On-line sequential injection dispersive liquid–liquid microextraction system for flame atomic absorption spectrometric determination of copper and lead in water samples

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ABSTRACT

A simple, sensitive and powerful on-line sequential injection (SI) dispersive liquid–liquid microextraction (DLLME) system was developed as an alternative approach for on-line metal preconcentration and separation, using extraction solvent at microlitre volume. The potentials of this novel schema, coupled to flame atomic absorption spectrometry (FAAS), were demonstrated for trace copper and lead determination in water samples. The stream of methanol (disperser solvent) containing 2.0% (v/v) xylene (extraction solvent) and 0.3% (m/v) ammonium diethyldithiophosphate (chelating agent) was merged on-line with the stream of sample (aqueous phase), resulting a cloudy mixture, which was consisted of fine droplets of the extraction solvent dispersed entirely into the aqueous phase. By this continuous process, metal chelating complexes were formed and extracted into the fine droplets of the extraction solvent. The hydrophobic droplets of organic phase were retained into a microcolumn packed with PTFE-turnings. A portion of 300 μL isobutylmethylketone was used for quantitative elution of the analytes, which transported directly to the nebulizer of FAAS. All the critical parameters of the system such as type of extraction solvent, flow-rate of disperser and sample, extraction time as well as the chemical parameters were studied. Under the optimum conditions the enhancement factor for copper and lead was 560 and 265, respectively. For copper, the detection limit and the precision (R.S.D.) were 0.04 $\mu g \, L^{-1}$ and 2.1% at 2.0 $\mu g \, L^{-1}$ Cu(II), respectively, while for lead were 0.54 $\mu g \, L^{-1}$ and 1.9% at 30.0 $\mu g \, L^{-1}$ Pb(II), respectively. The developed method was evaluated by analyzing certified reference material and applied successfully to the analysis of environmental water samples.

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1. Introduction

In recent years, pollution of the environment by large amount of toxic elements has been considered as a result of human activities. Exposure to these toxic elements imposes risks not only to human health, but also to plants, animals and microorganisms [1]. Therefore, the determination of various toxic metals such as lead and copper in environmental samples is very important task. Despite its significant analytical capabilities for metal determination, flame atomic absorption spectrometry (FAAS) often requires a suitable sample pretreatment step (preconcentration and/or separation), due to analyte low concentration and matrix effects [2,3].

Conventional liquid–liquid extraction (LLE) is the most widely used sample pretreatment technique in routine analyses for trace metal determination due to i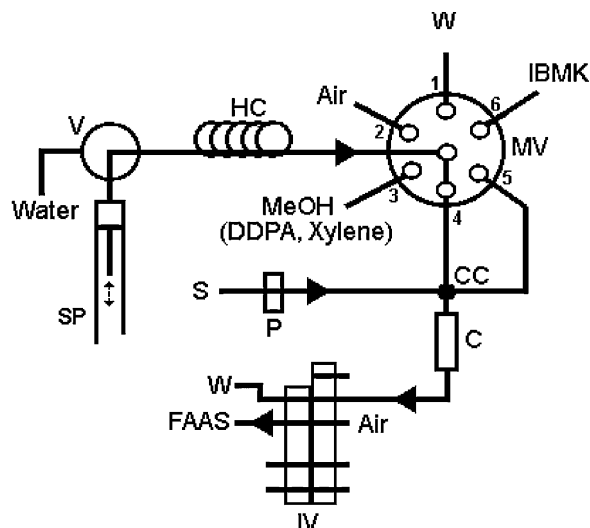ts simplicity and adaptability. However, some shortcomings like the use of large sample volumes and toxic organic solvents make LLE expensive, time-consuming, laborious and environmentally unfriendly. Sample pretreatment still remains to be the bottleneck for a large number of analytical procedures and often the one, which seriously hinders automation and miniaturization. The implementation of on-line LLE simulating the same sequences performed in batch procedures, offers advantages which are inherent in automatic methods [3]. Recently, efforts have been focused on the miniaturizing of the LLE procedure by reducing the organic solvent, leading to the development of microextraction methodologies [4,5].

The first attempt to miniaturize LLE in a flowing stream assembly was carried out by Liu and Dasgupta [6], while further research led to the development of the so-called single-drop microextraction (SDME) where a droplet of organic solvent was suspended at the tip of a microsyringe needle and immersed into the aqueous sample [7]. Psillakis and Kalogerakis [8] and recently Xu et al. [9] overviewed the principles of SDME as well as its latest developments and applications. An automatic sequential injection single-drop microextraction (SI-SDME) system for metal deter-

mination in environmental samples using ETAAS was developed recently by our group [5]. Cloud point extraction (CPE) procedure is based on the phase separation, which occurs in aqueous solutions of non-ionic surfactants, when heated above the so-called cloud point temperature. At this temperature the initial solution separates into a surfactant rich phase of small volume containing the metal trapped by organized structures and a bulk aqueous phase [10]. The principles and theoretical aspects as well as relevant applications regarding the use of micelle-mediated separation have been well documented in various interesting reviews [11,12].

On the other hand, homogeneous liquid–liquid extraction (HLLE) is a simple and powerful preconcentration technique for organic and inorganic analytes, which utilizes the phase separation from a homogeneous solution where no interface between the aqueous phase and organic solvent phase exist and the analytes are extracted into the sedimented phase. It can be considered that the surface area of the interface is infinitely large. Hence, vigorous shaking or mixing is not necessary. Generally, HLLE has been applied through two different modes: by the perfluorinated surfactant system [13–15] and the ternary component solvent one [16,17,18,19]. In these cases, phase separation is based on pH, on ion-pair formation or on the solubility difference among the solvents of a ternary system.

Recently, Assadi and coworkers [20] presented a microextraction technique, termed dispersive liquid–liquid microextraction (DLLME) based on ternary component solvent systems, similar to HLLE and CPE. An appropriate mixture of an extraction solvent and a disperser solvent with high miscibility in both aqueous phase and extractant is rapidly injected into the sample (aqueous phase) using a microsyringe. By this procedure a cloudy solution (high turbulence) is produced, which is consisted of fine droplets of extraction solvent dispersed throughout the aqueous sample. The nature of the disperser solvent can also have an influence on the mean droplet size and their distribution. After centrifuging of the cloudy solution, the sedimented phase at the bottom of a conical tube is removed manually by a syringe and analyzed by various analytical techniques like gas chromatography (GC) [21,22], high-performance liquid chromatography (HPLC) [23], electrothermal atomic absorption spectrometry (ETAAS) [24–27] and FAAS [28,29]. DLLME has been applied for preconcentration and determination of organic compounds as well as for metals [24–29] and metalloids [30] in water samples. The principal advantage of DLLME is that the surface area between extraction solvent and aqueous sample is very large, thus the equilibrium state is achieved quickly and the extraction time is very short. However, when DLLME is performed in manual mode, is limited to a small number of extraction solvents, since the required conditions, i.e. to have higher density rather than water, to form a stable cloudy solution and to be easily removed from the bottom of the conical vial after centrifugation, are met by a few organic solvents [31]. Also it suffers from the inherent problems of the manual procedures.

The aim of present work was to develop a novel automatic sequential injection dispersive liquid–liquid microextraction (SI-DLLME) system for metal preconcentration and determination using FAAS. To best of our knowledge, the automation of the DLLME was demonstrated for the first time. The effectiveness and efficiency of the proposed SI-DLLME system has been demonstrated for copper and lead determination in environmental water samples. Ammonium diethyldithiophosphate (DDPA) was selected as chelating agent due to its good selectivity in strong acidic medium. All main factors were investigated and optimized. The proposed method was evaluated by analyzing certified reference material and spiked environmental water samples.

Fig. 1. Schematic manifold for SI-DLLME metal determination by FAAS (step 4). For details see text and Table 1. S, sample; MeOH, solution containing 2.0% (v/v) xylene and 0.3% (m/v) DDPA; W, waste; P, peristaltic pump; SP, syringe pump; MV, multi-position valve; IV, injection valve in "load" position; V, valve in "out" position; HC, holding coil; C, microcolumn; CC, confluence connector.

2. Experimental

2.1. Instrumentation

The manifold and its operation for on-line sequential injection dispersive liquid–liquid microextraction (SI-DLLME) metal determination by FAAS is presented schematically in Fig. 1.

A FIAlab®-3000 sequential injection system (Alitea FIAlab, USA) equipped with an internally incorporated six-port multiposition valve (MV) and a syringe pump (SP, Cavro, Sunnyvale, CA) with a capacity of 1000 μL was adopted. The FIAlab®-3000 system was controlled by a personal computer and the FIAlab for windows v. 5.9.245 application software, written by FIAlab instruments (http://www.flowinjection.com).

A PerkinElmer, Norwalk, Connecticut, U.S.A. (http://las.perkinelmer.com) model 5100 PC flame atomic absorption spectrometer was exploited as detection system. Copper hollow cathode lamp (HCL) and lead electrodeless discharge lamp (EDL) was used as light source operated at 15 and 10 W, respectively. The slit was set at 0.7 nm, while the wavelength was set at 324.7 and 283.3 nm resonance line for copper and lead, respectively. A time-constant of 0.2 s was used for peak height evaluation. The flame conditions were adjusted to be slightly leaner than the recommended by the manufacturer, in order to compensate the effect of organic solvent isobutyl methyl ketone (IBMK), which serves as an additional fuel. The air and acetylene flow-rate were 10.0 and 0.9 L min^{-1}, respectively. By these conditions the nebulizer's free uptake rate was 5.6 mL min^{-1}. A flow spoiler was employed inside the spray chamber for better nebulization conditions.

A PerkinElmer Norwalk, Connecticut, U.S.A. model FIAS-400 flow injection analysis system was coupled to FAAS and SI system for automatic processing of the whole procedure. The FIAS-400 system was controlled by a personal computer and the AA Lab. Benchtop version 7.2 software program. The two programs (FIAlab and AA Lab.) were synchronized and activated in the same time. The FIAS-400 system consisted of two peristaltic pumps (only one was used in the proposed manifold) and a 5-port 2-position injection valve, IV. The connecting tubing between the IV and the FAAS nebulizer was as short as possible PTFE tube (20 cm length, 0.5 mm i.d.).

Table 1
Operational sequence of the SI-DLLME–FAAS on-line preconcentration method for copper and lead determination (for manifold details, see Fig. 1).

Step	V position	MV position	SP flow-rate	SP operation	IV position	P operation	Commentary
1	IN	2	$50\,\mu L\,s^{-1}$	Aspirate 200 μL	Load	OFF	Water into SP
2	OUT	2	$3\,\mu L\,s^{-1}$	Aspirate 5 μL	Load	OFF	Segment of air into HC
3	OUT	3	$30\,\mu L\,s^{-1}$	Aspirate 770 μL	Load	OFF	Disperser solvent into HC
4	OUT	4	$6\,\mu L\,s^{-1}$	Dispense 720 μL	Load	ON[a]	Cloudy solution formation/Extraction (120 s)/retention
5	OUT	1	$20\,\mu L\,s^{-1}$	Dispense 200 μL	Load	OFF	Discard of air segment
6	IN	1	$50\,\mu L\,s^{-1}$	Aspirate 600 μL	Load	OFF	Water into SP
7	OUT	6	$30\,\mu L\,s^{-1}$	Aspirate 300 μL	Load	OFF	IBMK into HC
8	OUT	5	$50\,\mu L\,s^{-1}$	Dispense 600 μL	Elution	OFF	Elution of microcolumn/measurement
9	OUT	6	$50\,\mu L\,s^{-1}$	Aspirate 500 μL	Elution	OFF	IBMK into HC
10	OUT	4	$50\,\mu L\,s^{-1}$	Empty	Elution	OFF	Washing of manifold

[a] Sample propulsion with flow-rate, $6.0\,mL\,min^{-1}$; V, valve; MV, multiposition valve; SP, syringe pump; IV, injection valve; P, peristaltic pump.

The microcolumn for retention of the extraction solvent, which includes the derivative chelate complex M–DDPA, was fabricated by firmly packing 180 mg of PTFE-turnings in a piece of narrow PTFE tube (40 mm length, 2.1 mm i.d./3.2 mm o.d.). PTFE-turnings were mechanically produced in our laboratory by lathe as described elsewhere [32] and the image of turnings has been given previously [33]. The PTFE-turnings were washed thoroughly by ethanol followed by $1\,mol\,L^{-1}$ HNO$_3$ and de-ionized water. The performance of the column was stable for at least 1000 cycles.

A VICI AG (Valco International) four-section "cross" type confluence connector made of PEEK, with 0.5 mm i.d. bore size was used for intermixing of aqueous (sample) and organic phase (methanol plus xylene). An Orion EA940 pH-meter was employed for the pH measurements being defined by NIST buffers.

2.2. Reagents and samples

All chemicals were of analytical reagent grade and were provided by Merck (Darmstadt, Germany, http://www.merck.de). Ultra-pure quality water was used throughout which was produced by a Milli-Q system (Millipore, Bedford, USA, http://www.millipore.com). All standard solutions were prepared immediately before use, by stepwise dilution of $1000\,mg\,L^{-1}$ Cu(II) and Pb(II) stock standard solution in HNO$_3$ $0.5\,mol\,L^{-1}$ (Merck Titrisol) to the required sub-μg$\,L^{-1}$ levels. The chelating agent, 0.3% (m/v) ammonium DDPA solution was prepared daily by dissolving appropriate amount of DDPA (Aldrich) in methanol without any further purification. IBMK was previously saturated with ultra-pure water.

Natural water samples were filtered through 0.45 μm membrane filters and acidified to $0.05\,mol\,L^{-1}$ HNO$_3$ (pH $\sim$ 1.4).

2.3. Procedure

The operation steps of the optimized on-line sequential injection dispersive liquid–liquid microextraction method (SI-DLLME–FAAS) for copper and lead determination are summarized in Table 1.

In step 2 and 3 segments of air and disperser solution (methanol containing 2.0% (v/v) xylene and 0.3% (m/v) DDPA) at volumes of 5 and 770 μL, respectively were aspirated into the holding coil (HC). In step 4 (Fig. 1), peristaltic pump (P) is activated in order to transport the sample towards the microcolumn (C) through the confluence connector (CC). In the same time syringe pump (SP) dispense the disperser solution through the port 4 of MV towards microcolumn through also CC. Hence, the disperser solution was injected into the moving sample solution, resulting thus a cloudy solution of fine droplets of extraction solvent (xylene). Under these conditions the metal complexes were extracted continuously from aqueous phase to organic phase (xylene). The extraction procedure performed for 120 s (extraction time). Meanwhile, the xylene droplets, which contain the metal complexes, were retained on the PTFE-turnings into

the microcolumn (C). In step 8, the injection valve (IV) was switched to the "Elution" position, so that the segment of 300 μL of IBMK was delivered through the microcolumn in order to elute the analyte. The eluent forwarded to the FAAS nebulizer for atomization and measuring. During the next steps a completed washing of the manifold were done. Five replicate measurements were made in all instances.

3. Results and discussion

For higher sensitivity, selectivity and precision for metal determination with the SI-DLLME method, the effect of the main parameters, like the type of disperser and extraction solvent, sample and disperser flow-rate, sample acidity, amount of chelating agent, sample ionic strength and extraction time, were studied and optimized thoroughly. Standard aqueous solutions $10.0\,\mu g\,L^{-1}$ Cu(II) and $100.0\,\mu g\,L^{-1}$ Pb(II) in pH $\sim$ 1.4 were used for the following experiments. In order to obtain effective microcolumn elution and atomization, a segment of 300 μL IBMK at $3.0\,mL\,min^{-1}$ flow-rate was used in any case, as they were optimized during preliminary experiments.

3.1. Effect of extraction solvent type and concentration

The selection of an appropriate organic solvent for SI-DLLME is very important task. It should able to form a cloudy solution system with water and disperser solvent. Also it should have highly immiscibility with aqueous phase (sample solution), high extraction efficiency, selectivity and hydrophobicity. Opposition to conventional (off-line) dispersive liquid–liquid microextraction systems, extraction solvent was not necessary to have higher density than water, due to the fact that the formation of the cloud solution took place in a moving stream and the retention of the extraction droplets on PTFE-turnings was based on its hydrophobicity. It means that the separation of the organic phase is based on the retention and not on the centrifuging. Chloroform, carbon tetrachloride, dichloromethane, hexane, benzene, xylene, toluene, IBMK, di-isobutyl ketone (DIBK) were compared in the extraction of copper and lead. The concentration of the examined extraction solvents in methanol was fixed at 2.0% (v/v). The obtained results of relative absorbance of copper and lead together with the solubility "in water" values of the extraction solvents, are given in Table 2. The results revealed that xylene achieved the highest extraction efficiency and therefore selected as optimum for all further experiments.

The concentration of the extraction solvent in methanol affects positively the volume of the droplets. On the other hand an increment in the droplets results in lower preconcentration factor, due to lower volume-ratio of aqueous to organic phase. In addition the volume of extraction solvent can affect the retention processes and finally the enhancement factor. The effect of the xylene concentra-

Table 2
Extraction efficiency of various extraction solvents (2.0%, v/v in methanol) for Cu(II) and Pb(II) determination by SI-DLLME–FAAS.

Extraction solvent	Solubility in water, % (m/m)	Relative absorbance, %	
		Cu(II)	Pb(II)
Chloroform	0.82	44	46
Carbon tetrachloride	0.08	86	89
Dichloromethane	1.60	32	35
Hexane	0.005	76	75
Benzene	0.18	60	58
Xylene	0.02	100	100
Toluene	0.05	94	91
IBMK	1.91	24	28
DIBK	0.05	97	96

tion on the absorbance was studied from 0.5 to 8.0% (v/v) and the results are illustrated in Fig. 2. Relative absorbance remained almost constant for xylene concentration from 0.5% up to 2.0%, while for higher concentrations it was gradually decreasing. In the absent of xylene the recorded absorbance was insignificant indicating that the retention of the metal complex on the column was not achieved, due to the presence of MeOH.

For better precision and higher enrichment factors a xylene concentration of 2.0% (v/v) was chosen as optimum for the following experiments.

3.2. Effect of disperser solvent type and flow-rate

The miscibility of the disperser solvent with the extraction solvent and aqueous phase (sample solution) is a significant criterion for its choice, in DLLME methods. Acetone, ethanol and methanol were examined for this purpose. Xylene concentration in any disperser solvent was 2.0% (v/v). Other experimental conditions were as in Fig. 2. The resulting data had shown no significant statistical differences between the studied solvents. Methanol was selected as disperser solvent in the subsequent experiments due to its accessibility and low-priced.

The flow-rate of disperser solvent affects on the sedimentation of the extraction solvent (fine droplets formation) and on the retention of them in the microcolumn. This consideration is based on the variation of the linear velocity of the liquid mixture by changing the flow-rate into the microcolumn. The influence of disperser solvent flow-rate on the absorbance was examined in the range of 0.12–0.72 mL min^{-1}. It was found that the absorbance was increased by increasing the flow-rate of methanol up to
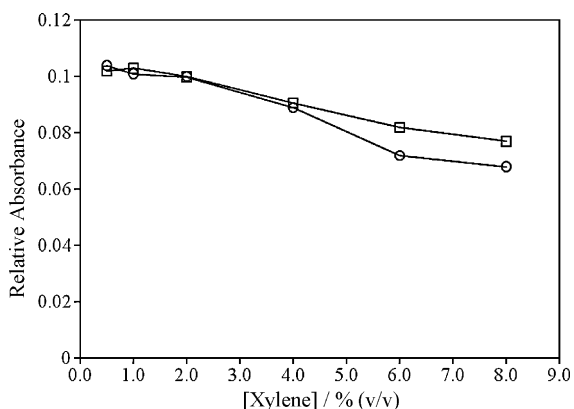
Fig. 2. Effect of the concentration of xylene on the absorbance of 10.0 µg L^{-1} Cu(II) (–□–) and 100.0 µg L^{-1} Pb(II) (–○–) obtained from SI-DLLME–FAAS. Extraction solvent: methanol containing 0.2% (m/v) DDPA and xylene; 5.1 mL min^{-1} sample flow-rate; 0.36 mL min^{-1} methanol flow-rate.
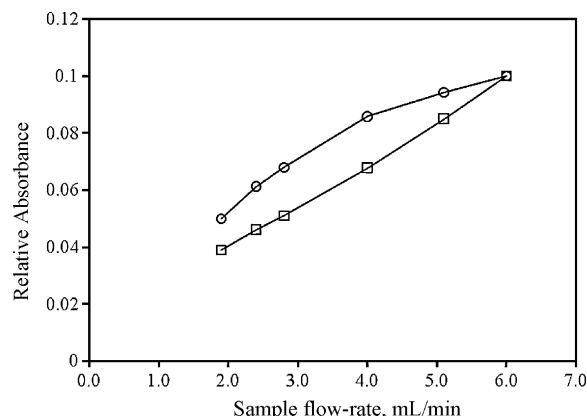
Fig. 3. Effect of the sample flow-rate on the absorbance of 10.0 µg L^{-1} Cu(II) (–□–) and 100.0 µg L^{-1} Pb(II) (–○–) obtained from SI-DLLME–FAAS. Extraction solvent: methanol containing 0.2% (m/v) DDPA and 2.0% (v/v) xylene; 0.36 mL min^{-1} methanol flow-rate.

0.36 mL min^{-1} and after that it was slightly decreased. Therefore, 0.36 mL min^{-1} was chosen as optimum.

3.3. Effect of aqueous phase flow-rate

The effect of aqueous phase (sample solution) flow-rate was evaluated in the range of 1.9–6.0 mL min^{-1}. Methanol flow-rate was fixed at 0.36 mL min^{-1}. Thereby, the ratio of aqueous to organic volume was increased by increasing the sample flow-rate. The recorded relative absorption was almost linearly within the studied range as it is shown in Fig. 3, implying that the contact time of fine droplets of extraction solvent with the PTFE-turnings was sufficient. This is an advantage because enables more sample to be loaded during a given loading time resulting in higher preconcentration factor. A flow-rate of 6.0 mL min^{-1} was selected for high sensitivity.

3.4. Sample acidity and DDPA concentration

Ammonium DDPA has been used successfully in on-line liquid–liquid extraction and solid phase extraction methodologies and compared with other dithiocarbamate reagents proved to be more selective and stable for cadmium, copper and lead in very low pH values [34]. The main advantage of the DDPA is that forms stable complexes with several metals even in strong acidic medium due to its resistance against hydrolysis, avoiding thus the addition of buffer solutions which are a significant source of contamination [35].

Among chemical variables, sample acidity, which is defined by the pH of the sample solution, plays a significant role in the overall performance of the on-line solvent extraction affecting the complex formation and the extraction efficiency. The effect of the pH on the absorbance was studied in the range 0.9–3.0 by adjusting it (in metal solution) with dilute nitric acid. The obtained results shown that, the absorbance was maximum and practically stable in a pH window varied between 0.9 and 1.6, while for higher values the absorbance was decreased gradually. Thus, samples and standards solutions were fixed to pH = 1.4 ± 0.1. This optimum pH value enables the use of the method directly in many aqueous samples after common acid preservation.

The effect of DDPA concentration in the disperser solvent (methanol) was studied in the range of 0.01–0.6% (m/v). The maximum absorbance was observed over the concentration range 0.15–0.6% (m/v). Given its competitive complexation with other coexisting ions in the real samples a DDPA concentration of 0.3% (m/v) was adopted for all further experiments.

Table 3
Effect of interferents on the recovery of 2.0 µg L^{-1} Cu(II) and 20.0 µg L^{-1} Pb(II) in water samples using SI-DLLME–FAAS.

Interferent	[Interferent]/[M(II)]	Recovery (%)	
		Cu(II)	Pb(II)
Al(III)	1000	97	96
Ag(I)	100	100	99
Cu(II)	100	–	95
Cd(II)	100	95	95
Co(II)	1000	99	100
Pb(II)	100	97	–
Fe(II)	1000	99	98
Fe(III)	1000	97	100
Zn(II)	1000	99	101
Mn(II)	1000	98	99
Ni(II)	1000	95	96
Hg(II)	100	96	95

3.5. Effect of ionic strength

The influence of ionic strength of the aqueous solutions on the absorbance was evaluated by adding various amounts of sodium chloride (NaCl) in the range 0–5.0% (m/v). The experimental conditions of the SI-DLLME method were the optimum. As it is shown by increasing the NaCl concentration no significant statistical differences were recorded throughout the studied range.

3.6. Extraction time

The process of mass transfer is time-dependent, while the extraction rate is reduced, as the system is going to the chemical equilibrium. Thereby, maximum absorbance is attained. However, in the on-line systems, the equilibrium is not necessary, as long as the extraction conditions are extremely reproducible.

The influence of the extraction time on the absorbance was investigated in the range 30–120 s. The results have shown, that the absorbance was increased almost proportional by increasing the extraction time. As a compromise between sensitivity and sampling frequency, an extraction time of 120 s was adopted throughout the experiments.

3.7. Interference studies

The effect of potential ions, encountered in natural water samples, on the recovery of 2.0 µg L^{-1} Cu(II) and 20.0 µg L^{-1} Pb(II) standard solution in the presence of various amounts of individual interfering ions, was examined. For this purpose the proposed SI-SLLME–FAAS method under the optimum conditions was adopted while a variation on the recovery greater than ±5% was considered as interference. The results are presented in Table 3. A significant advantage of DDPA is that it does not react with alkali, alkaline earth metals and others such as Mn, V, Ti, Co, Cr, Zn allowing the separation of major components of the matrix [34]. Moreover, the effect of common coexisting cations such as Ca(II), Mg(II), Ba(II),

Table 5
Analytical results of copper determination (dissolved fraction) in certified reference material and spiked natural waters samples with the SI-DLLME–FAAS method ([M] in µg L^{-1}).

Sample	Certified	Added	Found[a]	Recovery (%)
CRM 1643e	22.76 ± 0.31	–	22.15 ± 1.5	97.3
Tap water		–	0.82 ± 0.05	–
		2.00	2.75 ± 0.16	96.5
River water		–	1.94 ± 0.08	–
		2.00	3.85 ± 0.24	95.5
Coastal seawater		–	1.64 ± 0.11	–
		2.00	3.65 ± 0.26	100.5

[a] Mean value ± standard deviation based on three replicate measurements.

Table 6
Analytical results of lead determination (dissolved fraction) in certified reference material and spiked natural waters samples with the SI-DLLME–FAAS method ([M] in µg L^{-1}).

Sample	Certified	Added	Found[a]	Recovery (%)
CRM 1643e	19.63 ± 0.21	–	18.95 ± 0.9	96.5
Tap water		–	$<c_L$	–
		10.0	9.8 ± 0.4	98.0
River water		–	4.3 ± 0.3	–
		10.0	13.9 ± 0.7	96.0
Coastal seawater		–	5.0 ± 0.4	–
		10.0	14.5 ± 0.8	95.0

c_L: detection limit.
[a] Mean value ± standard deviation based on three replicate measurements.

Na(I) and K(I) were also investigated. No significant variation in the absorbance was observed at concentrations up to 1000 mg L^{-1}.

3.8. Analytical performance and analysis of natural waters

The performance data of the SI-LLME on-line preconcentration method for FAAS copper and lead determination under the optimum conditions are summarized in Table 4.

For a sample consumption 12.0 mL, the detection limit, based on 3 s criterion, was 0.04 and 0.54 µg L^{-1} while the precision (RSD) was 2.1% (at 2.0 µg L^{-1}) and 1.9% (at 30.0 µg L^{-1}) for copper and lead, respectively. The enhancement factor calculated by comparison the slops of the calibration graphs with and without preconcentration, was 560 and 265 for copper and lead, respectively.

The accuracy of the proposed method was evaluated, by the analysis of the certified reference material, NIST CRM 1643e (National Institute of Standard and Technology, Trace elements in water). The proposed method was applied to the analysis of tap water, river water and coastal seawater samples, which had been collected from Northern Greece. All the determinations were performed using aqueous standard solutions for calibration, without further matrix specific optimization for the samples. The certified

Table 4
Analytical performance characteristics of the SI-DLLME-FAAS method for copper and lead determination under the optimized conditions.

Parameter	Copper	Lead
Sample consumption (mL)	12.0	12.0
Extraction/preconcentration time (s)	120	120
Sampling frequency (h^{-1})	12	12
Enhancement factor	560	265
Linear range ([M] in µg L^{-1})	0.16–12.0	2.3–160.0
Detection limit (3 s), µg L^{-1}	0.04	0.54
Precision (R.S.D., $n = 10$) (%)	2.1 (2.0 µg/L)	1.9 (30.0 µg/L)
Regression equation ([M] in µg L^{-1}, $n = 9$)	$A = (0.0304 ± 0.00097)[Cu(II)] + (0.0014 ± 0.00598)$	$A = (0.0021 ± 0.00005)[Pb(II)] + 0.0014 ± 0.0045$
Correlation coefficient (r)	0.9993	0.9995

values and the analytical results are presented in Tables 5 and 6. The determined concentration of copper and lead were in good agreement with the certified values and the recovery ranged between 95.0 and 100.5% showing the good performance of the method in all type of natural waters samples.

4. Conclusions

A new on-line liquid–liquid microextraction technique for metal determination with FAAS was developed. The proposed method was based on the metal extraction from aqueous phase into the fine droplets of extraction solvent (cloudy solution), which were formed on-line from a ternary system (water–methanol–xylene). From commercial, economical and environmental point of view, SI-DLLME offers several important advantages: faster operation in micro-scale analysis, extremely low time, low cost and particularly low consumption of organic solvent thanks to the use of a syringe pump of SI manifold, simple manifold (no need of separation unit), high recovery and enhancement factor. In addition, the proposed system seems to have easier linkage to analytical methods. The results have shown that SI-DLLME is a precise, economic and reproducibly way for trace copper and lead determination in natural environmental samples.

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