Sequential determination of mono- and divalent copper in water by flow-injection analysis

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ABSTRACT

A simple and sequential method is presented for the fast speciation of copper in aqueous media. The use of a spectroelectrochemical flow-cell allows the quantification of individual copper oxidation states using a combination of spectroscopical and electrochemical techniques. The proposed method is based on a reverse flow injection system (r-FIA) for the spectrophotometric determination of Cu (II) with cuprizone, with a linear fit between 1.2 and 12 μg mL^{-1} and a detection limit of 0.11 μg mL^{-1}. Amperometric determination of Cu (I) is synchronized with the optical measurement to avoid interferences and has a linear behavior ranged from 0.04 to 0.8 μg mL^{-1} and a detection limit of 7.7 ng mL^{-1}.

KEYWORDS: Copper speciation, Cuprizone, Colorimetric determination, Flow Injection Analysis, Amperometric determination.

INTRODUCTION

Copper is an important trace element found in biochemical systems of interest, minerals and other natural systems. Although ~~his~~its natural state is as metal or compounds of Cu (II), there are certain materials of technological interest in which is present as Cu (I) like some spinels [1] or other superconducting compounds [2], catalysts [3,4] and more.

The most sensitive methods for analyzing copper traces are based on stripping voltammetry [5]. Other analytical techniques used are atomic absorption spectrometry [6,7] or potentiometry on ion-selective electrodes [8] among others [9,10]. Also, many articles have been published based on spectroscopic techniques for the determination of copper using specific [11] or nonspecific complexing agents, latter in combination with masking agents [12]. Some of these analytical methods have been adapted for flow determination [13-15~~] but most~~]. Most of them only determine one of the copper valences ~~of copper,~~, or the total amount of copper in sample by complete oxidation or reduction before analysis~~, or use~~. Other authors uses a sequence of both strategies to allow indirect quantitative speciation.

In this work, we report a new method for direct speciation of copper in aqueous samples. The use of a mixed ~~detector~~detection device allows the quantification of individual copper oxidation states independently through different techniques, colorimetric for Cu (II) and electrochemical for Cu (I). The proposed method is based on a reverse flow injection system (r-FIA) for the spectrophotometric determination of Cu (II) with cuprizone (N,N'- bis(cyclohexylideneamino) ethanediamide). Amperometric determination of Cu (I) is synchronized with the optical measurement to avoid interferences.

The aim of this paper is to demonstrate how the use of a mixed detector, which has been designed in our laboratories, facilitates and accelerates the analysis of multiple analyte that may be present simultaneously in certain types of samples and illustrate practical precautions to be taken into account to avoid cross-interference. This strategy allows the use of affordable instrumentation and involves low reagent consumption. Although solving the analytical problem is not the real purpose of the work, we have chosen a system that could be of interest, both environmental (polluted waters in rivers or swamps, drinking water) and biochemical (tissular

samples, foods), and has only been solved in the literature using complex sequences and indirect methods of analysis.

MATERIALS AND METHODS

Apparatus

All spectroscopic measurements were recorded on a Varian Cary 1E (Aligent Inc., Santa Clara, CA) double beam UV-Vis spectrophotometer, using a spectroelectrochemical flow-cell built by the authors which has been previously described [16]. The spectrophotometer used doesn´t allow the correction of Schlieren effect by simultaneously measurement of the absorbance at two wavelengths and subtracting them [17]. Therefore this effect must be prevented by choosing the adequate combination of reagents for the carrier and the injected solutions.

All electrochemical measurements were made using a BAS 100B (BASi, West Lafayette, IN) three-electrode potentiostat interfaced to a PC and connected to the afore-mentioned spectroelectrochemical flow-cell.

A Gilson Minipuls3 (Gilson Inc., Middleton, WI) peristaltic pump coupling to an Onmifit (Diba Industries Inc., Danbury, CT) six-way manual injection valve (Code #: 001106) has been used in the FIA manifold. All tubing was made in PTFE 0.8mm ID acquired in Supelco (Sigma-Aldrich).

Briefly, the spectroelectrochemical flow-cell was constructed with a piece of plastic material in which it was machined a flow channel. The flow inlet and outlet are two steel tubes, which also play the role of working and auxiliary electrodes respectively. Between them, the flow channel is delimited by ~~a pair of~~two optical windows ~~that allow~~allowing photometric measurement. Plastic part also houses a Ag/AgCl/NaCl(sat) reference electrode to complete the electrode set for electrochemical measurements. See Figure 1.

Reagents and solutions

All chemicals were of analytical reagent grade and Milli-Q (Millipore) deionised water was used throughout.

A 1000 µg mL^{-1} Cu (II) stock solution was prepared by dissolving 3.14 g of copper (II) acetate in water and diluted to 1L.

Cuprizone reagent solutions were prepared by dissolving 0.07 g in 7.14 mL of ethanol and diluted it to 50 mL with deionised water. Ultrasonic stirring and delicate heating during 15 min were necessary to obtain the complete dissolving of the reagent. Before the introduction of this solution in the FIA manifold, it was filtered too.

Ammonium citrate buffer solutions were prepared by dissolving 214.38 g of citric acid in water, subsequent adding 271.14 mL of ammonia solution (25%) and final making up to 1 L.

A 30 µg mL^{-1} Cu (I) stock solution was freshly prepared by taking 6 mL of a 500 µg mL^{-1} Cu (II) stock solution previously prepared and diluted it with deionised water. This solution is treated with a few crystals of hydroxylamine hydrochloride and excess of reductor was eliminated by boiling for 15 minutes. The Cu (I) stock solution is kept under nitrogen atmosphere to avoid reoxidation with air.

Solutions with different amounts of Cu (I) or/and Cu (II) are ~~daily~~ prepared every day by taking corresponding aliquots of stock solutions and diluting them in buffer solution.

FIA setup and procedure

According with previous bibliography [14], we adopt the reversed FIA approach to obtain the higher sensitivity in Cu (II) determination. The FIA system is a single-line manifold where the buffered sample is impelled continuously by a peristaltic pump and the adequate amount of cuprizone is injected on the stream by a six-port injection valve. Before reaching the flow cell on the spectrometer, a reaction coil of appropriate length is included to allow the formation of copper-cuprizone complex. To prevent the Schlieren effect and avoid ~~his~~its correction, the ammonia-citrate buffer is included in the carrier solution by mixing it with the sample before the analysis.

To determine Cu (I) in sample, an amperometric technique was selected. Time between cuprizone injection and spectrophotometer response is used to oxidize the monovalent copper that passes through the flow-cell and to register the current-time response. To prevent

interferences with Cu (II) determination, the oxidation potential required for electrochemical determination is switched on and off when needed. Figure ~~1~~ 2 shows the timing diagram for synchronizing determinations of both copper valences.

RESULTS AND DISCUSSION

Determination of divalent copper

Copper (II) determination is based on the quantitative formation of the blue chelate obtained from the reaction between the metallic ion and cuprizone (CPZ) in basic media~~. The stoichiometry of this chelate~~ [18~~] is~~]: Cu^{2+} + 2 CPZ → $Cu(CPZ)_2$ ~~and has a~~with maximum absorption at 595 nm. The colorimetric reagent is quite selective for Cu (II) permitting ~~his~~its determination without prior separation from complex matrices such as steels, alloys, natural waters, biological samples and more as have been demonstrated elsewhere by others authors [13,14]. The effect of the presence of monovalent copper on the determination of copper (II) will be discussed later.

The analytical magnitude used to quantify the copper (II) content was the area of peak in the absorbance response at 595 nm.

Optimization of FIA spectrophotometric method

As has been mentioned before, reversed FIA system was used to determine Cu (II) with cuprizone. Some of the parameters that affect the method features were investigated and selected to obtain the best results in determination of Cu (II) in absence of Cu (I).

According to previous literature [19,14] and preliminary ~~essays~~assays, the buffer and the cuprizone concentrations ~~are~~were 1.0 M and 5.13 mM respectively. Other operating parameters are listed below along with the results~~.~~:

i) Schlieren effect correction~~.~~

To avoid the light dispersion due to Schlieren effect (originated by the incomplete mixture of solutions with different concentrations or solvents) and its negative contribution to absorbance

registered, different compositions of carrier stream and injected solution were tried and compared the optical signal obtained. To minimize the necessary tests, we must take into account that the copper must be in the carrier, the cuprizone must be in the injected solution and the sum of both solutions should contain all the necessary reagents to obtain the colored complex. Table 1 summarizes the assays and ~~his~~its results. Figure ~~2~~ 3 shows the ~~shape of~~ resulting peak for the best three ~~best~~ options ~~and permit conclude that~~. To sum up, the Schlieren effect is totally corrected for the option V.

ii) Carrier flow rate~~.~~

Figure ~~3~~ 4 shows the different values obtained in absorbance by calculating the area of peaks when the carrier flow rate changes from 0.5 to 6.5 mL min^{-1}. A flow rate of 0.5 mL min^{-1} was chosen because higher area value is obtained and reagent consumption is fewer, this parameter also affects to analytical throughput ~~and maybe~~; therefore, a higher compromise ~~higher~~ value ~~can~~could be ~~taken account~~considered.

iii) Injected volume~~.~~

Figure ~~4~~ 5 shows the different values obtained for absorbance when the injected volume changes between 25 and 100 µL. ~~An~~The injected volume has a poor influence on the optical signal, so an injected volume of 25 µL was chosen because ~~good values for the area are obtained in this situation and~~the reagent consumption is fewer.

iv) Reaction coil length~~.~~

Figure ~~5~~ 6 shows the different values obtained for absorbance when the reaction coil length increases from 2 to 5.7 m. The higher length essayed was chosen because the best values for the area are obtained in this situation. Longer reaction ~~coil~~coils will ~~affects~~affect to analysis throughput ~~and do~~, not ~~significantly increases~~increasing the absorbance measured significantly.

v) pH.

Figure 6 7 shows the different values obtained for absorbance when the pH solution changes between 7 and 10 by modifying the citrate-ammonia proportion of buffer. As can be seen, the pH value has a poor influence on the signal registered while the pH value is within the appropriate range for blue quelate formation. A wider range of pH wasn't considered according to bibliographic information about the cuprizone blue quelate synthetic conditions. A value of 9.5 was chosen because this is the pH of the original buffer composition and the addition of other reagents is not necessary.

Analytical features

By taking into account the results of the operative parameters study previously described ~~variables study~~, the features of the FIA set-up have been evaluated under the following conditions: buffer pH, 9.5; buffer concentration, 1.0 M; cuprizone concentration, 5.13 mM; carrier flow rate, 0.5 mL min^{-1}; cuprizone injected volume 25 µL; reaction coil length, 5.7 m. For each standard solution, three peaks have been obtained, and the average of areas has been calculated. Under these conditions, a linear fit was derived between 1.2 and 12 µg mL^{-1}, with a typical equation: A = 2973.5 + 1090.5[Cu^{2+} (µg mL^{-1})] and a correlation coefficient r = 0.98, where A is the absorbance signal measured as area under peak.

The detection limit, estimated as three times the standard deviation of lower concentration measured divided by the slope, is 0.11 µg mL^{-1}. The quantification limit, derived from 10 times the standard deviation of the lower concentration measured divided by the slope, is 0.35 µg mL^{-1}. The R.S.D., obtained at the 4 µg mL^{-1} level (n=12), has a value of 3.82%. The analytical throughput of this system is 12 peaks h^{-1}. The buffered sample consumption is of 30 mL h^{-1} and 300 µL h^{-1} for the cuprizone solution.

Determination of monovalent copper

Copper (I) was determined by potentiostatic electrolysis, oxidizing to copper (II), while flow stream is maintained. Electrode potential must be carefully selected to avoid ~~interference~~possible interferences with other ions, ~~if present,~~ as Sn^{2+}, $[Co(NH_3)_6]^{2+}$ and Tl^{+}, whose standards potentials are ~~minor~~lower than Cu (II) / Cu (I) couple.

Optimisation of electrochemical flow method

To determine the potential used to oxidize copper (I), a hydrodynamic differential pulse voltammogram (DPV) was made while a 30 µg mL^{-1} buffered solution of copper (I) is flowing through the cell at roughly 0.5 mL min^{-1}. We have chosen a potential of 700 mV over the Ag/AgCl/NaCl(sat) reference electrode that corresponds to the voltammetric peak potential.

~~The analytical parameter, proportional to~~We can obtain information about the concentration of copper (I) in sample, ~~can be~~measuring the ~~recorded~~ total amount of charge consumed after a period of time, or also the current-limit reached after the transient electrode connection ~~transient~~. In both cases the electrode potential must be maintained for a long enough period to reach the steady state, which is achieved after 1 – 2 minutes. Our best results are obtained by sampling the current-limit for 5 to 10 seconds at the end of electrolysis and taking the average value, by this way also minimizing noise that could affect the data.

Other parameters such as flow rate, pH and others have not been analyzed and have been kept in the optimized values for copper (II) determination with a view to future integration of these two methods.

Analytical features

Watching the results of the study of variables described above, the features of the flow set-up have been evaluated under the following conditions: pH of the buffer, 9.5; buffer concentration, 1.0 M; buffered sample flow rate, 0.5 mL min^{-1}; electrolysis potential, 700 mV; electrolysis time, 1 minute; current sampling time, 10 seconds; reaction coil length, 5.7 m. For each standard solution, three results have been obtained, and the average has been calculated. Under these conditions, a linear fit was derived between 0.04 and 0.80 µg mL^{-1}, with a typical

equation: $I_{lim}(\mu A) = 8.52 + 66.43[Cu^{+} (\mu g\ mL^{-1})]$ and a correlation coefficient r = 0.99, where I_{lim} is the limiting current signal measured at steady state.

The detection limit, estimated as three times the standard deviation of lower concentration measured divided by the slope, is 7.7 ng mL^{-1}. The quantification limit, derived from 10 times the standard deviation of the lower concentration measured divided by the slow, is 26 ng mL^{-1}. The R.S.D., obtained at the 0.4 µg mL^{-1} level (n=12), have a value of 2.01%. The analytical throughput of this system is around 60 measurements by hour. The buffered sample consumption is 30 mL h^{-1}.

Speciation of copper (I) and copper (II) in mixture

According to the published information and the chosen operative conditions, both methods of quantification must be free of interferences due to the presence of copper ions in another oxidation state.

If the proposed sequential determination is made, the amperometric measures are performed in absence of cuprizone and determination of Cu (I) gives the same results even if there are different concentrations of Cu (II) (see figure ~~7~~8.a). On the other hand, the colorimetric measures are performed without applying oxidative potential ~~and also~~; therefore monovalent copper does not affects to the formation of colored chelate, whose concentration is proportional only to the Cu (II) contents of sample as can be seen in figure ~~7~~8.b

These results might offer the possibility of a simultaneous determination of both species. However, it is necessary to consider other precautions which are outlined below, as the effect of the presence of cuprizone in the electrochemical measures or the interferences that one of the analytical techniques used can produce in the other when both work simultaneously.

On the one hand, it is important to consider the influence of the electrode polarization on the spectroscopical measurements. Disturbances are not found in the spectroscopical baseline when the electrode is polarized or not. This is the expected result because the electrode is outside the optical pathway of the cell.

On the other hand, to verify whether the presence of cuprizone in the medium affects the electrochemical measurements, aliquots of this ligand were injected while a 4 µg mL^{-1} Cu (I) solution flowed through the cell and while the electrode was polarized. The same experience was repeated with a carrier solution containing 4 µg mL^{-1} of both oxidation state of copper.

The result of these experiments was a transitory decrease in the current-limit, with similar magnitude in both cases and minimal differences. To explain this result, an aliquot containing only the cuprizone solution's solvent was injected in the same conditions as before. Again, a similar decrease of the current-limit was observed. As conclusion, the main contribution to that decrease in current is the lower concentration of Cu (I) in the cell when the injected aliquot, free of the analyte, flows through it.

To explain the small differences observed in the aforementioned experiments, it could be ~~consider~~considered the influence of cuprizone in the redox potential of Cu (I) and the ~~possibility of~~possible formation of other electroactive species like $Cu^{II}(CPZ)_2$ and $Cu^{III}(CPZ)_2$. Yamamoto *et al*. [20], reports the redox potential of these species and compares them with the standard potential of Cu (I)/Cu (II) couple. However, these species can be easily avoided if the ligand is not ~~into the cell~~present when the electrode is polarized.

On the basis on the results obtained and the published information, it is desirable to apply the oxidation potential when cuprizone is not present in the solution, which does not allow the simultaneous determination of both species although sequential determination.

A single determination of both copper valences is completed at only 7 minutes. Therefore, the total throughput of the method is 8.6 measures by hour. The total buffered sample consumption is 30 mL h^{-1} and only 187.5 µL h^{-1} of cuprizone solution is needed. The amount of ammonium citrate buffer used ~~is dependent of~~depends on how concentrated ~~is~~ the sample is. It is necessary to consider both the dynamic range of the analytical method and the concentration of the sample. Normally, a ratio buffer/sample of 50% or less is enough, given the concentration used in the buffer solution, because the sample solution remains buffered.

CONCLUSIONS

A flow method for sequential analysis of free copper ions in aqueous solution is presented. The operational conditions are optimized for colorimetric determination of divalent copper with cuprizone and these parameters are used to take the electrochemical determination of monovalent copper.

The final operating conditions were: pH of the buffer, 9.5; buffer concentration, 1.0 M; cuprizone concentration, 5.13 mM; cuprizone injected volume 25 µL; buffered sample flow rate, 0.5 mL min^{-1}; reaction coil length, 5.7 m; electrolysis potential, 700 mV; electrolysis time, 1 minute; current sampling time, 10 seconds.

With ~~this~~these optimized parameters, a linear fit between 1.2 and 12 µg mL^{-1} was derived for the spectroscopical determination of Cu (II) with detection and quantification limits of 0.11 and 0.36 µg mL^{-1} respectively, and R.S.D. of 3.82%. For the electrochemical determination of Cu (I), a linear fit was derived between 0.04 and 0.8 µg mL^{-1}, with detection and quantification limits of 7.7 and 26 ng mL^{-1} respectively. A R.S.D. of 2.01% was determined.

~~The~~As is it summarized in Table 2, the proposed method offers dynamic range a bit lower than previously published spectroscopical flow methods for the Cu (II) determination [12,14,15] and slightly worse detection limits, although it still remains its application range around the maximum limit allowed regulated by WHO for this metal in drinking water [21] stated at 2 µg mL^{-1}. In return, it offers the possibility of copper speciation according to its oxidation state in a single measure, a type of analysis that may be of interest in biological tissues or in natural samples with low oxygen concentrations, in which the presence of both ions is it possible. Furthermore, the figures of merit of Cu (I) determination are clearly better than the Cu (II).

Although the analytical performance characteristics of the individual methods are comparable to previously reported, the possibility to quantify both valences in one shot is not yet reported at reviewed bibliography.

A further advantage of our method is the possibility of automating the process by synchronizing the two techniques, also using a very affordable instrumentation.

In conclusion, a simple and cheap sequential method is presented for the fast speciation of inorganic ions of copper in aqueous media, which requires small amounts of reagents. This allows include it in the “Green Chemistry” concept.

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Table 1. Schlieren effect correction

Assay	Carrier composition	Injection composition	Comments
I	Aqueous copper (II) [a]	Cuprizone solution + buffer[b]	Figure 2.a)
II	Aqueous copper (II) + ethanol[c]	Cuprizone solution + buffer	Bubble evolution and unstable baseline
III	Aqueous copper (II) + ethanol + buffer[d]	Cuprizone solution + buffer	No signal observed
IV	Aqueous copper (II) + buffer[e]	Cuprizone solution + buffer	Broad and noisy signals
V	Aqueous copper (II) + buffer	Cuprizone solution[f]	Figure 2.b)
VI	Aqueous copper (II) + ethanol + buffer	Cuprizone solution	Figure 2.c)

[a] 2 μg mL^{-1} of copper (II) in water
[b] 5.13 mM cuprizone in 1 M ammonia-citrate buffer and 14.28% ethanol (v/v)
[c] 2 μg mL^{-1} of copper (II) in water and 14.28% ethanol (v/v)
[d] 2 μg mL^{-1} of copper (II) in 1 M ammonia-citrate buffer and 14.28% ethanol (v/v)
[e] 2 μg mL^{-1} of copper (II) in 1 M ammonia-citrate buffer
[f] 5.13 mM cuprizone in water and 14.28% ethanol (v/v) as described in *Reagents and solutions*

Table 2. Copper determination methods in bibliography

Ref.	Analyte	Method	Reagent	LOD	Dynamic range	Sample
[5]	Cu (II)	AdS-LV	thiosemicarbazide	0.007 ng mL^{-1}	0.01 - 90 ng mL^{-1}	Food
[6]	Cu	atomic absorption spectroscopy	ammonium pyrollidine dithiocarbamate			Sea water
[7]	Cu	atomic absorption spectroscopy				Water
[8]	Cu(II)	ion selective electrode			0.05 - 2 µg mL^{-1}	White wine
[9]	Cu(I)	fluorometry	bathocuproine disulfonate	0.1 µM		Biological
[10]	Cu(I) Cu(II)	titrimetry	iodide			
[11]	Cu(II)	colorimetry	neocuproine	0.12 ng mL^{-1}		Water
[12]	Cu(II)	colorimetry	2,5-dimercapto-1,3,4-thiadiazole		0.1 - 20 µg mL^{-1}	Water, soil, biological
[13]	Cu(II)	FIA-colorimetry	cuprizone	0.13 µg mL^{-1}	- 20 µg mL^{-1}	Alloys
[14]	Cu(II)	FIA-colorimetry	cuprizone	13 ng mL^{-1}	0.06 - 4 µg mL^{-1}	Water
[15]	Cu(II)	MSFIA-colorimetry	zincon	0.1 ng mL^{-1}	- 100 ng mL^{-1}	Water
This work	Cu (I) Cu(II)	FIA-amperometry FIA-colorimetry	---------- cuprizone	7.7 ng mL^{-1} 0.11 µg mL^{-1}	0.04 - 0.8 µg mL^{-1} 1.2 - 12 µg mL^{-1}	Water

FIGURE CAPTIONS

Figure 1. Flow-cell diagram

Figure 2. Timing diagram for synchronized flow-determination of mono- and divalent copper

Figure ~~2~~3. Shape of the absorbance signals obtained with different compositions of the carrier and injected solutions: a) carrier: aqueous copper; injection: cuprizone + buffer. b) carrier: aqueous copper + buffer; injection: cuprizone. c) carrier: aqueous copper + buffer + ethanol; injection: cuprizone. Other operative conditions: pH = 8.5; reaction coil, 5.7 m; injected volume, 25 µL; flow rate, 3 mL min^{-1}; [Cu (II)] = 2 µg mL^{-1}

Figure ~~3~~4. Carrier flow rate effect on absorbance. Other conditions: injected volume, 25 µL; reaction coil length, 5.7 m; pH = 8.5; [Cu (II)] = 2 µg mL^{-1}

Figure ~~4~~5. Volume of injection loop effect on absorbance. Other conditions: reaction coil length, 5.7 m; carrier flow rate, 0.5 mL min^{-1}; pH = 9.5; [Cu (II)] = 2 µg mL^{-1}

Figure ~~5~~6. Reaction coil length effect on absorbance. Other conditions: carrier flow rate, 0.5 mL min^{-1}; injected volume, 25 µL; pH = 8.5; [Cu (II)] = 2 µg mL^{-1}

Figure ~~6~~7. pH effect on absorbance. Other conditions: injected volume, 25 µL; reaction coil length, 5.7 m; carrier flow rate, 0.5 mL min^{-1}; [Cu (II)] = 2 µg mL^{-1}

Figure ~~7~~8. Study of interferences due to the presence of both analytes in the carrier. a) Electrochemical response for 0.4 µg mL^{-1} of Cu (I) and several concentrations of Cu (II). b) Spectroscopic response for 4 µg mL^{-1} of Cu (II) and several concentrations of Cu (I)

Figure 1

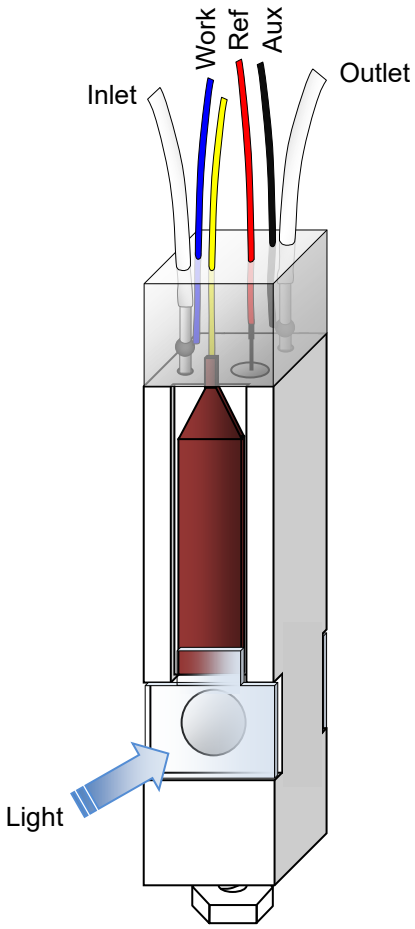


Figure 2

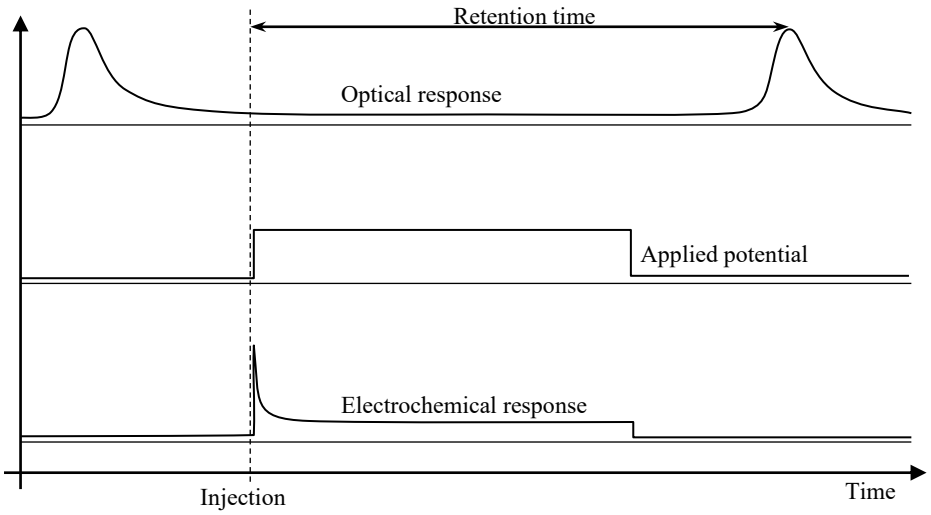

Retention time
Optical response
Applied potential
Electrochemical response
Injection
Time

Figure 3

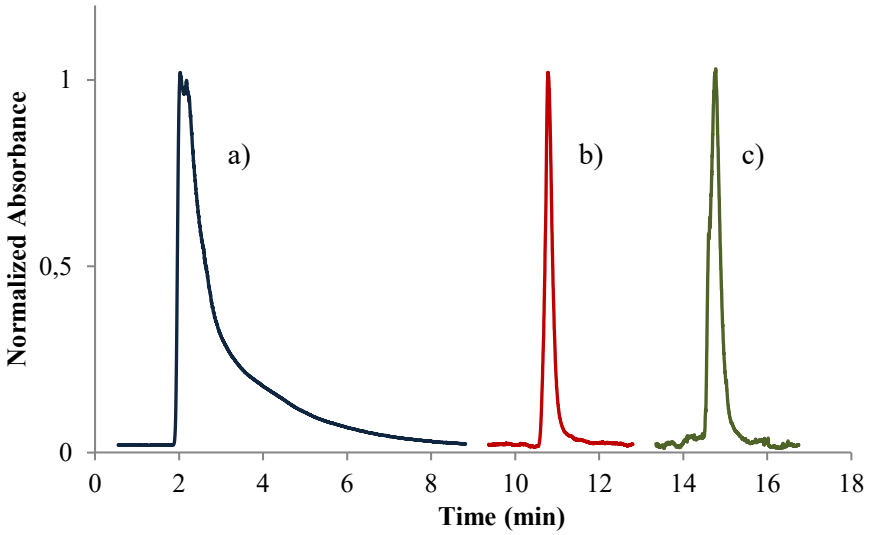


Figure 4

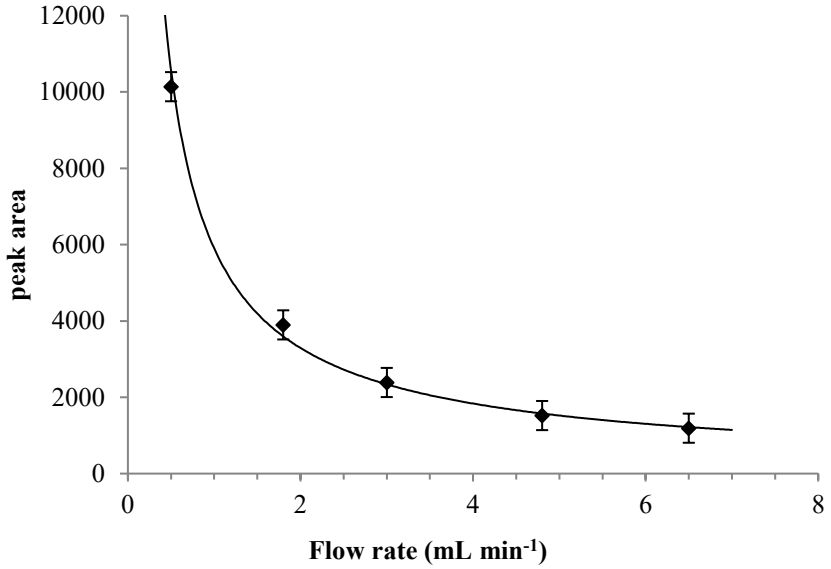

12000
10000
8000
6000
4000
2000
0
peak area
0
2
4
6
8
Flow rate (mL min-1)

Figure 5

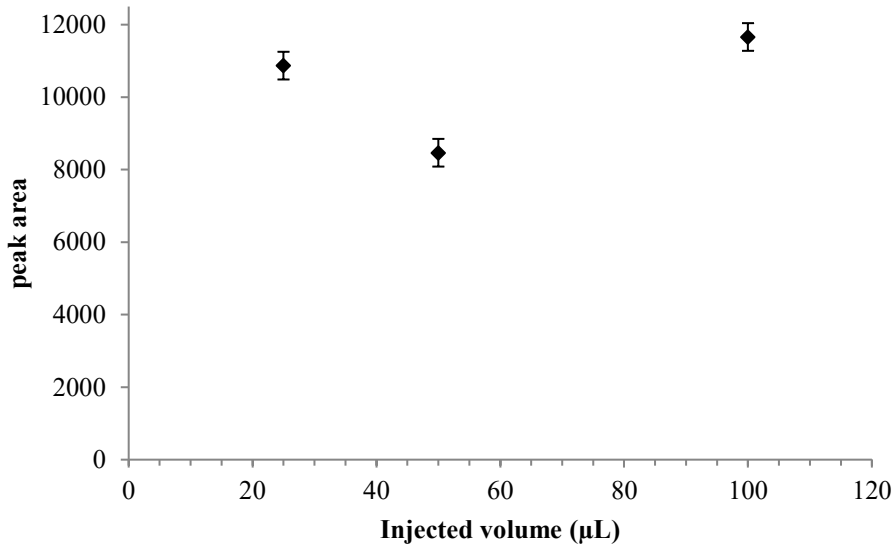

12000
10000
8000
6000
4000
2000
0
peak area
0
20
40
60
80
100
120
Injected volume (µL)

Figure 6

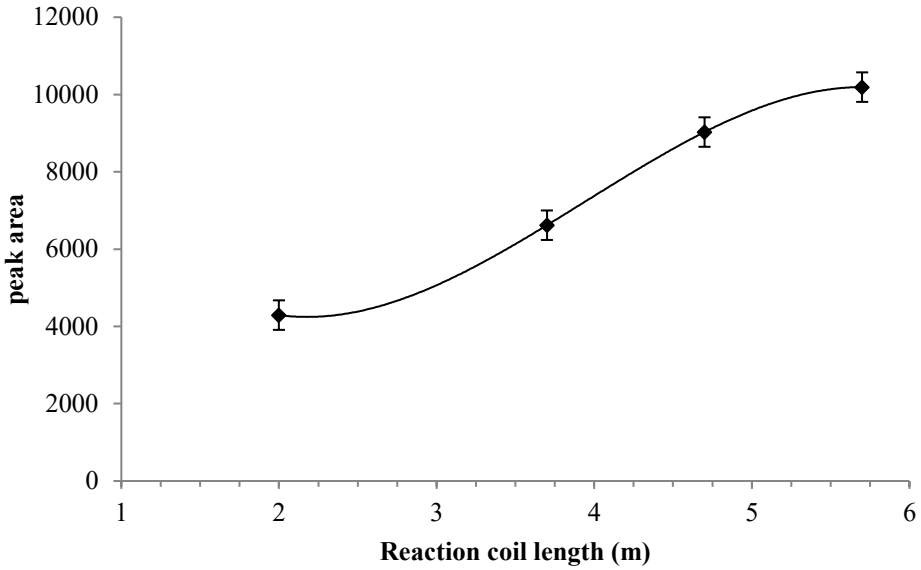

12000
10000
8000
6000
4000
2000
0
peak area
1
2
3
4
5
6
Reaction coil length (m)

Figure 7

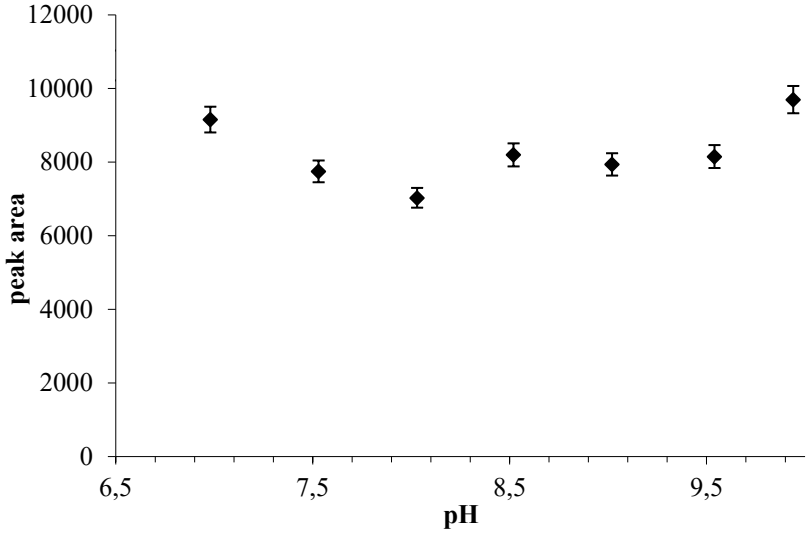
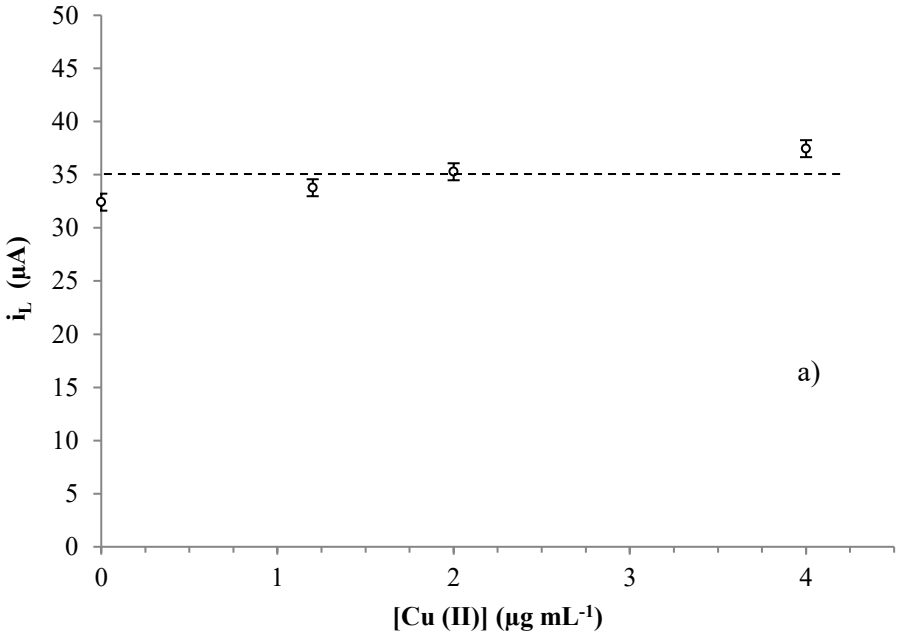
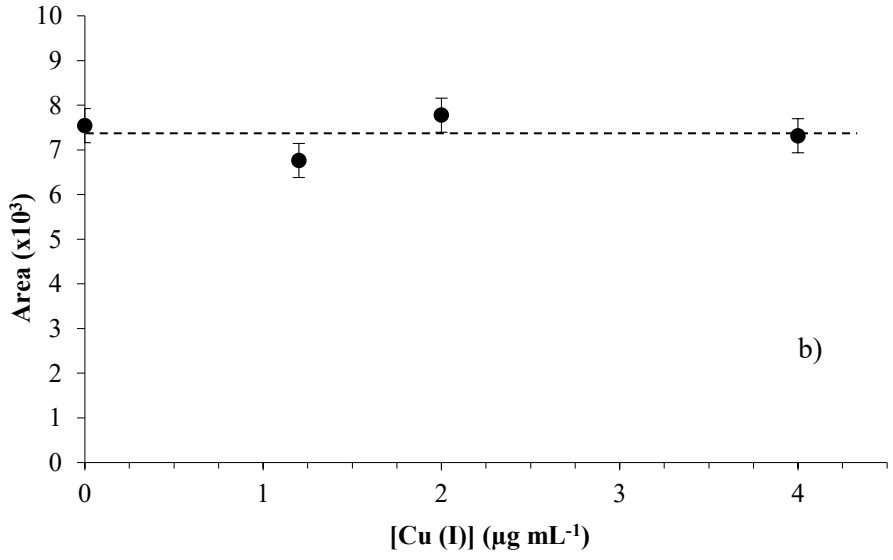
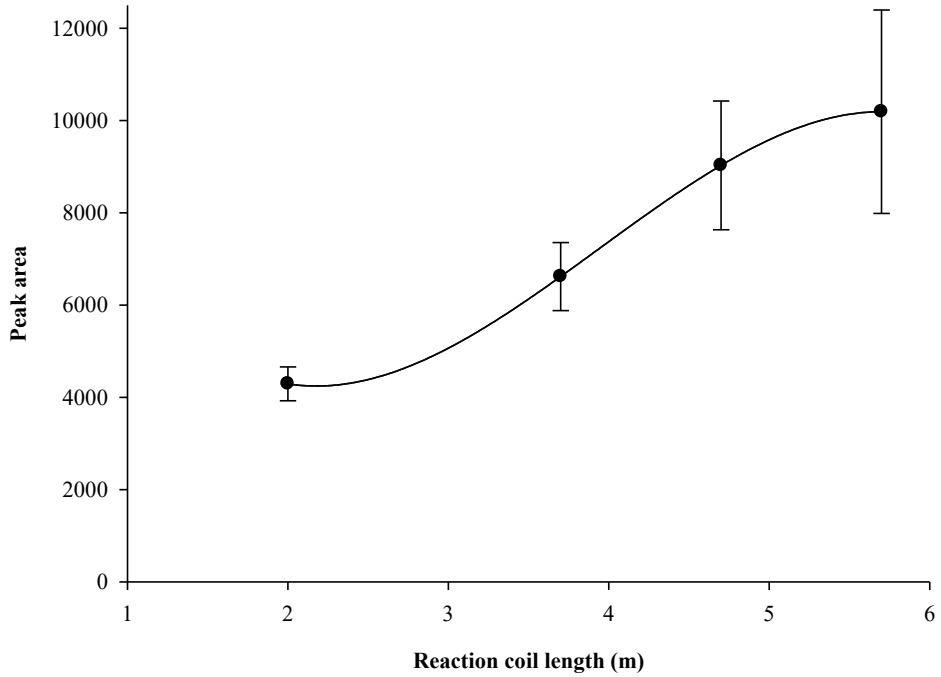
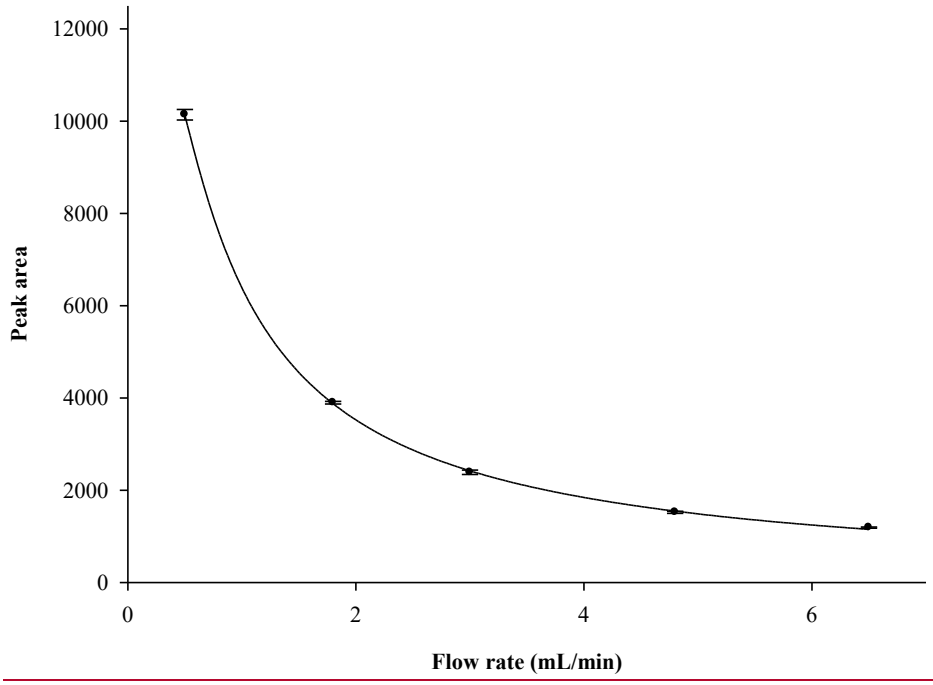


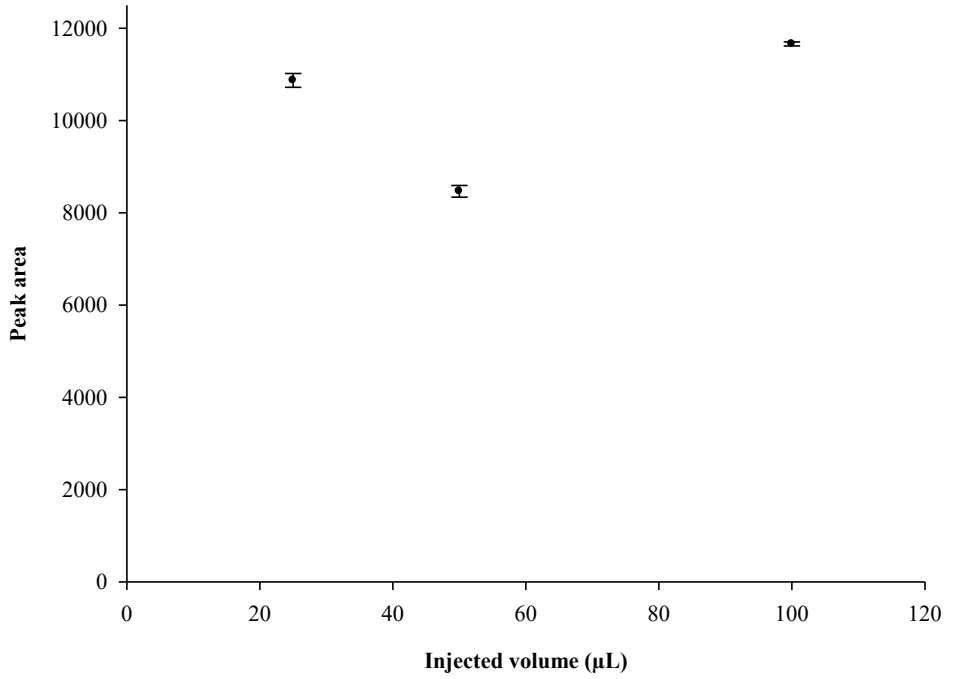
Figure 8

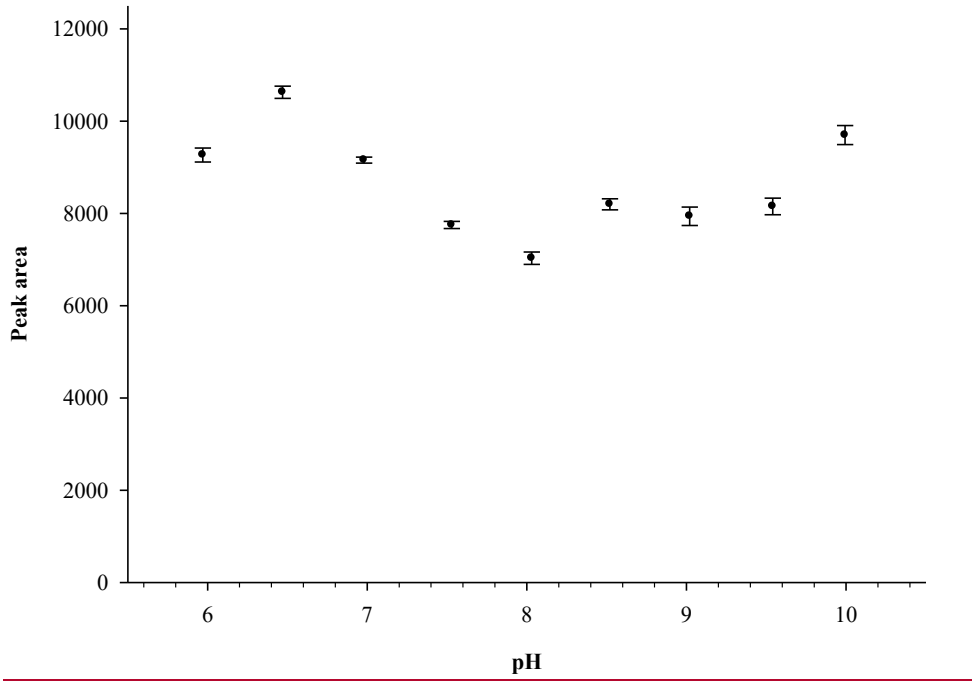

50
45
40
35
30
25
20
15
10
5
0
i_L (µA)
a)
0
1
2
3
4
[Cu (II)] (µg mL^{-1})


10
9
8
7
6
5
4
3
2
1
0
Area (x10^3)
b)
0
1
2
3
4
[Cu (I)] (µg mL^{-1})


12000
10000
8000
6000
4000
2000
0
Peak area
1
2
3
4
5
6
Reaction coil length (m)


12000
10000
8000
6000
4000
2000
0
Peak area
0
2
4
6
Flow rate (mL/min)


12000
10000
8000
6000
4000
2000
0
Peak area
0
20
40
60
80
100
120
Injected volume (µL)


12000
10000
8000
6000
4000
2000
0
Peak area
6
7
8
9
10
pH