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Automatic determination of copper by in-syringe dispersive liquid–liquid microextraction of its bathocuproine-complex using long path-length spectrophotometric detection

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ABSTRACT

The recently proposed concept of automatic in-syringe dispersive liquid-liquid microextraction was successfully applied to the determination of copper in environmental water samples. Bathocuproine was added to the organic phase as a selective reagent, resulting in the formation of a complex with copper. Dispersion was achieved by aspiration of the organic phase and then the watery phase into the syringe as rapidly as possible. After aggregation of the solvent droplets at the head of the syringe, the organic phase was pushed into a liquid waveguide capillary cell for highly sensitive spectrophotometric detection. The entire analytical procedure was carried out automatically on a multisyringe flow-injection analysis platform and a copper determination was accomplished in less than 220 s. A limit of detection of 5 nmol L^{-1} was achieved at an extraction efficiency $>90\%$ and a preconcentration factor of 30. A linear working range for concentrations of up to 500 nmol L^{-1} and an average standard deviation of 7% in peak height were found. The method proved to be well-suited for the determination of copper in water samples, with an average analyte recovery of 100.6%.

1. Introduction

Depending on its concentration, copper can be considered either essential or hazardous to life forms and plays a substantial role in the environment [1–7]. As a micronutrient, copper is responsible for the proper functioning of several metalloenzymes and related physiological processes. Toxicity is related to binding to thiole groups, oxidation processes and radical formation. Excessive intake of copper can cause accumulation especially in liver cells and cause, among others, hemolytic crisis and neurological disturbances [1,4].

An important alimentary source of copper is drinking and tap water [3]. In natural waters, copper concentration can be increased by domestic and industrial waste waters. Therefore, copper determination in both kinds of watery samples and methodological improvement are frequent and important objectives in analytical chemistry [2–10].

Due to the low concentrations of copper in environment, a preconcentration step is generally required [2–4,6,9,11]. Bathocuproine is a commonly used and highly selective reagent for copper determination giving an orange colored and hydrophobic complex, which can be easily extracted by liquid–liquid extraction (LLE) [12]. However, LLE has several disadvantages being a considerably lasting and laborious procedure and the requirement of a considerable amount of high-purity organic solvents, which are mostly harmful to health and environmental unsafe [2–6,8]. These difficulties have been addressed by the development of liquid phase microextraction techniques (LPME) [13], allowing analyte extraction and matrix removal with a minimal amount of solvents and often within a single preparative step. Depending on the way of bringing the organic solvent into contact to the aqueous phase, required supports, and modes of later phase separation, a variety of different techniques be distinguished, among others single-drop microextraction (SDME) [14], hollow fiber liquid phase microextraction (HF-LPME) [15], and recently developed dispersive liquid–liquid microextraction (DLLME) [16].

Briefly, a mixture of a water-immiscible extraction solvent and a so-called dispersing solvent, miscible in both phases, is rapidly injected into the watery sample. Hereby, the dissolution of the dispersing solvent in the sample leads to the disruption of the

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mixture into fine droplets and enormous increase of the contact surface between extraction solvent and sample being observable by the formation of a cloudy solution.

While SDME and HF-LPME require both long extraction times and careful handling of organic phase supports, DLLME yields fine extraction efficiencies using a very quick and simple procedure but to the disadvantage of a higher volume of organic solvent due to the required dispersion solvent [17].

DLLME has been applied to the determination of copper using, e.g. ionic-liquids as extractive solvent (IL-DLLME) [3], solvents lighter than that of water followed by phase separation based on solidification of a floating organic drop (DLLME-SFO) [18], and ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME) [19]. Methods, which have used DLLME as the preconcentration step for copper determination, are summarized in Table 1.

Besides the scientific effort of miniaturization of these techniques, in the recent years, a main focus has been drawn to their automation. A strategy for the automation of microextraction techniques is their implementation using flow techniques such as flow injection analysis (FIA) [20], sequential injection analysis (SIA) [21], or their consequent hyphenation being multisyringe flow injection analysis (MSFIA) [22]. Flow techniques are based on the handling of liquids in a tubing system denoted manifold where valves for solution injection or re-direction as well as processing devices such as columns or phase separators can be included. Controlled and reproducible solution handling (injection, mixing, and transport) enable a gain in reproducibility or the complete analytical procedure in addition to stand-alone operation and the possibility to develop monitoring applications.

Table 2 shows the brief evolution of different strategies based on flow techniques used recently for the development of on-line DLLME procedures. Since DLLME does not require renewable solid supports for the extraction solvent such as hollow membrane or capillary tubes, its automation using flow techniques is straightforward. However, there is a lack of DLLME automation while in contrast, reports on manual approaches of DLLME are rapidly increasing since 2006 [16].

To the best of our knowledge, the first automation of DLLME using SIA was done by Anthemidis et al. (2009) [2]. In this and following works [23,24], DLLME was achieved by the rapid injection of the extractant into the sample flow and the extraction solvent including formed target analyte complexes were retained on PTFE-turnings within a micro-column for phase separation. Later quantification was done by direct coupling to AAS techniques after elution with another solvent.

Andruch et al. (2012) [25] used two SIA systems, one for watery phases, one for organic phases, both coupled to one conical extraction cell, in which the DLLME procedure was accomplished. After phase separation by sedimentation, the extraction solvent was aspirated and pushed through the spectrophotometric detection flow cell.

While in the first approach, phase separation was done on a solid phase column, requiring additional time and eluent solvent, the second approach required a complex analyzer system for separated phase handing.

Melwanki et al. (2008) [26] used a syringe as an alternative extraction unit for DLLME followed by a semi-automatic LLE for back-extraction of the analyte to a fresh watery phase. Cruz-Vera et al. (2009) [27] performed in-syringe DLLME manually using a 10 ml plastic syringe as the extraction unit and a 1 ml glass syringe for the injection of the extracting and dispersing solvent mixture.

Fully automatic in-syringe DLLME was recently reported by Maya et al. (2011) [28] for the first time applying the MSFIA technique for extraction of benzo(a)pyrene and its subsequent chromatographic separation on a monolithic column and spectrophotometric determination.

In the present work, in-syringe DLLME technique was used for the fully-automatic determination of inorganic copper based on its reduction to Cu(I), formation of an extractable complex with bathocuproine, and the concomitant quantification by means of long path-length spectrophotometry. The former method and instrumentation was improved in several aspects. The proposed system proved to be a useful tool for the spectrophotometric detection of copper in natural waters.

Table 1
Comparison of various methods for determination of copper in water samples using DLLME techniques.

Detection	Chelating agent	*VS* [mL]	A	LOD [$\mu g\ L^{-1}$]	Concentration range [$\mu g\ L^{-1}$]	Reference
FAAS	8-HQ	5	No	3.0	50–2000	[10]
FAAS	No need	8	No	0.5	1–600	[32]
FAAS	TMK	10	No	0.45	2–50	[3]
FAAS	DDPA	12	Yes	0.04	0.16–12	[2]
FAAS	8-HQ	20	No	0.1	0.5–300	[18]
EFAAS	BAT	5	No	0.03	2–50	[9]
FO-LADS	BPDC	10	No	0.34	2–70	[6]
UV–vis*	8-HQ	5	No	10	10–4000	[8]
UV–vis	DIDC	5	No	5.0	20–90	[7]
UV–vis	DDTC	15	No	0.5	0–200	[4]
UV–vis	BCP-disulphonate	10	No	0.4	0–40	[11]
UV–vis	Neocuproine	5	No	0.33	1–200	[5]
UV–vis	Na-DDTC	40	No	0.05	0.5–50	[19]
UV–vis	BCP	3.75	Yes	0.34	0–32**	Proposed

VS: Volume of sample, A: Automation, 8-HQ: 8-Hydroxy quinoline, TMK: 4,4'bis (dimethylamino)thiobenzophenone, DDPA: Diethyldithiophosphate ammonium, BAT: S,S-bis(2-aminobenzyl)-dithioglyoxime, BPDC: 4-benzylpiperidineditiocarbamate potassium, DIDC: 1,3,3-trimethyl-2-[5-(1,3,3-trimethyl-1,3-dihydroindol-2-ylidene)-penta-1,3-dienyl]-3H-indolinum, DDTC: Diethyldithiocarbamate, BCP: Bathocuproine, Na-DDTC: Sodium diethyldithiocarbamate, EFAAS: Electrothermal atomic absorption spectrometry, FAAS: Flame atomic-absorption spectrophotometry, FO-LADS: Fiber optic-linear array detection spectrophotometry, UV–vis: Ultra-violet and visible spectrophotometry.

* Detection after separation with HPLC.

** Extension of the working range is possible by on-line dilution or by using a smaller sample volume.

Table 2
Applications of automatic dispersive liquid–liquid microextraction using flow techniques.

Flow technique	Detection	Analyte	DLLME	Phase separation	Reference
SIA	FAAS	Copper, Lead	Tubing	Column	[2]
SIA	ETAAS	Lead, Cadmium	Tubing	Column	[23]
SIA	FAAS	Silver	Tubing	Column	[24]
FIA	ETAAS	Selenium	Mixing chamber	Column	[17]
SIA	UV–vis	Tiocyanate	Mixing chamber	No need	[25]
MSFIA	UV–vis	Naproxen, Benzo(a)pyrene	Syringe	Injection loop	[28]
MSFIA	UV–vis	Copper	Syringe	Injection loop	[Proposed]

Detection techniques: ETAAS: Electrothermal atomic absorption spectrophotometry, FAAS: Flame atomic absorption spectrophotometry, UV–vis: Spectrophotometry.

2. Methods and materials

2.1. Reagents

Bi-distilled water obtained from a MilliQ Direct-8 purification system (Millipore Iberica S.A.U., Madrid, Spain) and analytical grade reagents were used throughout. The acetonitrile (ACN) was of HPLC grade quality, and the bathocuproine p.a. (BCP, 2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline) was purchased from Carlo Erba Reagents (Peypin, France).

A stock solution of 33.5 mg/20 mL bathocuproine in 1-butanol was prepared and the solution was stored in the dark bottle. A stock solution of $CuSO_4 \cdot 5H_2O$ of 1 mmol L^{-1} was prepared in water. Cu(II) standard solutions were prepared by appropriate dilution of the stock solution. As recommended elsewhere [10], a mixed reagent of 40% w/v sodium acetate and 10% w/v hydroxylammonium chloride was used for pH adjustment and reduction of Cu(II) to Cu(I).

2.2. Manifold configuration

The manifold used is depicted in Fig. 1. PTFE tubing was used with 0.8 mm and 1.5 mm inner diameters (id), respectively.

For liquid handling and distribution, a multisyringe pump (Multi-Buret 4S) and a valve module (V1+1) with one rotary 8-port selection valve and one rotary 6-port injection valve, both from Crison SL (Alella, Barcelona), were used. The multisyringe pump was equipped with 3 syringes (S1, S2 and S3) of 10 mL, 5 mL and 2.5 mL, respectively, from Hamilton (Bonaduz, GR, Switzerland).

The selection valve was used for the handling of solutions required for DLLME and for cleaning, while the injection valve served for separation of the organic phase after extraction and for introducing it into the solvent flowing towards the detection flow cell (see Section 2.5).

DLLME was carried out in S2, while S3 and S1 were for used for dilution of the extraction solvent and for propelling the mixture through the Liquid Waveguide Capillary Cell (LWCC), respectively. Since all syringes move simultaneously, the 3-way solenoid head valves shown are for connection to either the manifold in the ON position or to their respective liquid reservoir (S1, S3) for solution recycling or waste (S2) for discharge in the OFF position.

The head valve position of S2 was connected to the central port of the selection valve by PTFE tubing 15 cm in length and 0.8 mm id. External ports on the selection valve were connected to reservoirs for water (1), 1% nitric acid (2), ACN (3), reagent (5), sample (6) and extraction solvent mixture (7). At port 8, a dilution chamber (DC), constituted by 5 mL pipette tip, was placed. Port 4 was connected via a short PTFE tube to a 3-way confluence (T) made of Ultem (polyetherimide) for inline addition of ACN from S1 and further to the injection valve (LOAD). S3 was connected directly to the injection valve (INJECT) for pushing the organic phase, previously injected, through a mixing coil to the detection flow cell. The last selected injection loop (see Section 3.6) was 52 cm in length and 0.8 mm id.

A backpressure coil (BC) was placed at the outlet of the detection cell to increase the pressure within the capillary cell and to suppress gas bubble formation.

2.3. Detection cell and equipment

For detection a Liquid Waveguide Capillary Cell (LWCC) from World Precision Instruments Ltd. (Hitchin, Hertfordshire, UK) of 100 cm light path was used throughout. A bright-white LED was used as the light source and was directly mounted on one fiber optic port of the LWCC. For detection, the other fiber optic port of the LWCC was coupled to a miniature USB diode array spectrometer from Ocean Optics (Dunedin, FL, USA) via an optical fiber of 600 μm core diameter.

Spectrometer configuration was 40 ms integration time, averaged over 5 values, and a measuring frequency of 4 Hz. The difference between the absorbance values measured on a wavelength giving the maximal absorbance for the reaction product (478 nm) and a reference wavelength (580 nm) was used as the analytical signal. The reference wavelength allowed for the correction of analyte-unspecific intensity variations.

2.4. Software control and data handling

AutoAnalysis 5.0 software (Sciware SL, www.sciware-sl.com, Palma de Mallorca, Spain) was used for operating the flow instrumentation, data acquisition from the USB2000 spectrophotometer and data processing.

The program, written in Delphi and C, allows the definition and execution of instruction protocols, including the use of variables, loops, waiting steps and procedures on a Windows-based user interface. Detailed descriptions can be found elsewhere [29,30].

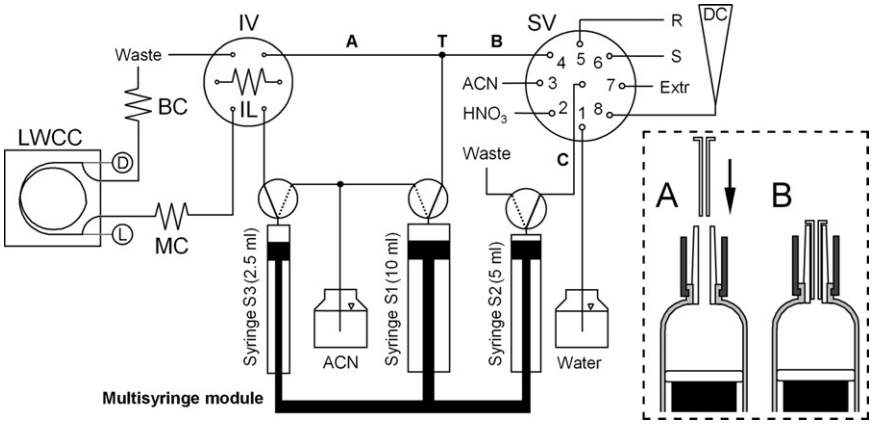

Fig. 1. MSFIA manifold developed in this work. Abbreviations used, A: Confluence, BC: Backpressure coil (50 cm, 0.8 mm id), D: spectrophotometer (Detector), DC: Dilution chamber, IL: Injection loop (52 cm, 0.8 mm id), IV: Injection valve, L: LED (light source), LWCC: Liquid waveguide capillary cell (connected light source and spectrometer not shown), MC: Mixing coil (16 cm, 1.5 mm id), SV: Selection valve, T: confluence of UTEM, ACN: Acetonitrile, Extr: Extraction solvent, R: Reagent, S: Sample. Further tubing lengths A, B, and C: 5 cm, 5 cm, 15 cm, respectively, 0.8 mm id each. A & B: Modification of the inlet of syringe 2 using a short PTFE tube (grew: brass, light grew: glass, white: PTFE, black: piston).

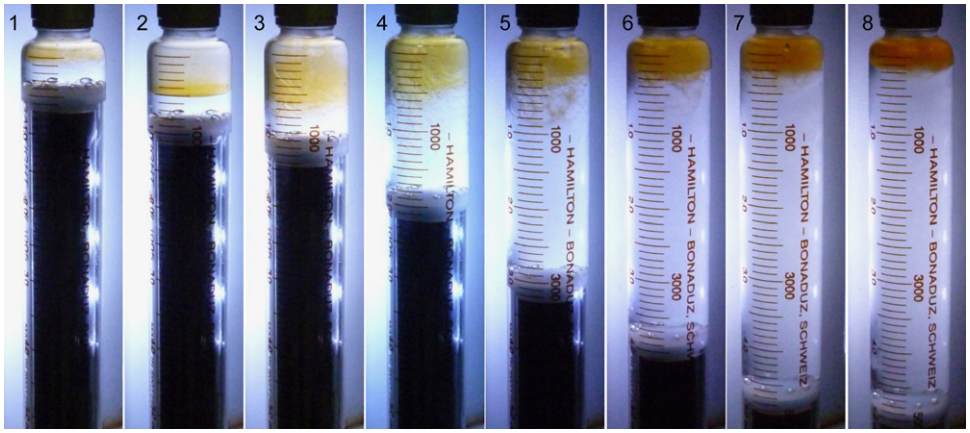

Fig. 2. Photo series of extraction and phase separation. 1: Aspiration of extractant, 2: Extractant fully aspirated, 3–7: DLLME (16 s in total) after 1.5 s, 4 s, 8 s, 12 s and 15 s, respectively, 8: after 30 s of phase separation. A 1 mmol L^{-1} copper standard was used for visualization.

2.5. Analytical protocol and flow method

The instrument is initialized by passing 1.25 mL of ACN from S3 through the detection cell for cleaning and subsequent blank measurement. Afterwards, the dilution chamber and the supply tubes could be cleaned if required, enabled on user demand. Finally, all syringes were emptied completely into their reservoirs (content re-circulation).

For the analytical protocol, first, 0.25 mL of reducing reagent and 3.75 mL of sample were thoroughly mixed, first by aspiration of both at the highest speed into S2 and then by pushing them into the dilution chamber, emptying S2 completely. After a reaction time of 10 s, 0.7 mL of the organic phase was aspirated into S2 followed by the complete contents of the dilution chamber at the highest speed, thus causing the dispersion of the organic phase in the sample volume.

After 30 s, the xylene droplets were suspended and combined at the top of the syringe and then dispensed through the confluence T, where ACN is added from S1. The major part is then used to fill the injection loop, followed by the injection of this volume into an ACN flow propelled by S3 and propelling it through the detection flow cell. Simultaneously, the watery phase and possible air bubbles are expelled from S2 as waste. The DLLME and phase separation is shown in a photo series in Fig. 2.

3. Results and discussion

3.1. System design

A compromise had to be made between a high concentration factor, i.e. a minimal volume of organic phase and maximum volume of sample, and sensitive and robust measurement, that is filling the LWCC as much as possible with organic phase and a homogeneous refraction index of the solution in the LWCC during measurement. Dilution of the organic phase with ACN prior to injection allowed these objectives to be fulfilled.

The volumes required to fill the injection loop completely were adjusted in order to inject the major part of the organic phase into the carrier of syringe 3, being ACN. The injection valve was used for the separation of the watery and organic phases.

In a previous work, it was found that the syringe series 1002TLL from Hamilton used on a multisyringe pump of more than 5 mL showed ideal dimensions for DLLME, while larger syringes did not show a sufficiently conical outlet to facilitate phase separation and complete expulsion of the organic phase. Since the organic phase tended to stick on the normal piston head made of PTFE, we used for S2 a piston from the "salt line" syringe series from the same company, where the piston head is made of Ultra High Molecular Weight Polyethylene, a less hydrophobic material.

The diameter of the entrance channel of S2 was diminished by placing a short PTFE tube into it. It enabled a higher flow velocity in the inlet of the syringe and consequently higher efficiency of the organic solvent dispersion.

3.2. Selection of the extraction solvent

The main requirements of the extraction solvent are a very low solubility in water, high-dissolving power for the target complex, and—in the presented work—a density lower than that of water. In the first work on in-syringe DLLME [28], n-octanol was used as the extraction solvent, but this showed several drawbacks. These were a high viscosity that inhibited the dispersion process and the fact that it stuck to surfaces, especially to the PTFE tubing walls and syringe piston head.

Cyclohexane, hexane, n-octanol, toluene, and xylene were tested in 1:9 v/v mixtures with ACN. Results from measurements of 100 nmol L^{-1} Cu(II) and blanks are given in Fig. 3A. Cyclohexane and hexane yielded peak heights about three-times lower than those of octanol. For cyclohexane, a baseline alteration was further observed and gas bubbles inhibited data evaluation.

With n-octanol, high peaks were obtained but peak-tailing, baseline alterations, and solvent accumulation on the head of the syringe piston were further observed, which could contribute to cross-over sample contamination. The phase separation and merging of the extraction solvent droplets at the head of the syringe is further slow for octanol due to the high viscosity, which resulted in an unacceptably low repeatability of measurement.

Toluene and xylene gave similar, well-defined peaks, but these were about 50% lower than the peaks obtained with octanol, while relations between the Cu(II) standard and blank signals were similar. In comparison with n-octanol, repeatability was further improved, with was reduced to the lower viscosity of the aromatic solvents and better led to better droplet combination. Due to its lower toxicity, vapor pressure and higher reproducibility compared with toluene, xylene was chosen as the extraction solvent for all further experiments.

3.3. Selection of the dispersing solvent

In DLLME, an additional solvent (disperser) soluble both in the extractant and water is required. The disperser is initially mixed with the extractant. Due to the highly turbulent mixture of both phases, the rapid dissolution of this mediator or dispersing solvent

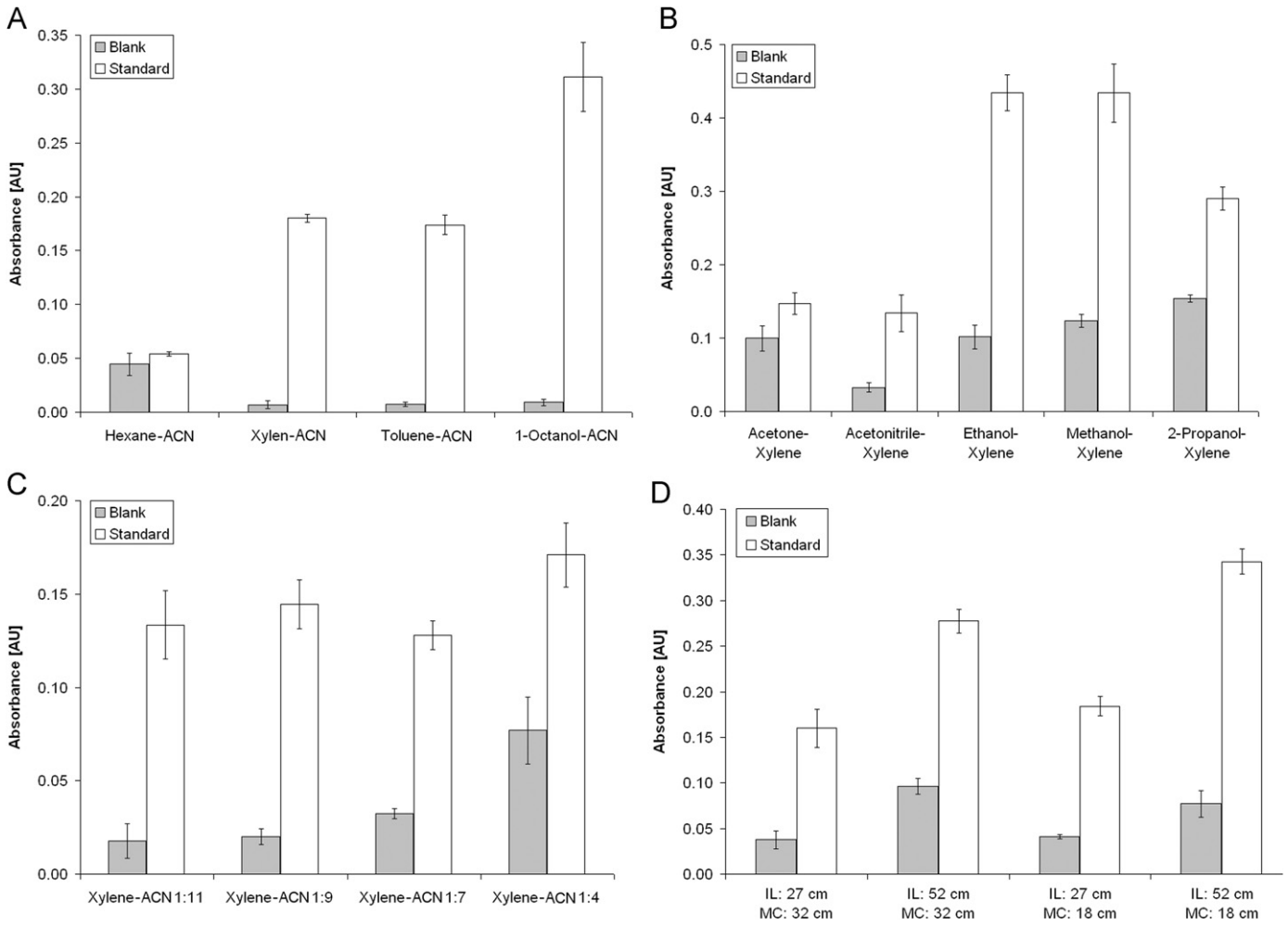

Fig. 3. Representations of the study of extraction solvent (A), dispersing solvent (B), Solvent ratio (C) and injection and mixing coil lengths (D).

causes the breaking up of the extractant into fine droplets observable by high turbidity and thus an enormous increase of the extraction surface area. Solvents typically used in DLLME were tested with xylene and n-butanol in a ratio of 1:0.6:8.4: acetone, ACN, ethanol, methanol and 2-propanol. Results are given in Fig. 3B for measurements of 100 nmol L^{-1} Cu(II) standard and blank.

Best results expressed as the peak height of the measured analytical signals were obtained for methanol and ethanol. The signal decrease of about a 30% was observed using 2-propanol, which was probably due to its higher water solubility and the increase of sample temperature due to exothermic dissolvation with concomitant increase of the formation of bubbles. However, the significant occurrence of small gas-bubbles during DLLME, which accumulated at the phase boundary layer and stacked further at the syringe piston head, led to poor reproducibility, unacceptable blank signals and baseline and peak distortions. In the case of 2-propanol, the blocking of the capillary cell was observed. Problems with gas-bubble formation and high blank values were also observed for acetone, while ACN gave similar signal heights but a lower blank value. Since the ratio between the Cu(II) standard and the blank signals was slightly higher for ACN than for ethanol or methanol and signal reproducibility was significantly better, ACN was further used as dispersing solvent.

3.4. Ratio of extraction and dispersing solvent

Different ratios of extraction and dispersing solvents were tested from 1:11 to 1:4 using an ACN-BCP stock solution. Results for measurements of the 100 nmol L^{-1} Cu(II) standard and the blank are given in Fig. 3C. Both the standard and the blank signals increased for higher amounts of xylene, and due to the best ratio of both and the best reproducibility, a 1:9 ratio was used further.

3.5. Concentration of bathocuproine

The influence of the bathocuproine (BCP) concentration on the signal peak height was studied in the range of 25 mg L^{-1} to 325 mg L^{-1} of extractant (data not shown). It was found that the blank values increased linearly, while a saturation curve with a characteristic concentration of 80 mg L^{-1} BCP was found. The highest difference between a 100 nmol L^{-1} Cu(II) standard and blank was found for a concentration of 280 mg L^{-1} of bathocuproine, and this concentration was used for all further experiments.

3.6. Lengths of injection loop and mixing coil

Prior experiments were carried out using an injection loop (IL) of 27 cm, 0.8 mm id, and a mixing coil (MC) of 32 cm, 1.5 mm id. The organic phase volume after phase separation and study of extractant composition was about 120 μL, and thus large enough to permit an increase of the injection volume by a factor of 2. Likewise, a half-length MC was tested in order to decrease the dispersion of the injected volume before entering the detection flow cell. A comparison of all four possible combinations is shown in Fig. 3D.

Both the Cu(II) standard and blank signals increased by a factor of about 2 upon using an IL longer than expected. With a shorter MC, a further increase of the standard signals was observed, while the effect on the blank signal was not significant. Therefore, both modifications, i.e. the longer IL and the shorter MC, were adopted for all further experiments. An even longer IL was considered to be impractical due to the higher probability to injected droplets of the watery phase or bubbles.

The dispersion factor of the injection volume was evaluated to be 3.2. For this, a 2 ppm rhodamine B solution in ACN was injected and the signal compared with the one obtained using the LWCC completely filled with the same solution.

3.7. Volume of extractant

Once the extractant composition and injection loop volume were optimized, the volume of extractant was studied in the range of 0.6 to 1 mL in order to reduce the final volume of the organic phase as much as possible and thus improve the DLLME enrichment factor of the analyte. Decreasing the volume of the extractant improved the tendency, sensitivity and peak height repeatability as shown in Table 3. However, with less than 0.7 mL, the appearance of air bubbles, apparent from baseline disruption, was increased, and therefore, 0.7 mL of extractant was chosen as the optimal volume.

3.8. Concentration of reducing agent and reaction time

The concentration of hydroxylammonium chloride used to convert Cu(II) into extractable Cu(I) complex was studied for 5, 10, 20 and 30% w/v for reaction times of 0, 10, 30 and 60 s in the dilution chamber. A higher yield was obtained throughout for 10 s, while for longer reaction times no further improvement was found. No significant difference was found in the signal yield using higher concentrations, thus the initial concentration of 10% w/v was kept for further experiments.

3.9. Concentration of sodium acetate

The influence of sodium acetate concentration on the reducing reagent for pH buffering was studied for 10, 20, 30, 40 and 70% w/v when using 0.25 mL of reagent to 3.75 mL of a 100 nmol L^{-1} Cu(II) standard and blank solution. The results are shown in Table 4. While the blank values decreased by about 25% towards the highest concentration, an equal increase in the standard peak signal was observed. This was explained by the higher extraction efficiency due to a salting-out effect and better phase separation, inhibiting the inclusion of water droplets in the organic phase. In fact, preparation of the highest concentration was highly impractical, so a concentration of 40% was used further.

Table 3
Results from the study of extractant volume given as mean and standard deviations ($n=3$). Conditions: 100 nmol L^{-1} Cu(II) standard, extractant: 10% v/v xylene, 5.75% n-buthanol, 8.25% v/v ACN, 280 mg L^{-1} bathocuproine, sample volume: 3.75 mL.

Volume extractant [mL]	Peak height [AU]	Relative change to "0.6 mL" [%]
0.6	0.445 ± 0.033	100
0.7	0.450 ± 0.059	97
0.8	0.415 ± 0.080	93
0.9	0.395 ± 0.051	89
1.0	0.381 ± 0.089	84

4. pH of reagent and acidity of sample

In a previous work using BCP for automatic but not-dispersive LLE, reduction of Cu(II) to Cu(I) and its subsequent complexation with BCP were found to be independent of the buffer pH in the range from 4 to 8 [31]. Therefore, the influence of the buffer was studied only in the small range from pH 4.7 to pH 6.5, adjusting the pH with acetic acid and sodium hydroxide. Since no significant differences in sensitivity or reproducibility were observed, the original conditions were maintained.

Nitric acid is a typical preservative for trace metals. Since the generally used 2% of concentrated nitric acid (65% w/v) was shown to be incompatible with the present approach, the influence of nitric acid in another addition to the sample was studied further. The highest concentration used was 0.4% v/v (41.2 mmol L^{-1}). The results are given in Table 5. It was observed that any addition higher than 0.1% v/v led to unacceptable loss of efficiency ($>5\%$). Therefore, the addition of 0.1% v/v of concentrated acid causing a sample pH of about 2 was applied further.

4.1. Flow rate for DLLME

The flow rate of sample aspiration for performing dispersive liquid–liquid microextraction was studied for 8, 10, 12 and 15 mL min^{-1}, the last one being the fastest possible for the configuration and instrumentation used herein. The signals decreased by only about 30% when using lower flow rates, indicating efficient dispersion of the chosen extractant. The highest flow rate was maintained as the best condition obtained.

4.2. Interference study and natural samples

The following compounds were tested by addition to a 100 nmol L^{-1} Cu(II) standard without any significant effect on the signal heights: NH_4Cl (100 μmol L^{-1}) and $CaCl_2$, $MgCl_2$, NaCl,

Table 4
Results from the study of sodium acetate given with mean and standard deviations ($n=3$). Conditions: 100 nmol L^{-1} Cu(II) standard and blank, extractant: 10% v/v xylene, 5.75% n-buthanol, 8.25% v/v ACN, 280 mg L^{-1} bathocuproine, sample volume: 3.75 mL.

Concentration of sodium acetate in reagent [% w/v]	Blank [mAU]	Standard 100 nmol L^{-1} [mAU]
10	53 ± 5	203 ± 15
20	38 ± 2	188 ± 16
30	37 ± 2	214 ± 12
40	41 ± 7	218 ± 16
70	33 ± 1	263 ± 24

Table 5
Results from the study of nitric acid concentration in a standard solution given as mean and standard deviations ($n=3$). Conditions: 100 nmol L^{-1} Cu(II) standard and blank, extractant: 10% v/v xylene, 5.75% n-butanol, 8.25% v/v ACN, 280 mg L^{-1} bathocuproine, sample volume: 3.75 mL.

Concentration HNO_3 [mmol L^{-1}]	Peak height [AU]	Relative change to "0.6 mL" [%]
0	0.211 ± 0.010	100
5.9	0.232 ± 0.019	99
11.8	0.215 ± 0.011	98
14.7	0.198 ± 0.002	95
17.6	0.206 ± 0.024	91
29.4	0.169 ± 0.026	82
41.2	0.161 ± 0.012	69

Table 6
Data from real sample analysis.

Type	pH & Conductivity	Added Cu Conc.[nmol L^{-1}]	Found Conc. DLLME [nmol L^{-1}]	Recovery [%]
Mineral water 1	pH 6.7	–	11.6 ± 1.1	107.6
Low mineralization	90 µS cm^{-1}	50	65.4 ± 2.7	
Mineral water 2	pH 7.2	–	41.7 ± 5.8	93.5
High Mineralization	442 µS cm^{-1}	50	88.4 ± 3.6	
River effluent	pH 8.4	–	34.2 ± 6.9	101.6
–	534 µS cm^{-1}	100	136 ± 6.9	
Fountain water	pH 7.9	–	192 ± 4.2	94.6
	1000 µS cm^{-1}	50	239 ± 11	
Well water	pH 7.8	–	174 ± 7	94.5
	643 µS cm^{-1}	100	269 ± 11	
Ground water	pH 7.8	–	9 ± 1	104.4%
	684 µS cm^{-1}	100	113.5 ± 7.1	

$Al_2(SO_4)_3$, and Na_2CO_3 (all 10 mmol L^{-1}). Transition metal cations Fe(III), Mn(II), Ni(II), and Pb(II) were tested in a final concentration of 1 µmol L^{-1}; and Ag(I), Al(III) and Zn(II) in a final concentration of 0.5 µmol L^{-1}. No significant alterations were observed apart from silver, where a 33% signal decrease was observed.

Various water samples, including mineral, well, fountain, river and ground water, were analyzed with the proposed analyzer system for comparison. The only sample pre-treatment was the addition of 0.1% v/v nitric acid before analysis, giving a final sample pH of about 2.

For estimating analyte recovery, measurements of spiked samples were further performed. The results are given in Table 6. The average recovery was 100.6%, and values ranged from 93.5 to 107.6%, indicating the applicability of the method for real sample analysis.

4.3. Method performance and final discussion

Using the proposed protocol, an enrichment factor of about 30 for DLLME and an extraction efficiency higher than 95% were calculated from the final organic phase volume after phase separation (120 µL), the dispersion factor[2] (3.2) of the injected organic phase, LWCC path length and the reported molar absorbance of the BCP-copper complex [10].

For a higher concentration range, either the sample could be diluted in the dilution chamber prior to extraction or a smaller sample volume could be used for the extraction procedure.

A linear concentration range between 10 nmol L^{-1} and 500 nmol L^{-1} was proven with Cu(II) standards. Limit of detection (LOD) and limit of quantification (LOQ) were calculated from the concentrations, yielding the three-fold and ten-fold standard deviations of ten subsequent blank measurements, respectively, which were 5.3 nmol L^{-1} and 17.7 nmol L^{-1} following a calibration function of $A = 1.77 \pm 0.20,\ 10^6\ \mu mol^{-1} \ldots C + 0.028 \pm 0.010$ ($n = 5$, day-to-day reproducibility).

The entire extraction and analysis procedure, including initial syringe cleaning with ACN and sample, can be accomplished in less than 220 s, enabling an injection frequency of 16 h^{-1}. Only 2.7 mL of ACN were required for each analysis: 0.55 mL in the extraction solvent, 0.5 mL for dilution in syringe 1, and 1.65 ml as carrier from syringe 3 to propel the organic phase to the detection cell. The required volume of xylene and butanol were 70 µL and 60 µL per analysis, respectively. A reduction of ACN consumption would be possible using a shorter LWCC detection cell.

[2] The dispersion factor is defined as the signal of the undiluted sample divided by the signal height obtained with the actual sample volume, which is diminished by the dilution within the carrier flow.

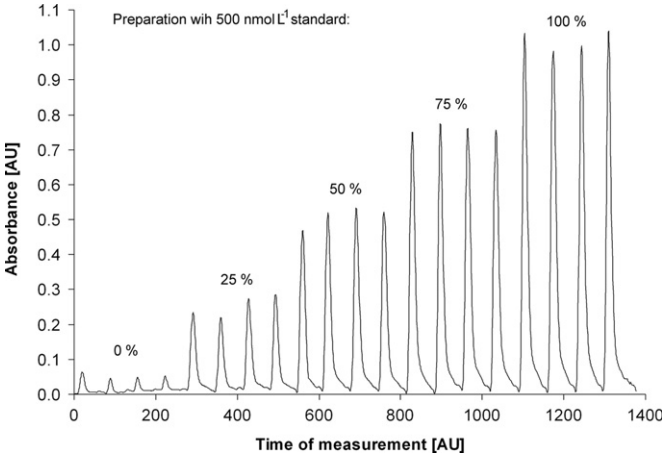

Fig. 4. In-system prepared calibration by dilution of a 500 nmol L^{-1} Cu(II) standard with MilliQ water in the dilution chamber in given ratios, with a final volume of 3.75 mL corresponding to concentrations of 125 nmol L^{-1}, 250 nmol L^{-1}, 375 nmol L^{-1} and 500 nmol L^{-1}.

In comparison with the previously reported manual and automatic approaches using DLLME summarized in Table 1. A similar or superior performance with respect to detection limits and linear working range was achieved.

A similar consumption of solvents was reported for manual approaches, as summarized in a recent review on DLLME, not mentioning the solvent consumed for cleaning or waste production by consumables. A further reduction of ACN without loss of sensitivity would be possible using an LWCC three times shorter and by omitting prior dilution of the organic phase. It might be further advantageous to increase the ratio between the reagent and the sample to decrease the potential effects of sample ion strength and pH. Consequently, a higher nitric acid concentration in the sample would be tolerable, and the possible influence of organic matter in the sample due to a decreased solubility of copper by formation of complexes would be lower.

It should be pointed out that by replacing a fraction of the sample volume with water as a supplement, in-system dilution is possible. This allows the extension of the linear working range as well as in-line preparation of Cu(II) standards or standard additions to natural samples, thus saving precious time. The usefulness of this approach is demonstrated with the peak examples of an in-system prepared calibration curve using only a 500 nmol L^{-1} Cu(II) calibration standard, as is seen in Fig. 4.

The presented method is focused on the determination of copper dissolvable at a of pH 2. A comparison of the presented

method and a standard method for copper determination such as ETAAS and ICP-AES was not carried out due to the digestion step required for the complete break-down of organic sample matrix in order to guarantee well comparability of results. Such digestion methods mostly require high acid concentrations, which can hardly be compensated by the addition of the buffer volume optimized in this work. Automatic copper extraction without prior digestion showed to be applicable to a variety of samples, however, future work would have to be directed to achieve robustness to digested samples to apply the method to samples with elevated organic content and complex matrices.

5. Conclusions

The recently described technique of in-syringe dispersive liquid-liquid microextraction was successfully applied for the fully-automatic copper determination, using bathocuproine as the selective reagent and subsequent spectrophotometric detection exploiting LWCC. The system proved to be well-suited or the analysis of copper in water samples. Fast analysis can be performed with similar or superior performance in respect to sensitivity, solvent consumption and working range as that of reported manual procedures applying DLLME or using commercial instrumentation. The high versatility of the automatic analyzer system was demonstrated by in-line standard preparation.

In this work we propose the first fully-automatic system able to accomplish the dispersive liquid–liquid microextraction of metals and its subsequent spectrophotometric detection, which in this case has been applied to the determination of copper in different kind of water samples. The applicability of the proposed system can be extended to other metals, which are able to react with ligands developing products extractable in organic solvents and detectable by spectrophotometry.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.05.063.

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