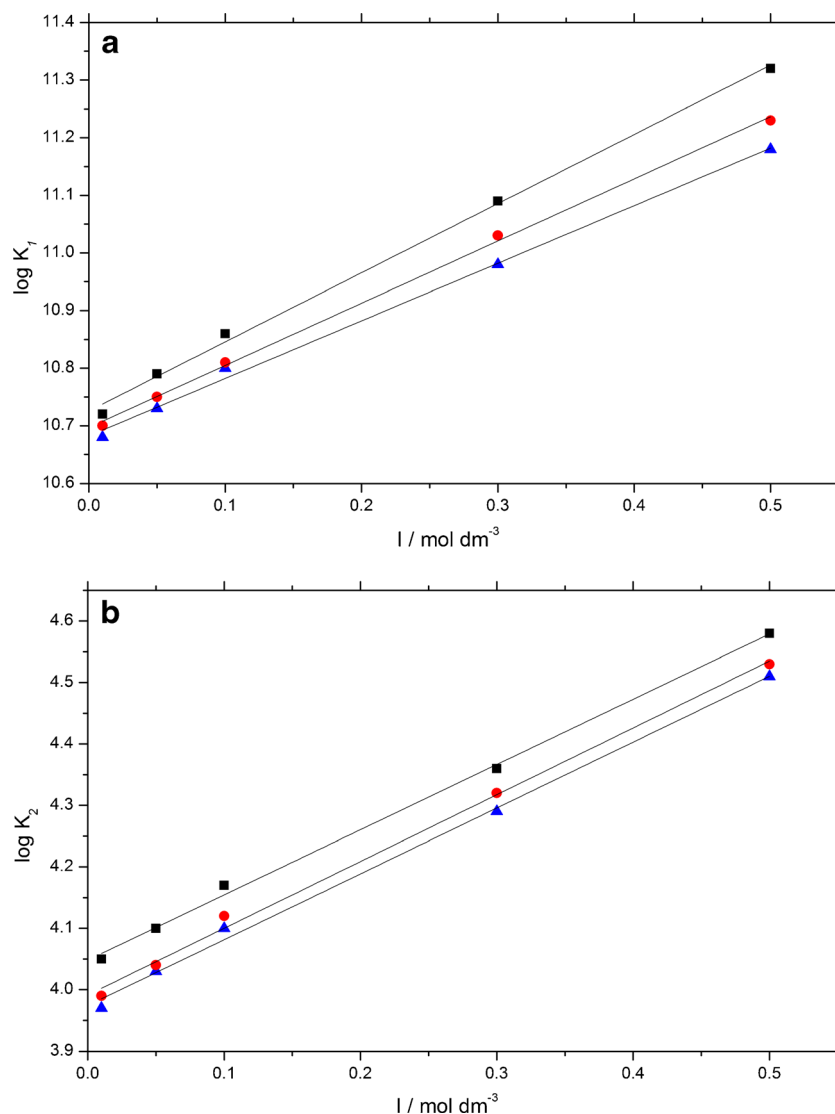
Table 5 Thermodynamic stability constants K_1^a and K_2^a for Cu(II) complexes with salicylate in different ILs

Ionic liquids	$\log K_1^a$	$\log K_2^a$	$\log \beta^a$
[C4mim][Sal]	10.73	4.05	14.78
[OHC$_3$mim][Sal]	10.68	3.97	14.65
[OHC$_2$OC$_2$mim][Sal]	10.70	3.99	14.69

$^a \log\beta = \log K_1^a + \log K_2^a$

E were significantly lower in ABS based on [HOC$_3$mim][Sal] and [HOC$_2$OC$_2$mim][Sal].

Since it is well-known that Cu(II) forms complexes with salicylate anion, stability constants of the formed complexes were determined in order to study influence of cation at complex stability. Stability constant dependence of the ionic strength is given in Fig. 3, and from extrapolation of the obtained results, thermodynamic stability constants (K_1^a and K_2^a) were evaluated and presented in Table 5.

As can be seen from Table 5 and Fig. 3, values of the stability constants for Cu(II)–salicylate complexes have similar values. These results indicated that there is no significant influence of ionic liquid cation on stability of Cu(II)–salicylate complexes.

Optimization of the ABS parameters

Based on described, ABS formed with [C$_4$mim][Sal] was chosen for further investigation of selective extraction and determination of Cu(II). The different parameters, such as IL and sample amounts, extraction, and centrifugation time, were optimized.

The effect of [C$_4$mim][Sal] volume (expressed as ratio of IL and aqueous sample volume) on partition coefficient and extraction efficiency is shown in Fig. 4. It can be seen that the extraction efficiency of Cu(II) in ABS based on [C$_4$mim][Sal] increases with increasing of IL/aqueous sample ratio and reaches 100% at their equal amounts. The partition coefficient of Cu(II) decreases with an increasing ratio of IL/aqueous sample. However, in the case of the same amount of IL and aqueous sample, the concentration of Cu(II) in aqueous phase after separation of two phases was lower than detection limits and practically the partition coefficient is infinite.

Figure 5 shows the influence of amount of aqueous sample on EF, the amount of the IL was fixed at 0.06 cm^3 and amount of K$_3$PO$_4$ was fixed to 2 cm^3 of 55% solution. EF increases from 6 to 54 with increasing volume of aqueous sample from 0.5 to 2 cm^3. It should be observed from Fig. 5 that experimentally obtained EFs for aqueous solution higher than 1.0 cm^3 are higher than calculated maximal EF for corresponding ratio of IL and aqueous sample. This effect is due to the fact that for higher volume of aqueous sample smaller volumes of IL-rich phase can be separated.

Also, the influence of extraction time was investigated in the range from 1 min to 4 h. After extraction of 1 cm^3 aqueous sample and 0.06 cm^3 of IL, the mean value of peak current of IL-rich phases obtained after different extraction time was 11.10 ± 0.52 nA. This result implies no influence of extraction time.

Based on the above results, the optimal experimental conditions are set as sample volume of 2 mL, ionic liquid volume of 0.06 mL, and concentration of K$_3$PO$_4$ 27.1% which ensure separation of two aqueous phases, 1 min extraction time under vigorous mixing using a vortex agitator at 2500 rpm and 10 min centrifugation at 10,000 rpm to ensure separation of phases.

Fig. 4 The effect of IL amount (expressed as ratio of IL and aqueous sample volume) on partition coefficient (P_{IL}, left axes) and extraction efficiency (E) of Cu(II) in the investigated ABS based on [C$_4$mim][Sal]: partition coefficient (blue square), extraction efficiency (red circle). Concentration of Cu(II) was 5 mg dm^{-3}
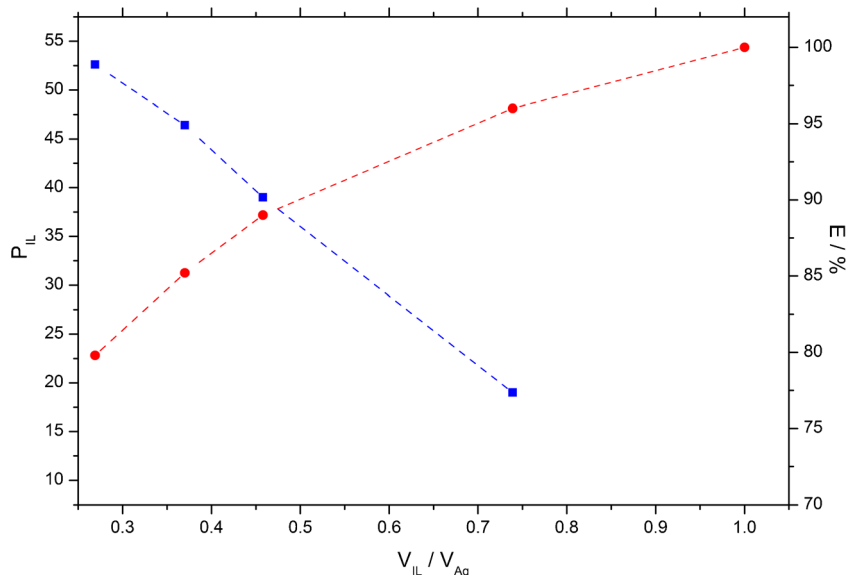
Fig. 5 The effect of aqueous sample volume on enrichment factor (EF) of Cu(II) in the ABS based on [C₄mim][Sal]. Legend: experimental values (blue square), calculated maximal EF (red circle). Concentration of Cu(II) was 5 mg dm^{-3}
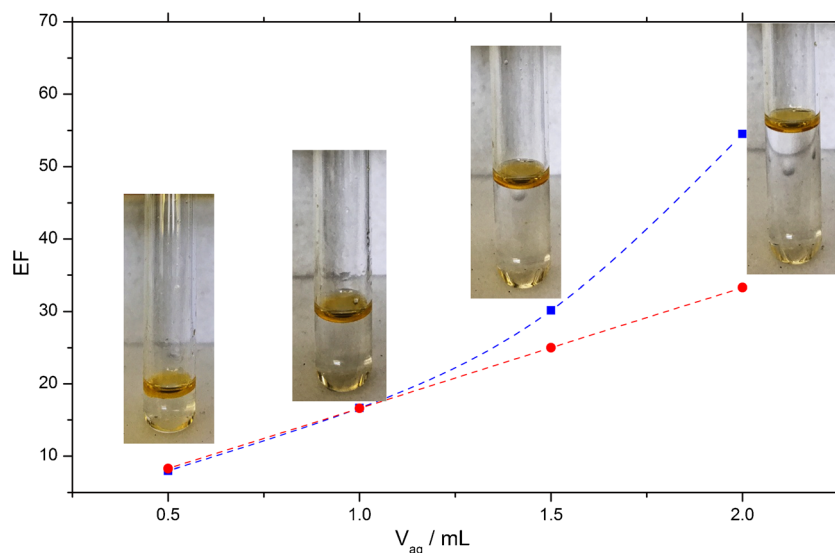
Analytical procedure of Cu(II) detection in environmental and biological samples

To validate the proposed selective extraction method based on ABS as sample preparation step for quantification of Cu(II) in wastewater and biological samples, linearity, selectivity, the limit of detection (LOD), limit of quantification (LOQ), and the uncertainty of the experimental procedure were studied under optimal conditions. Linearity was evaluated in two linear range by spiking de-ionized water with different amounts of Cu(II) to obtain the final concentration from 0.001 to 1 µg dm^{-3} and from 1 to 100 µg dm^{-3}. The linear determination coefficients were 0.9988 and 0.9996 for lower and higher concentration range, respectively. The calculated LOD and LOQ were 8 and 28 ng dm^{-3}, respectively. The obtained LOD is significantly lower than LOD (0.15 µg·dm^{-3}) obtained by ionic liquid-based single drop microextraction using 1-butyl-3-methylimidazolium hexafluorophosphate as a sample preparation step [34]. The uncertainty of the experimental procedure was tested with water samples spiked with the standard solution of Cu(II) (1, 10, and 100 µg dm^{-3}), analyzing each concentration level in five replicates. The obtained mean values of the recoveries and the standard deviation were 90.2 ± 8.2, 94.0 ± 6.1, and 97.8 ± 6.5 for 1, 10, and 100 µg dm^{-3}, respectively.

Due to the biological and environmental significance of copper, three real samples (tap water, wastewater, and 24-h urine) were analyzed under the optimal conditions to validate the applicability and accuracy of the proposed method. Each sample was analyzed in triplicate and the mean concentration was given in Table 6 together with calculated relative standard deviation (%). The precision of the experimental procedure, expressed as the relative standard deviation (%), was tested with the real samples (tap water, wastewater, and urine) analyzing each sample three times.

Conclusions

In this study, the applicability of the ABS based on task-specific ionic liquids as the sample pretreatment followed by differential pulse anodic stripping voltammetry for determination of Cu(II) in water and biological samples was demonstrated. Four hydrophilic ILs containing 1-alkyl-3-methylimidazolim cation ([C₄mim][Sal], [HOC₂OC₂mim][Sal], [HOC₃mim][Sal], and [HOC₃mim][Cl]) were synthesized and studied as suitable media for ABS formation and selective extraction of copper(II). Salicylate anion was selected in regard to its ability to form stable complexes with copper(II) with potential anti-inflammatory and antifungal activity. The cation ability of the targeted ILs with the salicylate anion to form ABS follows the order [C₄mim][Sal] > [HOC₂OC₂mim][Sal] > [HOC₃mim][Sal]. The oxygenated groups (in the form of hydroxide and/or ether group) as the part of the alkyl side chain of the imidazolium cation decrease the ability of IL to form ABS due to decreasing hydrophobicity. It was found that Cu(II) was extracted completely into an IL-rich phase in all investigated ABS based on IL with salicylate anion and selectively extracted in the ABS based on [C₄mim][Sal], whereas Zn(II), Cd(II), and Pb(II) were not partitioning in this system, as the

Table 6 Determined concentration of Cu(II) applying ABS based on [C4mim][Sal] as a sample pretreatment step before DPASV

Sample	pH	Cu^a, mg dm^{-3}	RSD, %
Tap water	6.1	0.979	1.7
Wastewater	2.6	15.22	2.4
24-h urine	6.7	0.085	7.8

^{a} Mean concentration obtained by analyzing three samples

advantages of ABS based on selected ILs can be pointed possibly to perform extraction in highly alkaline media (pH = 13) without hydroxide formation of the investigated divalent metal ions. Further investigation was focused on the optimization of extraction conditions of Cu(II) in order to reach maximal enrichment factor and low detection limits. The optimized extraction parameters are sample volume of 2 cm^3, ionic liquid volume of 0.06 cm^3, and concentration of K_3PO_4 27% which ensure separation of two aqueous phases, 1 min extraction time under vigorous mixing using a vortex agitator at 2500 rpm and 10 min centrifugation at 10,000 rpm to ensure separation of phases. It must be pointed out that this extraction procedure is cheap, selective, fast, simple, and achieves very low detection limit with no additional sample treatment before the application of the ABS. This work shows that the ABS based on task-specific ionic liquids can be considered as an alternative procedure to other extraction techniques for the determination of Cu(II) ions in water and some biological samples. It should be emphasized that TSILs are designed so that anion is responsible to form the complex with metal(II) and to prevent hydrolysis of metal cations at very strong alkaline pH, whereas cation is responsible for selective extraction of metal(II)–salicylate complexes.

Compliance with ethical standards Ethics approval for this study has been obtained from the Ethics Committee, Institute of Nuclear Sciences "Vinča," Belgrade, Serbia (Decision No. 1/2017). Informed consent was obtained from the individual participant who provided urine sample.

Conflict of interest The authors have declared no conflict of interest.

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