VILNIUS UNIVERSITY

INA RAZMISLEVIČIENĖ

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR THE DETERMINATION OF METALS

Summary of doctoral dissertation

Physical sciences, chemistry (03 P)

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The research has been carried out at Vilnius University during the years 2007-2012.

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VILNIAUS UNIVERSITETAS

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DISPERSINĖ SKYSTAFAZĖ MIKROEKSTRAKCIJA METALAMS NUSTATYTI

Daktaro disertacijos santrauka

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1. INTRODUCTION

Despite the development of highly efficient analytical techniques, most instruments cannot handle complex sample matrices directly and, as a result, a sample preparation step is commonly involved in an analytical procedure. Over the years, it has been realized that, in most cases, the operations associated to sample preparation are the major source of inaccuracy and imprecision on analysis in general, as well as the most time-consuming steps. Ideally, sample-preparation techniques should be selective, fast, easy to use, inexpensive and compatible with a range of analytical instruments. Extraction with its various modes is perhaps the most popular sample pre-treatment method. However, traditional and predominant liquid-liquid extraction is time-consuming, tedious and uses large amounts of potentially toxic organic solvent that is usually expensive because of its high purity necessary for analytical applications. Therefore, much effort has been devoted in recent years to exploring the possibilities of miniaturization of the extraction procedures to minimize or eliminate the limitations of the sample preparation.

The most recent microextraction technique, dispersive liquid-liquid microextraction (DLLME) is based on a ternary solvent component system involving an aqueous phase, a non-polar water immiscible solvent (extraction solvent) and a polar water miscible solvent (disperser solvent). In this technique, the appropriate mixture of extraction and disperser solvents is rapidly injected by syringe into an aqueous sample solution containing the analytes. The extraction solvent is dispersed into the aqueous sample as very fine droplets. Owing to the large surface area between extraction solvent and aqueous sample, the equilibrium is reached quickly and the extraction is almost independent of time.

However, despite the obvious merits of this technique, the majority of work on DLLME has so far been applied to extract and preconcentrate non-polar to medium polarity organic compounds with subsequent analysis by gas or liquid chromatography techniques. The application of DLLME to wider spectrum of the analytes and its coupling with other analytical techniques is still limited. For example, amongst all techniques currently used for the determination of trace amounts of metal species, inductively coupled plasma mass spectrometry (ICP-MS) appears to be one of the most attractive since it provides excellent detection power, with isotopic selectivity and high sample throughput. However, the organic solvents (usually chlorinated) required for the DLLME and the extremely small volumes (10-50 μL) of the extracts obtained for the analysis limits the combination of this technique with conventional ICP-MS. In contrast, these limitations can be considered as significant advantages by the combination of DLLME with ICP-MS utilizing laser ablation (LA) sample introduction. Until now, applications of LA-ICP-MS have principally been in the area of geological, environmental, and material science for determination of trace and ultratrace elements in solid and semi-solid samples. No significant effort has been made toward implementation of LA-ICP-MS for quantitative analyses of liquid samples.

Another limitation of DLLME technique is the limited number of the effective extractants. Generally, extraction solvent used in DLLME should have higher density than water, low solubility in water (*i.e.* high hydrophobicity) and should be compatible with analytical technique used. Therefore, only a few solvents, mainly chlorinated hydrocarbons, have been extensively used in DLLME. Although these highly volatile solvents are well compatible with gas chromatographic analysis, their use as sample diluents in liquid chromatography is restricted due to band broadening and deformation.

Ionic liquids (ILs) can be considered as an ideal alternative to chlorinated solvents. The unique properties of ILs such as tunable hydrophobicity, higher density than water, and excellent compatibility with liquid chromatography make them ideal candidates as extraction solvents for DLLME.

The aim of this work was to investigate and apply new DLLME systems coupled with LA-ICP-MS and ultra-performance liquid chromatography (UPLC) techniques for the preconcentration and determination of Cr(VI), Co(II), Cu(II) ir Ni(II) ions.

The following main tasks were set to achieve the aim:

1. Investigation of the extraction performance of Cr(VI) complex with ammonium pyrrolidine dithiocarbamate (APDC) by DLLME using carbon tetrachloride extractant.
2. Evaluation of the influence of LA parameters on the determination of ^{52}Cr and ^{53}Cr isotopes in extracts by “dried droplet” LA-ICP-MS.
3. Optimisation of IL-based DLLME and UPLC conditions for the extraction and determination of Cr(VI), Co(II), Cu(II) and Ni(II) ions complexed with APDC.
4. Comparison of the extraction performance of three IL-based DLLME approaches.

Novelty and actuality of the work:

By using “dried droplet” approach the DLLME technique was coupled for the first time with LA-ICP-MS method. This coupling significantly enhances the application areas of microextraction methods.

For the extraction of metal complexes a new ionic liquid - 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate – was proposed. It is heavier than water, exhibits low viscosity and due to ionic nature is well compatible with liquid chromatography.

For the first time the extraction performance of three IL-based DLLME approaches was evaluated and compared.

Statements for defense:

1. DLLME coupled with LA-ICP-MS technique significantly enhances the application areas of microextraction methods.
2. 1-Hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate ionic liquid is more effective extractant than conventional chlorinated hydrocarbons.
3. The mode of initiation of the dispersed phase in IL-based DLLME does not have an influence on the extraction performance of metal-APDC complexes.

2. EXPERIMENTAL

High resolution double-focusing sector field ICP mass spectrometer Element2 (Thermo Finnigan, Bremen, Germany) was used for the measurements. For the ICP-MS measurements medium (R = 4000) mass resolutions were employed. The isotopes chosen for the current work (^{52}Cr and ^{53}Cr) are the most commonly used for ICP-MS analysis because they are the most abundant without direct isobaric interferences from other elements. Laser ablation measurements were carried out using an UP-213 laser ablation system (New Wave, Freemont, USA). Laser ablation (LA) was performed with a Nd:YAG laser at a wavelength of 213 nm. The ablated material was transported by argon as a carrier gas into the plasma. Mass calibration of the instrument was verified by the analysis of the BCR 664 (glass) reference material purchased from the Institute for Reference Materials and Measurements. The LA system was operated in the single line scan mode, in which the sample moved horizontally at a constant speed while the laser was firing, respectively.

Ultra-performance liquid chromatography (UPLC) separations were performed on the Waters Acquity UPLC system (Waters, Milford MA) equipped with an Acquity UPLC PDA detector. An Acquity UPLC BEH C18 column (100 mm×2.1 mm i.d., 1.7 μm), maintained at 30 °C was used for the separations. Separations were performed under isocratic conditions at a flow rate of 0.5 mL/min using mobile phase composed of acetonitrile-water (70:30, v/v). Absorbance data were collected at 260 nm. Data collection and management was performed by Empower 2 build 2154 software (Waters).

3. RESULTS AND DISCUSSION

Dispersive liquid-liquid microextraction (DLLME) is a novel miniaturized sample-enrichment method developed in 2006. In this method (Fig.1), water-immiscible extraction solvent dissolved in a water-miscible dispersive solvent (*e.g.*, methanol, acetonitrile, acetone) is rapidly injected into an aqueous sample by a syringe. A cloudy solution containing fine droplets of extraction solvent dispersed entirely in the aqueous phase is formed. Extraction of the analytes from aqueous sample into the dispersed organic droplets takes place. After centrifugation, sedimented organic phase is removed using a microsyringe and analyzed. Simplicity of the operation, speed, low solvent volume, low cost, high recovery and high enrichment factor are some advantages of DLLME.

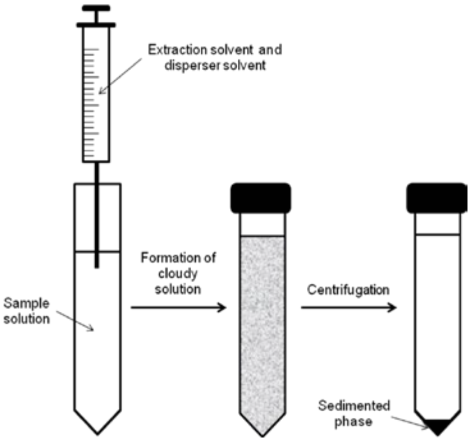

Fig. 1. Schematic diagram of DLLME procedure.

3.1. Determination of Cr(VI) by Dispersive Liquid-Liquid Microextraction and Dried-Droplet Laser Ablation ICP-MS

In this part of the work, a novel procedure is developed for the determination of ultra-trace amounts of Cr(VI) in waters via DLLME preconcentration of the Cr(VI) complex with ammonium pyrrolidine dithiocarbamate (APDC) and subsequent ICP-MS analysis of the extracts by using dried-droplet LA sample introduction technique.

Optimization of DLLME procedure. It is well known that under acidic conditions Cr(VI) is reduced easily by APDC to Cr(III) and produces uncharged complex, which is readily soluble in non-polar solvents. The complexation of Cr(III) ions, initially present in the sample, has been found to be inefficient due to the difficulty of displacing the coordinated water from the strongly hydrated Cr(III) by the APDC ligand. In the present work five variables were optimized for the DLLME of the Cr(VI)-APDC complex: sample pH, APDC concentration, volume of the extraction solvent, sample volume and matrix effects caused by salts and common transition metals. In our DLLME experiments carbon tetrachloride and methanol were selected as extraction and dispersion solvents, respectively. Preliminary DLLME experiments were performed with 5 mL of Cr(VI) standard solutions using 1 mL of methanol containing 50 μL of CCl_4 as extraction mixture. These experiments were performed using UPLC technique.

One of the critical variables for the formation of Cr(VI)-APDC complex and its extractability is acidity of the sample solution. A series of Cr(VI) solutions (10 and 100 μg/L) were prepared in H_3PO_4+CH_3COOH mixture (0.05 mol/L of each acid) and desired pH (from 2 to 6) was adjusted with NaOH. The maximum and constant signal intensities for Cr(VI) were observed in the pH range 2.5-4.0. In the next experiment, the effect of the APDC amount in the range of 0.01-0.5% (w/v) on the complexation/extraction performance of Cr(VI) at pH 2.5 was investigated. For the highest Cr(VI) concentration studied (200 μg/L), the signal intensity increased up to about 0.03% (w/v) APDC, reaching a plateau, which was considered as the complete complexation/extraction. Thus, 0.5 mL of 1% (w/v) APDC added to 5 mL of sample solution gives slightly lower than 0.1% APDC concentration which is enough for complete complexation.

In order to evaluate the effect of carbon tetrachloride volume on the extraction performance, extraction mixtures containing 1 mL of methanol and different volumes (from 30 to 80 μL) of CCl_4 were examined for Cr(VI) standard solutions (10 and 25 μg/L). Obtained results are presented in Fig. 2. As can be seen, the signal intensity of Cr(VI)-APDC chelate increased up to 50 μL of CCl_4, reaching a plateau at higher extractant volumes. Thus, 50 μL of CCl_4 was selected as optimum volume for all subsequent experiments.

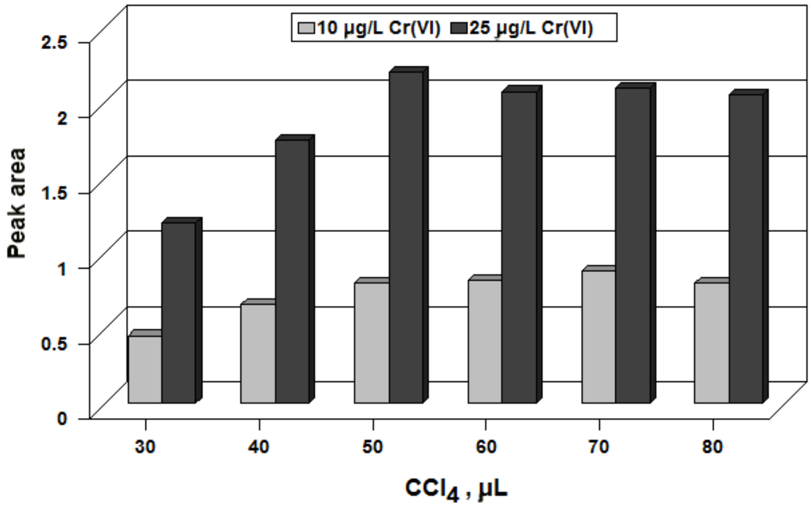

Fig. 2. Effect of CCl_4 volume on the extraction performance of Cr(VI)-APDC complex.

Fig. 3 shows the effect of sample volume on the amount of analyte extracted by a 50 μL of CCl_4. A gradual increase in peak area was observed with an increase in the sample volume up to 8 mL. At higher sample volumes the reduction of the extraction efficiency was observed.

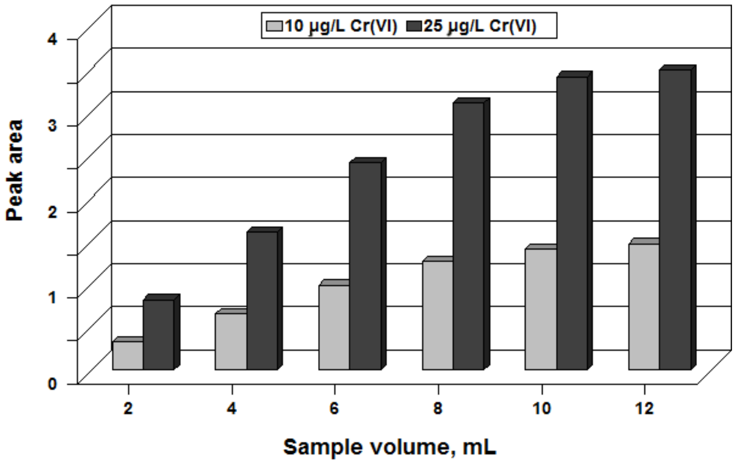

Fig. 3. Effect of sample volume on the extraction efficiency of Cr(VI)-APDC complex.

Deposition of the extracts. Polystyrene and glass plates were initially tested as substrates on which extracts of Cr(VI) standard were deposited. Different volumes (5, 7 and 10 µL) of the extract were deposited manually with a micropipette, dried at room temperature and pressure and imaged using the laser video display. However, the microdrops deposited on the glass substrate were quite irregularly shaped and sized and, most importantly, no dried spots were visible on the surface when using the laser video display. In contrast, the optical investigation of the microdroplet residues spotted on polystyrene surface revealed that these dry to give well visible, spherical and relatively uniform in diameter spots (Fig. 4). After the carbon tetrachloride had evaporated, polystyrene surface darkened. This may be due to excellent wetting and swelling of the hydrophobic polystyrene surface with carbon tetrachloride. In addition, the time required for complete drying of the sample droplet was significantly shorter using the polystyrene substrate compared to the same sample volumes deposited on glass surface (30-60 s *vs*. 3-5 min), most likely, due to partial permeability of polystyrene to halogenated hydrocarbons. Depending on the deposited volume of the extract, the spot diameters range approximately between 3.5 and 7.0 mm. No significant background (less than 100 counts/s) was measured for the Cr during ablation of the polystyrene plate, indicating no significant contribution to contamination from this substrate. A 7 µL extract volume was selected for all subsequent studies.

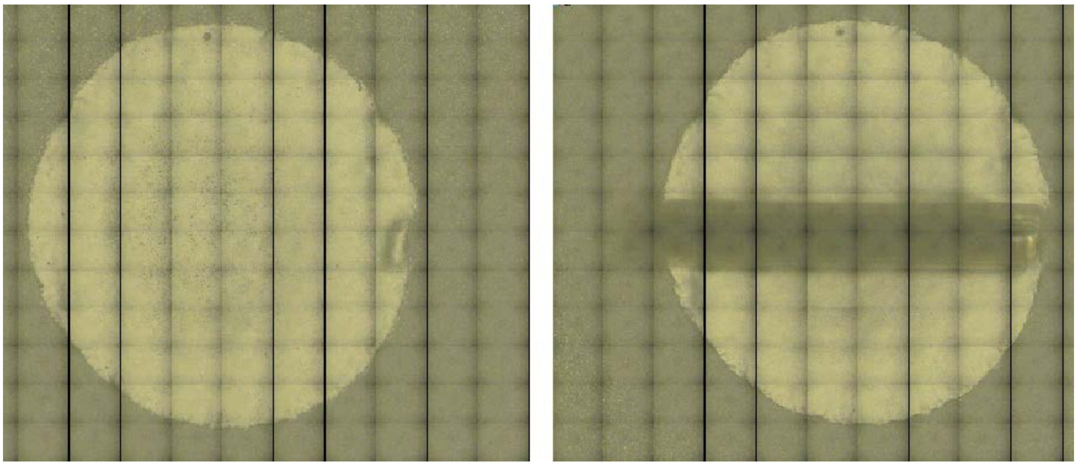

Fig. 4. Photographs of dried extract droplet (7 µL) before (left) and after (right) laser ablation.

Internal standardization. To improve precision of measurements, the use of an internal standard is required to correct possible instrumental drifts or changes in the ablation efficiency. The element chosen as internal standard has to be present in relatively high concentration so its measurement is as accurate as possible. One and perhaps the simplest way is the use of appropriate sample matrix compound. High levels of sulfur are present throughout the sample matrix and hence it was tested as internal standard. However, the obtained results showed that the signal variability was not reduced by the use of ^{32}S as an internal standard. By contrast, depending on Cr(VI) concentration, RSDs of 16-28% for ^{52}Cr/^{32}S and ^{53}Cr/^{32}S ratios were achieved, whereas without normalization the RSDs were even slightly lower (12-18% and 14-23% for ^{52}Cr and ^{53}Cr, respectively). Hence, the high RSDs of the chromium/sulfur ratios reflect the uneven distribution of these elements relative to one another in the dried extract droplet.

An alternative way for internal standardization in the proposed system might be an addition of an appropriate element to the initial sample solution prior to extraction. Platinum should be suitable for standardization purposes because it reacts with APDC and the chelate formed is extracted well into organic solvents. Moreover, natural waters do not contain measurable levels of platinum. In an additional experiment, the extraction performance of Pt-APDC chelate was checked and the obtained results showed that under above optimized DLLME conditions its extraction is reproducible. Fig. 5 illustrates how signal fluctuation during the line scan ablation can be corrected by use of platinum as an internal standard. As can be observed, the profiles of the Cr and Pt are quite similar indicating similar distribution of the both elements in the dried extract droplet.

Table 1 summarizes the RSDs in percent obtained for three Cr(VI) concentrations with no internal standard as well as with ^{195}Pt as internal standard. Depending on Cr(VI) concentration, about 3 times lower RSDs were achieved for ^{52}Cr/^{195}Pt and ^{53}Cr/^{195}Pt ratios.

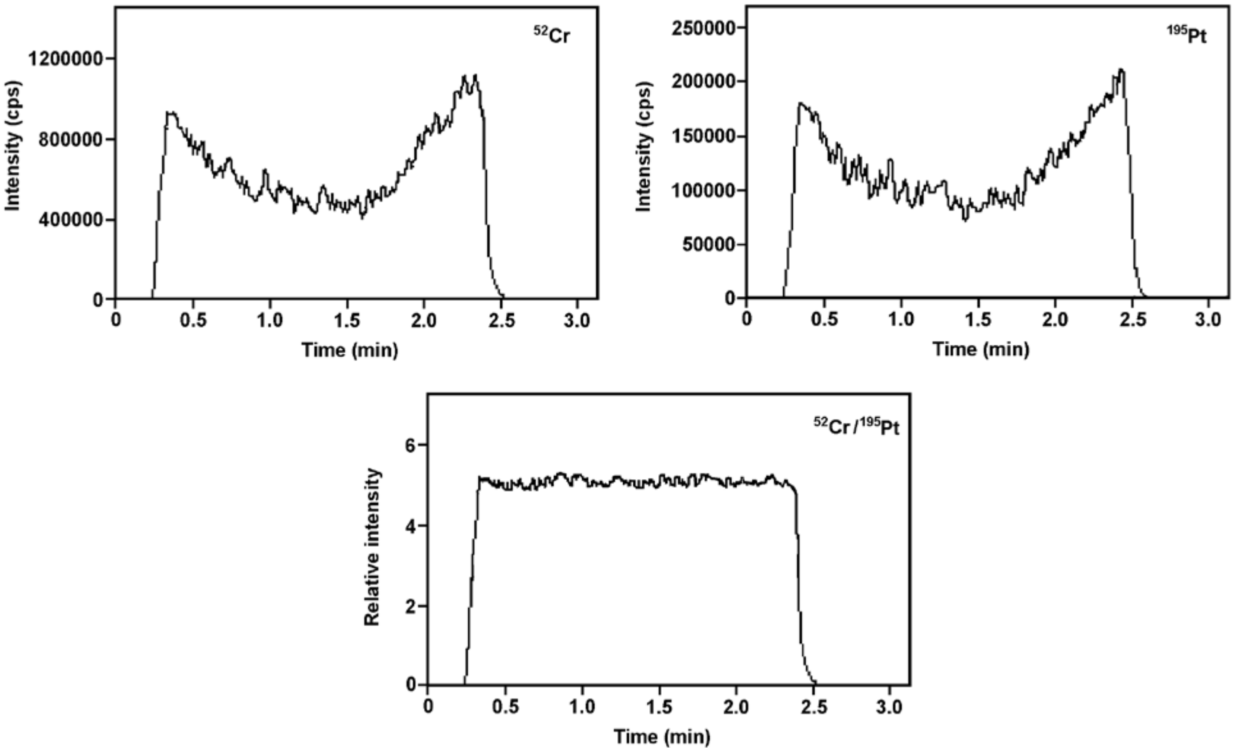

Fig. 5. Signal intensity profiles from a dried extract droplet obtained for ^{52}Cr, ^{195}Pt and for ^{52}Cr/^{195}Pt ratio.

Table 1. Relative standard deviations (%) without and with internal standard (six replicates)

Cr(VI), µg/L	^{52}Cr	^{53}Cr	^{52}Cr/^{195}Pt	^{53}Cr/^{195}Pt
5	18	21	5.4	6.5
25	14	23	4.3	4.6
100	11	16	4.0	4.8

Optimization of LA parameters. The parameters optimized were: ablation energy, laser beam diameter, pulse repetition rate and scanning speed. Each parameter was optimized regarding Cr signal intensities and precision. Optimized laser ablation parameters for the LA-ICP-MS system are summarized in Table 2.

Table 2. Optimized laser ablation parameters for the LA-ICP-MS measurements

Parameter	Optimized value
Laser energy	100% (3.4 mJ)
Laser beam size	100 μm
Pulse repetition rate	20 Hz
Scanning speed	30 μm/s

Analytical performance. Although LA-ICP-MS is very attractive, calibration remains a limiting factor when performing quantitative analyses on a wide variety of solid samples. Generally, to prevent matrix effects, the composition of the standards has to be as close as possible to that of the samples. However, the production of matrix-matched external standards for solid samples is costly and a completely homogeneous spiked solid often is impossible to achieve. By contrast, this is readily produced with a liquid. In addition, since most (especially ionogenic) sample matrix compounds are removed during microextraction, the extracts obtained from very different water matrices with similar composition might be formed. To prove this, two different series of external standards were used for calibration: one prepared in ultrapure water and the other prepared in high salinity synthetic sea water matrix. All standards contained 50 μg/L of Pt, which was used as an internal standard. The linearity of the external calibration curves was examined in the range from 0 to 250 μg/L. Detection limits were estimated from replicate measurements of six blank samples based on 3 times the standard deviation of measured concentrations. The results are summarized in Table 3. The ratios of calibration slopes in the saline matrix *versus* that in ultrapure water standard solutions are highly consistent and not significantly different from 1. This indicates that no additional matrix-matched standards are required to perform quantitative analysis. The detection limits are adequate for the speciation of Cr(VI) in natural waters.

Finally, the effect of potential interferents on the extraction and determination of Cr(VI) was assessed. The effect of K^+, Na^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}/Fe^{3+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} was studied. In these experiments, solutions containing 10 μg/L of Cr(VI) and the interfering ions were extracted and analyzed. An interference was defined to be significant if a variation of more than ±10% in the Cr(VI) signal was observed. The results showed that only higher (>1 mg/L) concentrations of Co^{2+}, Cu^{2+}, Fe^{3+} and Ni^{2+} caused significant depressive effects due to a competitive reaction with

APDC. These data show that the methodology is suitable for natural waters, where the levels of these elements (with the exception of Fe) are usually lower than those necessary to cause interferences. The interfering effect of Fe^{3+} could be reduced by increasing the amount of APDC or by masking of Fe^{3+} ions with EDTA prior to complexation with APDC. In this work, the first approach was adopted. We checked that by using a twice higher APDC amount (0.5 mL of 2% instead of 1%) the tolerance limit of Fe^{+3} ions was increased at least to about 10 mg/L.

Table 3. Analytical response characteristics of Cr using different external standards (six replicates)

Standards	Isotope of Cr	Slope, L/µg	R^2	Detection limit, µg/L
Matrix-matched	^{52}Cr ^{53}Cr	0.3719 0.0419	0.9988 0.9983	0.12 0.29
Ultrapure water	^{52}Cr ^{53}Cr	0.3768 0.0431	0.9985 0.9992	0.11 0.31

The DLLME-LA-ICP-MS method has been applied to the determination of Cr(VI) in tap and river water samples. A matrix spike recovery experiment was also performed in triplicate in both matrices. The obtained results are summarized in Table 4. The spike recoveries show acceptable performance of the method, with all of the recoveries within 7% of the nominal spike values.

Finally, certified reference material BCR-544 (lyophilized water; certified concentration of Cr(VI) 22.8 ± 1.0 µg/L) was analyzed in triplicate and the concentration determined was found to be 23.6 ± 1.6 µg/L. It is evident that good agreement with accepted value has been established for this material.

Table 4. Analysis of natural waters and recovery study (mean of three measurements ± standard deviation)

Sample	Cr(VI) found, µg/L	Cr(VI) added, µg/L	Cr(VI) found, µg/L	Recovery, %
Tap water	0.42 ± 0.04	0.50	0.89 ± 0.07	94.0
		2.00	2.48 ± 0.20	103
		10.0	9.73 ± 0.43	93.0
River water	n.d.	0.50	0.52 ± 0.03	104
		2.00	1.88 ± 0.15	94.0
		10.0	9.65 ± 0.51	96.5

3.2. Ionic liquid-based DLLME combined with ultra performance liquid chromatography for the determination of metal ions

Ionic liquids (ILs), resulting from the combination of organic cations (e.g., imidazolium, pyridinium, pyrrolidinium) and various anions, can be defined as organic salts that are liquid at or near room temperature, taking 100 °C as arbitrary reference. As a group of novel solvents, ILs possesses many significant advantages that make them ideal candidates as extraction solvents for DLLME. ILs exhibit "structural tuneability" in that their properties (*e.g.*, density, viscosity, hydrophobicity) can be easily tuned and manipulated by simply changing the combination of cations and anions. Furthermore, compared with commonly used chlorinated hydrocarbons, ILs are more compatible with conventional liquid chromatography.

In the second part of the work, imidazolium based ionic liquid were investigated as extraction solvents for DLLME of Cr(VI), Co(II), Cu(II) and Ni(II) chelates with APDC and subsequent analysis of the extracts by ultra performance liquid chromatography (UPLC).

UPLC separation. Initially, the chromatographic separation of four metal-APDC complexes was optimized. In view of the nature of the analytes, reversed-phase separation mode was selected for separation. In the first attempt, the retention properties of three UPLC columns (C18, C8 and phenyl) were compared for a mixture of four complexes in the isocratic elution mode with a 70:30 (*v/v*) acetonitrile/water mobile phase at a flow rate of 0.5 mL/min. Comparable trends in elution orders and peak shapes were obtained on all three phases but separation selectivity was slightly better for the C18 phase.

Next, the pH of the mobile phase was investigated in the range of pH 3.0-7.0 using appropriate phosphoric acid/phosphate and acetic acid/acetate buffer solutions. It was found that this parameter did not generate retention and/or selectivity shifts to a relevant extent: for all analytes the retention time and peak efficiency only fluctuated slightly (less than 10% variation) with pH most likely due to the lack of change in the ionization state of the analytes in the examined pH range. Based on these results buffer free mobile phase was selected for further experiments.

Finally, the effect of mobile phase flow-rate (from 0.2 to 0.8 mL/min) upon efficiency was studied. However, since no significant improvement in peak efficiency was noted for all compounds, further work was carried out at 0.5 mL/min mobile phase flow-rate. Performed experiments indicated that a mobile phase of acetonitrile-water (70:30, v/v) gave the best separation performance. As shown in Fig. 6, excellent separation of the analytes was achieved isocratically within less than 3 min.

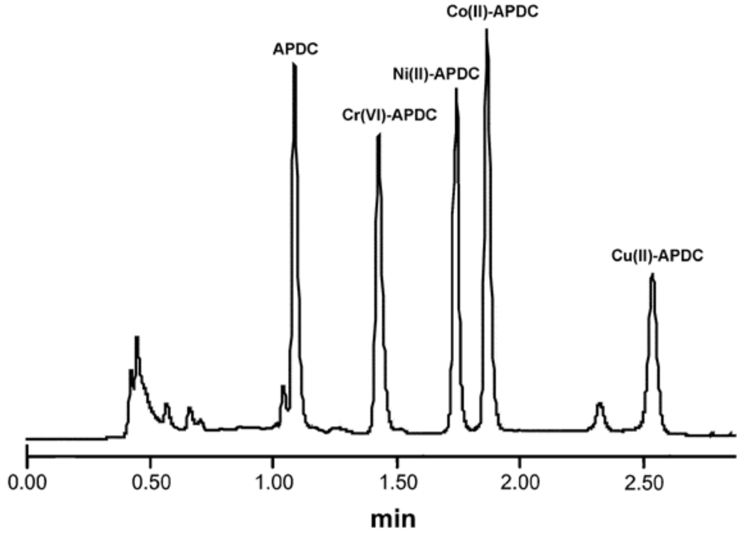

Fig. 6. UPLC separation of metal-APDC complexes under optimized conditions. Mobile phase - 70:30 (v/v) acetonitrile/water. Flow rate - 0.5 mL/min. UV detection at 260 nm.

Comparison of ILs. Until now, the most employed ILs in DLLME contain the hexafluorophosphate (PF_6^-) or bis[(trifluoromethyl)sulfonyl]imide (NTf_2^-) anions. Recently, a new class of ILs containing tris(pentafluoroethyl)trifluorophosphate (FAP) anions were developed by Merck. One of the most peculiar properties exhibited by these ILs is their exceptionally strong hydrophobic nature. It has been shown that the water uptake of these ILs is much less than that of ILs containing the NTf_2^- and PF_6^- anions. Furthermore, replacement of three fluorine atoms in the PF_6^- anion by pentafluoroethyl groups decreases drastically (~7-times) the viscosity of ILs.

To compare the extraction performance among different hydrophobic ILs, studies were performed on 1-hexyl-3-methylimidazolium ILs containing the NTf_2^-, PF_6^-, and FAP^- anions (Fig. 7). Density, viscosity and water solubility of these ILs are listed in Table 5.

Initial DLLME experiments were performed with 5 mL of aqueous standard solutions using 0.5 mL of methanol containing 50 μL of appropriate IL as extraction mixture. However, due to different water solubility of the ILs the volume of the sedimented phase also differed. In addition, for more viscous [HMIM][PF_6] it was difficult to withdraw the IL phase into the microsyringe for subsequent analysis. In order to overcome mentioned problems, all sedimented phases were diluted to 1 mL with acetonitrile. The results (Fig. 8) show that extraction efficiency of metal-APDC complexes increased in the order [HMIM][PF_6] < [HMIM][NTf_2] < [HMIM][FAP] which is consistent with the decrease in water solubility of these ILs.

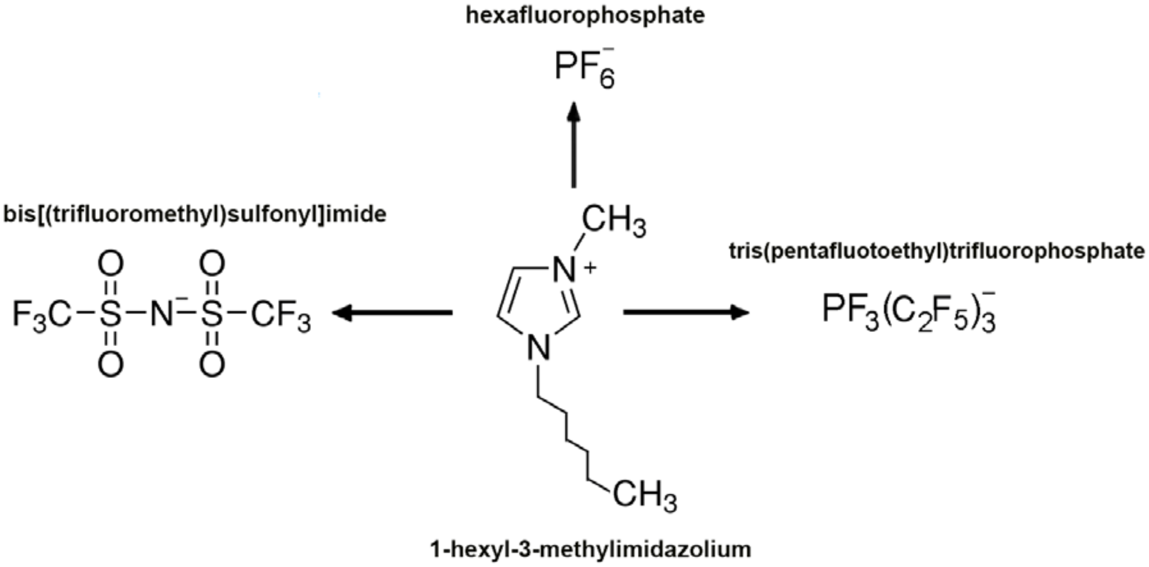

Fig. 7. Structures of ILs studied.

Table 5. Some physicochemical properties (at 25 °C) of ILs used in this study

IL	Density (g/mL)	Viscosity (mPa·s)	Water solubility (g/100 mL)
[HMIM][PF_6]	1.29-1.31	560-586	0.75
[HMIM][NTf_2]	1.33	70	0.34
[HMIM][FAP]	1.56	74	0.038

Optimization of complexation and DLLME conditions. In order to optimize complexation/extraction conditions, the effects of sample pH, the amount of APDC, the nature of disperser solvent, [HMIM][FAP] volume, and sample volume on the extraction efficiency of metal-APDC complexes were investigated. The analyte concentrations used for these experiments were 10, 50 and 100 μg/L.

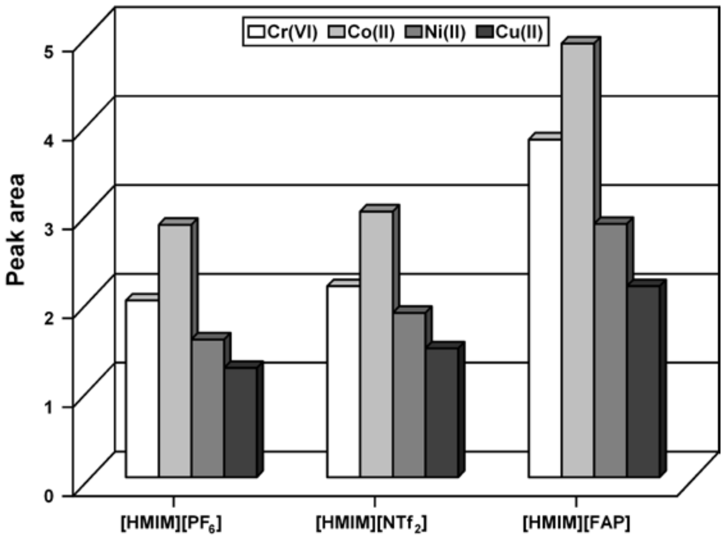

Fig. 8. Effect of IL nature on the peak areas of metal-APDC complexes (50 μg/L of each cation). Sample - 5 mL, pH 3.0. APDC concentration - 0.05% (w/v). Extraction mixture - 0.5 mL of methanol containing 50 μL of appropriate IL.

The pH of the sample solution is one of the important factors affecting the formation of complexes and subsequent extraction. A series of standard metal solutions were prepared in HCOOH+CH_3COOH mixture (0.05 mol/L of each acid) adjusted with NaOH to desired pH (from 2 to 6), treated with APDC (5 min), then extracted and analyzed. The complexation/extraction of Cr(III) under the same conditions was also investigated. Fig. 9 depicts the effect of pH on the peak areas of metal-APDC complexes. The maximum and almost constant signal intensities for all four complexes were observed in the pH range 2.5-4.0 confirming that the complexation requires a weakly acidic solution. When the sample pH was higher than 4, the peak area of Cr(VI) complex considerably decreased, most likely, due to slower reduction of Cr(VI) ions and, consequently, incomplete complexation. As expected, the Cr(III)-APDC complex was not detected in the whole range of pH values tested.

In the next experiment, the effect of the APDC amount in the range of 0.01-0.5% (w/v) on the complexation/extraction performance of the analytes at pH 3.0 was investigated. For the highest analyte concentration studied (100 μg/L), the signal intensity increased up to about 0.02-0.03% APDC, reaching a plateau, which was considered as the complete complexation/extraction. Hence, an APDC concentration of 0.1% was used to optimize other parameters. The time required for complete complexation of metal cations was also evaluated. The results showed that 1 min was enough for complete formation of metal-APDC complexes studied.

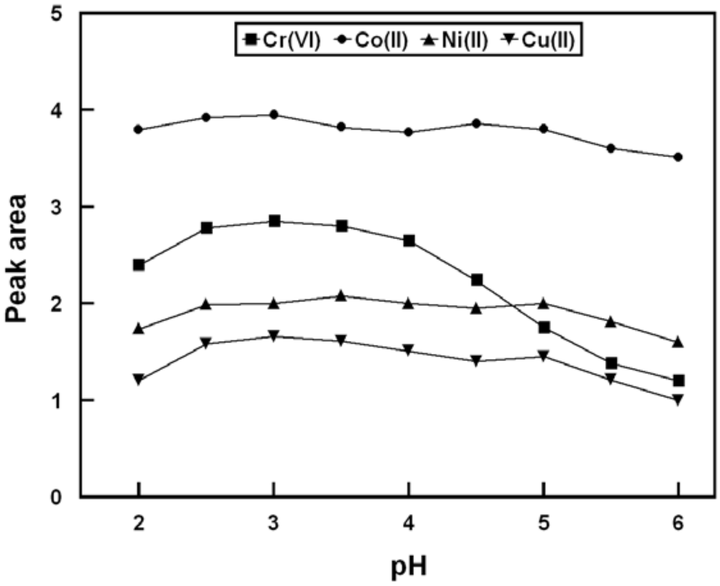

Fig. 9. Effect of sample pH on the on the peak areas of metal-APDC complexes. Sample - 5 mL. APDC concentration - 0.05% (w/v). Extraction mixture - 0.5 mL of methanol containing 50 μL of [HMIM][FAP].

Four common disperser solvents (acetonitrile, acetone, ethanol and methanol) were tested using 0.5 mL of each solvent containing 50 μL of [HMIM][FAP] as extraction mixture. However, there was no statistically significant difference between the extraction performance obtained by these solvents.

In order to evaluate the effect of [HMIM][FAP] volume on the extraction performance, extraction mixtures containing 0.5 mL of methanol and different volumes (from 20 to 100 μL) of [HMIM][FAP] were examined for standard analyte solutions. Fig. 10 shows the effect of IL volume on the recoveries (the percentage of the total amount of analyte which was extracted) of the analytes studied. The results indicate that 40 μL was the lowest amount of [HMIM][FAP] required to achieve satisfactory recoveries (≥80%) for all four analytes. Higher amounts of IL did not improve extraction efficiency. Nevertheless, in order to obtain higher extraction capacity for real samples, 80 μL of [HMIM][FAP] was selected as optimum volume for all subsequent experiments. It should be noted, that after centrifugation a part of sedimented phase always remains on the wall of a centrifuge tube. Therefore, measured recovery values are lower than true ones. The recoveries were enhanced up to 95-102% by washing of the tube wall with acetonitrile during extract dilution step. It should be noted, that this procedure can be avoided by direct (without dilution) UPLC analysis of the extracts.

Sample volume is another factor that affects the extraction efficiencies in microextraction techniques. It should be noted that the effects of sample volume are not only a function of the amount of the analytes in solution but also the solubility of the IL phase. Fig. 11 shows the effect of sample volume on the amount of analytes extracted by a 80 μL [HMIM][FAP]. A gradual increase in peak area was observed for all of the studied analytes with an increase in the sample volume up to 10 mL. At higher sample volumes the reduction of the extraction efficiency was observed.

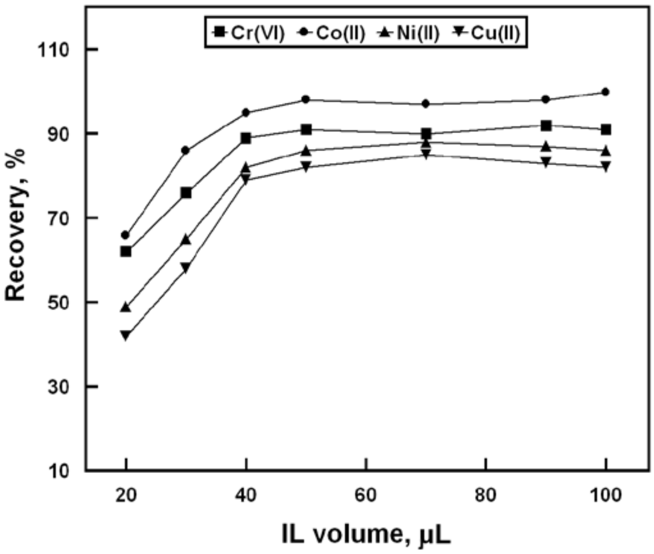

Fig. 10. Effect of [HMIM][FAP] volume on the recoveries of metal-APDC complexes. Sample - 5 mL, pH 3.0. APDC concentration - 0.1% (w/v). Extraction mixture - 0.5 mL of methanol containing appropriate volume of IL.

Finally, the effect of potential interferents on the extraction and UPLC determination of Cr(VI), Co(II), Cu(II) and Ni(II) was assessed. The effect of Cd^{2+}, Cr^{3+}, Fe^{3+}, Ca^{2+}, Mg^{2+} and Zn^{2+} ions was studied. In these experiments, solutions containing 50 and 100 μg/L of appropriate analyte and the interfering ions at different concentrations were extracted and analyzed. An interference was defined to be significant if a variation of more than ±10% in the analyte signal was observed. The results showed that only higher concentrations of transition metals caused significant depressive effects due to a competitive reaction with APDC: Cd^{2+}, Cr^{3+}, and Zn^{2+} could be tolerated up to about 5 mg/L and Fe^{3+} could be tolerated up to 1 mg/L. These data show that the methodology is suitable for natural waters, where the levels of these elements (with the exception of Fe) are usually lower than those necessary to cause interferences. The interfering effect of Fe^{3+} was reduced by increasing the amount of APDC added to the sample.

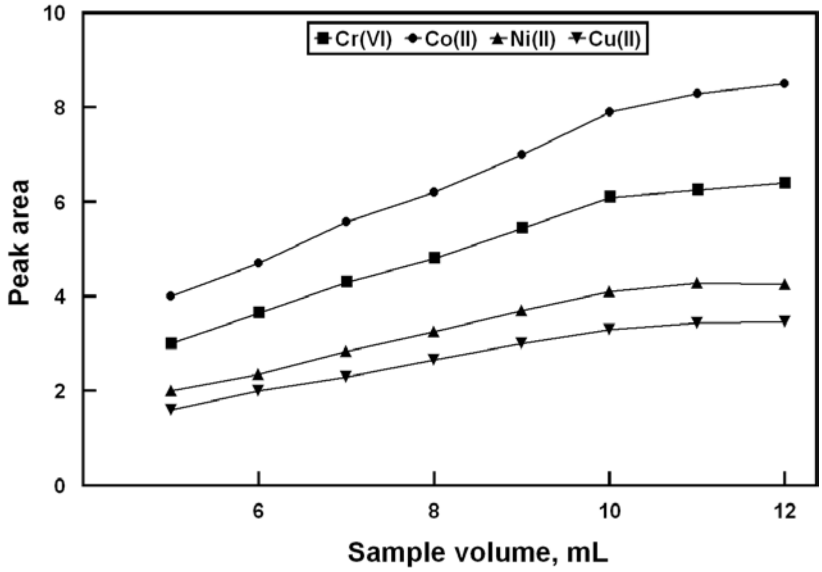

Fig. 10. Effect of sample volume on the peak areas of metal-APDC complexes. APDC concentration - 0.1% (w/v). Sample pH 3.0. Extraction mixture - 0.5 mL of methanol containing 80 μL of [HMIM][FAP].

Analytical performance. The use of an internal standard is required to enhance the recoveries and to improve precision of measurements. Phenyl-2-acetyloxybenzoate was selected for standardization purposes because under optimized UPLC conditions it is well separated from the analytes and is extracted well into organic solvents. Moreover, common water samples do not contain measurable levels of this compound. In an additional experiment, the extraction performance of phenyl-2-acetyloxybenzoate was checked and the obtained results showed that under above optimized DLLME conditions its extraction is quite reproducible.

The main analytical performance characteristics of the method are summarized in Table 6. Detection limits were obtained based on a signal-to-noise ratio of 3. The repeatability of the method, expressed as relative standard deviations (RSDs), was calculated for five replicates of the standard at an intermediate concentration of the calibration curve. The enrichment factors were calculated as the ratio of the analyte concentration in the final extract to that of the initial aqueous phase.

Table 6. Analytical performance characteristics

Analyte	Linear range (μg/L)	Correlation coefficient (*r*)	Limit of detection (μg/L)
Cr(VI)	1.5 - 200	0.9989	0.5
Co(II)	1.0 - 100	0.9992	0.3
Cu(II)	5.0 - 150	0.9975	2.0
Ni(II)	5.0 - 150	0.9958	1.5

Finally, the accuracy of the method was evaluated by analyzing two certified reference materials (CRM): natural water CRM NIST SRM 1643e with certified Co and Ni concentrations and lyophilized water CRM BCR-544 with certified Cr(VI) concentration. These CRMs also contain other ions commonly present in water samples. The obtained results are summarized in Table 7. It is evident that measured values are in good agreement with certified ones.

Table 7. Results (mean of three measurements ± confidence interval, P=0.95) for metal ion determination in certified reference materials

CRM	Analyte	Certified concentration (μg/L)	Measured concentration (μg/L)
NIST SRM 1643e	Co	27.1 ± 0.3	26.2 ± 2.4
	Ni	62.4 ± 0.7	64.8 ± 2.8
BCR-544	Cr(VI)	22.8 ± 1.0	21.5 ± 2.1

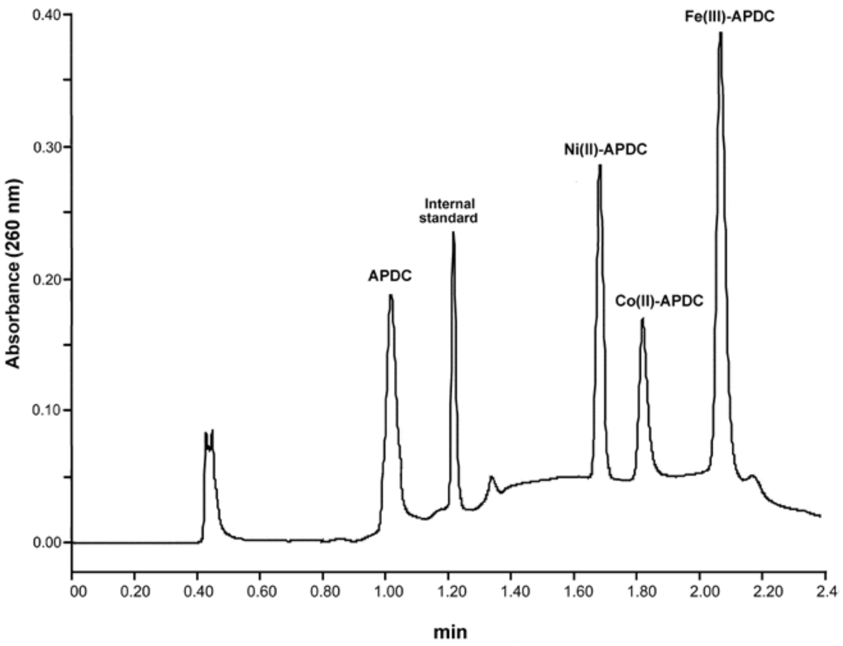

Fig. 11. Chromatogram of the extract of natural water CRM NIST SRM 1643e.

3.3. Comparison of different IL based DLLME techniques

One of the disadvantages associated with conventional DLLME, however, is that it uses relatively high volumes of a polar solvent such as methanol or acetonitrile to disperse the extraction solvent into the aqueous sample. This may result in lower extraction efficiency due to increased solubility of the analytes in aqueous sample solution. Thus, significant efforts have been made to disperse the IL phase effectively without the use of disperser solvent. One technique, so-called temperature-induced IL based DLLME, utilizes an increase in temperature to fully dissolve the IL in the sample solution followed by cooling and centrifugation to recover the IL phase.

Another addition to the list of IL-based DLLME techniques is ultrasound-assisted dispersive liquid-liquid microextraction. The technique is rather similar to temperature-induced-DLLME, the only difference being that instead of the initial heating step ultrasound was applied to dissolve the IL phase. However, a cooling process is still needed to obtain a turbid solution.

All the IL-based DLLME approaches are well established and can be applied to a wide spectrum of analytes. However, it should be highlight that there are no studies in which different IL-based DLLME techniques have been applied for the same analytes for comparative purposes. In this part of the work, the performance of three IL-based DLLME approaches, namely conventional solvent-induced, temperature-induced, and ultrasound-assisted was investigated and compared. [HMIM][FAP] was used as the extraction solvent for the enrichment of four metal-APDC complexes.

Solvent-induced DLLME. An aliquot of 5.0 mL of standard solution was placed into a 10-mL glass test tube with conical bottom. Then 0.5 mL of methanol (dispersive solvent) containing 30 μL of [HMIM][FAP] was injected rapidly into the sample solution by using a 2-mL syringe. A cloudy solution was formed in a test tube. The mixture was then centrifuged for 3 min at 5000 rpm. The upper aqueous phase was removed with a pipette, and the residue was diluted to 1 mL with acetonitrile and analyzed.

Temperature-induced DLLME. An aliquot of 5.0 mL of standard solution was placed into a 10-mL glass test tube with conical bottom. Then 30 μL of [HMIM][FAP] was added and the solution was heated with the temperature controlled at about 60-70°C.

IL was dissolved completely in the sample solution and mixed with the solution entirely. The tube was thereafter cooled at about -7 °C for about 10 min and a cloudy solution was formed. The mixture was then centrifuged for 3 min at 5000 rpm. The upper aqueous phase was removed with a pipette, and the residue was diluted to 1 mL with acetonitrile and analyzed.

Ultrasound-assisted DLLME. An aliquot of 5.0 mL of standard solution was placed into a 10-mL glass test tube with conical bottom. Then 30 μL of [HMIM][FAP] was added and the solution was sonicated for 15 min at room temperature. The IL was dissolved completely in the sample solution and nearly homogenous solution was achieved. The tube was thereafter cooled at a -7 °C for about 5 min and a cloudy solution was formed.

Obtained results (Fig. 12) showed that all three IL-based DLLME approaches gave almost identical extraction performance (≤5%). Similar results were obtained for higher concentration (100 μg/L) of the analytes. In comparison with conventional solvent-induced DLLME, for temperature-induced and ultrasound-assisted DLLME techniques an additional heating/ultrasonification and cooling steps are required. Taking into account that those steps are relatively time consuming (up to 20-30 min), conventional solvent-induced DLLME mode should be preferred for practical use.

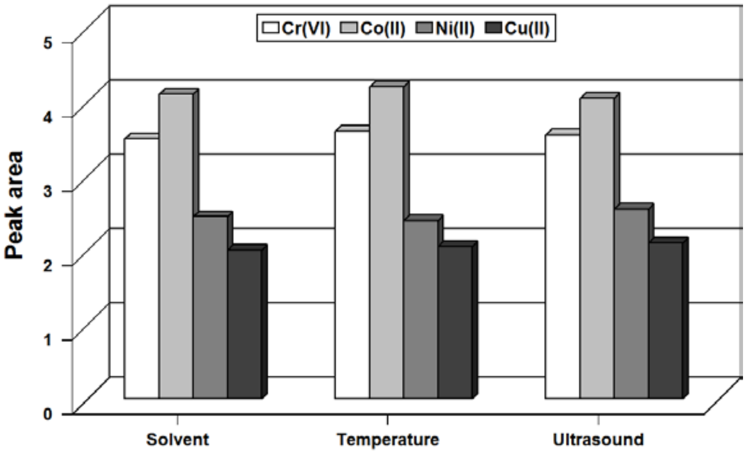

Fig. 12. Effect of IL-based DLLME mode on the extraction performance of metal-APDC chelates (50 μg/L each).

CONCLUSIONS

1. Extraction performance of six halogenated solvents was compared for DLLME of Cr(VI)-APDC chelate. CH_2Cl_2 and $CHCl_3$ do not form dispersed phase due to relatively high solubility in aqueous sample. Other solvents (CCl_4, C_6H_5Cl, C_6H_5Br and $C_6H_4Cl_2$) exhibited very similar extraction properties.
2. Quantitative extraction of Cr(VI)-APDC chelate was obtained at $V(CCl_4) \geq 50$ µL. By using higher than 2 mL volumes of disperser solvent (methanol) extraction efficiency decreases due to increased solubility of the chelate in aqueous-methanolic mixture. The highest values of enrichment factors were obtained by extraction from 8 mL sample volumes.
3. The microdrops of extract deposited on the glass substrate were quite irregularly shaped and sized and no dried spots were visible. In contrast, the microdroplet residues spotted on polystyrene surface gave well visible, spherical and relatively uniform in diameter spots. This may be due to excellent wetting and swelling of the hydrophobic polystyrene surface with carbon tetrachloride.
4. Cr(VI)-APDC distributes in the dried extract droplet non-uniformly: the RSDs (n=6) obtained for ^{52}Cr and ^{53}Cr ranged from 11 to 23%. Using ^{195}Pt as internal standard, about 3 times lower RSDs were achieved for ^{52}Cr/^{195}Pt and ^{53}Cr/^{195}Pt ratios.
5. Laser ablation parameters were optimized regarding ^{52}Cr and ^{53}Cr signal intensities: ablation energy - 100 %, laser beam diameter – 100 µm, pulse repetition rate – 20 Hz, scanning speed – 30 µm/s.
6. Developed DLLME-LA-ICP-MS system was applied to determine Cr(VI) in tap and river samples. Proposed methodology yields sufficiently low detection limits (0.11 and 0.31 µg/L for ^{52}Cr and ^{53}Cr, respectively) with spike recoveries 93-103%.
7. Excellent UPLC separation of four metal-APDC complexes (Cr(VI), Co(II), Cu(II) and Ni(II)) was achieved isocratically within about 2.5 min. Complete complexation of metal ions was observed in the pH range 2.5-4.5 by using 10-fold excess of APDC.
8. Extraction efficiency of metal-APDC complexes with different imidazolium ionic liquids increased in the order [HMIM][PF_6] < [HMIM][NTf_2] < [HMIM][FAP] which is consistent with the increased hydrophobicity of these solvents. Quantitative extraction was obtained at V([HMIM][FAP]) ≥ 40 µL. Compared to CCl_4, ionic liquid exhibited higher extraction efficiency.
9. IL-based DLLME-UPLC method yields detection limits ranged from 0.5 to 1.5 µg/L. The accuracy of the method was evaluated by analyzing two certified reference materials and measured values were in good agreement with certified ones.
10. Three IL-based DLLME approaches (solvent-induced, temperature-induced and ultrasound-assisted) gave almost identical extraction performance (≤5%). The fastest and simplest conventional solvent-induced DLLME mode should be preferred for practical use.

The List of Original Publications by the Author

Articles in journals:

1. **I. Razmislevičienė**, A. Padarauskas, B. Pranaitytė, E. Naujalis. Determination of Cr(VI) by dispersive liquid-liquid microextraction and dried-droplet laser ablation ICP-MS. *Current Analytical Chemistry*, 6 (2010) 310-315.

2. **I. Razmislevičienė**, V. Olšauskaitė, A. Padarauskas. Ionic liquid-based dispersive liquid-liquid microextraction combined with ultra performance liquid chromatography for the determination of metal ions. *Chemija*, 22 (2011) 197-203.

Published contributions to academic conferences:

1. **I. Razmislevičienė**, A. Padarauskas, B. Pranaitytė, E. Naujalis. Dispersive liquid-liquid microextraction of Cr(VI) followed by ultra-performance liquid chromatographic analysis. Lietuvos 9-osios chemikų konferencijos "Chemija-2009" pranešimų tezės, Vilnius, 2009 spalio 16 d., p. 42.

2. A. Padarauskas, **I. Razmislevičienė**, J. Aukštkalnytė. Dispersive liquid-liquid microextraction followed by ultra-performance liquid chromatography for the determination of metals. Abstracts of the IUPAC International Congress on Analytical Sciences ICAS 2011, May 22-26, Kyoto, Japan, 2011, P. 23P071.

3. **I. Razmislevičienė**, V. Olšauskaitė, A. Padarauskas. Ionic liquid-based dispersive liquid-liquid microextraction: a comparative study. Abstracts of the 10th International Conference of Lithuanian Chemists "Chemistry 2011", October 14-15, 2011, Vilnius, P. 91.

Other publications:

1. Ž. Daunoravičius, **I. Juknaitė (Razmislevičienė)**, E. Naujalis, A. Padarauskas. Simple and rapid determination of denaturants in alcohol formulations by hydrophilic interaction chromatography. *Chromatographia*, 63 (2006) 373-377.

2. **I. Razmislevičienė**, R. Baltuškonytė, A. Padarauskas, E. Naujalis. Determination of fumaric and maleic acids by capillary electrophoresis. *Chemija*, Vol. 19, No 3-4 (2008) 31-35.

3. Ž. Černova, **I. Razmislevičienė**, A. Padarauskas. Determination of melamine in milk powder by capillary electrophoresis. *Chemija*, 20 (2009) 231-235.

4. Ž. Daunoravičius, **I. Juknaitė (Razmislevičienė)**, A. Padarauskas. Determination of denaturants by hydrophilic interaction chromatography. Konferencijos "Neorganinių medžiagų chemija ir technologija" pranešimų medžiaga, Kaunas, 2006, 62-63.

5. **I. Razmislevičienė**, A. Padarauskas. Capillary electrophoretic determination of melamine. Lietuvos 9-osios chemikų konferencijos "Chemija-2009" pranešimų tezės, Vilnius, 2009 spalio 16 d., p. 43.

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2000-2004 studies at the Faculty of Natural Sciences, Vilnius Pedagogical University – Bachelor of Science in Chemistry.

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DISPERSINĖ SKYSTAFAZĖ MIKROEKSTRAKCIJA METALAMS NUSTATYTI

SANTRAUKA

Šioje daktaro disertacijoje apibendrintų mokslinių tyrimų tikslas – ištirti ir pritaikyti naujus Cr(VI), Co(II), Cu(II) ir Ni(II) koncentravimo ir nustatymo metodus apjungiant dispersinę skystafazę mikroekstrakciją (DSME) su lazerinio išgarinimo induktyviai sužadintos plazmos masių spektrometrijos (LA-ICP-MS) bei ultraefektyviosios skysčių chromatografijos (UESCh) metodais.

Darbe ištirtos Cr(VI) komplekso su amonio pirolidinditiokarbamatu (APDC) dispersinės skystafazės mikroekstrakcijos sąlygos. Dėl palyginti didelio tirpumo vandenyje CH_2Cl_2 ir $CHCl_3$ netinkami mikroekstrakcijai. Kitų keturių tirpiklių (CCl_4, C_6H_5Cl, C_6H_5Br ir $C_6H_4Cl_2$) ekstrahuojančios savybės labai panašios. Cr(VI)-APDC kompleksas išekstrahuojamas kiekybiškai, kai CCl_4 tūris $\geq$50 µL. Naudojant didesnius nei 2 mL dispergento (metanolio) tūrius, ekstrakcijos efektyvumas blogėja, greičiausiai dėl padidėjančio komplekso tirpumo vandens-metanolio mišinyje. Maksimalūs sukoncentravimo laipsniai pasiekiami ekstrahuojant iš 8 mL tūrio mėginių.

Optimizuotos lašo išgarinimo sąlygos LA-ICP-MS analizei. Stiklo paviršiuje susidaro labai nereguliarios formos ir dydžio lašas, o jam išdžiuvus, sausa liekana beveik nematoma. Polistireno paviršiuje gaunami sferinės formos, panašaus dydžio, žymiai greičiau išdžiūstantys lašai, o sausa liekana puikiai matoma ekrane. Nevienoda padėklo medžiagos prigimties įtaka susijusi su tuo, kad hidrofobinis ekstrahentas (anglies terachloridas) gerai drėkina ir brinkina hidrofobinį polistireno paviršių. Išdžiovinto ekstrakto liekanoje Cr(VI)-APDC kompleksas pasiskirsto labai netolygiai: ^{52}Cr ir ^{53}Cr izotopų signalų intensyvumo santykinis standartinis nuokrypis (n=6) siekia 11-23%. Vidiniu standartu panaudojus Pt(II), signalų intensyvumo atsikartojamumas pagerėjo maždaug 3-5 kartus. Optimizuotos lazerinio išgarinimo sąlygos ^{52}Cr ir ^{53}Cr izotopų nustatymui: lazerio galia 100 %, kraterio skersmuo – 100 µm, šūvių skaičiaus per sekundę – 20 Hz, skenavimo greitis – 30 µm/s. Apjungta dispersinės skystafazės mikroekstrakcijos ir lazerinio išgarinimo ICP-MS sistema panaudota Cr(VI) nustatymui. Cr(VI) aptikimo ribos siekia 0,11 µg/L ^{52}Cr izotopui ir 0,31 µg/L ^{53}Cr izotopui, o Cr(VI) išgavos iš vandentiekio ir upės vandens mėginių sudaro 93-103%.

Ultraefektyviosios skysčių chromatografijos metodu optimizuotose izokratinės eliucijos sąlygose Cr(VI), Co(II), Cu(II) ir Ni(II) kompleksai su APDC visiškai atskiriami per 2,5 min. Optimali pH sritis metalų reakcijai su APDC – 2,5-4,5. Pilnam metalų kompleksavimui būtinas ne mažesnis nei 10-kartinis APDC perteklius.

Palyginus trijų hidrofobinių 1-heksil-3-metil imidazolio joninių skysčių ([HMIM][PF_6], [HMIM][NTf_2] ir [HMIM][FAP]) ekstrahuojančias savybes nustatyta, kad efektyviausiai metalų kompleksus ekstrahuoja hidrofobiškiausias [HMIM][FAP]. Visi kompleksai išekstrahuojami kiekybiškai, kai [HMIM][FAP] tūris $\geq$40 µL. DSME joniniu skysčiu efektyvesnė už ekstrakciją identiškose sąlygose anglies tetrachloridu. DSME-UESCh metodu nustatytos metalų aptikimo ribos siekia 0,5-1,5 µg/L. Dviejuose etaloniniuose vandens pavyzdžiuose išmatuotos metalų koncentracijos atitiko sertifikuotas koncentracijų vertes.

Nustatyta, kad dispersinės fazės inicijavimo būdas neturi įtakos Me-APDC kompleksų ekstrakcijos joniniais skysčiais efektyvumui. Todėl geriausiai mikroekstrakcijai naudoti greičiausią ir paprasčiausią inicijavimo tirpikliu būdą.