An automated system for liquid–liquid extraction based on a new micro-batch extraction chamber with on-line detection
Preconcentration and determination of copper(II)

Maria Celeste Teixeira Diniz a, Orlando Fatibello Filho b, Jarbas J.R. Rohwedder a, ∗

a Analytical Chemistry, Instituto de Química, Universidade Estadual de Campinas, CP 6154, 13083-970 Campinas-SP, Brazil
b Departamento de Química, Universidade Federal de São Carlos, Rod. Washington Luiz, Jd. I.235, CP 676, 13560-970 São Carlos-SP, Brazil

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Abstract
An automated system to perform liquid–liquid extraction is proposed, in which the effective mixture (the intimate contact) between the aqueous phase and the organic phase, as well as the separation of the phases, are carried out in a micro-batch glass extraction chamber. Sample, reagents and organic solvent are introduced into the glass extraction chamber by a peristaltic pump using air as carrier. The detection of the extracted species from the aqueous phase is made in a small volume (120–150 μl) of isobutyl methyl ketone (MIBK). The system allows enrichment factors of 2–10-fold. The proposed automatic system was evaluated for Cu(II) extraction based on complex formation between copper(II) and 1-(2′-pyridylazo)naphthol (PAN) in MIBK. When a volumetric ratio of 2:1 (aqueous:organic) was implemented, copper was detected in the concentration range of 100–1600 μg l⁻¹ (r = 0.9995) with a relative standard deviation of 2% (200 μg l⁻¹, n = 5) and a detection limit of 20 μg l⁻¹. The analytical curve was linear over the concentration range 25–500 μg l⁻¹ (r = 0.9994) when a volumetric ratio of 10:1 was employed. With this ratio, the detection limit was 5.0 μg l⁻¹ and the relative standard deviation was 6% (50 μg l⁻¹, n = 5).

Keywords: Liquid–liquid extraction; Micro-batch extraction chamber; Copper

1. Introduction
Recent advances in analytical instrumentation enable the analysis of many samples at high speed. However, despite these advances it is still often necessary to use separation and preconcentration methods prior to the quantitative determination, mainly because of the low-level concentration common in toxic species determinations. Better detection limit values might be obtained by a complete separation of the species of interest [1]. Thus, for determination of samples of low analyte content, it is necessary to incorporate a preconcentration step in the analytical procedure. Solvent extraction is one of the most widely used method for preconcentration and separation of trace elements because of its simplicity, speed, and wide scope. In analytical applications solvent extraction may serve the following three purposes: preconcentration of trace elements, elimination of matrix interferences, and increased selectivity. Manual liquid–liquid extraction procedures are usually very tedious, involving a large consumption of solvents and chemical reagents and are subject to potential contamination from the atmosphere and the chemical glassware. In addition, the classic manual liquid–liquid extraction process requires manipulation of significant volumes of hazardous and/or toxic organic solvents. Automation of this type of sample pretreatment can greatly reduce operation time, especially when handling large numbers of samples [2]. The disadvantages related to the classic liquid–liquid extraction process can be reduced by performing the extraction in automated systems, decreasing the potential for sample contamination, which is critical in trace analysis.

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E-mail address: jarbas@iqm.unicamp.br (J.J.R. Rohwedder).

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A great variety of flow systems have been demonstrated for the automation of the liquid–liquid extraction process. Segment flow analysis (SFA), proposed by Skeggs [3] in 1957, was the first flow system employed in liquid–liquid extraction. Karlberg and Thelander [4] and Bergamin et al. [5] independently proposed a system to carry out liquid–liquid extraction in flow injection systems (FIA). Based on this idea, different flow injection solvent extraction systems (FI-SE) were proposed in a large number of analytical procedures [6–15]. Monosegmented continuous-flow analysis (MCFA), proposed by Pasquini and Oliveira [16], has been evaluated for automatic control of liquid–liquid extraction involving single phase [17] and film extractions [18]. Sequential injection analysis (SIA) [19,20] and multicommutation [21], whose principal characteristics are the use of a single pumping channel, has also been employed for on-line liquid–liquid extraction and preconcentration.

However, liquid–liquid extraction can be automated without a continuous flow. Sweekle and Dasgupta [22] introduced in 1988 a new alternative in terms of a automated liquid–liquid extraction using a batch-type microscale liquid phase analyzer. In this approach, the content of the reaction chamber, aqueous and organic phases, were effectively mixed and the phase separation and detection happened inside this reaction chamber. A batch automated liquid–liquid extraction and detection system employing an organic solvent drop was proposed by Liu and Dasgupta [23]. In this system, an organic solvent drop with a volume of approximate 1.3 μl is suspended inside a flowing aqueous solution from which the analyte is extracted. The absorbance signal is directly monitored in the organic phase. After analytical determination the organic drop is removed and replaced by a new one.

In this work, a new automatic batch-type system is proposed to evaluate liquid–liquid extraction and preconcentration. In this system a micro-batch extraction chamber is used to simulate the manual liquid–liquid extraction procedure. To guarantee the reproducibility of the proposed liquid–liquid extraction system, the manifold was fully automated using miniature solenoid valves and optical sensors. Furthermore, the stirring, solvent injection and detection were controlled by computer. This proposal was evaluated for the preconcentration and determination of copper based on the formation of a complex between copper(II) and 1-(2′-pyridylazo)naphthol (PAN) and its extraction to isobutyl methyl ketone (MIBK).

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade, and distilled and deionized water was used throughout the experimental work. Copper stock solution (1000 mg L⁻¹) was prepared from the metal in a nitric acid medium. Standard solutions (S) containing from 10 to 1600 μg L⁻¹ were prepared by appropriate dilution with water of aliquots of the stock solutions. The extraction reagent (SV), 0.01 mol L⁻¹ PAN, was prepared by dissolving 6.232 g of PAN (1-(2′-pyridylazo)naphthol) in 250 ml of isobutyl methyl ketone. Tetraborate solution at a concentration of 0.1 mol L⁻¹ (SR1) was prepared by dissolving 5.0312 g of sodium tetraborate in water, with a final volume of 250 ml. Sodium sulfate, at a concentration of 0.50 mol L⁻¹ (SR2) was prepared by dissolving 17.75 g of
Table 1
Sequence for liquid–liquid extraction

<table>
<thead>
<tr>
<th>Step</th>
<th>Function</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>Agitation</th>
<th>Detection</th>
<th>Air</th>
<th>Water</th>
<th>Syringe</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Start</td>
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<td>Off</td>
<td>Off</td>
<td>Off</td>
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<td>Pump</td>
<td>Recycle</td>
<td>Off</td>
</tr>
<tr>
<td>2</td>
<td>Solvent injected</td>
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<td>Off</td>
<td>Off</td>
<td>Off</td>
<td>Off</td>
<td>Pump</td>
<td>Recycle</td>
<td>Off</td>
</tr>
<tr>
<td>3</td>
<td>Extraction</td>
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<td>Off</td>
<td>Off</td>
<td>On</td>
<td>Off</td>
<td>Pump</td>
<td>Recycle</td>
<td>Off</td>
</tr>
<tr>
<td>4</td>
<td>Phase separation</td>
<td>Off</td>
<td>Off</td>
<td>Off</td>
<td>Off</td>
<td>Off</td>
<td>Pump</td>
<td>Recycle</td>
<td>Off</td>
</tr>
<tr>
<td>5</td>
<td>Aqueous phase aspiration</td>
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<td>Off</td>
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<td>Pump</td>
<td>Recycle</td>
<td>Off</td>
</tr>
<tr>
<td>6</td>
<td>Detection</td>
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<td>On</td>
<td>Off</td>
<td>Off</td>
<td>Off</td>
<td>Recycle</td>
<td>Pump</td>
<td>Off</td>
</tr>
<tr>
<td>7</td>
<td>First washing step</td>
<td>On</td>
<td>On</td>
<td>Off</td>
<td>On</td>
<td>Off</td>
<td>Recycle</td>
<td>Pump</td>
<td>Off</td>
</tr>
<tr>
<td>8</td>
<td>Second washing step</td>
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<td>On</td>
<td>Off</td>
<td>On</td>
<td>Off</td>
<td>Pump</td>
<td>Pump</td>
<td>Off</td>
</tr>
<tr>
<td>9</td>
<td>Chamber exhausting</td>
<td>Off</td>
<td>Off</td>
<td>On</td>
<td>Off</td>
<td>Off</td>
<td>Pump</td>
<td>Recycle</td>
<td>Off</td>
</tr>
</tbody>
</table>

sodium sulfate in water, with a final volume of 250 ml. The sodium tetraborate solution was used as buffer to keep the pH at 9 and the sodium sulfate solution was used to avoid formation of an emulsion, improving phase separation.

2.2. Extraction system with on-line detection

The diagram of the proposed automatic system assembled to evaluate the liquid–liquid extraction procedure is shown in Fig. 1. This system was configured in order that the standard solution and the reagent solution were injected simultaneously [24] while the organic solvent was injected using a syringe (Multichannel Syringe Pumps Cole-Parmer 74900 Series). For the propulsion of the solutions, a peristaltic pump was employed (Ismatec MP-13R). This system is controlled by a microcomputer through a parallel interface (Advantech 711S) using a program written in Visual Basic 3.0. The detection system used a homemade detector having a green (560 nm) light emitting diode (LED) and a photodiode (Cen- tronic, OS15K).

The operational sequence of the liquid–liquid extraction procedure is listed in Table 1.

- At the beginning of the extraction cycle valves V1–V3 and the magnetic stirrer are off. In this way, an air carrier stream is pumped through the system, step 1 (Table 1).
- When commuting the proportional injector to the injection position, sample and reagents solutions are carried to the extraction chamber E (Fig. 1). When this mixture reaches the optical sensor OS1, syringe Sy is automatically turned on and a pre-defined volume of the organic phase (Vorg) is added, step 2 (Table 1).
- After solvent, sample and reagent insertion into the chamber, the agitation of the mixture is started, step 3. Thus, analyte present in the sample is extracted and preconcentrated (Fig. 2a).
- The agitation is stopped, allowing phase separation inside the extraction chamber, step 4 (Fig. 2b). The aqueous phase is removed by turning on valve V3, step 5.
- Valves V1 and V2 are turned on, pumping distilled water to the system, filling the chamber, and transporting the organic phase towards the detector. When the organic phase is sensed by the optical sensor OS2, analytical data acquisition is started, step 6 (Fig. 2c). At the end of signal registration, the organic phase is discarded through the upper exit, carried by the water stream.
- The washing of the chamber is a two step operation. In the first step, water is pumped for 60 s (step 7). In the second step, water and air are pumped simultaneously by turning off valve V1 (step 8). Agitation is on during all the washing cycle.
- After this, valves V1 and V2 are turned off and air is again pumped through the system. Simultaneously, valve V3 is turned on to remove the residual washing solution present in the extraction chamber, step 9 (Fig. 2d).

The two extraction chambers illustrated in Fig. 2 were made in glass with total capacities of 1.4 and 2.1 ml, two...
tube with 0.8 mm of inner diameter were coupled to the extraction chambers, one in the lateral part of the entrance of the solutions (standard, reagent and solvent) and the other, in the lower part, for removing the solution and for washing the chamber. The upper exit consists of a glass tube with 4 mm o.d. and 2 mm i.d. Around it is located the detection system.

2.3. Influence of the stirring time on the extraction efficiency

The stirring time, necessary to attain the equilibrium distribution of copper between the organic and aqueous phase, was investigated by employing 600 μl aliquots of 100 μg l$^{-1}$ Cu(II) standard solution, 60 μl of 0.1 mol l$^{-1}$ tetraborate solution, 50 μl of 0.5 mol l$^{-1}$ sodium sulfate solution and 120 μl of the MIBK organic phase containing 0.01 mol l$^{-1}$ PAN. The mixture was submitted to stirring for different time intervals from 10 to 90 s.

2.4. Effect of the aqueous/organic volumetric ratio on the preconcentration of Cu(II)

In all procedures, the enrichment factor was improved by increasing the volumetric ratio between the aqueous phase ($V_{aq}$) and organic phase ($V_{org}$). The aqueous volume referred in this work corresponds only to the sample or standard solution volume. In this study, the volume of the standard solution (SV) was kept at 600 μl and the volume of the organic phase (SV) was: 60, 67, 75, 100, 120, 150 or 300 μl, thus, the $V_{aq}:V_{org}$ ratios of 10:1, 9:1, 8:1, 6:1, 5:1, 4:1 and 2:1 were obtained. The additional volume of reagents (sodium tetraborate and sodium sulfate) was as described in Section 2.3. Using the extraction chamber with a total capacity of 1.4 ml, standard solutions containing 50 and 100 μg l$^{-1}$ and a stirring fixed time of 30 s were employed.

2.5. Efficiency of the extraction in the automatic system compared with manual extraction

Manual extraction was carried out to verify and compare the copper(II) extraction efficiency with that of the automatic liquid–liquid extraction system. In a separation funnel of 25 ml were added 6 ml of 1000 μg l$^{-1}$ Cu(II) standard solution or deionized water, 600 μl of the reagent solution SR1 and 500 μl of the reagent solution SR2. These solutions were agitated for 2 min and soon afterwards 1 ml of organic phase (MIBK) containing 0.01 mol l$^{-1}$ PAN was added to this mixture, and it was agitated again for 2 min. After promoting intimate contact between the phases, the separation funnel was allow to rest for complete separation of the phases. A 5 ml of the aqueous phase was used to carry out the copper determination by anodic stripping voltammetry and a deposition time of 30 s.

The automatic system procedure was carried out using 600 μl of 1000 μg l$^{-1}$ Cu(II) standard solution, 60 μl of reagent solution SR1 and 50 μl of reagent solution SR2, 100 μl organic phase (MIBK) containing 0.01 mol l$^{-1}$ PAN. In this study, the extraction system presented an aqueous/organic volumetric ratio of 6:1, the same value as used in the manual procedure. It was necessary to carry out 10 automatic extractions to obtain 5 ml of the aqueous phase. This solution was then used to carry out copper determination by anodic stripping voltammetry (ASV), as described above.

2.6. Determination of copper in certified reference materials

A 0.5 g Certified Reference Material of bovine liver CRM (No. 185) was weighed in triplicate into a Teflon microwave vessel and 5 ml of concentrated nitric acid were added. The vessel was placed in a microwave digestive furnace (DGT 100 Oplus system from Provecto Analítica) and submitted to a digestion program as previously reported [25]. After total digestion, the solution was neutralized using a sodium hydroxide solution and it was transferred to a 100 ml volumetric flask. The volume was completed with Milli-Q® water. River and lake samples from the Amazon region (Brazil) were spiked with 100–300 μg l$^{-1}$ Cu(II) standard solution to perform recuperation tests.

3. Results and discussion

Some steps necessary for obtaining good precision of the analytical signal were evaluated during the preliminary optimization of the system. To improve the precision and to avoid memory effects, it was observed that after the extraction and preconcentration step (step 2), all the aqueous phase should be removed from the extraction chamber before the organic phase is transported towards the detector. In order to remove all organic phase from the chamber air segmented water streams were pumped through the chamber. It was demonstrated that the segmented stream is more efficient than a non-segmented water stream.

3.1. Effect of stirring time interval on extraction efficiency

The effect of the stirring time interval, varying from 10 to 90 s, on the extraction efficiency of 100 μg l$^{-1}$ Cu(II) standard solution by 0.01 mol l$^{-1}$ PAN in isobutyl methyl ketone was evaluated using the extraction conditions reported in Section 2.3. As can be seen from Fig. 3, there is an increase of the analytical signal up to 30 s stirring time, which then remains constant up to 90 s. This study shows that the min-

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[This text has been translated for the purpose of the exercise, as it appears to be in a non-English language, possibly due to a misunderstanding or error in the processing of the original document.]
Fig. 3. Influence of the stirring time interval on the extraction efficiency of the 100 μg Cu(II) l⁻¹ standard solution.

imum stirring time needed for maximum extraction is 30 s. This value was employed in all other experiments.

3.2. Influence of the total volume on the analytical signal

The variation of the analytical signal caused by the variation of the total volume inside the extraction chamber was evaluated starting from two different volumetric ratios of aqueous-organic phase (V_{aq}:V_{org}—6:1 and 2:1) using the extraction chamber having a volume of 1.4 ml. The experiments carried out using the 6:1 volumetric ratio were made with three volumes levels inside the extraction chamber (405, 607.5 and 810 μl). The experiments carried out using the 2:1 volumetric ratio allowed for a larger variation of total volume to be investigated (252.5, 336.7, 550, 757.5 and 1010 μl). These experiments indicated that the total volume, keeping the V_{aq}:V_{org} ratio constant, do not affect the analytical signal in a significative way.

3.3. Effect of the aqueous:organic volumetric ratio on the preconcentration of Cu(II)

The effect of the aqueous:organic volumetric ratio, ranging from 2:1 to 10:1, on the preconcentration of Cu(II) standard solutions with concentrations of 50 and 100 μg l⁻¹ was evaluated. An extraction chamber of 1.4 ml was employed. In this study, the volume of Cu(II) standard solutions was fixed at 600 μl and the organic solvent volume was varied from 60 to 300 μl, as shown in Fig. 4. The additional volume of reagents (sodium tetraborate and sodium sulfate) was as described in Section 2.3. For preconcentration purposes, the enrichment factor should be higher than one when the volume of organic phase is smaller than that of aqueous phase. As can be seen in Fig. 4, by decreasing the organic phase volume in relation to the aqueous phase volume, fixed at 600 μl, an exponential increase of the analytical signal is observed. The experiment shows that the highest analytical signal was obtained for the largest volumetric ratio V_{aq}:V_{org}, in an other words, by using 600 μl of aqueous phase and 60 μl of the organic solvent. The signal acquisition shown in Fig. 5 is carried out when the organic phase is passing through the detector. As the speed of the pump kept constant during all the analysis procedure, if the volume of the organic phase is very small (<60 μl), few analytical signals are obtained. For this reason, although the highest detectability has been obtained using 60 μl of organic solvent, it was decided to use a larger volume due to a compromise with precision. Therefore, another extraction chamber with a larger inner volume (2.1 ml) was employed so that it would be possible to work with a higher ratio, such as 10:1, using a higher total volume such as 1400 μl of the standard solution and 140 μl of organic

Fig. 4. Effect of the aqueous:organic volumetric ratio on the preconcentration of standard solution of 50 or 100 μg Cu(II) l⁻¹ solution.

Fig. 5. Typical signal obtained with the proposed. The insert portions refer to (a) air, (b) analytical signal due to organic phase passage through the detector, (c) water; (1) air–organic phase interface and (2) organic–aqueous interface.
solvent volume. Under these conditions, a relative standard deviation lower than 6% was obtained. This experiment also showed that the analytical signal increases in proportion to the volumetric ratio increase for either 50 or 100 ml.

3.4. Efficiency of the extraction in an automatic system compared with manual extraction

The residual copper present in the aqueous phase after extraction, found by anodic stripping voltammetry (ASV), allows calculation of the extraction efficiency for manual and for automatic procedures. It was assumed that the copper present in the aqueous residual was not extracted to the organic phase. In this way, it was confirmed that the intimate contact between aqueous phase and organic phase achieved in the proposed system permits the high detectability necessary to work with samples having very low copper contents.

3.5. Analytical characteristics of the system

From measurements made using different volumetric ratios, three analytical curves were obtained presenting a linear behavior over distinct concentration ranges, as shown in Table 2. The detection limits are defined as $DL = 3S_D/n$, where $DL$, $S_D$, and $n$ are the detection limit, standard deviation of the blank and slope of the analytical curve, respectively. The determination frequency was 14 h$^{-1}$, using volumetric ratios of 2:1, 5:1 or 10:1.

A bovine liver CRM (No. 185) was analyzed by the proposed method. The result, expressed in mg kg$^{-1}$ of copper, and its precision ($n = 3$) are presented in Table 3. It can be seen that the result is in good agreement with the certificate value. This confirms that the method can be satisfactorily applied to real samples. In addition, the proposed method was applied in recovery tests for water samples from rivers and lakes of the Amazon region (Brazil). The results for the assays are present in Table 3, showing a recovery between 97.9 and 103.7% for the proposed method.

4. Conclusions

The automated liquid–liquid extraction system proposed allows control with good performance, of all extraction steps regarding preconcentration and/or solvent extraction such as: (a) efficient mixture between the organic and aqueous phases, (b) easy separation of the phases, (c) detection of the content of the organic phase without its isolation, and (d) reduction of organic solvent consumption. All these steps are carried on a very reduced volumetric scale (<1.5 ml) and with repetitivity lower than 6%. With this system, it is possible to carry out 14 extraction per hour. Another characteristic of this alternative procedure is that all the steps, except detection, occur in the extraction chamber, without the use of phase segmentors and phase separators, contributing to decrease the associated error produced by each step and improving the quality of the results. The preconcentration factor and, therefore, the detectability of the method is established by the volumetric ratio between the organic and the aqueous phase. In this way, preconcentration factors from 2- to 10-fold or even more can be obtained and should be selected considering the concentration range of the analyte in the sample. On the other hand, in the application of the method described here, the detectabil-
ity was restricted due to the short optical length used (i.d. 2 mm).

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