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Full Paper

Electrocatalytic Oxidation and Determination of Metoprolol using a Bimetallic Cu-Ni Sensing Platform

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Abstract- Metoprolol tartrate (MET) is a selective β-adrenergic antagonist used in the treatment of cardiovascular diseases. MET overdose can affect the heart, blood pressure, breathing, and nervous system. Hence, sensitive and easy methods are required for its determination. In this work, we report a simple approach for fabrication of a bimetallic Cu/Ni modified carbon paste electrode (Cu/Ni/CPE) for electrocatalytic oxidation and determination of the metoprolol drug. At first, using the electrodeposition method, Ni was deposited on the carbon paste electrode at room temperature; then, a galvanic replacement reaction was applied for the replacement of some nickel atoms with Cu atoms. Effective parameters on electrode response to metoprolol were optimized. In optimized conditions, the bimetallic modified electrode exhibited higher electrocatalytic activity for metoprolol than a single metal modified electrode: Cu/Ni/CPE has a wider linear range and higher sensitivity to metoprolol respect to Ni/CPE. The surface of Cu/Ni/CPE was characterized by field emission scanning electron microscopy (FE-SEM) and Energy-dispersive X-ray spectroscopy (EDX). The linear dynamic range of this modified electrode for metoprolol is 1×10^{-5} to 7×10^{-4} M, and the limit of detection was determined to be 2.9 µM. Finally, this modified electrode was applied for the determination of metoprolol in human plasma and tablet samples, and acceptable results were obtained. For comparison, spectrophotometry was used as the reference method.

Keywords- Metoprolol; Determination; Electrodeposition; Galvanic replacement reaction; Modified electrode

1. INTRODUCTION

Metoprolol is a cardioselective β-blocker medication used to treat high blood pressure, heart problems, and migraine, also as β-blockers doping agent is a forbidden drug for athletes [1]. Metoprolol is the most commonly used beta-blocker drug and is the sixth most prescribed medication in U.S. [2].

Due to the widespread use of MET, its measurement and quantification in biological fluids, in pharmaceutical, toxicological, doping, and clinical chemistry research is essential. Various analytical methods for determination of MET are described in the literature, such as spectrophotometry [3,4], HPLC [5-8] and capillary electrophoresis [9]. All of these techniques often suffer from high cost of equipment, high amount of reagents, time-consuming pretreatment and extraction steps; Therefore, cheaper, simpler, and faster methods could be interesting alternatives. Compared to other analytical methods, electrochemical methods have several advantages, including simplicity, low cost, and high sensitivity and selectivity [10], and usually do not require complex sample preparation steps [11]. According to our investigation, few electrochemical sensors for metoprolol measurement have been published in the literature [10-19].

Electrochemical methods for analyte determination are usually based on the use of modified electrodes. One of the methods for preparing a modified electrode is to immobilize metal particles on the electrode surface. Electrodeposition is a method in which metal particles are produced and simultaneously immobilized on the electrode surface. The properties of the resulting metal coating can be controlled by changing the deposition variables [20-21]. This method can also be performed at room temperature and reduces the high cost of preparing metal catalysts [22].

In this paper, we proposed a Cu/Ni bimetallic modified electrode for the electrochemical sensing of MET. According to the review of Kannan [23], bimetallic materials are better catalysts than monometallic counterparts. For preparation of a bimetallic modified electrode, at first Ni-modified CPE was prepared using electrochemical deposition, and then a galvanic replacement reaction was used for replacement of some Ni atoms with Cu atoms. Considering the greater reduction potential of Cu^{2+}/Cu (E^o = + 0.34 V) with respect to Ni^{2+}/Ni (E^o= − 0.25 V), by immersing Ni/CPE in Cu^{2+} solution, the galvanic replacement reaction can be carried out as follows [24,25]:

$$Ni\ (s) + Cu^{2+}\ (aq) \longrightarrow Ni^{2+}\ (aq) + Cu\ (s) \quad (1)$$

In industry, galvanic replacement reaction has been widely used for preparation of open circuit boards [26-28]. This reaction can also be used to make modified electrodes by preparing bimetallic catalysts. These types of catalysts were used for various electrochemical reactions such as uric acid and glucose oxidation [29], methanol, ethanol, and ethylene glycol oxidation [30-34], oxygen and hydrogen evolution reactions [35-37], and some reduction reactions [38].

2. EXPERIMENTAL SECTION

2.1. Chemicals and apparatus

All chemicals were of analytical reagent grade and used as received. Doubly distilled water was used as solvent. Graphite powder (particle diameter: 0.10 mm) and paraffin oil (density: 0.88 g/cm^3) from Fluka were used to prepare the carbon paste electrode. Metoprolol tartrate and Metoral tablets were purchased, respectively, from Sigma-Aldrich and a local pharmacy (Iran). All other chemicals were purchased from Merck. The human plasma sample was obtained from healthy volunteers from a clinical diagnostic lab and stored under refrigeration.

A potentiostat/galvanostat (Sama 500-C Galvanostate-Potentiostate, Sama, Iran) was used for electrochemical studies. A conventional voltammetry cell containing three electrodes: a carbon paste electrode as the working electrode, a Pt wire as the auxiliary electrode, and Ag|AgCl as the reference electrode was used. FESEM images and EDX patterns of electrodes were obtained using MIRATESCAN-XMU (Czech Republic).

2.2. Preparation of Ni/CPE and Cu/Ni/CPE

Carbon paste electrode (CPE) was prepared from a mixture of conducting graphite powder and paraffin oil as a pasting liquid, according to our previous work [39].

CPE modification with Ni particles was achieved by potentiostatic electrodeposition. For this purpose, CPE was placed in 0.5 M H_3BO_3 solution containing 0.1 M $NiSO_4$. 6 H_2O, and an appropriate concentration of SDS, and a reduction potential was applied to it for a certain period of time (t_{ed}). Different applied potentials, different electrodeposition times, and different SDS concentrations were studied. Then Ni/CPE was washed with distilled water and was placed in 0.1 M $CuSO_4$ solution for a period of time (galvanic replacement time, t_{gr}). Two procedures with some differences have been used to produce Cu/Ni/CPE, which are described in the next section.

2.3.Preparation of standard solutions and real samples

Due to the nature of the modifiers, all electrochemical studies were performed in 0.1 M NaOH solution (nickel, which is the main catalyst in the oxidation of metoprolol, in alkaline solution has the best response) [40]; therefore, the stock solution of metoprolol was prepared with ultrapure water and was diluted with 0.1 M NaOH as appropriate. To prepare the pharmaceutical sample, 10 metoprolol tablets were weighed and ground into powder in a hand mortar, a certain amount of this powder was weighed, dissolved in electrolyte solution, sonicated for 20 min, and then filtered by centrifugation, the filtrate was transferred to a 25 mL flask, and diluted up to the mark with 0.1 M NaOH solution.

3. RESULTS AND DISCUSSION

3.1. Electrochemical oxidation of MET on CPE and Ni/CPE and effect of SDS addition

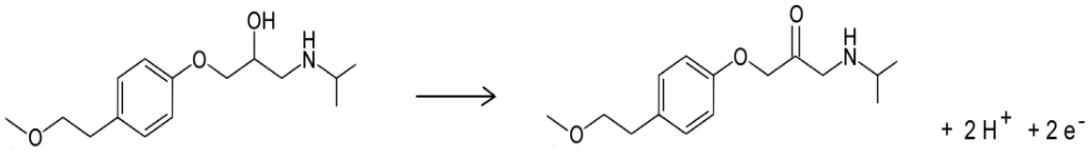
The main panel of Figure 1A shows the cyclic voltammogram of CPE and Ni/CPE in the absence and presence of MET in NaOH solution. Comparison of cyclic voltammograms a and b shows that the addition of MET only slightly increased the current, and MET doesn't have an obvious oxidation peak at the surface of CPE.

Figure 1, curve c shows the cyclic voltammogram of the Ni/CPE in a 0.1 M NaOH solution. This modified electrode was prepared by the electrodeposition method described in the experimental section, and SDS was used as an additive in the electrodeposition solution; a pair of oxidation-reduction peaks was obtained. The anodic peak with a potential of about 0.52 V, and the cathodic peak with a potential of about 0.41 V are ascribed to the NiOOH / $Ni(OH)_2$ redox couple (equation 2). After adding 1 mM MET to NaOH solution, it is observed that the anodic peak current has increased and the cathodic peak current has decreased (Figure 1, curve d). These observations confirm the electrocatalytic oxidation of metoprolol at the surface of Ni/CPE according to the EC′ mechanism as follows [41,42]:

$$Ni(OH)_2 + OH^- \longleftrightarrow NiOOH + H_2O + e^- \qquad E \qquad (2)$$

$$NiOOH + \text{metoprolol} \longrightarrow Ni(OH)_2 + \text{Product} \qquad C' \qquad (3)$$

According to the literatures [15,18], the oxidation of metoprolol, probably occurs through the hydroxyl group and with loss of two electron and two proton (Scheme 1).



Scheme 1. Proposed mechanism for the oxidation of metoprolol

For comparison, cyclic voltammograms of Ni/CPE prepared in a surfactant-free solution were also recorded in the absence and presence of metoprolol (Inset of Figure 1A). Comparison of the two electrodes (Figure 1B) shows that adding SDS to the electrodeposition solution can improve the electrode response to metoprolol. The effect of surfactants in improving the catalytic ability of electrodeposited metals has also been previously reported by Zolfaghari [43] and Bamirdi [44].

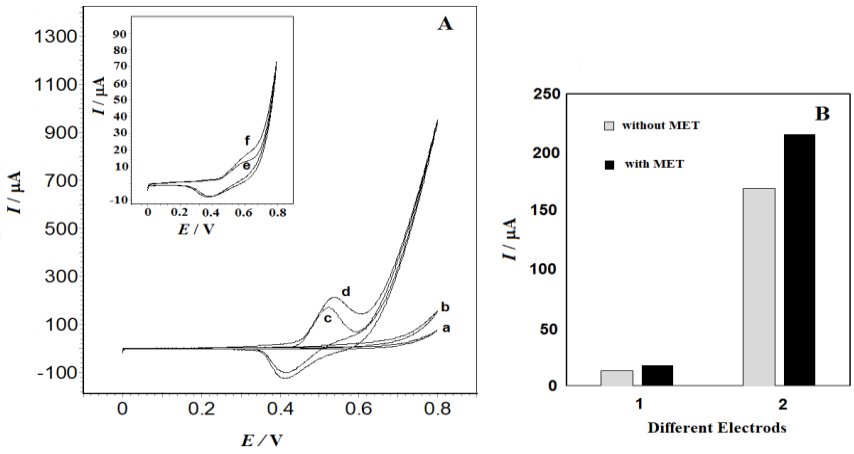


Figure 1. A) Main Panel: cyclic voltammograms of CPE in 0.1 M NaOH solution a) in the absence and b) in the presence of 1 mM metoprolol. (c) such as (a), and (d) such as (b), for Ni/CPE prepared with SDS addition in the electrodeposition solution. Inset: (e) such as (a), and (f) such as (b), for Ni/CPE prepared without SDS addition in the electrodeposition solution. B) Comparison of anodic peak current for different Ni/CPE prepared: 1) without SDS addition and 2) with SDS addition, in the absence and presence of metoprolol.

3.2. Effect of galvanic replacement in Cu^{2+} solution on electrode response

For preparation of a bimetallic modified electrode, two procedures were applied. In the first procedure, after electrodeposition of Ni on CPE, the prepared Ni/CPE electrode was washed with water and immediately immersed in 0.1 M Cu^{2+} solution for 5 min. During this step, Ni metal particles on CPE surface were replaced by Cu metal according to the chemical equation of (1); then the electrode was rinsed with water, and its cyclic voltammogram in NaOH solution was recorded. As can be seen in Figure 2A, curve (a) the anodic and cathodic peaks due to $Ni(OH)_2$/NiOOH redox couple (that can be seen in the voltammogram of c in Figure1) were removed. These show that galvanic replacement of Cu atoms was done successfully.

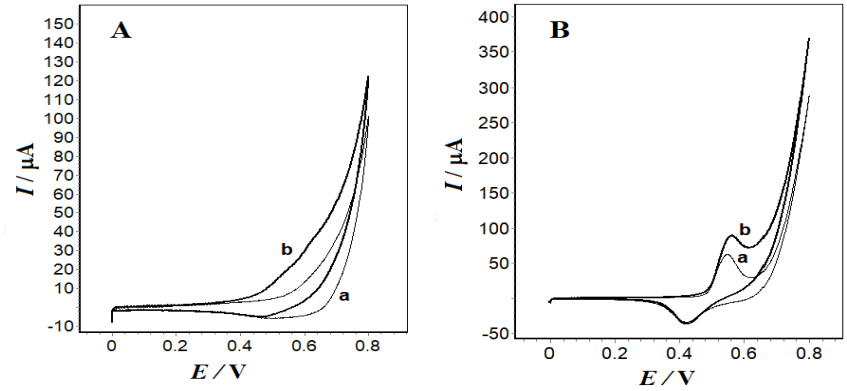


Figure 2. Cyclic voltammograms of Cu/Ni/CPE prepared with A) first procedure and B) second procedure in 0.1 M NaOH solution a) in the absence and b) in presence of 0.4 mM metoprolol.

But contrary to our expectations, this bimetallic electrode shows a weaker response to MET (Figure 2A, curve b); in fact, the current was increased slightly, but because of the low amount of $Ni(OH)_2$, there is no obvious oxidation peak, and determination of anodic peak current is difficult.

Therefore, we decided to examine another procedure to partially galvanic replacement of surface Ni atoms with Cu, instead of complete replacement. In the second method, Ni/CPE after preparation was rinsed and immersed in 0.1 M NaOH solution and successive cyclic voltammograms were recorded to some of Ni atoms change to $Ni(OH)_2$, and then the electrode was rinsed with water and placed in Cu^{2+} solution for galvanic replacement of remained Ni atoms with Cu atoms. In this procedure Ni atoms which were changed to $Ni(OH)_2$, cannot be replaced with Cu (because the galvanic replacement reaction only occurs between copper ions and nickel atoms with zero oxidation state [24]) Therefore, oxidation and reduction peak due to $Ni(OH)_2$/NiOOH redox couple can be observed (Figure 2B, curve a) and its response to MET have obvious anodic peak (Figure 2B, curve b). Our primary studies show that Cu/Ni/CPE, which was prepared with the second procedure, has a better response to MET respect to Ni/CPE. A complete comparison of these two electrodes will be studied after their optimization in the following sections.

Previously, Maity and coworkers [45] reported galvanic replacement of Ni by Pd in $Ni(OH)_2$/Ni nanostructure. They used a galvanic replacement reaction to quantify metallic Ni dispersed in $Ni(OH)_2$. They show that a bimetallic $Ni(OH)_2$/NiPd modified electrode has better electrocatalytic activity for methanol oxidation respect to a Pd modified electrode.

3.3. Optimization of effective parameters

Parameters affecting the electrode response to metoprolol, including SDS concentration, applied potential value, nickel deposition time, and galvanic replacement time, should be optimized.

3.3.1. Optimization of SDS concentration

Electrochemical deposition was performed with different SDS concentrations. Then, each electrode was washed with water, and its cyclic voltammograms were recorded in 0.1 M NaOH solution in the absence and presence of MET. The difference between the anodic peak current of the modified electrode in the absence and presence of MET (ΔI_p) was determined for each concentration of SDS. Figure 3A shows that with the addition of SDS concentration, the amount of ΔI_p was increased, and a concentration of 2 mM is the optimum concentration for this electrode.

3.3.2. Optimization of electrodeposition potential

In this step, electrochemical deposition of nickel on the CPE surface was performed at different potentials, and the response of the prepared electrodes was recorded in the absence

and presence of 0.5 mM MET. Figure 3B shows the graph of ΔI_p as a function of the applied potential. As can be seen, the best response is for the electrode prepared at a potential of -0.4 V, so this potential was chosen as the optimal value for nickel deposition.

3.3.3. Optimization of electrodeposition time

To determine the optimal time for nickel deposition, a carbon paste electrode was placed in the deposition solution and a potential of -0.4 V was applied to it for different times. The response of the prepared electrodes was recorded in the absence and presence of metoprolol. As can be seen in Figure 3C, the electrode prepared for a period of 20 s showed the best response to metoprolol.

3.3.4. Optimization of galvanic replacement time

In this step, various galvanic replacement times were used for preparation of Cu/Ni/CPE according to the second procedure described in section 3.2. Cyclic voltammograms of each electrode in the absence and presence of MET were recorded. As can be seen in Figure 3D, the electrode which prepared with 2 min galvanic replacement shows the best response to MET.

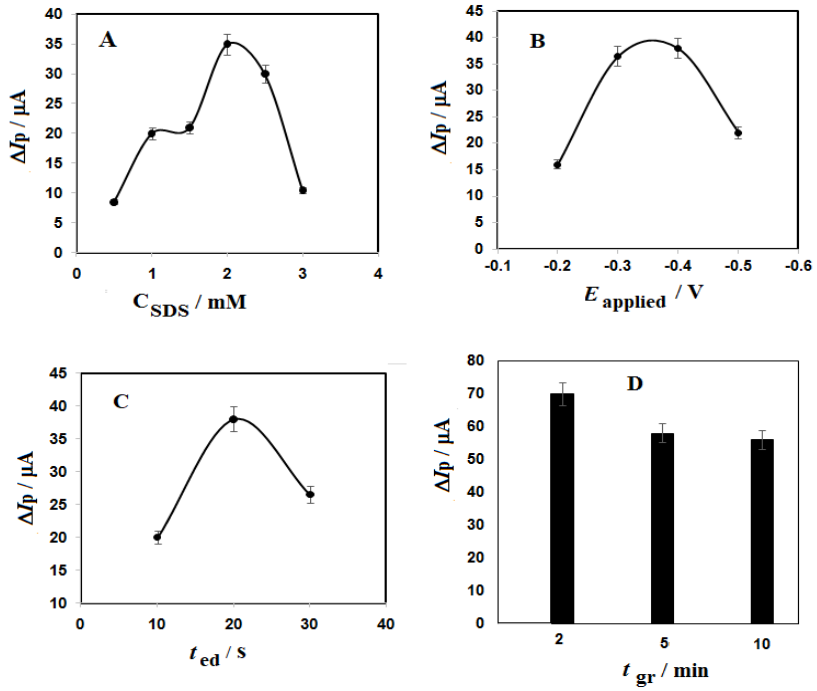


Figure 3. Effect of a) SDS concentration, b) applied potential, c) electrodeposition time and d) galvanic replacement time, on response of prepared Cu/Ni/CPE to MET oxidation in 0.1 M NaOH solution

3.4. The morphology and composition of electrodes

Figure 4 shows the FE-SEM images of CPE (Figure 4.a), Ni/CPE (Figure 4.b and c) and Cu/Ni/CPE (Figure 4.d). As can be seen, the shape of the surface in these electrodes is changed,

and in the case of Ni/CPE, particles with rich nickel can be seen. Although in the case of Cu/Ni/CPE the Cu particles aren't obvious; but EDX analysis (Figure 5) and map analysis of Cu/Ni/CPE (Figure 6) confirm the existence of Cu.

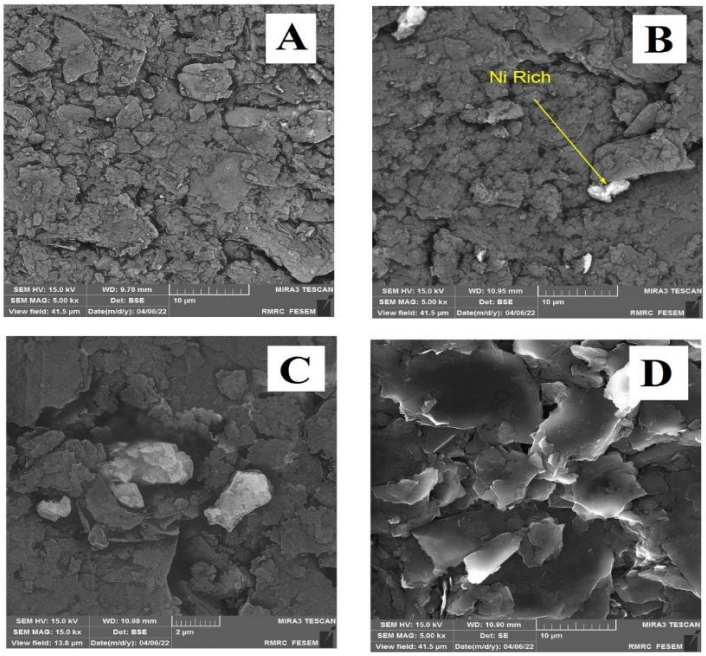


Figure 4. FE-SEM images of A) CPE, B) Ni/CPE, C) Zoomed image of Ni/CPE and D) Cu/Ni/CPE

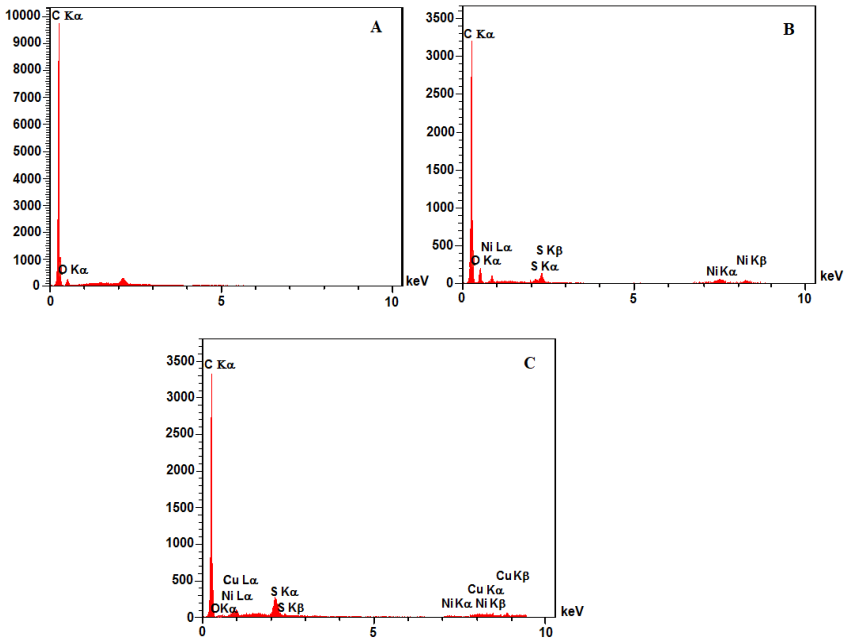


Figure 5. EDX of A) CPE, B) Ni/CPE and C) Cu/Ni/CPE

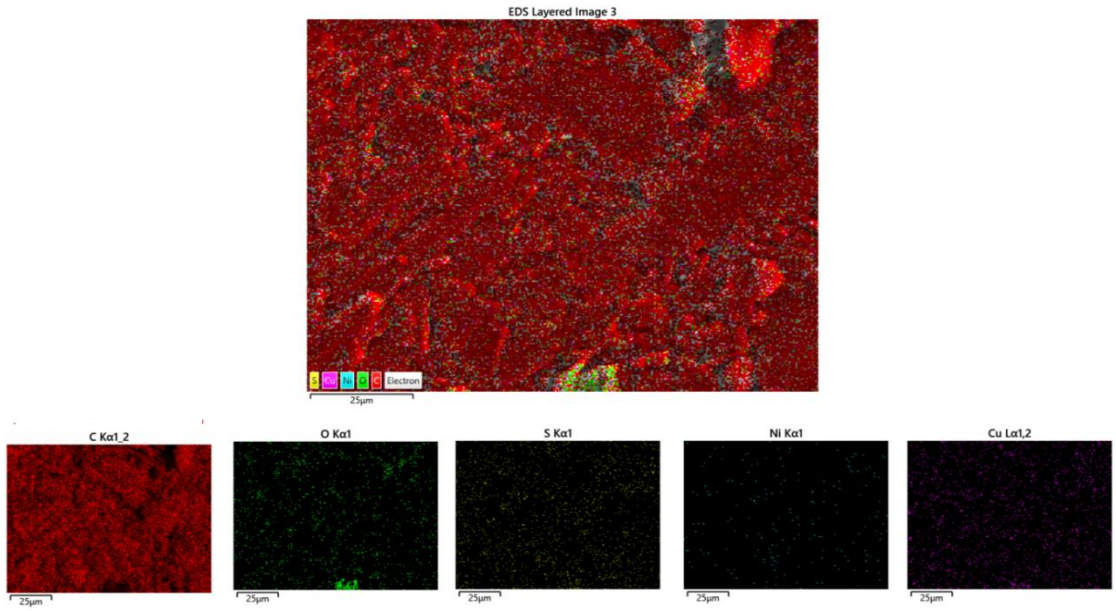


Figure 6. Map imaging of Cu/Ni/CPE

3.5. Electrochemical behavior of modified electrode

3.5.1. Response of modified electrode to $K_4[Fe(CN)_6]$ as a probe

Figure 7 shows the electrochemical response of different electrodes in 0.1 M KCl solution containing 5 mM $K_4[Fe(CN)_6]$. As can be seen, in comparison with CPE and Ni/CPE, Cu/Ni/CPE shows the lowest amount of ΔE_p (peak-to-peak separation potential) and the higher peak current. This can be due to the higher surface area and also higher electron transfer kinetics in Cu/Ni/CPE.

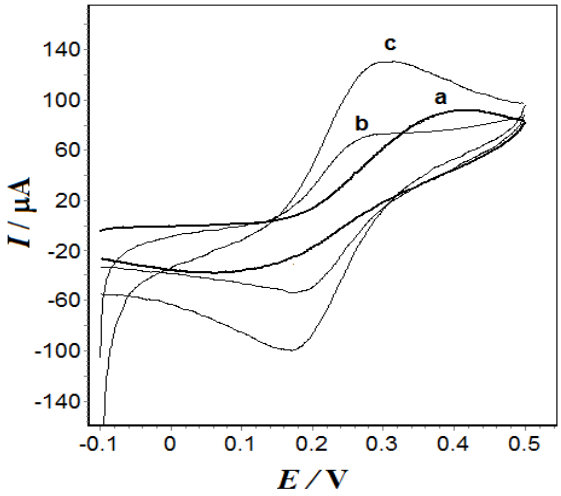


Figure 7. Cyclic voltammograms of a) CPE, b) Ni/CPE and c) Cu/Ni/CPE in 0.1 M KCl solution containing 5 mM $K_4[Fe(CN)_6]$ solution at a scan rate of 50 mV s^{-1}

3.5.2. Chronoamperometric studies

Figure 8A shows the double potential step chronoamperograms for Cu/Ni/CPE in the presence of different concentrations of metoprolol. To record these chronoamperograms, anodic and cathodic potential steps (each for 10 seconds) were applied to the modified carbon paste electrode to induce oxidation and reduction processes, and the changes in anodic and cathodic currents were recorded during these times.

Inset of Figure 8A shows the Chronocoulogram obtained by integrating the chronoamperograms in the absence and presence of metoprolol. As can be seen from this Figure, in the absence of metoprolol, the anodic and cathodic passed charges (associated with the oxidation and reduction of $Ni(OH)_2$/NiOOH redox couple) are approximately equal. However, in the presence of metoprolol, the anodic charge increases and the cathodic charge decreases, which is a confirmation of the electrocatalytic process of metoprolol oxidation on the Cu/Ni/CPE surface.

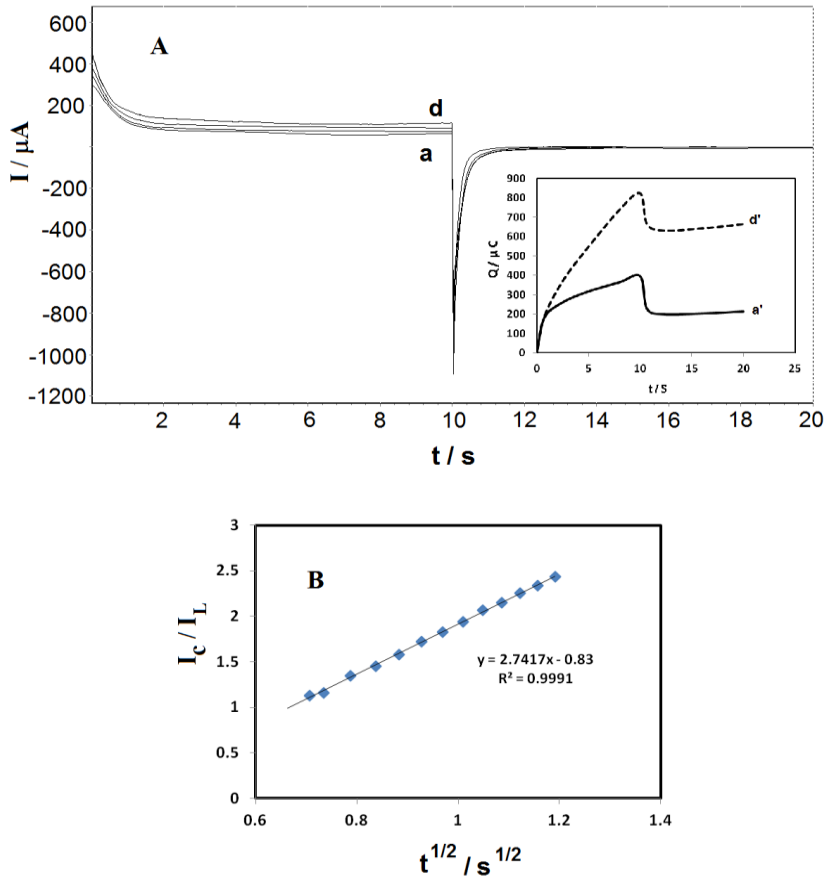


Figure 8. A) Chronoamperometry with double potential step on the Cu/Ni/CPE surface in the absence (a) and presence of (b) 0.2, (c) 0.4 and (d) 0.6 mM metoprolol in 0.1 M NaOH solution. The first and second potential steps are 600 mV and 400 mV, respect to the reference electrode. (Inset) Dependence of Q (µC) vs. t. (a') and (d') were obtained by integrating chronoamperograms (a) and (d), respectively. B) Plot of I_C/I_L vs. $t^{1/2}$ obtained from the data of chronoamperogams of (a) and (d)

It is possible to determine the rate constant of the electrocatalytic oxidation reaction of metoprolol using chronoamperometric measurements using the following equation [46]:

$$I_C/I_L = \gamma^{1/2}[\pi^{1/2}\mathrm{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}], \quad (4)$$

In this equation, I_C is the electrochemical current in the presence of a metoprolol and I_L is the limiting current in the absence of metoprolol at the surface of the modified electrode. Also, γ is defined as kC_ot where C_o, k and t are the analyte concentration (mM), catalytic reaction rate constant (cm^3/mol s) and the time elapsed (s), respectively. In cases where λ is greater than 1.5, equation (4) simplifies to:

$$I_C/I_L = \gamma^{1/2}\pi^{1/2} = \pi^{1/2}(kc_ot)^{1/2} \quad (5)$$

The I_C/I_L versus $t^{1/2}$ graph was plotted using the data from two chronoamperograms, one in the absence of metoprolol and the other in the presence of 0.6 mM metoprolol (Figure 8B). The value of k was calculated using the slope of this graph to be 3.98×10^6 cm^3/mol s.

3.6. Dependence of modified electrodes response to MET concentration

Comparison of Cu/Ni/CPE and Ni/CPE (prepared in optimized conditions) responses to MET oxidation was shown in Figure 9. As can be seen, in Cu/Ni/CPE, the amount of increase in anodic peak current with the addition of MET is more than Ni/CPE. Electrochemical responses of Cu/Ni/CPE in the presence of different concentrations of MET were recorded (Figure 10A). A linear concentration range of 1×10^{-5} to 7×10^{-4} M with the slope of about 120 μA/mM was observed and amount of LOD was calculated 2.9×10^{-6} M. Similar study was done for Ni/CPE (not shown); comparison of the plot of ΔI_p versus concentration of MET (Figure 10B) show that Cu/Ni/CPE has higher sensitivity to MET, wider LDR and lower LOD respect to Ni/CPE.

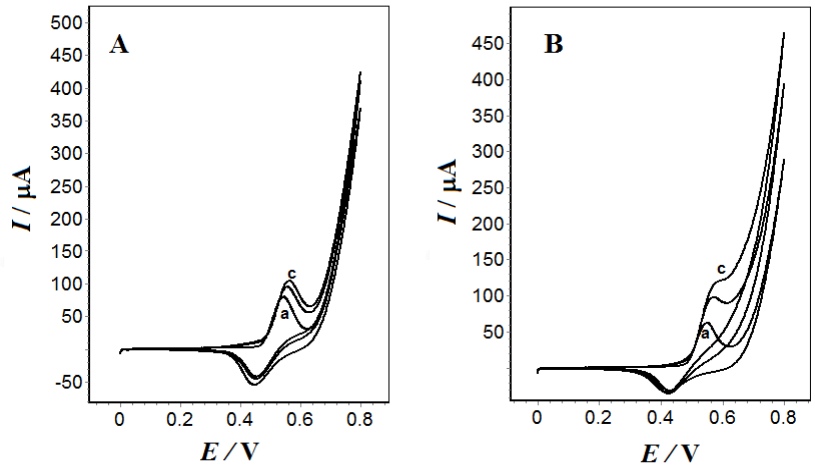


Figure 9. Cyclic voltammograms of A) Ni/CPE and B) Cu/Ni/CPE in 0.1 M NaOH solution containing (a) 0, (b) 0.2, (c 0.4 mM of MET concentration

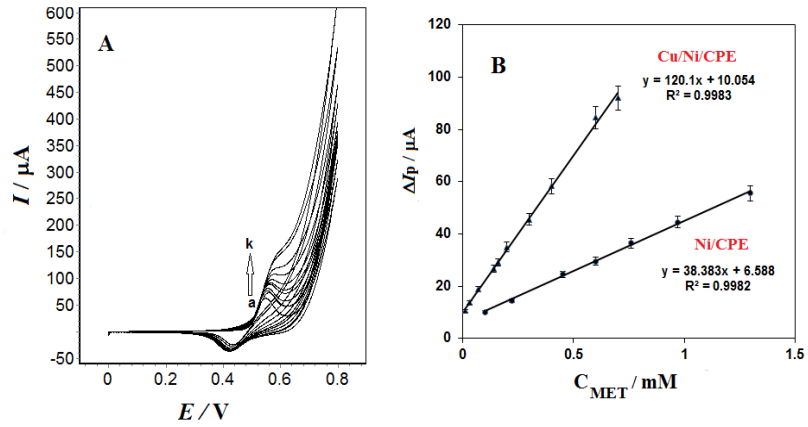


Figure 10. A) Cyclic voltammograms obtained for different concentrations of MET at the surface of Cu/Ni/CPE: (a) 0, (b) 0.01, (c) 0.03, (d) 0.07, (e) 0.14, (f) 0.16, (g) 0.2, (h) 0.3, (i) 0.4, (j) 0.6 and (k) 0.7 mM, B) Plot of ΔIp *vs.* metoprolol concentration for Cu/Ni/CPE and Ni/CPE

Some analytical performance of Cu/Ni/CPE was compared with other modified electrodes previously proposed for MET determination (Table 1). Although the LOD of Cu/Ni/CPE is inferior to most of reported electrodes (except references [17]), the linear range at the surface of Cu/Ni/CPE is wider than most of reported electrodes. Moreover, preparation of this electrode is simple, and contrary to adsorptive stripping methods, no time is needed for accumulation of analyte at Cu/Ni/CPE, and the response of the electrode to MET can immediately be recorded. Among the different electrodes that have been reported for determination of MET, the GCE and HMDE [14] are the simplest ones, but their dynamic range is narrow, and HMDE is not favorable because of Hg toxicity.

Table 1. Analytical parameters of different developed sensors used in the determination of MET

Electrode	**Electrochemical method**	**LDR (µM)**	**LOD (µM)**	**Ref.**
GRE/PtNps/NFN	Ads-DPV	0.0144 – 7.5	0.0043	[10]
MIP/MWCNTs/PGE	DPV	0.06 – 490	0.0029	[12]
BDD	DPV	0.38 – 22	0.034	[13]
GCE	Ads-DPV	0.62 – 3.25	0.19	[14]
HMDE	SWV	3.18 – 16.9	0.96	[14]
NH_2-HMS-CPE	DPV	1– 60	0.23	[15]
Poly(AN-co-PTSA)	SWV	40 – 1500	37.9	[17]
MnO_2NPs/SPCE	DPV	0.006 – 0.01	0.005	[18]
NiONPs/PPy/GCE	DPV	5 – 1600	0.018	[19]
Ni/CPE	CV	100 – 1300	30	This work
Cu/Ni/CPE	CV	10 – 700	2.9	This work

The reproducibility of Cu/Ni/CPE preparation and its application for MET determination is expressed in terms of relative standard deviation (RSD), which is calculated to be 5.5% for five electrodes and 0.4 mM MET solution. Also, the repeatability of the proposed sensor was examined by performing four measurements with a single electrode in a 0.4 mM MET solution. The RSD for these measurements was found to be 4%.

3.7. Determination of Metoprolol in real samples

In order to evaluate the applicability of the proposed sensor for determination of MET in real samples, it was used for determination of MET in tablet and human plasma samples using the standard addition method. All samples were diluted with 0.1 M NaOH solution, and a known amount of MET was spiked into the plasma solution. The tablet samples were also analysed by direct UV-spectrophotometric method (λ_{max}= 224 nm) as a reference method (Figures 11A and 11B). Results were compared statistically by t-test (for the accuracy) and F-test (for the precision). The results are summarized in Table 2.

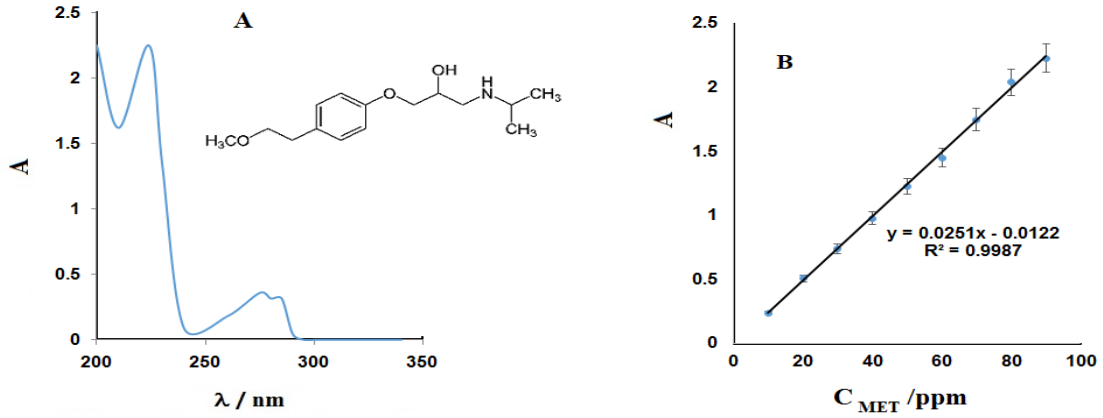


Figure 11. A) Ultraviolet absorption spectrum of MET in Phosphate buffer solution (80 ppm, pH = 7). B) Calibration graph of MET

Table 2. Results of metoprolol determination in real samples using Cu/Ni/CPE

Sample	Labeled (mg)	Spiked (µM)	Found by proposed method*	Found by UV method*	Recovery (%)	t_{exp}	F_{exp}
tablet	50	-	51.6±1.5	51.2±0.8	-	1.06	3.57
plasma	-	60	57.2	-	95.3±3.2	-	-

*Average ± relative standard deviation. Theoretical values at 95% confidence level, F = 6.39, t = 2.31 for spooled standard deviation for n_1= n_2 =5

4. CONCLUSION

In this work, we introduced a bimetallic Cu/Ni modified electrode as a sensor for the determination of metoprolol. The electrochemical behavior of the modified electrode was investigated using cyclic voltammetry and chronoamperometry. Experimental results showed that this modified electrode has a good ability in electrocatalytic oxidation of metoprolol in an alkaline solution. Studies showed that changes in electrode preparation conditions affect the electrode response to metoprolol; therefore, in order to achieve the best electrocatalytic response, the variables involved in the preparation of the sensor were investigated, and the optimal conditions were determined. In the optimized condition, the modified electrode shows acceptable LOD and Linear range. Preparation of this sensor was low cost, simple, and fast, and unlike chemical methods for producing catalysts, it did not require high temperatures or large amounts of reagents. This modified electrode can be used for the measurement of metoprolol in pharmaceutical and biological samples.

LIST OF ABBREVIATIONS

LDR = linear dynamic range
LOD = limit of detection
GRE = graphene
PtNps = Pt nanoparticles
NFN = nafion
MIP = molecularly imprinted polymer
MWCNTs = multi wall carbon nanotubes
PGE = pyrolitic graphite electrode
BDD = boron-doped diamond
GCE = glassy carbon electrode
NH_2-HMS = amino-functionalized mesostructured silica
HMDE = hanging mercury drop electrode
AdS = adsorptive stripping
DPV = differential pulse voltammetry
SWV = square wave voltammetry
Poly (AN-co-PTSA) = Poly(aniline-co-p-toluene sulfonic acid)
CPE = carbon paste electrode

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Declarations of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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