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Highly Selective Electrochemical Detection of Copper (II) Using *N,N'*-bis(acetylacetone)ethylenediimine as a Receptor

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The highly selective, indirect detection of copper (II) ions at a bare glassy carbon electrode, in the presence of *N,N'*-bis(acetylacetone)ethylenediimine, is reported here. The developed method is based on the electrochemical signal of the complex formed between the above mentioned ligand and the copper (II) ions. The nature of the formed complex was investigated by spectrophotometric and electrochemical methods. The developed electrochemical method is fast, cheap, easy to perform and it proved to be very selective for copper (II). Using, the new method a linear response was obtained for Cu (II) ions in the concentration range of 10 µM to 2 mM, with a limit of detection (LOD) of 0.635 ppm. The optimized electrochemical method was successfully used for the detection of copper (II) ions from real samples. It was also demonstrated that the occurrence of the anodic oxidation peak for the complex was strongly dependent on the immobilization technique of the ligand at the surface of the electrode.

Keywords: selective Cu (II) detection; bare glassy carbon electrode; *N,N'*-bis(acetylacetone)ethylenediimine; indirect detection; electroactive complex; flow injection analysis

1. INTRODUCTION

Copper is an essential micronutrient, part of several proteins involved in a variety of biological processes, required for growth, development and homeostasis, playing a central role in the biochemistry of every living organism. Low copper status has been associated with anaemia, bone demineralization, depigmentation of the skin and the hair, poor immune response and cardiovascular

effects [1]. In order to treat copper deficiency, many food supplements containing copper (II) ions have been elaborated and are commercially available.

At the same time, copper in excess can be toxic, its toxicity being typically expressed by the development of liver cirrhosis with episodes of haemolysis and damage to renal tubules, to the brain and to other organs [2].

The common methods for the detection of copper (II) and other metals include liquid chromatography [3], electrophoresis [4], spectrophotometry [5], solid-phase extraction coupled with atomic absorption spectroscopy [6], atomic emission spectroscopy [7] and inductively coupled plasma mass spectrometry [8]. However, even if these methods present high sensitivity and selectivity, they are expensive, time-consuming and cannot be used for *in situ* analyses. Therefore, there is a growing interest in developing electrochemical methods for the detection of copper ions in different matrices.

Electrochemical detection of heavy metals presents many advantages [9], such as good sensitivity, high selectivity without any prior separation (due to complexation by organic molecules containing coordinating sites), fast analytical response making them useful for flow analysis [10] and alert systems, ease of use (simple and low cost equipment, few analytical steps) and it offers the possibility of outside laboratory analyses, using a portable "pocket" potentiostat.

Among the electrochemical methods for the detection of heavy metals, the most used ones are the anodic stripping voltammetry, the biosensors and the potentiometry. The latter one is widely used, the membrane-based and the solid-contact ion-selective electrodes (ISEs) representing the largest group among potentiometric sensors [11]. The ionophore is a key component of the ISEs, assuring the selectivity of the sensor for a certain ion. For example, a carbon paste electrode (CPE) modified with etioporphyrin I has been used for the detection of Cu^{2+} in the range of 1.28 μM – 12.8 mM [12] and a potentiometric sensor based on bis(acetylacetone)propylenediimine combined with anion localizing agent (sodium tetraphenyl borate) and solvent mediators (dibutyl butyl phosphonate, tri-*n*-butyl phosphate and chloronaphthalene) was able to detect Cu (II) in the range of 10 μM to 100 mM [13]. The potentiometric sensors are relatively inexpensive, easy to use, they can measure both positive and negative ions, but in some cases, they can lack selectivity, sensitivity and they need complex fabrication and frequent calibrations for good precision levels [14,15].

The analysis of heavy metal ions with biosensors relies on enzyme inhibition or activation. For example, Cu (II) was detected in the range of 0.05 to 4 mM using an amperometric biosensor based on the inhibition of acetylcholinesterase [16] and in the range of 0.05 – 1 mM using a membrane with ascorbate oxidase (copper-depending enzyme) onto a flow-through oxygen electrode, based on the apoenzyme reactivation method [17]. Biosensors present the advantages of specificity, but they can exhibit a limited lifetime, low sensitivity, low reproducibility and lack of selectivity in the case of the inhibition-based enzyme biosensors, as some enzymes are inhibited by several metals and even other pollutants (18).

Due to its high sensitivity, with LODs of nM, the most employed electroanalytical method for the detection of heavy metals is the anodic stripping voltammetry (ASV) [9,19]. ASV involves two steps: the accumulation of the analyte within or at the working electrode by applying a reduction potential (the electrodeposition step), followed by the electrochemical measurement (the stripping step). The preconcentration step leads to long analysis time, reaching, sometimes, ten minutes [20] and

to a certain lack of selectivity, since other electroactive species, having the potential reduction in the same range as the metallic ion to be analyzed, strongly interfere. Different electrode materials (bismuth film [21], gold [22] or carbon electrodes [23]) have been employed, as working electrodes, to replace the highly toxic hanging mercury drop electrode (HMDE) [24] or mercury film electrodes [25], and many substitute methods have been developed. Using chemically modified electrodes (CMEs), these methods involve the preconcentration of the analyte on the electrode surface, followed by the electrochemical analysis. The method based on the complexation of ions consists in the immobilization of a receptor at the surface of the electrode, which preconcentrates the analyte, facilitating its detection. Several molecular receptors, like EDTA [26], oligopeptides [27] or polymeric film [28], have been studied for the preconcentration of copper (II) ions. Compared to mercury electrode, this method presents better selectivity, due to the specifically designed receptor, but it involves a more or less complicated fabrication of the modified electrodes, with reproducibility and reusability issues and a prolongation of the analysis time up to 20 min [29].

Although there are many papers on the use of chemical receptors for the electrochemical detection of metal ions, few of them rely on electroactive ligands for indirect determination of the metals, like the use of a solution of sodium thiopentone for the indirect detection of Mg (II), this cation being able to increase the cathodic peak of the ligand [30].

As far as we know, this is the first report on the indirect detection of copper (II) ions based on the electrochemical signal at a bare glassy carbon electrode of the complex of Cu^{2+} ions with *N,N'*-bis(acetylacetone)ethylenediimine (Figure 1), a highly selective ligand. The developed electrochemical method, enables a very selective, fast, low cost, suitable for flow and *in situ* detection of copper (II), with sensitivity comparable with that achieved by some biosensors and potentiometric sensors. The nature of the formed complex was investigated by spectrophotometric and electrochemical methods. The optimized electrochemical method was successfully used for the detection of copper (II) ions from real samples. In order to modify the electrode with the ligand, different immobilization strategies were envisaged and the results demonstrated the occurrence of the anodic oxidation peak for the complex was strongly dependent on the immobilization technique of the ligand at the surface of the electrode. It was also demonstrated the suitability of the developed method for flow injection analysis.

Figure 1. *N,N'*-bis(acetylacetone)ethylenediimine and its complex with copper (II) ion

2. MATERIALS AND METHODS

2.1. Reagents and materials

Copper (II) chloride anhydrous, zinc (II) nitrate hexahydrate, lead (II) nitrate, iron (III) nitrate nonahydrate, potassium chloride, lithium perchlorate, glacial acetic acid, phosphoric acid and nitric

acid were purchased from Acros, cobalt (II) nitrate hexahydrate, chromium (III) nitrate nonahydrate, iron (II) sulphate heptahydrate, manganese (II) chloride tetrahydrate, mercury (II) nitrate monohydrate, aluminium (III) chloride hexahydrate, calcium (II) nitrate tetrahydrate, magnesium (II) chloride hexahydrate, potassium hexacyanoferrate (II) trihydrate, potassium hexacyanoferrate (III) from Merck and nickel (II) nitrate hexahydrate, cadmium (II) nitrate tetrahydrate, monosodium and disodium phosphate, polyethyleneimine (MW 60000) (PEI), Nafion solution (5%) and methanol from Sigma-Aldrich.

All reagents were of analytical grade and were used as received. All solutions were prepared with ultrapure water (18.2 MΩ, Millipore Simplicity) and all glassware was rinsed before use with a 10% HNO_3 solution followed by ultrapure water, in order to avoid metal contamination.

2.2. Spectrophotometric analyses

Spectrophotometric analyses were performed using a SPECORD 250 PLUS UV-VIS spectrophotometer (Analytik Jena AG, Jena, Germany), with 1 cm cells, recording the ultraviolet (UV) spectral scans between 200 and 350 nm and the visible (Vis) spectral scans between 400 and 780 nm, with a 1 nm slit, 1 nm wave length step and a speed of 5 nm s^{-1}.

2.3. Electrochemical measurements

The electrochemical experiments were performed using an AUTOLAB PGSTAT 302N (Ecochemie, The Netherlands) equipped with the associated NOVA 1.10 software. The glassy carbon electrode (GCE) with a geometrical surface area about 0.12 cm^2 and carbon paste electrode (CPE), used as working electrodes in the conventional three-electrode cell, along with Ag/AgCl KCl 3 M (SSCE) as reference electrode and a Pt wire as counter electrode, were purchased from BAS Inc. (West Lafayette, USA). Before each analysis, the GCE was polished using an alumina suspension and polishing cloth.

The carbon paste (CP) was prepared by hand-mixing graphite powder with melted solid paraffin in a ratio of 9:1 (m/m). The CPE was constructed by filling the Teflon cavity (d = 4 mm; h = 5 mm) with the homemade carbon paste, the electric contact being provided by a copper wire. The surface was then manually smoothed against paper until a shiny surface was obtained.

The pH of the solutions was determined with a ChemCadet pH-meter.

The solution to be analyzed containing 0.1 M electrolyte, 0.01 M ligand and Cu (II) ions was prepared daily, just before the experiments and it was used undearated.

The cyclic voltammetry (CV) experiments were performed in the conventional three-electrode cell of 5 mL, in static mode, using a bare GCE as working electrode and at scan rate of 0.1 V s^{-1}.

For the quantitative analyses, differential pulse voltammetry (DPV) was employed. After varying both the pulse height (PH) and pulse width (PW), the following DPV parameters were employed: potential window between 0.5 V_{SSCE} to 1 V_{SSCE}, scan rate of 0.01 V s^{-1}, PH of 0.1 V and PW of 25 ms.

For the tests on the electrodes modified with the ligand, the solution to be analyzed no longer contained the ligand, only electrolyte and Cu (II) ions.

2.4. Synthesis of N,N'-bis(acetylacetone)ethylenediimine

N,N'-bis(acetylacetone)ethylenediimine was obtained in accordance with the literature data [31] by reaction of ethylenediamine with 2,4 pentanedione (acetylacetone) in a molar ratio of 1:2. 330 μL (5 mmol) anhydrous ethylenediamine were added dropwise to 1 mL (10 mmol, 2 eq.) acetylacetone under agitation. The reaction is spontaneous and exothermic, leading to the evaporation of the water formed during the reaction. The light yellow coloured product solidified when it was cooled and the colourless ligand was obtained after two times crystallization from water. The ^{1}H NMR spectrum of the obtained product was in accordance with the literature [31].

2.5. Preparation of the modified electrodes

The modification of the GCE by drop-coating was performed by applying 25 μL of a solution containing 20 mg *N,N'*-bis(acetylacetone)ethylenediimine in 1 mL methanol. Afterwards, the electrode was left to air-dry.

The immobilization of the ligand at the surface of the electrode through a covalent bond was obtained by performing 10 scans of cyclic voltammetry (CV), sweeping the potential between 0 to 1.2 V_{SSCE}. The CVs were performed in a static mode, in a three-electrode cell, containing 10 mM of ligand in a 0.1M $LiClO_4$ solution.

The incorporation of the ligand in PEI was performed by mixing 20 mg ligand with 5 mL or 1 mL PEI solution (5 mg mL^{-1} prepared in 50:50 (v/v) ethanol/water mixture). The modification of the electrode with the PEI solution containing ligand (4 mg/mL or 20 mg/mL) was performed by applying two drops of 5 μL of the PEI solution onto the electrode and air-drying after each application.

The modification of the GCE with the Nafion film incorporating the ligand was done by applying 10 μL of an ethanolic solution containing 1% Nafion and 4 mg/mL ligand. This solution was the result of mixing together 400 μL ethanolic solution of the ligand (5 mg/mL) and 100 μL ethanolic solution of Nafion (5%).

The incorporation of the *N,N'*-bis(acetylacetone)ethylenediimine in the carbon paste was assured by mixing together priory made 475 mg CP with 10% paraffin, 25 mg ligand and 2 mL methanol or by mixing together 80 mg CP, 20 mg ligand and 0.5 mL methanol. These were heated and mixed until the complete evaporation of the methanol, leading to a 5% or a 20% ligand containing CP. Then, the modified CPE was obtained according to the procedure described above.

2.6. Real samples analyses

The presence of Cu (II) ions in the unspiked tap water was verified by performing a DPV analysis in a 0.1 M $LiClO_4$ solution, prepared with tap water. The 0.1 M $LiClO_4$ tap water solution was

then spiked with 10^{-2} M $CuCl_2$ stock solution to obtain 10^{-4} M, 2×10^{-4} M, 3×10^{-4} M, 4×10^{-4} M and 5×10^{-4} M spiked tap water. The concentration of copper (II) ions was determined by the standard addition method (n=4).

The same procedure was applied to an oral solution containing copper (II) ions (Oligosol from Labcatal, Montrouge, France). Using the declared concentration in copper (725.2 µg Cu^{2+} / 2 mL oral solution), a solution of 10^{-4} M of copper in ultrapure water was prepared by diluting the Oligosol solution. It was analyzed by DPV and the concentration of copper was then determined by the standard addition method (n = 4).

2.7. Flow injection analysis

A homemade flow injection cell was used to demonstrate the applicability of the developed method for flow injection analyses. The solution containing 0.1 M $LiClO_4$ and 0.01 M ligand was percolated by the Heidolph Pumpdrive 5201 peristaltic pump, with a flow rate of 1.6 mL min^{-1}, through an Eppendorf tube adapted to accommodate the three electrodes (GCE, SSCE and Pt). The injection of Cu (II) solutions with concentrations of 10^{-4} M, 10^{-3} M and 10^{-2} M was achieved with an injection valve with an injection loop of 50 µL. The electrochemical analysis consisted in a chronoamperometry, with the applied potential of 0.85 V_{SSCE} for 15 min.

3. RESULTS AND DISCUSSIONS

3.1. Spectrophotometric analyses

In order to evaluate the chelating capacity of *N,N'*-bis(acetylacetone)ethylenediimine towards copper (II) ions in aqueous solutions, UV spectrophotometry analyses were performed (Figure 2). The spectrum of the aqueous solution of the ligand, of the copper (II) acetate ($CuAc_2$) and of the ligand in the presence of copper (II) acetate were recorded for pH 3 to 7 in acetate buffer 0.1 M, and the medium of pH 8 was achieved by adding diethylamine to an aqueous solution.

The ligand spectra vary with the pH of the solutions: in an acidic solution of pH 3 the spectrum of the ligand showed only one peak, at 320 nm, while in solutions of pH 4 to 7 the absorbance increases in the region 300-320 nm with a small peak at 300 nm and a larger one at 320 nm and in alkaline medium the peak at 300 nm is as large as the one at 320 nm.

The reaction between *N,N'*-bis(acetylacetone)ethylenediimine and Cu (II) ions is also influenced by the pH. At pH 3, the absence of any change in the spectra of the ligand when $CuAc_2$ was added suggests that little to no interactions take place at this pH between the molecule and the Cu (II) ions take place at this pH, the protonation of the amino groups preventing the complexation reaction.

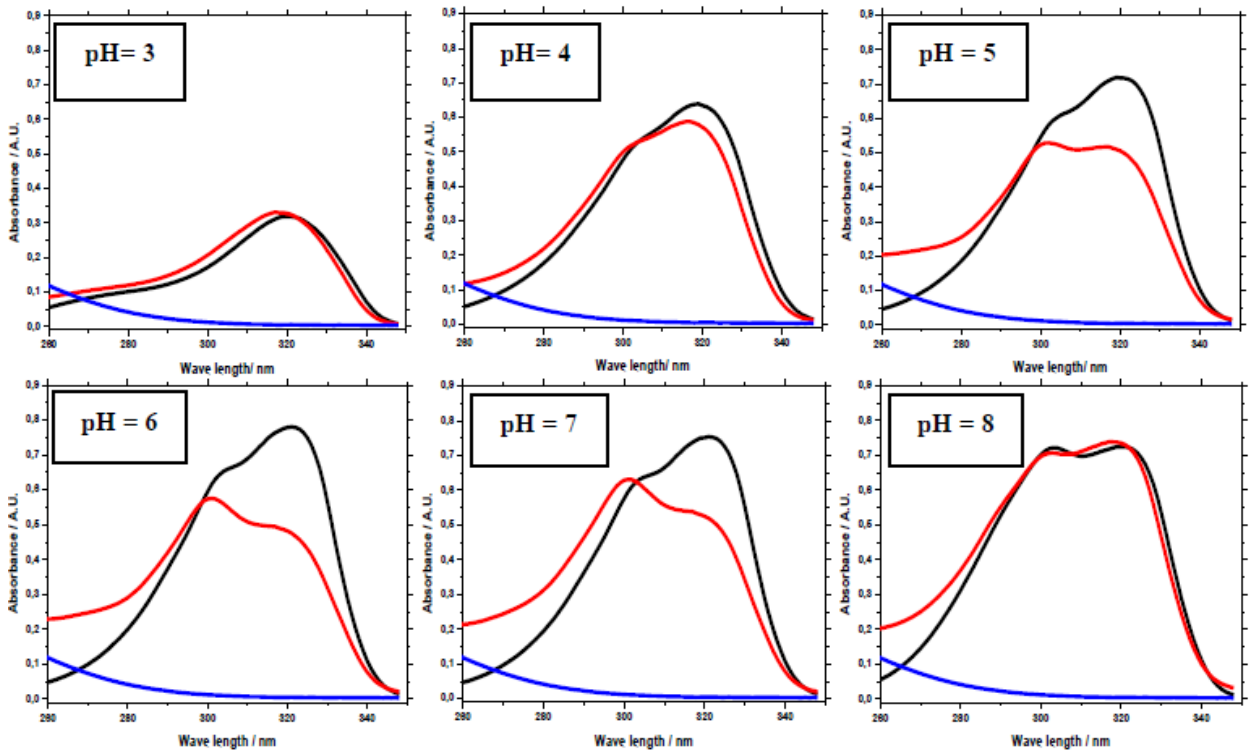

Figure 2. UV spectra of aquous solutions of 5×10^{-5} M $CuAc_2$ (—), of 5×10^{-5} M *N,N'*-bis(acetylacetone)ethylenediimine (—) and of 5×10^{-5} M *N,N'*-bis(acetylacetone) ethylenediimine in the presence of 5×10^{-5} M $CuAc_2$ (—) at different pHs.

The complexation reaction is facilitated by the increase of the pH of the reaction medium, since the spectra change, obtaining an increase of the peak at 300 nm and a decrease of the peak at 320 nm. This is in agreement with previously reported studies on copper (II) complexes formed with other ligands containing within their molecules N and O atoms [32]. These changes in the spectrum of the ligand are not as clear as in the case of other spectrophotometric analyses of complexes, where a clear shift of the peak was obtained [33], hence the impossibility to develop a UV spectrophotometric detection method. The strongest interactions seem to occur at pHs between 6 and 7. Taking this into consideration, a neutral medium was used for the electrochemical experiments. The UV spectrophotometry analyses did not show the formation of the complex with copper (II) at pH 8. Even if it was expected for the chosen receptor to present the best complexing capacity in its basic form, at pH 8 there were no differences in the spectrum of the ligand in the presence of $CuAc_2$. This can be explained by the precipitation of $Cu(OH)_2$, formed at this pH for concentrations of $CuAc_2$ of 5×10^{-5} M.

Some kinetics studies were performed in acetate buffer 0.1 M, pH 7. The ligand was left in contact with the Cu (II) ions for 1, 5 or 10 min prior to the recording of the spectrum. There were no significant differences between the three spectra, demonstrating that the complexation reaction is fast.

Besides proving that the molecular receptor is capable to complex copper (II) in aqueous solutions, the UV spectrophotometry analyses also showed its great selectivity for the copper (II) complexation. The spectra recorded for solutions of acetate buffer 0.1 M, pH 6, containing the ligand

and Co^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Al^{3+}, Mn^{2+} or Hg^{2+}, showed no changes in the spectrum of the ligand, proving that the chosen ligand presents a good selectivity for the complexation of Cu^{2+} ions in aqueous solutions.

The formation of the copper (II) complex was confirmed as well by Vis spectrophotometric experiments (Figure 3). By adding Cu^{2+} ions to an aqueous solution of the receptor the solution becomes violet, but only in the case of concentrated solutions. The Vis spectrum shows the presence of a peak around 550 nm, but this peak, specific for the formed complex, was not used for spectrophotometric determination, because of its lower molar absorption coefficient, compared to the UV spectrum.

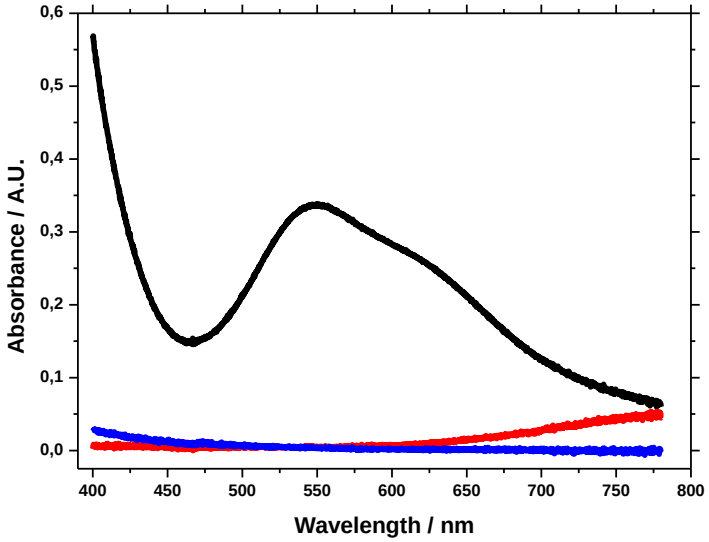

Figure 3. Vis spectra of aqueous solutions of 10^{-2} M ligand alone (—), of 5×10^{-3} M Cu^{2+} (—) and 10^{-2} M ligand in the presence of 5×10^{-3} M Cu^{2+} (—)

3.2. Electrochemical analyses

3.2.1. Investigation of the anodic oxidation of the complex

The Cu (II) complex, formed in aqueous solution, presents a characteristic oxidation peak at 0.69 V_{SSCE}, allowing the Cu (II) analysis by electrochemical techniques.

This peak was investigated by adding ligand to a solution of copper (II) chloride (Figure 4A) and by adding Cu^{2+} ions to a ligand solution (Figure 4B). In the first case, the successive additions of the ligand led to the decrease of the copper oxidation peak from 0.1 V_{SSCE} and of the copper reduction peaks from -0.05 V_{SSCE} and -0.175 V_{SSCE}. Furthermore, the peak from 0.69 V_{SSCE}, corresponding to the oxidation of the complex, increased. At greater concentrations of the ligand, both the oxidation and the reduction peaks for copper disappeared and an increase of the current after 1 V was observed, due to the oxidation of the ligand still left unreacted with the Cu (II) ions, but no clear peak was observed, as in the case of the ligand solution (Figure 4B). In the second case, the successive additions of the Cu (II) ions led also to the increase of the peak from 0.69 V_{SSCE}, but no decrease of the peak from 1.1 V is

observed, suggesting that there still remains ligand that is not involved in the complex. A peak for copper oxidation is obtained only in the presence of a great concentration in Cu^{2+}, the peak being very small.

It is interesting to notice that the peak from 0.69 V_{SSCE} is obtained even if the CV was performed between 0.5 V_{SSCE} and 1 V_{SSCE} (data not shown), confirming the anodic oxidation of the amino group [34] of the ligand from the complex and refuting the possibility that the oxidation peak from 0.69 V_{SSCE} might be due to the oxidation of the priory electrochemically reduced copper.

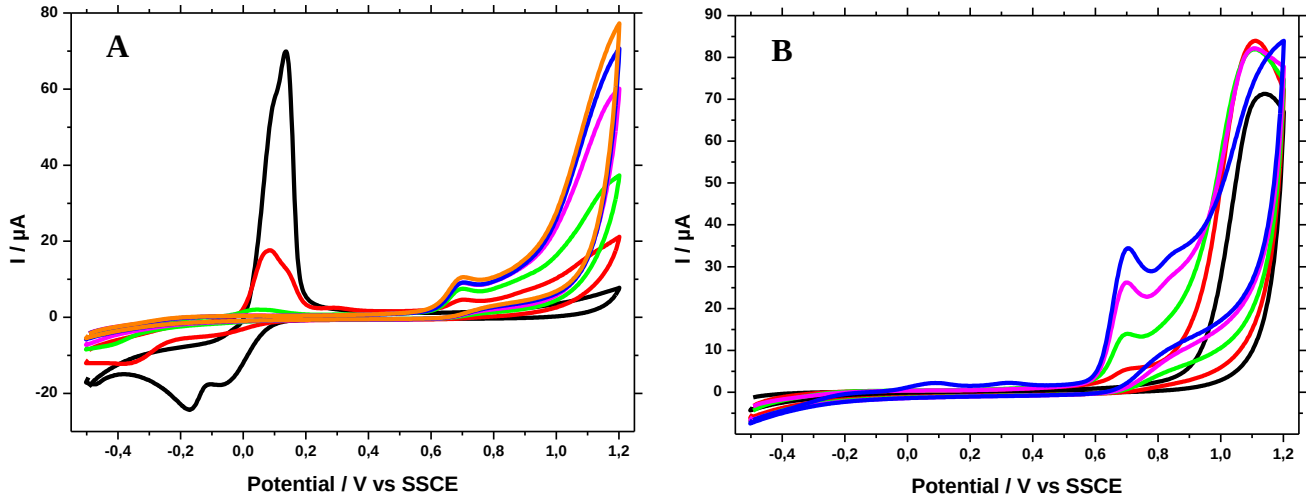

Figure 4. A) CVs at GCE in 5 mL solution Cu^{2+} 1 mM in $LiClO_4$ 0.1 M (—) and 50 (—), 100 (—), 150 (—), 200 (—) and 250 (—) µL ligand solution (90 mM); **B)** CVs at GCE in 4.5 mL solution of $LiClO_4$ 0.1 M with 15 mg ligand (—) and 0.1 (—), 0.5 (—), 1 (—)and 2 (—) mL 10 mM Cu^{2+} solution. Scan rate 0.1 V s^{-1}

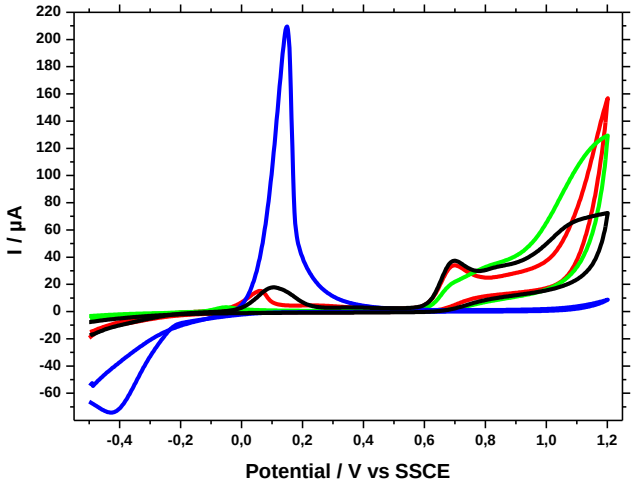

Figure 5. CVs at GCE in a solution containing 10 mM ligand and 5 mM Cu^{2+} of 0.1M $LiClO_4$ (—), 0.1M KCl (—), 0.5 M phosphate buffer pH=6.9 (—) and 0.5 M phosphate buffer pH=5 (—). Scan rate 0.1 V s^{-1}

The voltamperommetric analyses confirmed the dependence of the formation of the complex upon the pH of the medium: at pH lower than 5, conditions in which the complex does not form, no peak at 0.69 V_{SSCE} was obtained (Figure 5), proving the need for the complex formation in order to obtain this oxidation peak. Therefore, different electrolytes of neutral pH were tested and the best results were obtained with $LiClO_4$ 0.1 M, which was used for the further analyses as the supporting electrolyte.

The different electrochemical behavior of the ligand alone and of that involved in the copper (II) complex was pointed out by performing 10 cycles of CV on GCE in a solution of 0.1M $LiClO_4$ containing ligand with and without Cu^{2+} ions.

The voltammogram of the *N,N'*-bis(acetylacetone)ethylenediimine presents an oxidation peak at 1.1 V_{SSCE} , corresponding to the irreversible oxidation of one or both of the amino groups from its molecule. The oxidation of the amino group involves the loss of one electron, with the formation of a highly reactive radical, capable to attack the surface of the electrode, leading to its passivation [34]. This possibility is sustained by the disappearance of this peak after the first scan (Figure 6A). The voltamperometric analysis of the Cu (II) complex presents an extra peak at 0.69 V_{SSCE}, besides the peak at 1.1 V_{SSCE}. Even if both of the oxidation peaks are irreversible, the peak from 0.69 V_{SSCE} behaves differently than the one from 1.1 V_{SSCE}, since it persists during the second to tenth scan, suggesting that the ligand from the complex presents a different reactivity towards the electrode.

The passivation of the electrode by the oxidation of the ligand was confirmed by CV analyses of a potassium hexacyanoferrate (II) / potassium hexacyanoferrate (III) (ferro/ferricyanide) solution. Figure 6B shows that, compared to the signal obtained on a bare GCE, an important decrease of the peak for the ferrocyanide is obtained only in the case of GCE modified by 10 cycles from -0.5 V_{SSCE} to 1.2 V_{SSCE} in a 10 mM ligand solution. When the potential window was narrower or when Cu (II) ions were added to the ligand solution, no significant passivation was observed, showing the importance of the process occurring at 1.1 V_{SSCE} in the passivation of the electrode.

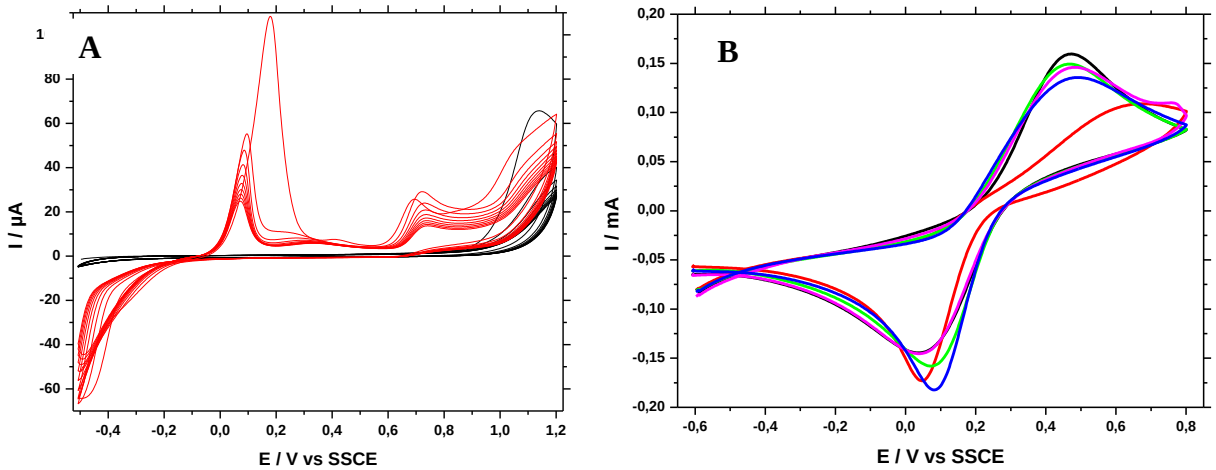

Figure 6. A) CVs at GCE in 10 mM ligand solution in 0.1 M $LiClO_4$ without (—) and with 5 mM Cu^{2+} (—); **B)** CVs of 10 mM ferro/ferricyanide in 0.1M KCl at bare GCE (—) and modified GCEs by 10 cycles (-0.5 V to 1.2 V) (—) and (-0.5 V to 0.85 V) (—) in a 10 mM ligand solution and by 10 cycles (-0.5 V to 0.85 V) (—) and (-0.5V to 1.2V) (—) in a (10 mM ligand and 5 mM Cu^{2+}) solution. Scan rate 0.1 V s^{-1}

3.2.2. Influence of the scan rate

In order to determine the processes involved in the electrochemical oxidation of the Cu (II) complex at the GCE, the influence of the potential scan rate on the electrochemical response of the complex was investigated by cyclic voltammetry. As can be seen from Figure 7, the anodic peak currents increase with the increase of the scan rate. Moreover, the anodic peak currents (Ipa) are proportional to the square root of the scan rate (inset of Figure 7), and the linear regression equation is expressed as Ipa (μA) = $0.0878359 \cdot v^{1/2}$ ($mV^{1/2}$ $s^{-1/2}$) - 2.785873 (R^2 = 0.995). This result indicates that the oxidation of the complex is controlled by its diffusion towards the electrode surface.

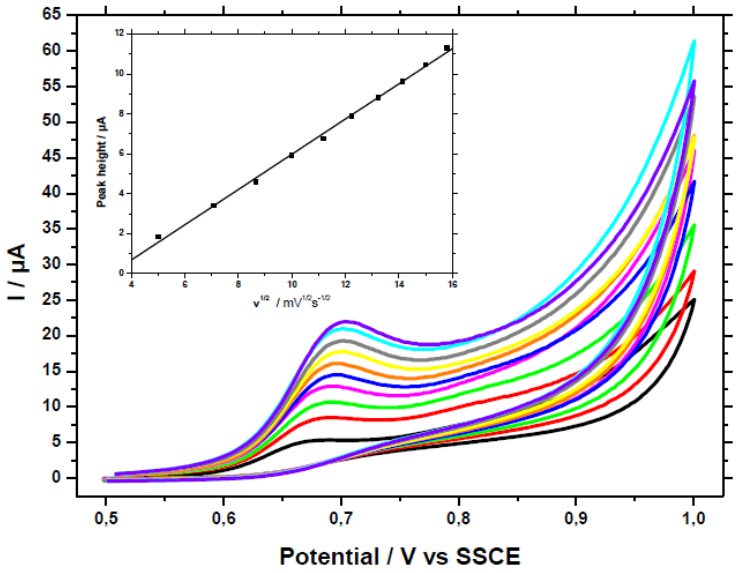

Figure 7. CVs at GCE in a solution of 0.1M $LiClO_4$ containing 10 mM ligand and 1 mM Cu^{2+} at different scan rates: 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 mV s^{-1}(from bottom to top). Inset: linear variation of the oxidation peak current versus the square root of the scan rate. Error bars are based on the measurement of two samples

3.2.3. Interferences studies

In order to evaluate the selectivity of the method, the capacity of different cations to form a complex with the *N,N'*-bis(acetylacetone)ethylenediimine was tested, complex which, during the voltamperommetric analysis, would lead to an anodic oxidation peak. From the tested cations (Zn^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Mn^{2+}, Al^{3+}, Ca^{2+}, Mg^{2+}), only Ni (II) ions led to the formation of a peak, but a very small one. It is interesting to notice that the formed Ni (II) ligand complex presents the oxidation peak at almost the same potential (0.65 V_{SSCE}) as the copper (II) complex (Figure 8), proving that this peak is due to the electrochemical oxidation of the ligand involved in a cationic complex.

The electrochemical analysis of different solutions containing 10 mM *N,N'*-bis(acetylacetone)ethylenediimine, 5 mM Cu (II) ions and 5 mM of each studied cation, showed no

influence on the oxidation peak for Cu (II) complex from the presence in solution of any other cation, proving the high selectivity of the developed method.

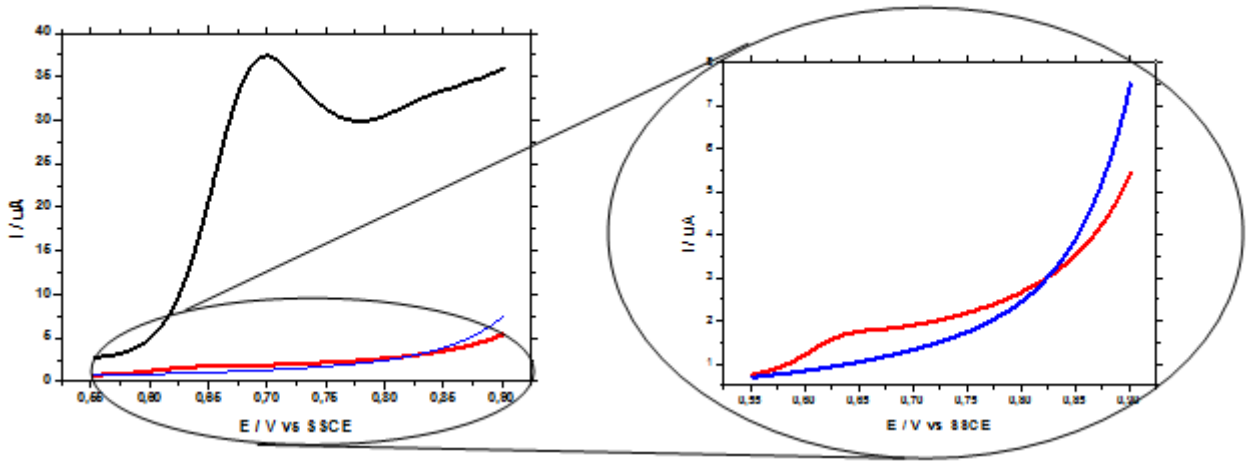

Figure 8. CVs at GCE in a solution of 0.1M $LiClO_4$ containing 10 mM ligand and 5 mM Cu^{2+} (—), 10 mM ligand and 5 mM Ni^{2+} (—) and 10 mM ligand alone (—). Scan rate 0.1 V s^{-1}

3.3. Calibration curve and limit of detection

In order to obtain the calibration curve, DPV analyses were performed, employing the optimal parameters.

The fact that the developed method does not employ stripping voltammetry is advantageous from two points of view: first, it significantly shortens the analysis time, the deposition step from a stripping voltammetry analysis being usually long [20] and secondly, it avoids the interferences from other cations that can be reduced at the applied deposition potential [9]. Furthermore, the step of removing the oxygen by purging nitrogen into solution is not needed, since no reduction potential is applied, making the analysis time short.

A linear correlation was obtained between the peak height and the Cu (II) molar concentration of the samples in the range of 10 μM – 2 mM, a plateau being reached for concentrations greater than 3 mM (Figure 9).

The limit of detection (LOD) was determined as the lowest concentration giving rise to the signal St satisfying Eq.1:

$$St \geq Sb + 3\sigma \quad (1)$$

where St is the gross analyte signal, Sb the field blank signal and σ the standard deviation of five blank determinations [35]. We found a LOD of 0.635 ppm, a value which is a little higher than other LODs reported in the literature, but in the same range as others, as seen in Table I. Overall the obtained LOD is lower than the WHO guidelines for the drinking water of 2 ppm [36], underlining the possibility to apply the developed method for the monitoring of the quality of the drinking water.

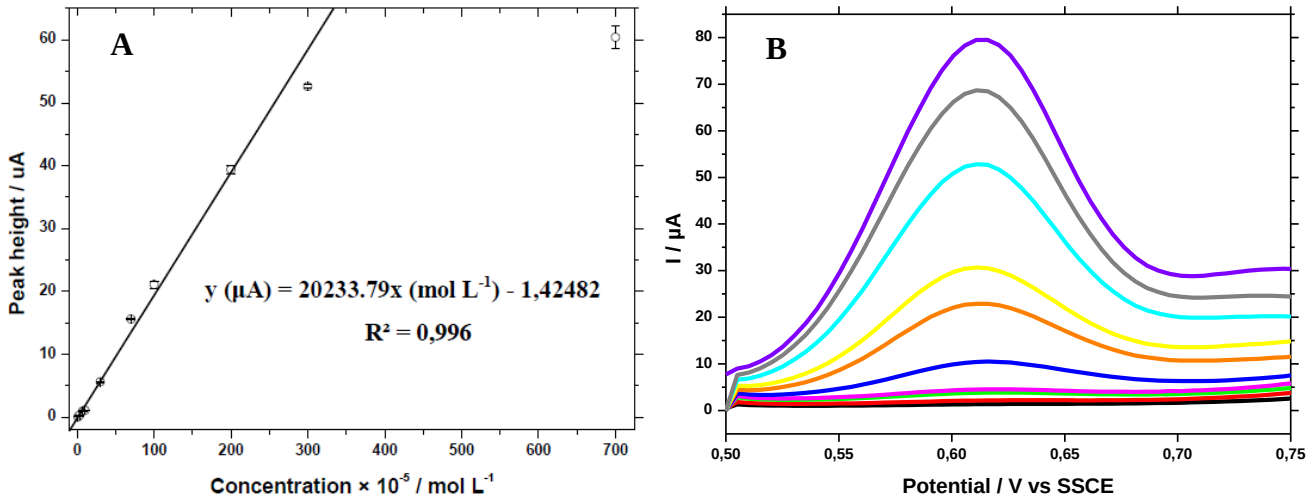

Figure 9. A) Calibration plot: peak height variation for copper (II) concentrations between 0.01 mM to 7 mM. Error bars are based on the measurement of three samples; **B)** DPVs at GCE in a solution of 0.1M $LiClO_4$ containing 10 mM ligand and 0.01, 0.03, 0.07, 0.1, 0.3, 0.7, 1, 2, 3 and 7 mM Cu^{2+} (from bottom to top). Scan rate 0.01 V s^{-1}, PH 0.1 V, PW 25 ms

Table I. Comparison of the developed method with the previous literature reports

No	Electrode	Method	Detection range (mol L^{-1})	LOD (mol L^{-1})	Ref
1.	HMDE	ASV	$0 – 3.15 \times 10^{-6}$	4.25×10^{-8}	[24]
2.	Solid paraffin-based carbon paste electrode modified with 2-aminothiazole organofunctionalized silica	Preconcentration at open circuit for 20 min followed by DPSV analysis	7.5×10^{-8} - 2.5×10^{-6}	3.1×10^{-8}	[37]
3.	4-carboxyphenyl-grafted screen-printed electrode	Preconcentration at open circuit for 10 min followed by DPSV analysis	7.5×10^{-9}– 1.8×10^{-7}	5×10^{-9}	[38]
4.	A sensor array comprising potentiometric chemical sensors with solvent polymeric and chalcogenide glass membranes	Potentiometric detection	-	2×10^{-10} M	[39]
5.	Au, Pt and C SPEs modified by drop coating with solution containing the self-assembled peptide nano fibrils	ASV	$15 - 50 \times 10^{-6}$	-	[40]
6.	PVC membrane electrode based on bis(acetylacetone)propylene diimine combined with sodium tetraphenyl borate and dibutyl	Potentiometric detection	1.0×10^{-5} - 1.0×10^{-1}	7.8×10^{-8}	[41]

	butyl phosphonate				
7.	Screen-printed graphite and graphite/epoxy electrodes modified with 1,3-disubstituted calix [4]arenes and acetylcholinesterase in a Nafion film	Amperometric detection based on enzyme inhibition biosensors	5×10^{-5} – 4×10^{-3}	-	[16]
8.	Hybrid type of enzyme membrane covalently fixing alkaline phosphatase and ascorbate oxidase onto a flow-through oxygen electrode	Amperometric detection based on apoenzyme reactivation biosensor	5×10^{-5} – 1×10^{-3}	-	[17]
9.	Bare GCE	The detection method developed in this work	1×10^{-5} - 2×10^{-3}	10^{-5}	

3.4. Real samples analyses

In order to evaluate the performance of the analytical system for practical analytical applications, the determination of Cu^{2+} was carried out in tap water spiked with the analyte and in a food supplement containing copper (II) (Table II). Since the Cu^{2+} concentration from these real samples was determined without any pretreatment of the sample, the standard addition method was employed to compensate the matrix effect from the real sample.

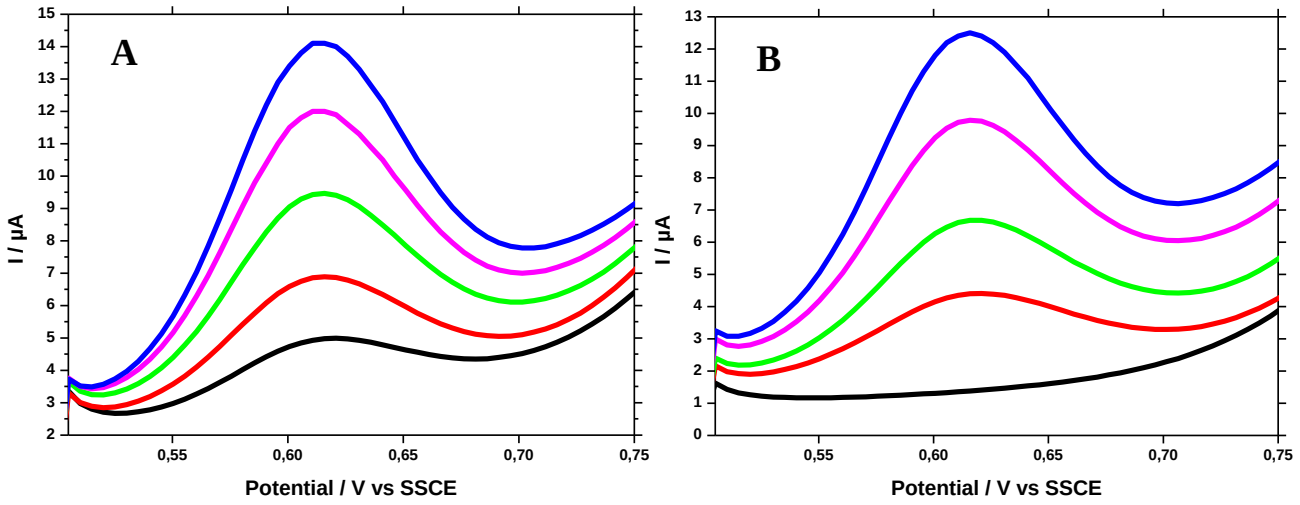

Figure 10. A) DPVs at GCE in a solution of 0.1M $LiClO_4$ containing 10 mM ligand and 100 µM Cu^{2+} (from diluted food supplement) (—) and extra 100 µM Cu^{2+} (—), 200 µM Cu^{2+} (—), 300 µM Cu^{2+} (—) and 400 µM Cu^{2+} (—) from standard addition; **B)** DPVs at GCE in tap water containing 0.1M $LiClO_4$ and 10 mM ligand (—) and extra 100 µM Cu^{2+} (—), 200 µM Cu^{2+} (—), 300 µM Cu^{2+} (—) and 400 µM Cu^{2+} (—) from standard addition. Scan rate 0.01 V s^{-1}, PH 0.1 V, PW 25 ms

As seen in Figure 10, in both cases, no important matrix effect was observed and good correlations between the amounts determined and the initial sample were obtained with recovery percentages of 91.2% ± 9.2% and 98.8 % ± 3.3 % for the food supplement and for the spiked tap water, respectively.

Table II. Determination of Cu (II) concentration in real samples

Sample	Real concentration (mM)	Measured concentration (mM)	Recovery (%)	RSD (n=4)
Spiked tap water	**0.1**	**0.0988**	**98.8**	**3.3**
Food supplement	**0.1**	**0.0912**	**91.2**	**9.2**

3.5. Reproducibility tests

To demonstrate the reproducibility of the developed method, a solution containing 10 mM ligand and 5×10^{-4} M Cu (II) ions was analyzed successively for 10 times using a GCE polished before each analysis. In this case, the value of relative standard deviation (RSD) was 2,008%, which indicates that this simple cleaning procedure is enough for the assurance of a good repeatability. If the GCE was only rinsed with water between the 10 successive analyses, the value of RSD slightly increases (3,832%), but this very simple procedure for the cleaning of the electrode can still be used, with reliable results. This last procedure could be useful for flow analyses using the developed method.

3.6. Electrochemical detection of Cu (II) at electrodes modified with N,N'-bis (acetylacetone)ethylenediimine

The capacity of the ligand immobilized at the surface of the electrode to form the complex was also tested, along with the obtainment of the oxidation peak at 0.69 V_{SSCE} in this case. For this purpose, different immobilization strategies were envisaged: drop coating of a concentrated methanolic solution of the ligand, covalent grafting by anodic oxidation of the ligand, incorporation in conducting polymers (PEI and Nafion) and in carbon paste.

The results obtained after analyses of Cu^{2+} solutions using these modified electrodes showed that the occurrence of the oxidation peak at 0.69 V_{SSCE} was strongly dependent on the immobilization technique of the ligand: the anodic oxidation peak was obtained only when the receptor was able to pass into solution, emphasizing the need of the ligand to adopt its optimum conformational structure in order to obtain the complex that leads to the formation of the oxidation peak.

The electrode modified by one drop of 25 µL ligand solution (20 mg ligand / 1 mL methanol) used for the analysis of 5 mM copper (II) solution led to the occurrence of a large peak (Figure 11), due to the formation of a highly concentrated layer of ligand solution in the proximity of the electrode.

Unfortunately, due to the complete pass of the receptor from the surface of the electrode into solution, the electrode modified in this way was of single use.

An oxidation peak was also obtained when the ligand was incorporated in PEI or in CP. PEI was chosen for the modification of the electrode because it is a conductive polymer, with good dispersive properties [42], offering the possibility of easy modification, by simply incorporating the modifier in the PEI solution. PEI is a cationic polymer, being used for the entrapment of biological materials in the development of biosensors [43] and rarely for the analysis of heavy metal cations. Therefore, it was expected that the Cu^{2+} cations would be repulsed, impairing the formation of the Cu^{2+}-receptor complex, but due to the partial solubility of the PEI film in the copper (II) solution, the ligand was allowed to enter the solution and the oxidation peak was observed.

Particularly in their modified forms, CPEs present a great interest for determining heavy metals by accumulation, being easily modifiable by simply adding the modifier directly to the paste material, without the need of rigorous chemical methods, with low background current and adsorption-extraction capabilities [44]. The oxidation peak obtained with CPE modified with the ligand could be explained by the morphology of the modified CPE surface, allowing the formation of the complex at and into the electrode.

In the case of immobilization of the ligand through PEI film or in CP, the increase of the amount of immobilized ligand led to the increase of the oxidation peak (Figure 11).

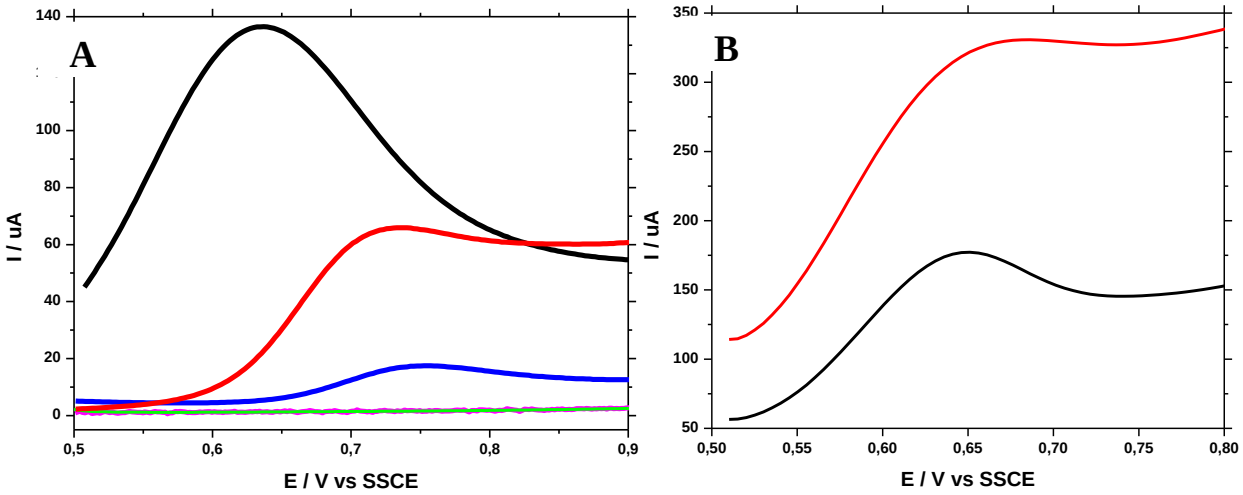

Figure 11. A) CVs at GCE modified by drop-coating with 25 µL ligand methanolic solution (20 mg /mL) (—), by applying 2 × 5 µL of the PEI solution (5 mg mL^{-1}) incorporating the ligand (4 mg/mL)(—) or (20 mg/mL)(—), by electrografting the ligand (—) or by applying 10 µL of an ethanolic solution with 1% Nafion and 4 mg/mL ligand (—) in a 0.1M $LiClO_4$ solution with 5 mM Cu^{2+}. Scan rate 0.1 V s^{-1}; **B)** DPVs at CPEs containing 5% (—) or 20% (—) ligand in a 0.1M $LiClO_4$ solution with 5 mM Cu^{2+}. Scan rate 0.01 V s^{-1}, PH 0.1 V, PW 25 ms

The Nafion polymer is often used in electroanalysis of aqueous solutions considering that it is insoluble in water, hydrophilic, permeable with size-exclusion properties, being able to prevent fouling of the electrode surface. Opposite to PEI, the Nafion, a perfluorosulfonated ionomer, presents

negatively-charged groups at pH > 5, facilitating the accumulation of Cu^{2+} at the surface of modified electrode [45]. But despite this, because Nafion is not soluble in water, no oxidation peak for the complex was obtained. The same lack of oxidation peak from 0.69 V_{SSCE} was observed in the case of the covalent immobilization of the ligand at the surface of the electrode by anodic oxidation. These results prove that it is of great importance that the ligand molecule is allowed to adopt a structural conformation capable to complex the Cu^{2+} ions. The smallest stress on the molecule of the receptor impairs either the formation of the Cu^{2+}-ligand complex or its electrochemical oxidation.

The large peak obtained with the electrode modified by drop-coating and the larger peaks obtained in the case of electrodes modified with greater amounts of the ligand (PEI films and PC) suggest that the performances of the developed method could be improved if a different solvent was used, in which the ligand would be more soluble.

3.7. Flow injection analysis

Presenting a fast response, the developed electrochemical method is suitable for analyses in flow. Using a homemade flow injection cell, adapted for the same three electrodes used in the static cell (GCE, SSCE and Pt), we were able to prove that the method can be adapted for monitoring flow analyses. The current intensity increased after each injection of Cu (II) solutions, leading to sharp, reproducible peaks, proportional with the concentration of the injected solution (Figure 12). After each peak, the baseline was reached easily, without any extra-steps, the flow of the solution containing 0.1 M $LiClO_4$ and 0.01 M ligand being enough to regenerate the bare GCE. In order to increase the amplitude of the peaks for diluted solutions, an improved flow cell will be developed.

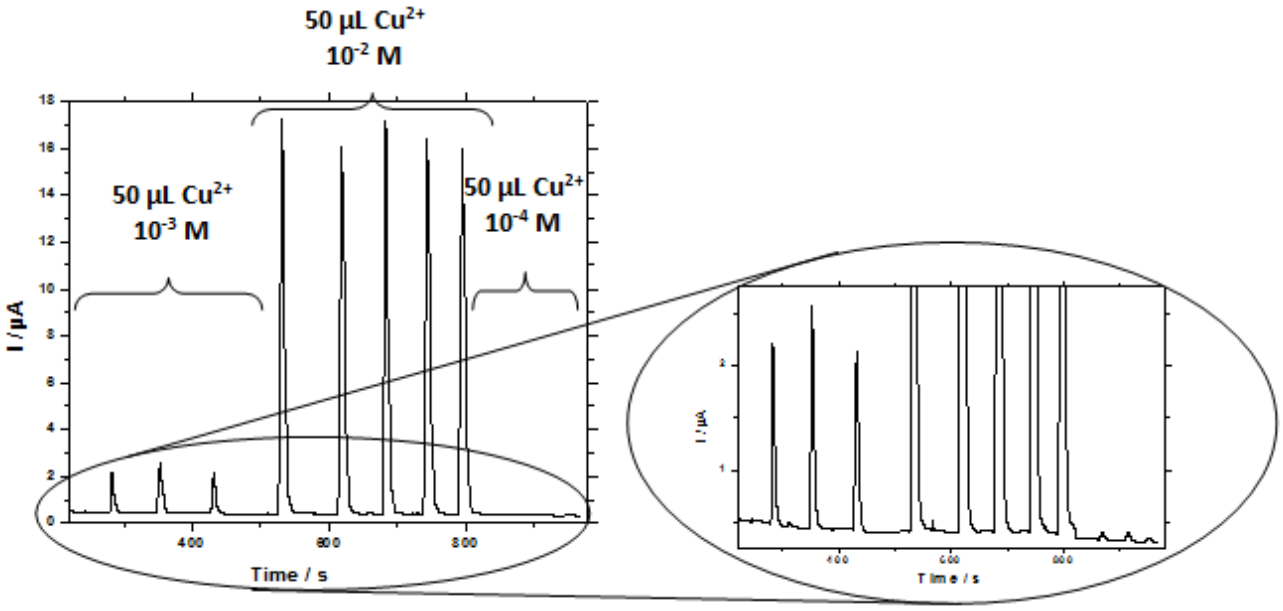

Figure 12. Flow injection analysis of a solution containing 0.1 M $LiClO_4$ and 0.01 M ligand (1.6 mL min^{-1}) with 50 µL injections of 10^{-4} M, 10^{-3} M and 10^{-2} M Cu (II) solutions. E_{ap}=0.85 V_{SSCE}

4. CONCLUSIONS

A new, indirect detection method of Cu (II) ions was developed based on the electrochemical signal of the Cu (II) complex formed with *N,N'*-bis(acetylacetone)ethylenediimine in aqueous medium. The spectrophotometric and electrochemical experiments showed the great selectivity of the chosen receptor for copper (II) cations. The factors influencing the anodic oxidation peak for the complex were investigated. The new method was sensitive enough for the successful determination of Cu (II) from real samples, with little influence from the matrix.

It was also demonstrated that, in the case of the ligand immobilized at the surface of the electrode, it is of great importance that the ligand molecule is allowed to adopt a structural conformation capable to complex the Cu (II) ions in order to obtain the anodic oxidation peak.

The developed electrochemical method, by employing the electrochemical signal of the described complex, proved to be rapid, easy to perform, suitable for flow analyses and highly selective.

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