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Interfacing a microchip-based capillary electrophoresis system with a microwave induced plasma spectrometry for copper speciation

Research Article

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Abstract: A microchip-based capillary electrophoresis (μCE) system was interfaced with a microwave induced plasma optical emission spectrometry (MIP-OES) to provide copper species separation capabilities. This system uses an extremely low flow demountable direct injection high efficiency nebulizer (D-DIHEN) sited directly at the liquid exit of the chip. A supplementary flow of buffer solution at the channel exit was used to improve nebulization efficiency. A small evaporation chamber has been incorporated into the interface in order to prevent the losses associated with traditional spray chambers, allowing the entire aerosol sample to enter the plasma. Syringe pumps were used to manipulate the flow rate and flow direction of the sample, buffer, and supplementary buffer solution. Sample volumes of 25 nL can be analyzed. With application of an electric field up to 500 V cm^{-1}, species such as Cu(II) and $Cu(EDTA)^{2-}$ were separated in acidic solution within 90 s using a 26 mm long separation channel etched in a glass base. Resolution of the Cu(II) and $Cu(EDTA)^{2-}$ peaks was 1.1 using the chip-based μCE-MIP-OES system.

Keywords: *Microwave induced plasma spectrometry • Capillary electrophoresis • Micronebulizer • Chip technology • Copper species*

1. Introduction

Microwave-induced plasma (MIP) is a small, low power plasma that exhibits high excitation efficiency for metal and non-metal elements [1,2]. The technique of MIP optical emission spectrometry (OES) would be excellent for the separation of elements; however due to the small size and low thermal temperature of the plasma (compared to its high excitation capability) it cannot be easily interfaced to liquid separation techniques such as high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE). This problem could be overcome by using lab on a chip technology for the separation, as this provides rapid separation with nanoliter volumes of liquid. Laboratory on a chip devices provide an excellent opportunity for rapid on-chip pretreatment of samples, separation for speciation studies and the analysis of very small sample volumes.

The coupling of CE with analytical atomic spectrometry provides a very sensitive, element specific detection method in conjunction with high separation efficiency [3]. However, interfacing this technique remains challenging, primarily due to flow incompatibility. Microfabricated analytical systems using electrophoresis as a separation mechanism have been shown to drastically reduce separation times and to simplify instrumental design. Another advantage of microchip technology is that pre-etched channel networks can be used to provide several flow streams, omitting tube connectors and thus reducing dead volumes. In most microchip CE (µCE) systems, sample injection is achieved by the electrokinetic method [4]. Song *et al.* [5,6] and Pearson and Greenway [7] reported elemental speciation using µCE which was successfully interfaced with inductively coupled plasma mass spectrometry (ICP-MS) *via* a commercial low flow micronebulizer and miniaturized spray chamber. Hui *et al.* [8] were the first to report an

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on-chip nebulizer. A chip-based electrophoresis system was interfaced to inductively coupled plasma optical emission spectrometry (ICP-OES) by means of a cross-flow nebulizer.

In the present work, an attempt was made to interface chip-μCE to microwave-induced plasma optical emission spectrometry (MIP-OES). Copper was chosen as the target to demonstrate the performance of the developed hybrid technique. Copper species (Cu(II) and $Cu(EDTA)^{2-}$ were baseline-separated within 90 s using a 26 mm long separation channel. The results show that under an appropriate deposition potential, the inert $Cu(EDTA)^{2-}$ complex becomes electroactive, which makes EDTA a suitable competing ligand for determination of copper speciation in solution. The analytical performance of the chip-based μCE-MIP-OES setup was examined.

2. Experimental procedure

2.1. Instruments and apparatus

An Echelle grating optical emission spectrometer (PLASMAQUANT 100, Carl Zeiss, Jena, Germany) using fibre-optical light-guides and photomultiplier tubes (PMT) and a TE_{101} microwave plasma cavity assembly was used, and was essentially the same as previously described [9].

The MIP resonant cavity was used as an excitation source, as previously discussed [9]. The plasma is viewed axially with the axis of the plasma perpendicular to the plane of the entrance slit. Since the plasma torch, cavity, generator and gas flow have been described in detail in previous work [9], they will not be discussed again here, but only briefly summarized. The microwave generator is connected by means of a flexible cable to the rectangular resonant cavity of the TE_{101} design. The torch is a quartz capillary made of Suprasil (Heraeus, Hanau, Germany). Instrument settings and operational parameters used for the experimental MIP-OES system are summarized in Table 1.

2.2. Sample introduction systems

Four different micronebulizers were assessed: the Demountable Direct Injection High Efficiency Nebulizer (D-DIHEN) (Analab, Strasbourg, France), MiraMist CE (MMCE, Burgener Research Inc., Mississauga, Canada), Micromist (Glass Expansion, Switzerland) joined with Quasi-Direct Injection system for very low uptakes (QuDIN, EPOND, Vevey Switzerland) and Microflow Concentric Nebulizer DS-5 (CETAC, Omaha, USA) equipped with a low-volume (8 mL) spray chamber.

The waste was removed by the peristaltic pump. The gas flow rate was controlled by means of a mass flow controller (DHN, Warsaw, Poland) with a pressure regulator and with a single pass. Argon was used as the nebulizing carrier gas and as the plasma gas, while helium/argon was used as the plasma gas.

Table 1. Instrumental parameters for the μCE-MIP-OES system.

Mounting	**Czerny-Turner in tetrahedral set-up**
Focal length/mm	500
Spectral range/nm	193-852
Order lines	28th-123rd
Microwave frequency/MHz	2450
Microwave power/W	160
Microwave cavity	TE101 rectangular, water cooled
Microwave generator	700 W, MPC-01 (Plazmatronika Ltd., Wrocław, Poland)
Plasma viewing mode	Axial
Plasma torch, axial position	Quartz tube, 3.0 mm i.d., air cooled
Argon flow rate/mL min^{-1}	150
Plasma supporting helium flow rate/ mL min^{-1}	200
Plasma form	Annular
Nebulizer supplementary buffer flow rate/ μL min^{-1}	5.0
Carrier buffer flow rate/ μL min^{-1}	0.5
CE potential/ kV	2.2
Sample flow rate/ μL min^{-1}	0.5
Sample injection time/s	3.0
Sample volume/nL	25
Read	On-peak
Integration time/s	0.1
Background correction	Fixed point
Determination	Simultaneous
Wavelength/nm (line type)	Cu 324.754 (I)

2.3. Chip capillary electrophoresis

Experiments were carried out on two laboratory-built μCE systems (Chip1, Chip2), which were composed of a 30 kV high voltage power supply (HVPS Spelman, Spišská Nová Ves, Slovakia) and three microsyringe pumps (Ascor, Warsaw, Poland) that were capable of providing 0.4-100 μL min^{-1}. The flow rate of the solution

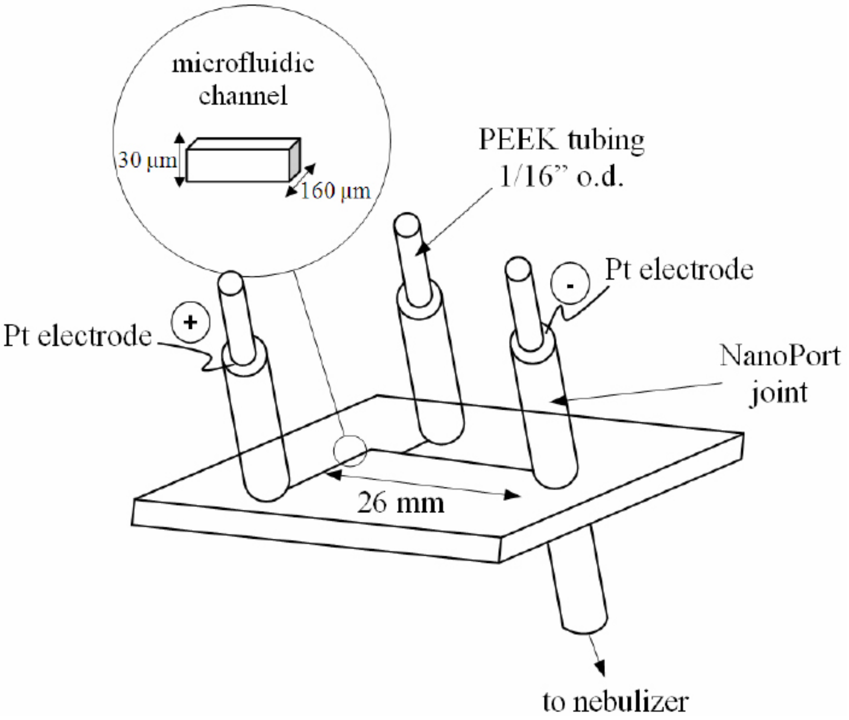

Figure 1. Schematic diagram of the microfluidic chip.

was 0.5 µL min^{-1} to inject sample and buffer solution into the separation channel *via* a NanoPort Assemblies N-124 joint (IDEX Health and Science, Oak Harbor, USA) modified to connect PEEK tubing of 1/16" OD (Supelco, Bellafonte, USA). These overcome the pulsation introduced through the use of peristaltic pumps at low flow rates. The microchips used in this µCE-MIP-OES system consisted of two (35×17×2.2 mm) glass plates and were fabricated by the Wrocław University of Technology, Department of Microengineering and Photovoltaics, from borosilicate glass by a photolithography/wet etching process. The design of the microchip, with the channel layout, is shown in Fig. 1. The third microsyringe pump was used to introduce the supplementary buffer flow at the channel exit. The flow rate of the supplementary buffer solution was 5 µL min^{-1}. The gas flow rate was controlled by means of a mass flow controller (DHN, Warsaw, Poland). An external argon source was needed for the nebulization, as the MIP-OES is not able to provide the pressure required by this interface.

The microchip was interfaced to the MIP-OES *via* a commercially available, low flow rate D-DIHEN nebulizer, as shown in Fig. 2, using a NanoPort Assemblies N-124 joint. The uptake flow rate of the nebulizer was about 5 µL min^{-1}. A QuDIN miniature evaporation tube-shaped spray chamber fitted directly with the quartz plasma torch was used to obtain high sample introduction efficiency with the low flow rate nebulizer. The experimental conditions of the chip-based µCE system are summarized in Table 1. The instrument can be run in either a continuous flow mode to give a quick assessment of the instrument stability and the µCE separation, or in time resolved acquisition mode for quantification of the peak areas.

A schematic diagram of the entire experimental sample introduction system set-up (µCE-MIP-OES) is shown in Fig. 3.

2.4. Reagents, chemicals and gases

Compressed, pure argon and helium gases (N-50 purity, 99.999%) obtained from BOC GAZY (Poznań, Poland) were used as plasma gases. A 0.01 mol L^{-1} stock solution of EDTA was prepared by dissolving an appropriate quantity of EDTA purchased from FLUKA (≥99%, Steinheim, Germany). The mixed standard of Cu(II) and Cu(EDTA)$^{2-}$ was prepared by adding an appropriate amount of EDTA solution to a Cu(II) standard solution (Merck, Darmstadt, Germany) to form the required molar ratio of the two species. The HNO_3 (69%, v/v) acid used was of the highest quality grade (Trace pure, Merck, Germany). A solution consisting of 15 mM nitric acid was used as the carrier electrolyte for copper speciation. Water was initially deionized (Model DEMIWA 5 ROSA, Watek, Czech Republic) and then doubly distilled in a quartz apparatus (Heraeus Bi18, Hanau, Germany).

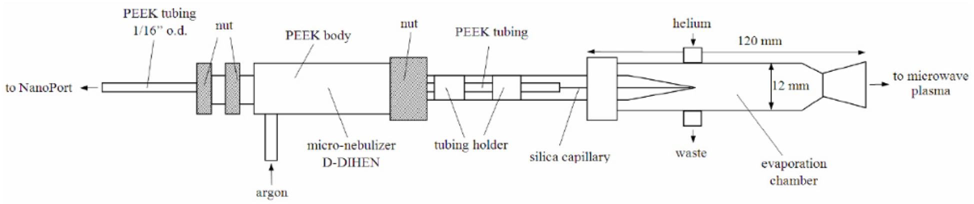

Figure 2. Schematic diagram of the interface micro-nebulizer/mini spray chamber.

2.5. Simplex optimization procedure

A simplex optimization approach was undertaken to establish the best conditions for liquid nebulization, transport and excitation. The optimized parameters are listed in Table 2, along with the ranges over which optimization experiments were possible and conducted. In practice, the ranges were judiciously selected for each parameter in turn, taking into account the practical problem of maintaining a stable emission signal.

Simplex optimization experiments were performed using the Multisimplex AB software package (Karlskrona, Sweden). The optimization was carried out on each nebulizer in order to establish the “real” experimental conditions. A QuDIN spray chamber was used on all these experiments. The net signal-to-background ratio (S/B) was taken as the criterion of merit. Some preliminary univariate experiments (screening) were performed prior to the simplex optimization in order to establish the boundary values of each parameter. Three measurements for each variable were conducted at the value of interest. Between each experiment, a blank corrective experiment was run to ensure stable and repeatable results.

The optimum conditions obtained from this procedure were then used to run standard element solutions.

2.6. Sample injection

Liquid flow was delivered to the sample and carrier reservoir by two syringe pumps. A third syringe pump was used to deliver the make-up flow at 5 μL min^{-1}, which produced the maximum sample signal intensity, hence satisfying the nebulizer uptake requirements. Liquid was pumped to all reservoirs at 0.5 μL min^{-1} in order to purge the chip for approximately 5 min before checking the flow from each reservoir by monitoring the copper signal (Cu, sample, carrier, make-up).

3. Results and discussion

One of the problems with previous designs of microchip sample introduction systems for plasma spectrometry has been the low transport efficiency of the sample into the plasma [7]. In the present experiments, a bulb-shaped spray chamber of relatively large volume was used. The large chamber may broaden the CE peak. Since the liquid flow rate in the chip is very low, it should be possible to achieve 100% transport of the sample out of the end of the chip to the microwave plasma. In order to achieve this, a small single-pass spray chamber with a volume of 10 mL was used in the following study.

The optimization of wavelength was not carried out because the wavelength used for the determination was pre-selected by the producer of the polychromator.

Preliminary analytical performance of the Ar/He-MIP was examined by measuring the S/B ratio of copper. However, substantial optimization of the gas parameters for the analyte was not undertaken, as this information was readily available from the literature on excitation and ionization conditions for MIP-OES with pneumatic nebulization [10] (and references cited therein). The comparison of these parameters obtained for mixed plasma with those presented for pure argon plasma and helium plasma with pneumatic nebulization shows that mixed plasma achieved a better detection limit than pure plasma gases. In addition, the mixed Ar + He MIP exhibits higher tolerance to water loading. As a result, an Ar/He-MIP was selected for all subsequent experiments, with a plasma gas composition of *ca.* 40% Ar and 60% He; this is in agreement with results presented earlier [11].

3.1. Simplex optimization of operational variables

Two different types of experimental variables affect the method: first, variables controlling the emission response in the microwave plasma, that is, the microwave forward power of the microwave generator; second, variables such as the argon carrier flow and sample uptake rate that regulate sample transport. Following univariate searches for the optimum values of applied power, nebulizing-carrier gas flow rate and make-up solution uptake rate, a multivariate simplex optimization was used to establish the optimum experimental parameters for the low detection limits of copper. For each nebulizer, the optimization was completed in 18 steps, which took approximately 2 h. These values were chosen based on the recommendations given in the literature and preliminary experiences with solution nebulization by the MIP-OES method. The effectiveness of the simplex

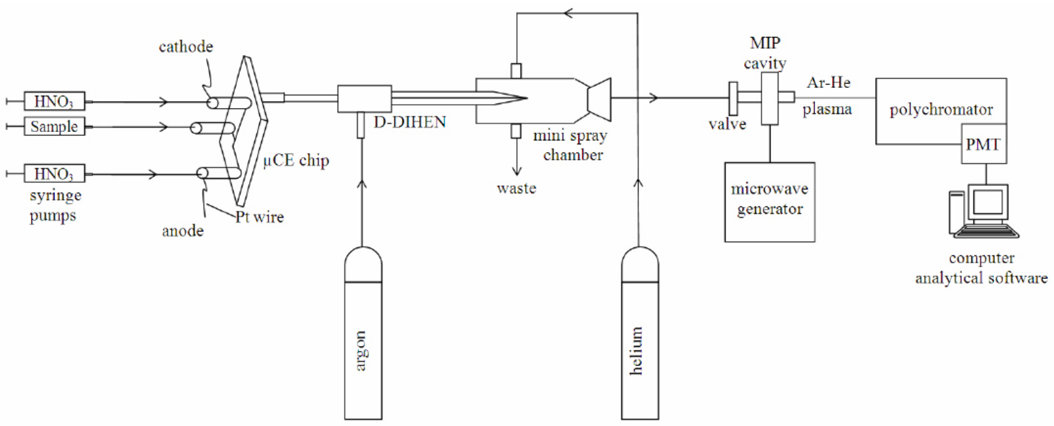

Figure 3. Component diagram of the elaborated microchip based µCE-MIP-OES system (not to scale).

Table 2. Optimum operating conditions for MIP-OES measurement[a] of copper obtained by simplex and univariate methods.

Parameter (variable)	Boundary limits of parameters, range				Univariate method				Simplex method			
	D-DIHEN	DS-5	MICROMIST	MMCE	D-DIHEN	DS-5	MICROMIST	MMCE	D-DIHEN	DS-5	MICROMIST	MMCE
Forward power, W	100-180	100-180	100-180	100-180	160	150	160	160	155	154	157	155
Sample carrier argon flow rate, mL min^{-1}	50-250	800-1200	200-1000	500-1100	150	800	700	900	150	660	860	720
Plasma helium flow rate, rate, mL min^{-1}	100-300	100-300	80-300	100-300	200	250	160	200	220	224	165	212
Sample liquid uptake rate (pumped), µL min^{-1}	1-9	1-9	1-9	1-9	5	4	8	7	5	4	8	8
Electrolyte acidity, pH	1.5-2.8				1.8							
CE voltage, kV	1.0-4.0	-	-	-	2.2	-	-	-	-	-	-	-

[a] *Response, peak height of the element emission intensity.*

procedure was confirmed with univariate searches, which assisted in verifying that the optimum lay near the simplex value. The optimized parameters are listed in Table 2.

3.1.1. Nebulizer

Detection limits obtained on a single-element basis for the various nebulizers employed in this work are compared. A comparison between detection limits obtained by micro-nebulization (D-DIHEN, DS-5, Micromist, MMCE) for the copper line tested is shown in Table 3. The limits of detection (LOD) calculated using the IUPAC recommendation (based on a $3\sigma_{\text{blank}}$ criterion) and obtained by use of the optimized operating conditions are summarized in Table 3, based on the raw, unsmoothed data.

The D-DIHEN provides lower limits of detection than the other nebulizers for copper evaluated in an axially-viewed microwave plasma with a 1% nitric acid matrix.

The detection limits presented in Table 3 were calculated from the standard deviation (3σ) of six measurements of the known injection volume of the blank solution. The precision of replicate determinations was calculated from the RSD (%) of the mean of six replicate measurements of element standard using a mass 50-fold higher than the LOD. The precision of the D-DIHEN is similar or slightly better than that of the other nebulizers. The precision of the copper determination ranges from 6 to 10% for the liquid original samples (evaluated as peak height) and is probably largely due to the instability of the microwave plasma source. These values can be considered satisfactory, especially owing to the large number of parameters governing the performance of the analytical technique. In other words, this reflects the cumulative imprecision of all of the sample nebulization, transfer of aerosols, excitation and detection steps.

3.1.2. Microwave forward power

The MIP is normally operated at low power levels in the range of 50 – 150 W. In this work, the stable Ar/He plasma could be maintained at a level of greater than 100 W forward power. Between 100 and 180 W, neither the intensities of spectral lines nor the S/B ratios showed a dependence on the power that would point to a pronounced optimum. In addition, the stability of the background and line signals did not significantly

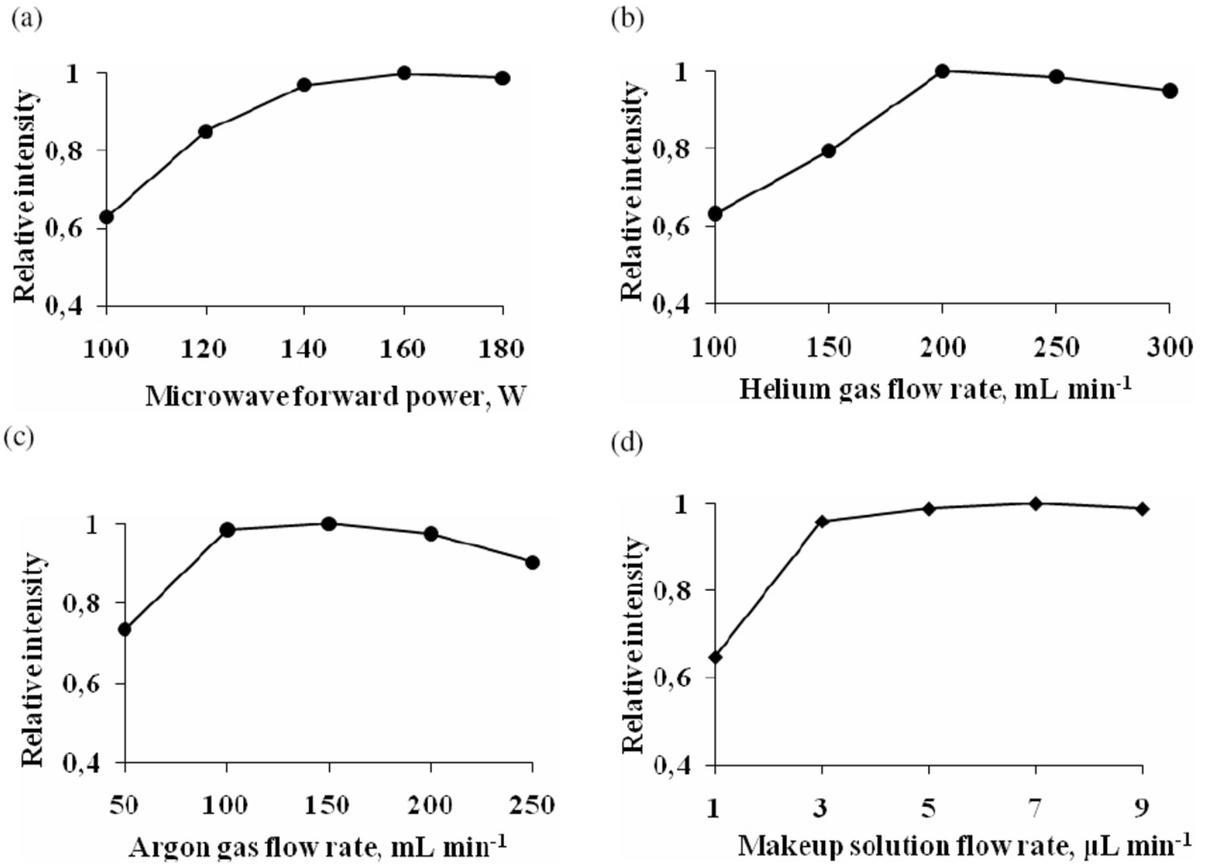

Figure 4. Effect of the variables on the copper normalized emission intensity for µCE-MIPOES system. Influence of (a) microwave forward power; (b) helium gas flow rate; (c) argon gas flow rate; (d) makeup solution flow rate on the emission intensity for continuous injection of 10 mg L^{-1} Cu (II) through make-up channel without application of voltage.

vary with power in the stated range. In general, for two analytical lines of studied elements, S/B ratios usually tend to level off after the microwave power approaches 160 W (Fig. 4a). The intensities of spectral lines also level off, but more slowly. As a result, an optimized power of 160 W was selected as an acceptable value and a practical working range.

3.1.3. Argon carrier and plasma helium/argon flow rates

The effect of the plasma (support) helium gas flow rate was optimized in our experiments and was selected based upon previous experience and in order to maintain the plasma stability and plasma shape. Stable operation of the plasma was obtained at gas flow rates of 200 mL min^{-1} (Fig. 4b).

It was also observed that the carrier Ar gas stream flow rate has a more significant influence on the emission intensities than the plasma support gas flow rate. The carrier Ar gas affects the formation of the plasma channel (an annular configuration) [11], the residence time of the analyte in the plasma, and the aerosol generation and transport efficiency [12]. To optimize the carrier (nebulizing) argon gas flow for copper determination, the optimum total flow for copper was estimated in the range of 50 – 250 mL min^{-1}. It was observed that the carrier argon stream flow rate has a significant influence on the emission intensities and thus proved to be a critical parameter. In general, it was observed that when

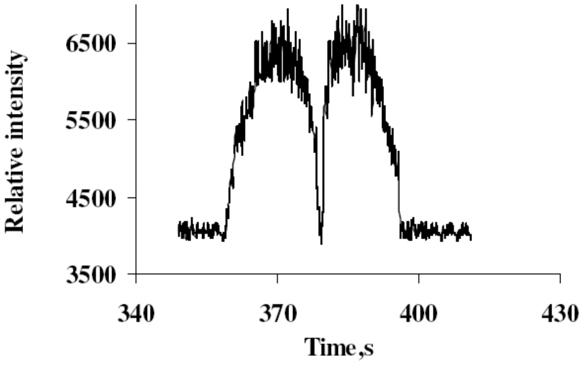

Figure 5. Separation of Cu (II) and Cu(EDTA)2- in microchip based µCE-MIP-OES system. Sample injection 3s. CE potential 2.2 kV.

Table 3. Limits of detection (LOD) for copper and nebulizers tested. Continuous solution injection through make-up channel without application of voltage.

Nebulizer	ngmL^{-1}	pg	% RSD
D-DIHEN	0.7	3.5[a]	6
DS-5	2.7	10.8[b]	8
MICROMIST	1.2	6.0[c]	7
MMCE	8.9	62.3[d]	10

[a] *Continuous injection of 5 µL solution*
[b] *Continuous injection of 4 µL solution*
[c] *Continuous injection of 8 µL solution*
[d] *Continuous injection of 7 µL solution*

Table 4. Microchips comparison for use in µCE-MIP-OES system.

Parameter		Chip1	Chip 2
Channel width, µm		160	130
Channel depth, µm		30	15
Chanell length, mm		26	26
LOD, ng mL^{-1}	**Cu (II)**	2.5	4.3
	Cu(EDTA)$^{2-}$	2.8	4.5
LOD, pg[a]	**Cu (II)**	0.06	0.11
	Cu(EDTA)$^{2-}$	0.07	0.11
Resolution		1.1	0.7
RSD, %	**Cu (II)**	7	11
	Cu(EDTA)$^{2-}$	9	13
Linear range, µg mL^{-1}	**Cu (II)**	0.02-50	0.1-50
	Cu(EDTA)$^{2-}$	0.03-50	0.2-50

[a] *for sample injection of 25 nL*

the flow rate ranged between 50 – 250 mL min^{-1}, the emission intensities reached maximum at 150 mL min^{-1}, and with further increase of the flow rate above these values, the emission intensities decreased for copper (Fig. 4c). The maxima are the result of opposite effects of nebulizing gas flow on aerosol characteristics and transport and interaction of aerosol with the plasma. Increasing the nebulizing gas flow rate commonly causes a shift of both primary and tertiary drop size distributions to smaller droplet sizes. This in turn leads directly to higher analyte and solvent transport rates. However, these two transport rates exert opposite trends on net signal intensity. In addition, the higher the nebulizing-carrier gas flow, the smaller the residence time of droplets in the plasma. Therefore, the overall effect is shown as a maximum behaviour. Therefore, in this study, a 150 mL min^{-1} argon carrier flow rate was chosen.

3.1.4. Flow rate of make-up liquid

The make-up solution (buffer solution) also proved to be important for this work. When the sample pumping rate was greater than approximately 5 µL min^{-1}, it was found that the signal intensities would not increase further and began to level off (Fig. 4d). For pneumatic nebulization, the higher the liquid flow, the more the primary drop size distribution is shifted to larger drop sizes. Nevertheless, the absolute amount of aerosol volume contained in smaller drops increased. Therefore, the higher the liquid flow, the higher the analyte and solvent transport rates, so a sample flow uptake rate of 5 uL min-^{1} was chosen.

3.2. Electrophoretic separation of Cu(II) and Cu(EDTA)$^{2-}$ by the µCE-MIP-OES

For the further assessment of the hybrid system, the separation of Cu(II) and its complex with EDTA was investigated. The ion mobilities of Cu(II) and Cu(EDTA)$^{2-}$ are similar and the ions carry the same charge. To simplify the carrier flow profile, 15 mM nitric acid (pH=2) was chosen as the electrolyte. Also, the initial forms of the cations in the study are better preserved in acidic condition, because when the pH increases, Cu(II) is likely to hydrolyse and to lose its effective charge. The degree of separation of the ions is an indicator of the resolving power of the µCE-ICP-OES system. The hydrodynamically controlled flow system eliminates the discrimination, so that even oppositely charged ions such as Cu(II) and Cu(EDTA)$^{2-}$ can be analyzed in one run. Fig. 5 shows the electropherogram of the Cu(II) and Cu(EDTA)$^{2-}$ mixture. The influence of the applied voltage on the separation of Cu(II) and Cu(EDTA)$^{2-}$ was investigated from 1000 to 4000 V. As expected, by increasing the applied voltage, the migration time and half-peak width decrease; the two ions are clearly beginning to separate on application of 2200 V, and the separation was completed within 90 s. The half widths of the peaks were 17 s and 13 s for Cu(II) and Cu(EDTA)$^{2-}$, respectively. The resolution of the elution peaks was approximately 1.1.

3.3. Analytical figures of merit

The analytical performance characteristics were evaluated for Cu(II) and Cu(EDTA)$^{2-}$. Detection limits were calculated on the basis of the blank signals plus three times the standard deviations. The analytical characteristics are given in Table 4. The achieved limits of detection (LOD) of2.5 and 2.8 ng mL^{-1} for Cu(II) and Cu(EDTA)$^{2-}$, respectively, are slightly better than the LOD achieved for Cu(II) and Cu(EDTA)$^{2-}$ in an electrophoresis chip with inductively coupled plasma mass spectrometry (3.2 and 3.1 ng mL^{-1}, respectively, reported by Song *et al.* [6]).

4. Conclusions

In this work, a microchip based capillary electrophoresis system was interfaced with MIP-OES. Sample and buffer solution flows in the μCE chip were manipulated using a pair of syringe pumps for flexible control of solution flow rate and flow direction. The feasibility for copper speciation was demonstrated by the separation of different species of copper in 90 s, within a 26 mm long separation channel. The microchip system currently requires manual filling with background buffer and sample.

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