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Copper Electrochemical Detection with Hybrid Mesoporous Silica-Gly-Gly-His Modified Electrodes

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A carbon paste electrode modified with mesoporous silica functionalized with the tripeptide Gly-Gly-His was prepared, characterized and used for copper (II) quantitative determination in aqueous media. The metal ion was preconcentrated in Britton-Robinson buffer (pH 2.56) at -0.800 V (vs. SCE electrode) and then determined by linear sweep anodic stripping voltammetry. The influence of some different parameters such as the working solution pH, the scan rate, the preconcentration potential and the preconcentration time were studied. Under the optimum conditions, the calibration curve was linear in the range $5 \cdot 10^{-7}$-$1 \cdot 10^{-5}$ mol$\cdot$L^{-1}. The effects of potential interfering ions were studied. The prepared electrode was used for copper direct determination in tap water samples with accurate results.

Keywords: functionalized mesoporous silica modified electrode, Gly-Gly-His, voltammetry, copper

1. INTRODUCTION

Monitoring the presence of heavy metals at very low levels in aquatic ecosystems and soils is of great importance in recent years due to the increased demand for reliable means of detecting metal contaminants in environmental matrices.

Electrochemical stripping analysis is one of the electrochemical methods that afford high sensitivity and sufficient accuracy for the detection of trace metals. Stripping methods that use chemically-modified electrodes present high selectivity for the specific ions of interest [1,2].

Carbon paste still represents one of the most popular electrode materials and one of the most flexible substrates with almost unlimited applicability in basic research, highly specialized investigations, as well as in practically oriented electroanalysis [3-5].

Ordered mesoporous silica are materials in which silica walls grew around the self-assembled template to form periodic mesoporous substrates that display a narrow, controlled pore-size distribution in well-organized structures. Beside MCM-41, MCM-48 and SBA-15, hexagonal mesoporous silica (HMS) is one of the most important mesoporous material used as adsorbent, separation membrane, catalyst support. This material could be functionalized with a variety of organic groups, combining – in a single solid – mechanical stability of the inorganic skeleton with reactivity of the organic moiety.

Silica-modified carbon paste electrodes (CPEs) represent an important subclass of chemically modified electrodes (CMCPEs) with widespread applicability to the determination of inorganic ions, complexes and molecules, including successful attempts in environmental speciation due to the unique properties of these materials that could be combined with electron transfer process [6-9]. Some examples of functionalized silica are available for the detection of Cu(II) at CMCPEs with amine- [10], carnosine- [11], sulphonic acid- [12], cyclam- [13] or acetyl-acetone- [14] functionalized silica.

Peptides are effective ligands for many metal ions due to the great number of potential donor atoms through both the peptide backbone and amino acid side chains. Peptide modified electrodes are used for detection and quantification of metal ions in natural samples [15-19]. The copper ion specific tripeptide Gly-Gly-His was immobilized on poly(3-thiophenacetic) conductive polymer [20] or covalently attached to a 3-mercaptopropionic (MPA) modified gold electrode [21] and used for Cu(II) detection.

The present study will focus on a new organic-inorganic hybrid made of HMS mesoporous silica and the copper selective peptide Gly-Gly-His. The hybrid mesoporous silica was used as an electrode modifier for determining copper(II) by linear sweep anodic stripping voltammetry from aqueous solutions, after preconcentration by selective binding to the peptide centers. To our best knowledge, however, no attempt was made so far to exploit the binding properties of Gly-Gly-His immobilized on mesoporous silica support when incorporated into a carbon paste electrode.

2. EXPERIMENTAL

2.1. Reagents

All the solutions were prepared with purified water obtained using a Millipore Mili-Q system. All the reagents were of analytical grade and used without further purification.

The progress of reactions and purity of products were evaluated on thin layer silica gel chromatographic plates (Merk, Kieselgel 60 F_{254}) with ethyl acetate-hexane or chloroform-methanol mixtures of eluents. Dodecylamine (Aldrich), tetraethoxyorthosilane (TEOS) (Fluka), 3-aminopropyl-triethoxysilane (APTES) (Aldrich) and tripeptide Gly-Gly-His (Aldrich) were used for synthesis and functionalization of the HMS silica.

As stock copper solution, „Certipur” (Merck, Cu^{2+} 1000 $\mu g \cdot L^{-1}$) solution was used. The working standard solutions were prepared on a daily by dilution to appropriate volumes. The supporting electrolyte for the voltammetric experiments was a Britton-Robinson buffer solution.

In order to prepare the carbon paste electrodes, graphite powder (with particles diameter < 20 μm) (Fluka) and paraffin oil (Merck) were used.

The tap water samples were collected just before the analysis was done. The samples were acidified with Britton-Robinson buffer solution (pH 2.56).

2.2. Apparatus

Nuclear magnetic resonance spectra (^{1}H NMR) were recorded using a Bruker DRX-400 spectrometer working at 400.13 MHz. The solid state ^{29}Si magic angle spinning (MAS) NMR spectra were recorded on a Varian VNMRS 400 MHz solid spectrometer with 7.5 mm rotors. Low-angle X-ray diffraction (XRD) patterns were acquired on a Bruker AXS D8 diffractometer by using Cu Kα radiation and Ni filter. N_2 adsorption–desorption isotherms were measured at -196°C with a Micromeritics ASAP 2010 instrument. The sample was previously degassed under vacuum at 40°C for 12 h. Specific surface area was calculated by the Brunaur, Emmett, Teller Isotherm Model (BET) and the mesopore volume was determined from the isotherm at the end of capillary condensation. The pores size distribution was obtained from the desorption branch using the Barret–Joyner–Halenda (BJH) method and the Harkins-Jura standard isotherm. Fourier transform infrared spectroscopy (FTIR) spectra of all samples were performed in KBr pellets using a Bruker Alpha spectrometer.

The voltammetric measurements were made using a voltammetric analyzer Voltalab PST 050 Radiometer with a VoltaMaster 4.0 software. A 20 mL capacity thermostated glass cell at 25^{o}C was used. A carbon paste modified with HMS-Gly-Gly-His was the working electrode, a saturated calomel was the reference electrode (SCE) and a platinum wire was the auxiliary electrode. For the solutions deaeration high purity argon (Linde Gas 5.0) was used. For the stripping step linear sweep anodic stripping voltammetry was used.

2.3. Synthesis of the Silica-Peptide Hybrid Material (HMS-Gly-Gly-His)

Gly-Gly-His (**I**, Scheme 1) is a naturally occurring copper ion specific tripeptide that could be coordinated to the metallic ion through four of its five N atoms and consequently forming a stabile chelate combination [22].

The sililated peptide (*APTS-Gly-Gly-His,* **II**, Scheme 1) was synthesized following a protocol presented in the literature [23] with some changes, by amide coupling between the amino group of APTS and the carboxyl group of the histidine aminoacid. The terminal and imidazole amino groups of the peptide have been protected previously by tert-butyloxycarbonyl (Boc) substituents. In order to avoid the hydrolysis of the triethoxysilane, which would lead to uncontrolled polymerization, it is compulsory to avoid the presence of water or alcohols even in traces. For this reason, isopropenyl-chloroformiate was chosen as coupling agent, leading to the formation of easily removable by-products such as carbon dioxide and acetone during the reaction. Hydrochloric acid generated during the synthesis is trapped with N-methyl-morpholine and removed thereafter by treatment with diethyl ether.

Synthesis of di-Boc-Gly-Gly-His

Di-tert-butyl dicarbonate (0.04 mole) was added to a solution containing 0.017 mole Gly-Gly-His dissolved into a mixture of 10 mL water, 20 mL dioxane and 10 mL 1N NaOH, at 0^0C. After stirring for 30 min. at room temperature, the solution was concentrated under reduced pressure to 50% of its initial volume, the solution was acidified with 1N HCl to pH 2 at 5^oC and the organic phase was extracted with ethyl acetate (30 mL). The extracted organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was washed with Et_2O.

IR: ν_{NH}: 3200-3400 cm^{-1}, ν_{CN}: 1614 cm^{-1}, ν_{CO}: 1652 cm^{-1}, ν_{CO}: 1681 cm^{-1}.

^{1}H RMN ($CDCl_3$): δ = 1,40 ppm and 1,60 ppm (CH_3(Boc), s), δ = 2,40 ppm ($CH_2\alpha$, d, *J* = 7), 3,10 (CH_{2im}, 1H, dd, *J* = 8 and 16), 3,25(CH_{2im}, 1H, dd, *J* = 4,5 and 16), 3,45 ($CH_2\beta$, m), 4,65 (CH, m), 5,25 (NH, m), 6,75 (NH, d, *J* = 7), 7,25 (CH_{im}, s), 8,15(CH_{im}, s,1H).

Synthesis of (triethoxysilyl)alchylamido modified peptide (APTS-Gly-Gly-His)

To a solution of 0.001 mole di-Boc-Gly-Gly-His in 50 mL tetrahydrofurane (THF), were added 0.01 mole methylmorpholine under N_2 atmosphere. After cooling the solution at -15^0C, in the reaction vessel were added a mixture of 0.01 mole of isopropenyl chloroformiate and 0.01 mole of APTS in 10 mL THF. The solution was warm up at room temperature and after the precipitate filtration the solution was evaporated under reduced pressure. The residue was washed with Et_2O and a white solid was obtained.

IR: ν_{NH} : 3200-3400 cm^{-1}, ν_{CN}: 1614cm^{-1}, ν_{CO}: 1652cm^{-1}, ν_{CO}: 1681cm^{-1}.

^{1}H RMN (CD_3OD): δ = 0,60 ppm (CH_{2a}, t, *J* = 6), 1,35(CH_3, t, *J* = 9) , 1,50 (CH_3(Boc), s), 1,60 (CH_{2b}, m), 1,65 (CH_3(Boc), s), 2,50 ($CH_2\alpha$, m), 2,95 (CH_{2im}, 1H, dd, *J* = 8 and 16), 3,15(CH_{2im}, dd, *J* = 4,5and 16), 3,25 ($CH_2\beta$, m), 3,45 (CH_{2c}, m), 3,85 (CH_2,q, *J* = 9), 4,65 (CH, m), 5,60 (NH, m), 7,10 (NH, m), 7,30 (CH_{im}, s), 8,05(CH_{im} , s).

Synthesis of HMS-Gly-Gly-His

The hybrid HMS-Gly-Gly-His (Fig. 1) was obtained by the sol-gel procedure which consists of a copolymerization under controlled conditions at ambient temperature of the APTS-Gly-Gly-His in the presence of the silica source TEOS, using ethanol as co-solvent and DDA as a structure directing agent and as a base catalyst, too. Dodecylamine (0.304 g) was dissolved in a mixture of ethanol (3.8 mL) and H_2O (6 mL) and stirred for 30 min. TEOS (1.39 g) was thereafter added drop wise under vigorous stirring, and after another 30 min. 0.5 g APTS-Gly-Gly-His were added. The mixture was stirred for 20 h at room temperature. The resulting precipitate was filtered and air dried. The product was then washed for the surfactant removal by Soxhlet extraction with ethanol for 24 h, and then dried for 24 h at room temperature and for 1h at 100^0C.

IR: ν_{NH}: 3200-3400 cm^{-1}, ν_{CN}: 1614 cm^{-1}, ν_{CO}: 1652 cm^{-1}, ν_{CO}: 1681 cm^{-1}.

^{13}C (solid) RMN : δ 10; 20; 30; 40; 120-140; 160; 175.^{29}Si (solid)NMR: δ -110.

Removal of the Boc protecting group

Cleavage of the Boc groups was achieved by treating the hybrid solids with a mixture of 1% trifluoroacetic acid (TFA), 2.5% triisopropylsilane (TIS) and 2% H_2O in CH_2Cl_2 for 30 min. at room temperature. The solid was filtered and washed with the same solvent.

^{13}C (solid) RMN : δ 10; 20; 30; 40; 120-140; 160; 175. ^{29}Si (solid)NMR: δ -110.

2.4. Preparation of the modified carbon paste electrodes

Organofunctionalized HMS modified carbon paste electrodes (MCPE) were prepared by homogenous mixing HMS-Gly-Gly-His with the graphite powder at different mass ratios, the optimum one proving to be 1:6. After that, the paraffin oil was added, mixing thoroughly to obtain a homogenous paste. The carbon paste electrode was obtained by packing the paste into a plastic tube (2 mm interior diameter). The external electrical contact consisted in a copper wire. The surface of the electrode was smoothed on a bond paper. To regenerate the electrode surface an excess of paste was pushed out of the tube, this excess was removed and than the electrode surface was mechanically polished.

In the Figure 1 the schematic representation of the carbon paste electrodes modified with the functionalized hexagonal mesoporous silica (HMS-Gly-Gly-His) is presented.

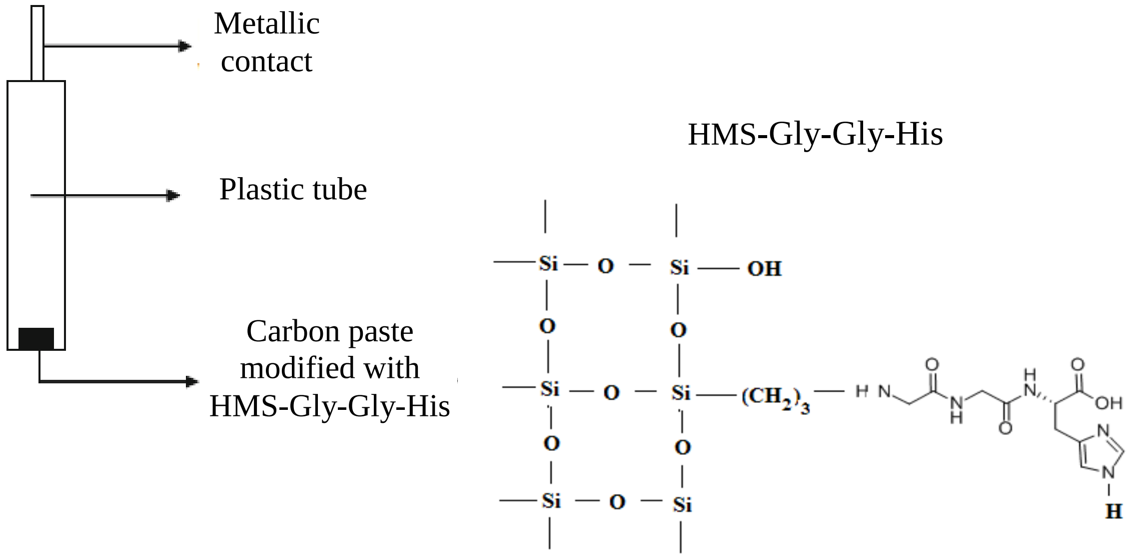


Figure 1. Schematic representation of the HMS-Gly-Gly-His modified carbon paste electrode

2.5. Procedure for Cyclic Voltammetry (CV) and Linear Sweep Anodic Stripping Voltammetry (LSASV) analysis

The supporting electrolyte used for the copper determination was a Britton-Robinson buffer solution (pH 2.56). The solutions were deaerated with high purity argon for 5 min. Cyclic voltammograms were recorded for a $1\cdot10^{-4}$ mol·L^{-1} Cu^{2+} solution in Britton-Robinson buffer (pH 2.09 – 7.00), scanning the potential between -0.5 V and +0.5 V.

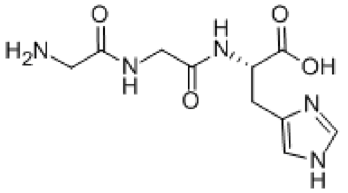
Linear scan anodic stripping voltammetry (LSASV) technique was used for the copper quantitative determination by scanning the potential in the positive direction. The measurements were carried out after a preconcentration step, in which the solution was stirred. The preconcentration time was 180 s and the preconcentration potential was -0.8 V.

3. RESULTS AND DISCUSSION

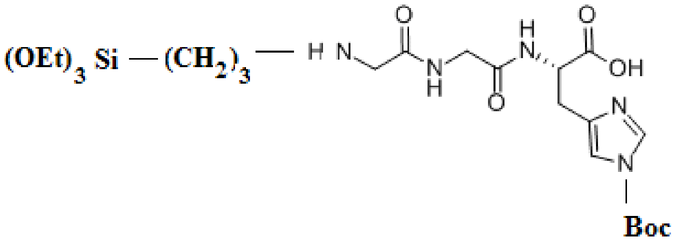
3.1. Characterization of the HMS and of the hybrid HMS-Gly-Gly-His

A new organic-inorganic hybrid made of HMS mesoporous silica and Gly-Gly-His tripeptide was synthesized and used thereafter for the construction of a modified carbon paste electrode. The advantage of the mesoporous silica is that the number and distribution of the silanol groups that participate to the functionalization could be much easily controlled. Furthermore, the HMS silica allows a conformation of the chelating ligand inside the mesopores of the inorganic support that is much favourable for the complexation of the copper ions comparatively with an amorphous silica functionalized with the same ligand. Despite the hexagonal mesoporous silica (HMS) are less ordered than the MCM-41 and SBA-15 materials, they are much easier and straightforward to synthesize due to the use of a cheap and neutral template, i.e. long chain amines, which does not require the need of strongly acidic conditions [24]. For the HMS silica synthesis, a fixed DDA:TEOS:H_2O:EtOH ratio of 1:4:200:50 was used knowing that this ratio is important for the control of the morphology and of the silica pore structure [25,26].

The new hybrid material was synthesized by sol-gel process, by co-condensation of the tripeptide with TEOS, at room temperature (Scheme 1 and Figure 1). The Gly-Gly-His amino groups were protected and the tripeptide was thereafter modified by the sililating agent APTS in order to react with the silica network polymer.



(I) Gly-Gly-His



(II) APTS-Gly-Gly-His

Scheme 1. Molecular structure of copper selective tripeptide and its sililated derivative

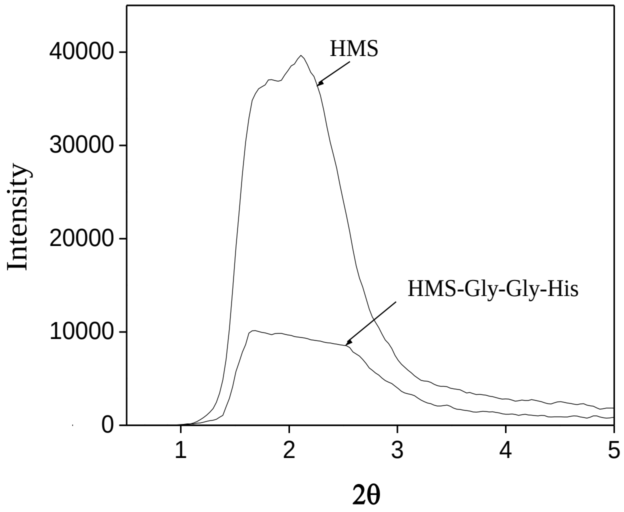


Figure 2. Powder X-ray diffraction patterns of pure and modified HMS silica

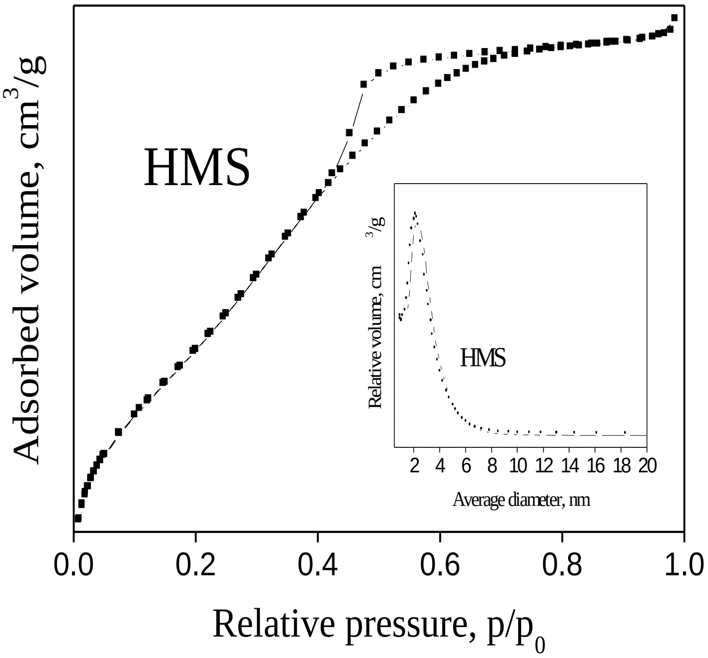


Figure 3. N_2 adsorption-desorption isotherms of the HMS support and the corresponding pore size distribution (insert)

The nitrogen adsorption isotherm at -196°C of the calcined HMS material (Figure 3) is of type IV, featuring a sharp step corresponding to the filling of the ordered mesopores in the p/p_o range of 0.1-0.5 and a H1 hysteresis loop at p/p_o of 0.5 and therefore is typical of mesoporous materials. The surface area decreased for the HMS-Gly-Gly-His sample with 49.56%, comparatively to the HMS support. These textural results confirm that the grafted species are also located inside the mesopores and not only on the outer surface.

3.2. Electrochemical behavior of Cu^{2+} using cyclic voltammetry (CV) on HMS-Gly-Gly-His modified carbon paste electrodes

The effect of pH on the cyclic voltammetric response of the carbon paste electrode modified with organofunctionalised silica was studied. The cyclic voltamogramms for $1\cdot10^{-4}$ mol·L^{-1} Cu^{2+} solution were recorded in Britton-Robinson buffer with different pH values between 2.09 and 7.00 (figure 4). Well defined voltammetric peaks were obtained in all cases, except for pH 7.00 and the anodic peak appeared at potential values that ranged between 0.001 and 0.113 V vs. SCE. The anodic peak current increased as the pH changes from 2 to 2.56, reaching a maximum at the last value. This is probably due to the fact that in more acidic medium the organofunctionalised silica is preferentially in the protoned form. In the 2.56-7.00 pH range the current decreased. This is argued by the fact that at higher pH values, the precipitation of the insoluble copper hydroxide takes place [28].

In conclusion, the acid solution prevents the hydrolysis of the Cu(II) ions and the pH value of 2.56 was used in all subsequent experiments.

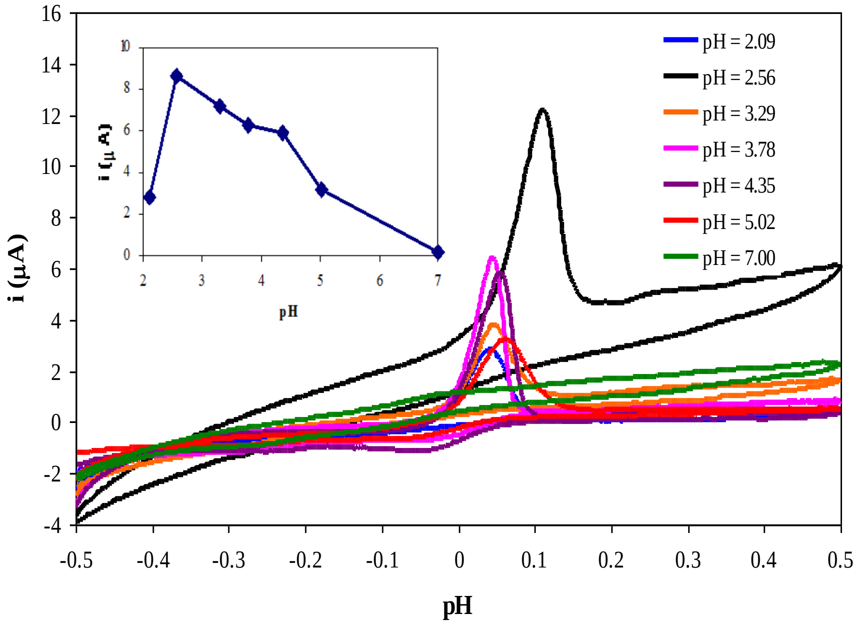


Figure 4. Graphical representation of the peak current intensity vs. pH values for a solution containing $1\cdot10^{-4}$ mol·L^{-1} Cu^{2+} in Britton-Robinson buffer

3.3. Electrochemical behavior of Cu^{2+} on HMS-Gly-Gly-His modified carbon paste electrodes using linear scan anodic stripping voltammetry

The recorded voltamogramm for $1\cdot10^{-5}$ mol·L^{-1} Cu^{2+} solution in Britton-Robinson buffer (pH 2.56) showed one anodic peak at 0.112 V vs. SCE when the HMS-Gly-Gly-His modified electrode was used (Figure 5(b)). When using the HMS silica modified electrode the anodic peak appeared at 0.078 V (Figure 5(a)).

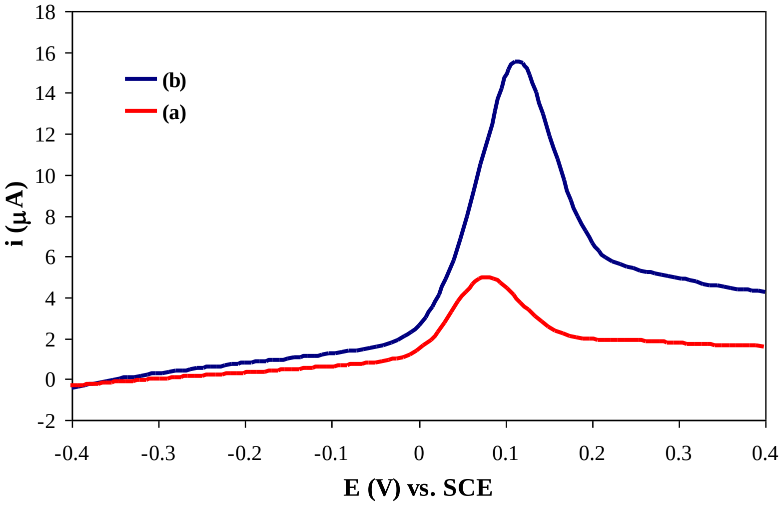


Figure 5. Linear sweep anodic stripping voltammograms obtained for $1\cdot10^{-5}$ mol·L^{-1} Cu^{2+}, at a scan rate of 100 mV/s, in Britton-Robinson buffer (pH 2.56): a) HMS silica modified and b) HMS-Gly-Gly-His modified carbon paste electrode (preconcentration potential -0.800 V vs. SCE, preconcentration time 180 s).

For the HMS-Gly-Gly-His modified electrode the peak is very well defined, sharp and more than three times bigger than that obtained for the HMS modified electrode. The better sensitivity of the HMS-Gly-Gly-His modified electrode towards the Cu^{2+} is due to the presence of the specific tripeptide which selectively binds the copper ions.

The parameters that affect the voltammetric peak current: the preconcentration potential, the preconcentration time and the scan rate were optimized.

3.3.1. The preconcentration potential influence

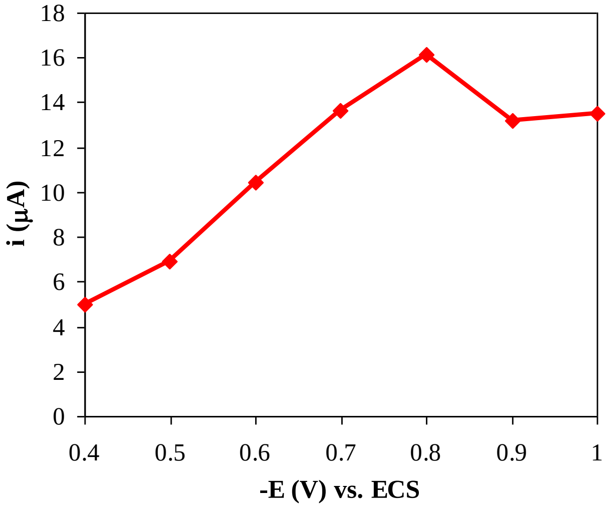


Figure 6. The influence of the preconcentration potential on the peak current intensity for a $3\cdot10^{-5}$ mol·L^{-1} Cu^{2+} solution in Britton Robinson buffer (pH 2.56), 120 s preconcentration time

Preconcentration potential values between -1.000 and -0.400 V vs. SCE were investigated for a solution containing $3\cdot10^{-5}$ mol·L^{-1} Cu^{2+} and the obtained results are presented in Figure 6. It can be observed that the optimal value of this potential is -0.800 V vs. SCE. This value was chosen as preconcentration potential in all further experiments.

3.3.2. The preconcentration time influence

The dependence of the peak current with the preconcentration time (30 ÷ 360 s) for a $3\cdot10^{-5}$ mol·L^{-1} Cu^{2+} solution was also studied (Figure 7). The peak current increased with the increase of preconcentration time between 30 and 180 s, after that remaining nearly constant. This is due to the electrode surface saturation. Therefore, for all the subsequent measurements the preconcentration time of 180 s was used.

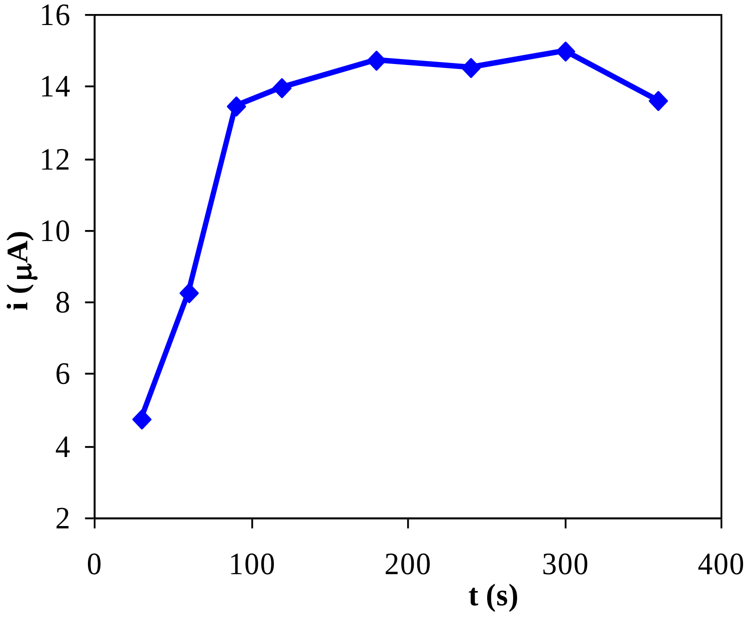


Figure 7. The influence of the preconcentration time on the peak current intensity for a $3\cdot10^{-5}$ mol·L^{-1} Cu^{2+} solution in Britton Robinson buffer (pH 2.56), preconcentration potential -0.800 V vs. SCE

3.3.3. The scan rate influence

The peak current was evaluated depending on the scan rate value ranging between 25 and 250 mV·s^{-1}. From the stripping voltamogramms (figure 8) it can be observed that the signal increases with the scan rate increase, but at scan rates higher than 100 mV·s^{-1} a significant increase on the magnitude of the peak current was not observed. Given this fact, the shapes of the voltamogramms and how well they are defined, the chosen value of the scan rate for further studies was 100 mV·s^{-1}.

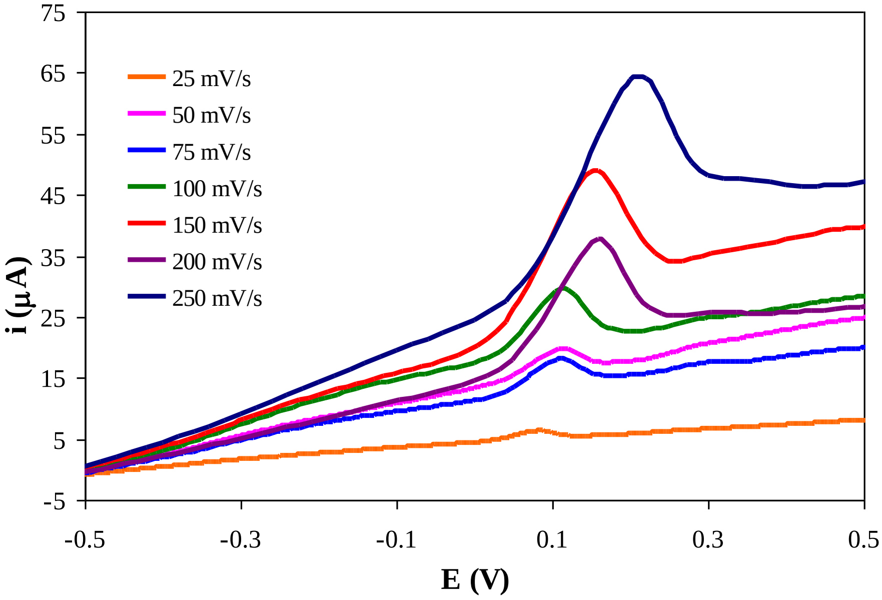


Figure 8. The influence of the scan rate on the peak current for a $3\cdot10^{-5}$ mol·L^{-1} Cu^{2+} solution in Britton Robinson buffer (pH 2.56), preconcentration potential -0.800 V vs. SCE, preconcentration time 120 s

3.3.4. The linearity, the detection limit, the quantification limit, precision

The concentration range for which the peak current intensity increased linearly with the Cu^{2+} concentration is $5\cdot10^{-7} – 1\cdot10^{-5}$ mol·L^{-1}. The regression equation is: i_p=1.33 c–0.16 and R^2=0.9901.

The *detection limit* (LD) and the *quantification limit* (LQ) can be calculated using different approaches [29]. In the present study two of them were used:

LD = $3s_{c\,min}/b$ and LC = $10s_{c\,min}/b$ (1)

LD = $3s_a/b$ and LC = $10s_a/b$ (2)

where $s_{c\,min}$ is the standard deviation of the smallest concentration from the calibration curve, s_a is the standard deviation of the intercept and b is the calibration curve slope.

The obtained detection limits are: $4.00\cdot10^{-7}$ mol·L^{-1} for (1) and $4.55\cdot10^{-7}$ mol·L^{-1} (for 2), respectively. The quantification limit values are: $1.33\cdot10^{-6}$ mol·L^{-1} for (1) and $1.52\cdot10^{-6}$ mol·L^{-1} for (2), respectively.

The precision of the method was evaluated on the repeatability level, expressed as percentage relative standard deviation (RSD%). The value obtained for the solutions containing $5\cdot10^{-6}$ M Cu^{2+} under the optimized conditions was: 4.28 %.

The influence of the various metallic species (Pb^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Cr^{3+}, Ni^{2+}) present in the solution at different concentrations (the same, half and double compared to the Cu^{2+} concentration) on the copper peak current was studied. The obtained results are presented in the Table 1.

There is a decrease or an increase of the Cu^{2+} signal in the presence of different interfering ions. In the presence of Pb^{2+} appears another well defined peak, thus demonstrating that the two elements can be determined simultaneously.

From the above it follows that all evaluated cations interfere with the copper response. However, when real samples are analyzed these interferences can be diminished using the standard addition method.

3.3.5. Interferences

Table 1. The effect of the presence of different cations on the Cu^{2+} response using LSASV under optimized conditions ($5\cdot10^{-6}$ mol·L^{-1} Cu^{2+}).

Interfering cation	Interfering cation concentration $c\cdot10^{-6}$ mol·L^{-1}	(%) from Cu^{2+} signal
Pb^{2+}	2.5	47.1
	5.0	62.7
	10.0	29.8
Zn^{2+}	2.5	162.7
	5.0	125.0
	10.0	107.0
Cd^{2+}	2.5	132.9
	5.0	56.8
	10.0	108.2
Mn^{2+}	2.5	84.4
	5.0	96.4
	10.0	103.8
Cr^{2+}	2.5	68.5
	5.0	51.7
	10.0	86.6
Ni^{2+}	2.5	60.6
	5.0	97.3
	10.0	65.7

3.3.6. Analytical applications

The application of the proposed modified electrode in real sample analysis was investigated by measurement of Cu^{2+} concentration in tap water. Since the amount of Cu^{2+} was under the detection limit of the method, the recovery experiments were performed by measuring the LSASV responses for the samples with known added concentration of Cu^{2+} (1 μM). The results are presented in Table 2. The mean value of the recoveries is 99 % with a RSD% = 5.89 %.

Table 2. Recovery analysis of Cu^{2+} in tap water samples.

Water sample replicate	Cu^{2+} concentration Spiked (μM)	Cu^{2+} concentration Found (μM)	Recovery (%)
1	1.00	0.94	94 %
2	1.00	1.07	107 %
3	1.00	0.97	97 %
4	1.00	0.96	96 %

4. CONCLUSIONS

A Gly-Gly-His functionalised silica modified carbon paste electrode for determination of copper was developed. The obtained results showed that the presence of the modified silica improves the obtained signal and this electrode is an alternative for the ASV determination of copper after optimizing parameters such as pH value, scan rate, preconcentration potential and time. A precise and reproducible copper determination from water samples can be done using standard addition method, taking into account the sample matrix and the concentration level of potential interfering ions.

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