

VALIDATION AND OPTIMIZATION OF AN *IN-SITU* COPPER-MODIFIED GLASSY CARBON ELECTRODEKLODIAN XHANARI^{1,2}, ŽAN ŠAŠEK¹, BARBARA PETOVAR¹, MATJAZH FINŠGAR¹¹*University of Maribor, Faculty of Chemistry and Chemical Engineering, Smetanova ulica 17, 2000 Maribor, Slovenia*²*University of Tirana, Faculty of Natural Sciences, Boulevard "Zogu I", 1001 Tirana, Albania*

Square wave anodic stripping voltammetry (SWASV) technique is one of the most used electrochemical techniques in the determination of the trace amounts of heavy metals in different environments. This is due among other reasons to the fact that this method offers low detection limit, high selectivity and precision at relatively low cost. Modified film electrodes have been found as good replacement to the mercury electrodes. In this work different deposition potentials have been used to optimize an in situ-prepared copper-film glassy carbon electrode (CuFE). In addition, a preliminary study using electrochemical impedance spectroscopy (EIS) was performed to investigate the characteristics of the CuFE sensor.

Keywords: heavy metal analysis; copper film electrode; square wave anodic stripping voltammetry.

INTRODUCTION

The determination of trace amounts of heavy metals in different systems has always been of great interest for the research community due to their high toxicity and ability to bioaccumulate. Moreover, their impact is not only on the environment, but it is also closely connected to the human health. The exposure to the heavy metals is mainly through the alimentary chain (Gumpu *et al.*, 2015; Bansod *et al.*, 2017). Several possible mechanisms (depending also on the specific heavy metal) have been proposed on how these elements affect the human health (Gumpu *et al.*, 2015; Tchounwou *et al.*, 2012). The generation of the DNA leading to its damaging, depletion of protein sulfhydryl and lipid peroxidation are among few of the known effects of these metals (Gumpu *et al.*, 2015; Tchounwou *et al.*, 2012; Valko *et al.*, 2005; Labuda *et al.*, 2005).

Different techniques have been employed to determine the concentration of the heavy metals in different matrixes including soil, air, water and biological samples. The selection of the method is closely dependent on the concentration of these elements (in the ppb range) which in response requires high sensitivity and selectivity of the method (Bansod *et al.*, 2017). Spectroscopic techniques, including atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), X-ray fluorescence spectrometry (XRF) and inductively coupled plasma-optical emission spectrometry (ICP-OES) have been extensively used in the determination of the heavy metals in different matrixes (Pohl, 2009; Silva *et al.*, 2009; Sitko *et al.*, 2015; Losev *et al.*, 2015). Electrochemical techniques have also found a wide range of applications in trace heavy metals determination. Based on the change of different electrical signals caused by the presence of these elements several techniques have been developed (Bansod *et al.*, 2017; Lu *et al.*, 2018; Ariño *et al.*, 2017; Alves *et al.*, 2017; Cui *et al.*, 2015; Yasri *et al.*, 2011; Town *et al.*, 2001; Gao *et al.*, 2013). The square wave anodic stripping voltammetry (SWASV) is an affordable (low cost) technique that offers low detection limit, high selectivity, accuracy and precision (Gumpu *et al.*, 2015; Bansod *et al.*, 2017; Lu *et al.*, 2018).

A large number of materials has been employed as the working electrode in the electrochemical techniques, trying to restrict the use of mercury drop electrodes (Ariño *et al.*, 2017). Film electrodes, prepared from the electrodeposition of different elements,

like Bi, Sb and Pb, on the surface of a glassy carbon electrode are among the most commonly used (Korolczuk *et al.*, 2005; Petovar *et al.*, 2018; Makombe *et al.*, 2016). Only a few research groups have reported on the use of copper film electrodes (CuFE) for trace heavy metal determination. Jovanovski *et al.* (2015) reported on the preparation of an *in situ* CuFE for the determination of Hg(II) and Pb(II) using the SWASV technique and 120 s accumulation time. The determination of trace levels of Zn(II) in blood serum was also reported by Pei *et al.* (2014) using a disposable copper-based electrochemical sensor. In this work copper was used not only as working electrode, but as counter and reference (Cu/CuCl₂) electrode as well.

In this work we report on the *in situ* preparation and validation of a copper modified film electrode, in 0.1 M HCl and 0.4 M NaCl containing 0.5 mg/L Cu(II) in the determination of Pb(II). The accumulation time was 60 s. The linear range of the method at three different potentials (*i.e.* at -0.8, -0.7 and -0.6 V) is given and the accuracy and precision of the obtained results is proven. Electrochemical impedance spectroscopy was used to understand the performance of CuFE, with and without additions of 15 and 40 µg/L Pb(II). The results were compared with the bare GCE.

EXPERIMENTAL

A PalmSens3 EIS potentiostat/galvanostat controlled by PSTrace 5.4 software (PalmSens, Houten, the Netherlands) was employed to perform all the electrochemical measurements at room temperature. The three-electrode electrochemical cell and the working (GCE, Cat. No.6.1204.300), reference (Ag/AgCl filled with saturated KCl) and counter (Pt wire) electrodes were all provided by Metrohm (Herisau, Switzerland). Al₂O₃ powder (Buehler, Illinois, USA) was used to polish the working electrode surface prior to the electrochemical measurements. All the potentials mentioned in this work are given in reference to the Ag/AgCl(saturated KCl) electrode.

KCl provided by Carlo Erba Reagents (Val de Reuil, France) and K₃[Fe(CN)₆] purchased from Sigma Aldrich (St. Louis, Missouri, USA) were employed to test the reversibility of the working electrode. Cyclic voltammetry measurements at different sweep rates were performed in 1.0 M KCl solution containing 10 mM K₃[Fe(CN)₆].

Ultrapure water (resistivity of 18.2 MΩ) obtained with a Milli-Q system (Millipore Corporation, Massachusetts, USA), was used to dilute all the solutions. The atomic absorption standard stock solutions (1000 mg L⁻¹) of Pb(II) and Cu(II) were provided by Merck (Darmstadt, Germany). HCl was provided by Carlo Erba Reagents (Val de Reuil, France).

A 0.1 M HCl and 0.4 M NaCl solution containing 0.5 mg/L of Cu(II) with and without additions of the analyte, *i.e.* Pb(II), was used to prepare the *in situ* CuFE.

The application of a potential of 0.3 V vs. Ag/AgCl for 30 s prior and after the SWASV measurements assured the electrochemical cleaning of the electrode through oxidation of all the employed metals. The preconcentration step was performed at -0.8 or -0.7 or -0.6 V vs. Ag/AgCl deposition potential. The accumulation was performed for 60 s. The solution was stirred during the preconcentration step at approximately 300 rpm, but not during the measurement and equilibration step. The latter lasted 15 s. Analysis was performed with a SW excitation signal after the preconcentration and equilibration step at three different potentials, *i.e.* -0.8 or -0.7 or -0.6 V vs. Ag/AgCl. The EIS measurements were carried out at the same potential as the deposition potential ($E_{\text{meas}} = E_{\text{dep}}$) and at the open circuit potential ($E_{\text{meas}} = E_{\text{ocp}}$), preserving also the preconcentration step used during the method validation procedure. A signal with 10 mV amplitude and 5 point per decade was used to record the EIS response in the frequency range from 50 kHz to 50 mHz.

RESULTS AND DISCUSSION

Analytical Performance of CuFE

Limit of Quantification

The limit of quantification (LOQ) was determined based on the signal-to-noise ratio (S/N) as a Pb(II) concentration at which $S/N \geq 10$. This was repeated for three deposition potentials, *i.e.* -0.8 , -0.7 , and -0.6 V vs. Ag/AgCl. The determined LOQ for all three deposition potentials used was found to be $2.00 \mu\text{g/L}$.

Selectivity of the Method

Figure 1 shows a voltammogram measured using CuFE. The peak for Pb(II) develops at approximately -0.4 V vs. Ag/AgCl. The stripping signal of Cu is represented by two peaks located at approximately -0.2 and 0.25 V vs. Ag/AgCl. On that basis, we can conclude that the method is selective towards Pb(II) and Cu(II). An intensive hydrogen evolution starts at approximately -0.7 V vs. Ag/AgCl as seen in Figure 1.

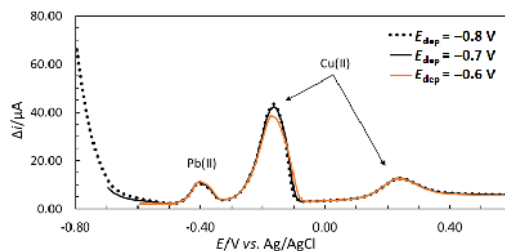


Figure 1. Voltammograms measured at three different deposition potentials using CuFE in 0.1 M HCl and 0.4 M NaCl containing $38.5 \mu\text{g/L}$ Pb(II).

Linearity of the Method

Figure 2 presents the linear ranges by employing different deposition potentials. The method showed linear response in the mass concentration range from 2.0 to $38.5 \mu\text{g/L}$ for the -0.6 V vs. Ag/AgCl deposition potential. A wider mass concentration range was determined when the deposition potential of -0.7 and -0.8 V vs. Ag/AgCl was employed. The linear range in this case was found to be from 2.0 to $56.6 \mu\text{g/L}$. The linearity was accepted when the correlation coefficient R^2 was higher than 0.99 ($R^2 > 0.99$).

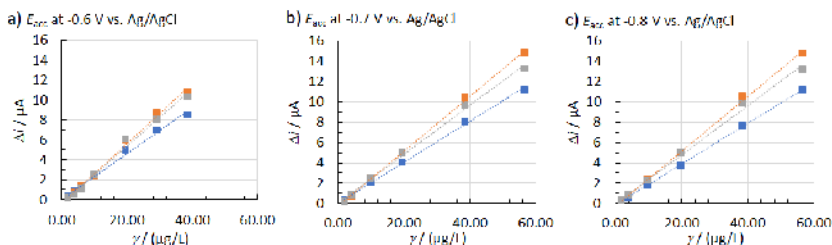


Figure 2. Linearity of the method for Pb(II) determination at different deposition potentials using an *in situ*-prepared CuFE.

Accuracy and Precision

Accuracy and precision were evaluated based on the recovery and relative standard deviation (RSD) values, respectively. The 0.1 M HCl and 0.4 M NaCl was spiked with certain Pb(II) concentration and the analysis using CuFE was repeated at least 6 times. An average recovery value and RSD was calculated. All three methods showed accurate (recovery in the range of 80.0–120.0%) and precise (RSD \leq 20.0%) results.

Electrochemical Impedance Spectroscopy (EIS) Measurements

The Nyquist plots showing the EIS response of the CuFE in 0.1 M HCl and 0.4 M NaCl, containing 0.5 mg/L Cu(II), measured either at the same potential as the deposition potential ($E_{\text{meas}} = E_{\text{dep}}$) or at the open circuit potential ($E_{\text{meas}} = E_{\text{oc}}$), are given in Figures 3a–c and 4a–c, respectively. In order to understand the performance of CuFE, the EIS spectra of the bare GCE in the same electrolyte, with and without additions of the analyte, for measurements performed at E_{dep} and E_{oc} (Figure 3d–f and Figure 4d–f) are also given. In all cases the EIS spectra were recorded at -0.8 , -0.6 and -0.3 V vs. Ag/AgCl, with or without addition of 15 and 40 $\mu\text{g/L}$ Pb(II).

As seen in Figures 3 and 4 the EIS response obtained at -0.6 and -0.8 V vs. Ag/AgCl, independently of the addition of analyte, is presented as depressed semicircles. This indicates that the system is under kinetic control. The same behaviour is not seen in the case of the bare GCE measured at E_{dep} , without addition of Pb(II) (Figure 3f) and with 15 $\mu\text{g/L}$ Pb(II) (Figure 3d).

The real part of impedance (Z_{real}) of all the CuFE systems (with or without addition of the analyte) measured at E_{dep} (Figure 3) decreased at the most negative potential (*i.e.* at -0.8 V vs. Ag/AgCl) compared with that of the bare GCE at the same conditions. This decrease is even more pronounced in the case of the measurements performed at E_{oc} (Figure 4) where it can be observed also for measurements performed at -0.6 V vs. Ag/AgCl. It is important to notice that on one hand the real part of impedance contributes to the polarization resistance of these systems. On the other hand, the sensitivity of the method is inverse proportional to the polarization resistance (Petovar *et al.*, 2017). This implies that the sensitivity of the method at the most negative potential increased when using CuFE.

No clear influence of the concentrations of the analyte on the impedance values can be observed for all the studied systems (Figures 3 and 4) independently if measured at E_{dep} or E_{oc} .

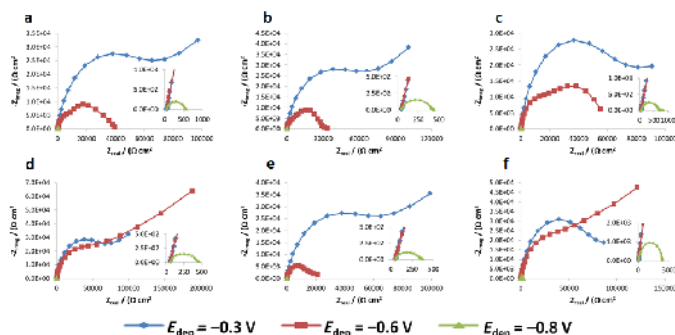


Figure 3. The Nyquist plots of the CuFE (a–c) and bare GCE (d–f), recorded in 0.1 M HCl and 0.4 M NaCl at the same potential as the deposition potential, with (a, d) 15 $\mu\text{g/L}$, (b, e) 40 $\mu\text{g/L}$ and (c, f) without additions of Pb(II).

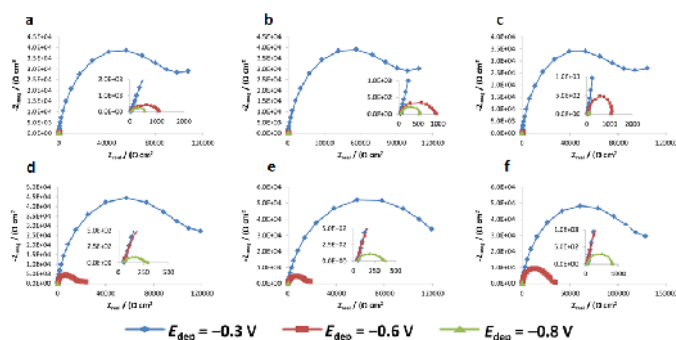


Figure 4. The Nyquist plots of the CuFE (a–c) and bare GCE (d–f), recorded in 0.1 M HCl and 0.4 M NaCl measured at open circuit potential, with (a, d) 15 $\mu\text{g/L}$, (b, e) 40 $\mu\text{g/L}$ and (c, f) without additions of Pb(II)

Figure 4 shows that for measurements performed at E_{oc} , the impedance of all the systems (with or without addition of Pb(II), bare GCE or CuFE) it is significantly decreasing when the measured potential shifts towards more negative values. The decrease of the impedance measured at the most positive potential (*i.e.* at -0.3 V vs. Ag/AgCl) and at the most negative potential (*i.e.* at -0.8 V vs. Ag/AgCl) it is of approximately three orders of magnitude. A similar behaviour of the EIS response with the change in value of the measured potential is generally seen also in the case when measurements were performed at E_{dep} (Figure 3) for both the bare GCE and the CuFE. However, the EIS spectra of the bare GCE with no additions of Pb(II) (Figure 3f) and with 15 $\mu\text{g/L}$ Pb(II) (Figure 3d) did not follow the above mentioned trend.

CONCLUSIONS

This work presents the validation and optimization of a *in situ*-modified copper glassy carbon electrode used for the determination of Pb(II). The CuFE was prepared 0.1 M HCl and 0.4 M NaCl solution containing 0.5 mg/L of Cu(II) and used for the determination of two different concentrations of Pb(II), *i.e.* 15 and 40 $\mu\text{g/L}$.

The results obtained from the square wave anodic stripping voltammetry (SWASV) measurements performed at three different potentials (*i.e.* at -0.8 , -0.7 and -0.6 V vs. Ag/AgCl) showed that the method was linear in the range 2.0–38.5 $\mu\text{g/L}$ for the -0.6 V vs. Ag/AgCl deposition potential, but the linearity at -0.7 and -0.8 V vs. Ag/AgCl was found to be from 2.0 to 56.6 $\mu\text{g/L}$. The limit of quantification at all three potentials was determined to be 2.00 $\mu\text{g/L}$. The accuracy and precision of the obtained results was also confirmed.

Preliminary electrochemical impedance spectroscopy (EIS) measurements performed at -0.8 , -0.6 and -0.3 V vs. Ag/AgCl showed that the systems are under kinetic control for measurements performed at the most negative potentials.

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