

Modified Glassy Carbon Electrode with Mesoporous Silica-Metformin/Multi-Walled Carbon Nanotubes as a Biosensor for Ethinylestradiol Detection

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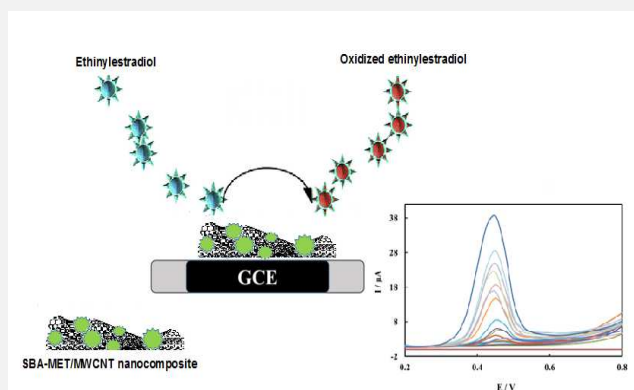


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ABSTRACT

The aim of this study was related to the determination of ethinylestradiol (EE) via electrochemical methods. Since the EE determination has approximately been impossible at low concentrations, the glassy carbon electrode (GCE) was modified with a mixture of metformin (MET), mesoporous silica (SBA-15), and multi-walled carbon nanotubes (MWCNT). Due to the high existence of the NH₂ functional group in MET, this material can act as a modifier by creating a chemical bond by adsorption in SBA mesoporous, and it subsequently can measure EE. Experimental investigations have shown that the modified electrode has had a repeatable response toward EE evaluation in real samples. Moreover, the physicochemical properties of the manufactured electrode were investigated via FT-IR, field-emission scanning electron microscopy (FESEM), and transmission electron microscope (TEM). Also, the electrochemical characteristics were studied through cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. The related result has indicated a considerable increment in current intensity. At optimal conditions, the modified electrode demonstrated linear voltammetric responses in the ranges of 0.005-200 μM and a low detection limit of 1.68×10^{-9} M for EE estimation.



Keywords: Electrochemical sensor, ethinylestradiol, metformin, multi-walled carbon nanotubes

1. Introduction

Environmental pollutants have recently been labeled as endocrine disruptor chemicals (EDCs) which have indicated deleterious effects on the endocrine systems of living organisms. Also, these materials have affected the growth, health, and reproduction of humans and other animals, and may operate as cancer inducers [1]. EDCs were made up of a variety of chemical groups, including synthetic and natural steroid estrogens. In detail, EE, a synthetic estrogen extensively utilized as an oral contraceptive and regarded as an effective estrogenic, belongs to the EDCs categorization [2].

The EE can also develop negative effects, containing fibrinolysis and faster coagulation. Furthermore, considering the estrogen concentration, the utilization of blended hormonal contraceptives has indicated an augmented threat of venous thrombosis. So, as a pharmaceutically active complex, the EE can move into aquatic media via animal and human excretion. Consequently, the environmental residues can cause intense effects on biota and humans, involving the feminization of creatures and the subsequent decline in reproduction rates or growth of carcinomas and sperm production decrease in males [3]. Hence, the evaluation of EE both in environmental samples and in pharmaceutical formulations is significant. There were numerous analytical approaches for EE estimation in different sample matrices, containing underground water, surface water, wastewater, biological fluid, and pharmaceutical formulations [4]. Due to the low concentration of EE in water contents in the range of ng/L, the usual methods involve a former step of pre-concentration and separation. The methods include solid-phase

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extraction and evaluation via high-efficiency liquid chromatography, combined with mass spectrometric determination [5]. Although these methods improve sensitive manners, they have numerous limitations, requiring both expensive apparatus and large sample volumes, being time-consuming, and preventing high velocity. Besides mentioned approaches, electrochemical sensors were also commonly used in the estimation of EE [6-8]. Moreover, the appropriate properties of electrochemical systems such as low measurement limits as well as their low acquisition costs have made them extensively applicable. In addition, the proper production of electrochemical sensors which can catalyze EE oxidation is really important because of the negligible electrochemical characteristics of EE at the conventional electrode surface [9].

Recently, carbon nanotubes were widely utilized to make biosensors and sensors. Carbon nanotubes can reduce determination limits in electrochemical sensors, using their astonishing benefits, containing a substantial increment of electron transmission rate, and over-potential reduction of the electrode [10]. Additionally, multi-walled carbon nanotubes (MWCNTs) possess fascinating physicochemical behaviors, involving ultra-light mass, a well-ordered construction with a great aspect ratio, high thermal conductivity, metallic or semi-metallic properties, high electrical conductivity, high mechanical strength, and a high surface area [11, 12].

Furthermore, the electrons transfer between the electrodes and the electroactive materials introduces great conditions for producing electrochemical biosensors and sensors. For instance, Nodehi et al. [6] evaluated the EE via the Au/Fe₃O₄@TA/MWNT/GCE sensor. They illustrated the fabrication and design of a sensitive, inexpensive, and selective electrochemical sensor for the detection of EE. Additionally, Li [13] presented a voltammetric procedure for EE estimation using a GCE. Furthermore, Joanna Smajdor [14] investigated a voltammetric method using continuous current in order to determine EE.

One of these materials is mesoporous silica SBA-15. SBA-15 can be utilized for the creation of stable biosensing structures, and it is a capable applicant for the immobilization of different substances considering its porosity, great surface-to-area ratio, thermal stability, and monotonous pore size distributions [15, 16].

Now, the essence of this work was related to Metformin utilization in pill form as an anti-diabetic drug under the brand name metformex and metformin hydrochloride C₄H₁₁N₅. Moreover, this drug was used to treat type 2 diabetes which has not been dependent on insulin and affects the liver. So, it can reduce glucose absorption and increase the effect of insulin on the body [17]. Likewise, metformin has numerous NH₂ functional groups which provide the conditions for its usage as a modifier at the electrode surface. Also, it is a suitable substrate for the stabilization of nanoparticles by providing chemical bonding or adsorption. Hence, compounds named mesoporous were prepared for metformin utilization as an electrode surface modifier. This is due to their individual properties, including high surface-to-volume ratio, controllable particle size, and great biocompatibility and biodegradability, which is why they are used as drug carriers [18]. Thus, using metformin associated with SBA-15 as nanoporous silica and multi-walled carbon nanotubes, an extraordinary modifier was prepared for the GCE. SBA-15 can provide a high level of adsorption due to its porosity. Besides, despite many factor groups in metformin structure, it causes more interaction between the analyte and the surface of the modified electrode [19, 20]. In this study, to measure EE, a modified GCE was fabricated and introduced based on the interaction of SBA metformin and multi-walled carbon nanotubes. The modified electrode has indicated high capability in detecting and measuring EE at low concentrations [18].

2. Materials and methods

2.1. Materials

Ferric chloride (FeCl₃·6H₂O), Ferrous chloride (FeCl₂·4H₂O), EE (C₂₀H₂₄O₂), Multi-walled carbon nanotubes, Metformin (C₄H₁₁N₅), Sodium dihydrogen phosphate (NaH₂PO₄), Sodium hydrogen phosphate (Na₂HPO₄), Ethanol (C₂H₅OH), Ethylacetate (C₄H₈O₂), EE (C₁₈H₂₄O₂), Glucose (C₆H₁₂O₆), Magnesium chloride (MgCl₂), Ascorbic acid (C₆H₈O₆), Urea (CH₄N₂O), Bisphenol A (C₁₅H₁₆O₂), Bisphenol AF (C₁₅H₁₀F₆O₂), Bisphenol Bp (C₂₅H₂₀O₂), Hydrochloridric acid (HCl) and Sodium hydroxide (NaOH) were purchased from Merck Chemical Co (Germany). Also, SBA-15 was prepared by Kermanshah University. In addition, no further purification was used to supply standard solutions and analysis. Also, double distilled water was used in the experiments.

The pH of the aqueous solution was measured by a digital pH meter (Metrohm, PHS-3BW model, Swiss) equipped with a glass combination electrode. The cyclic voltammograms data were recorded using a polarograph (Metrohm VA Computrace 797 model, Swiss). Moreover, a TESCAN-XMU FESEM and TEM system with an acceleration voltage of 15 kV were utilized to examine the surface morphology and structure of SBA-MET, respectively.

2.2. Preparation of SBA-MET/MWCNT/GCE

The electrochemical studies were performed utilizing a modified GCE. SBA-15 is functionalized with metformin and prepared by Kermanshah University. Firstly, modified GCE was prepared using 1mg of SBA-MET and 1mg MWCNT dispersed in 1 mL ethanol and sonicated for 20 min. Afterward, the solution is located in the oven for 5 min at 25 °C. The surface of GCE was polished with alumina slurries, rinsed thoroughly with ethanol and fourth-ionized water, sonicated in ethanol for 5 min, and allowed to dry at ambient temperature. Eventually, 3 μ L of the MET/MWCNT/GCE suspension was dripped onto the GCE surface, and modified electrodes were dried at 25 °C for electrochemical determination.

3. Results and discussion

3.1. Characterization of SBA-MET

The surface morphology and characterization of the SBA-MET structure were performed by FESEM and TEM images. **Figures 1a** and **1b** show FESEM and TEM images of the SBA-MET. As can be seen, the SBA-MET has homogeneous agglomerated spherical particles and highly porosity morphology. Therefore, all images have shown successful preparation of SBA-MET [16].

Figure 1c shows the FT-IR spectrum of SBA-15 and SBA-MET. As can be observed, a characteristic peak was observed through stretching vibrations Si-O-Si bands at 1052 cm^{-1} [15, 21]. The C = NH and C = NC bands were detected at 1670 and 1650 cm^{-1} , respectively. Also, N-H stretching mode was obtained at 3350 cm^{-1} and N-H bending mode at 1450 cm^{-1} . Therefore, in Figure 1C, all spectra have shown successful preparation of SBA-MET [22].

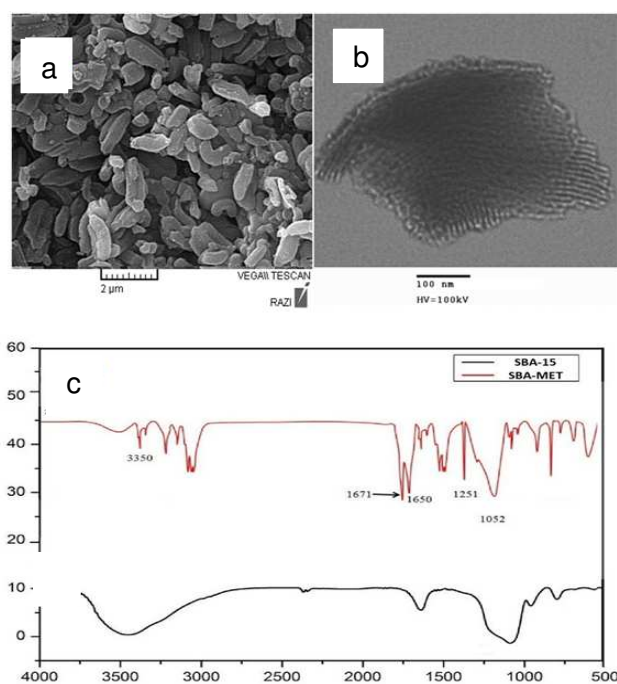


Figure 1. (a) FESEM and (b) TEM images of SBA-MET sample. (c) FT-IR spectra of SBA-15 and SBA-MET samples.

3.2. Electrochemical characterization of modified electrodes

To estimate the electrochemical properties and effective surface area of the GCE and different modified electrodes, the electrochemical probe 5.0×10^{-3} M $\text{K}_3\text{Fe}(\text{CN})_6$, 5.0×10^{-3} M $\text{K}_4\text{Fe}(\text{CN})_6$, and 0.1 M KCl was used.

Hence, Randles–Sevcik equation (Eq.1) was applied to express the relation between the redox current response of an electrochemical reagent and the potential scan rate.

$$I_p = (2.69 \times 10^5) An^{3/2} D^{1/2} C \sqrt{v} \quad (1)$$

where, I_p is the anodic peak current, and A refers to the effective surface area of the electrode (cm^2). n is the number of exchanged electrons for the redox reaction. Also, D and C are the diffusion coefficient and concentration of $\text{K}_4\text{Fe}(\text{CN})_6$, respectively. Additionally, the potential scan rate value (v) is 7.6×10^{-6} cm.s^{-1} .

Figure 2, illustrates the cyclic voltammograms of GCE, MWCNT/GCE, SBA-MET/GCE, and SBA-MET/MWCNT/GCE electrodes. As can be seen, as the potential scan rate increases from 10 to 300 mV/s, the intensity of the anodic and cathodic peak currents of $\text{K}_3/\text{K}_4\text{Fe}(\text{CN})_6$ increases. According to the Randles–Sevcik equation, the surface area of GCE, MWCNT/GCE, SBA-MET/GCE, SBA-MET/MWCNT/GCE were calculated as 0.24, 0.25, 0.32, 0.44 cm^2 , respectively. According to the obtained results, modified GCE with MWCNT, SBA-MET, and SBA-MET/MWCNT enhances the effective surface area of the electrode. Therefore, it can be said that the modified electrode is much more powerful than the previously modified forms in this method.

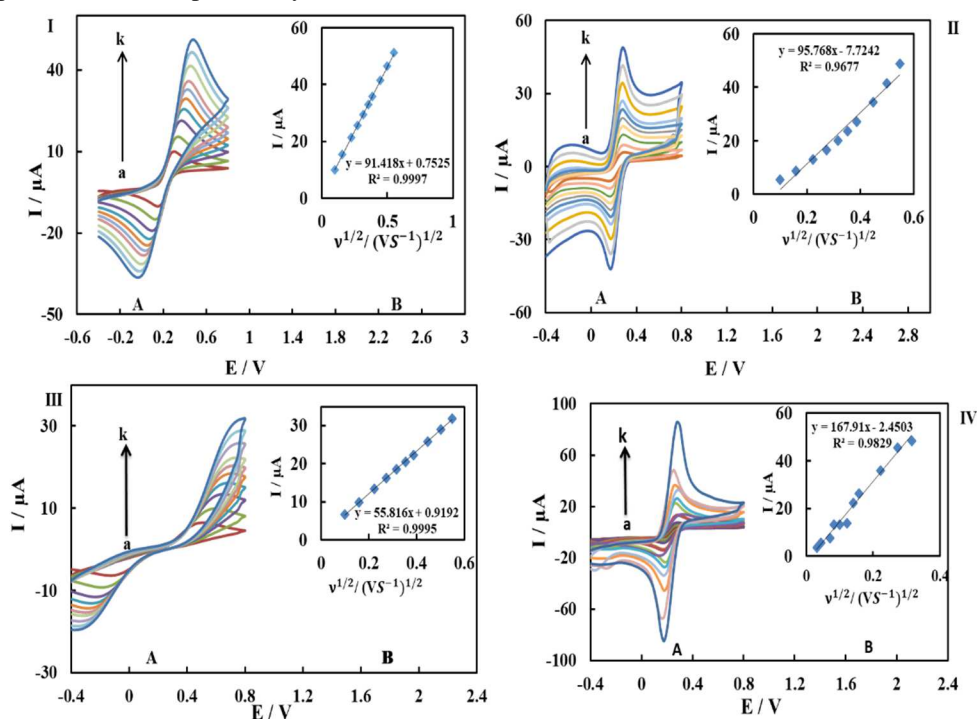


Figure 2. Cyclic voltammograms of (I) GCE, (II) MWCNT/GCE, (III) SBA-MET/GCE, (IV) SBA-MET/MWCNT/GCE electrodes at 5mM $\text{K}_3\text{Fe}(\text{CN})_6$, 5mM $\text{K}_4\text{Fe}(\text{CN})_6$ and 0.1 M KCl at different scan rates a) 10, b) 25, c) 50, d) 75, e) 100, f) 125, g) 150, h) 200, j) 250, k) 300 mV / s. Insets of Figures; anodic current changes versus root of the scan rates.

Electrochemical impedance spectroscopy (EIS) is a beneficial technique in electrode surface modification investigations. Hence, to perform EIS measurements, a bare GCE was placed in a solution that had 5.0×10^{-3} M $\text{K}_3\text{Fe}(\text{CN})_6$, 5.0×10^{-3} M $\text{K}_4\text{Fe}(\text{CN})_6$, and 0.1 M KCl. Subsequently, it was reformed in different stages. Figure 3 shows the Nyquist plots of the probe solution at the GCE, SBA-MET/GCE, MWCNT/GCE, and SBA-MET/MWCNT/GCE. The related values can be evaluated via the semicircular diameter of the Nyquist diagram. The semicircular diagram can be attributed to the kinetic constraints (R_{ct}) of the electrochemical reaction at high frequencies. Furthermore, at low frequencies, the linear part of the diagram corresponds to the electrode process under diffusion control. According to the graph, **Figure 3a** is related to GCE, and it has high resistance against electron

transfer due to the lack of a rectifier on its surface. Likewise, **Figure 3b** indicates the MWCNT/GCE impedance spectrum with the lowest electron transfer resistance. **Figure 3c** demonstrates that the presence of amine groups in metformin has improved the electron transfer resistance of the MWCNT/GCE ratio after modification of the GCE surface with MET. In addition, **Figure 3d** depicts the SBA-MET/MWCNT/GCE, whose resistance has risen due to the presence of organic groups in metformin.

In addition, compared to the modified MWCNT electrode, the SBA-MET/MWCNT/GCE transfer resistance increment can be attributed to the SBA presence on the electrode surface, which has slightly insulated the electrode surface. However, due to the existence of MWCNT, the resistance becomes less when it exposes to the bare GCE. So, the impedance results well prove the presence of MWCNT and SBA located on its surface. Therefore, after MWCNT and SBA-MET/MWCNT placement on the GCE surface, the semicircular diameters have decreased, suggesting that MWCNTs have improved the conductivity of the electrode and made the electron transfer easier

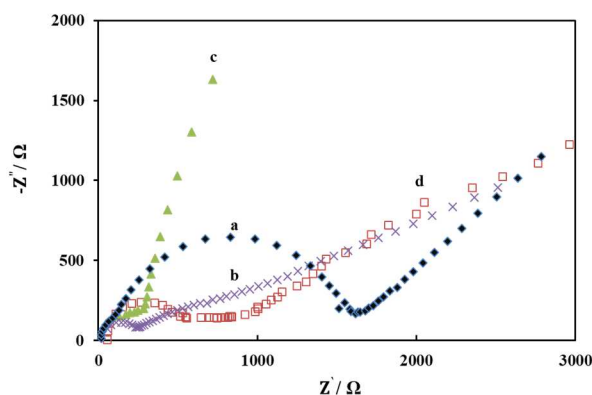


Figure 3. Electrochemical impedance spectroscopy of (a) GCE, (b) MWCNT/GCE, (c) SBA-MET/GCE, and (d) SBA-MET/MWCNT/GCE at 5mM $K_3Fe(CN)_6$, 5mM $K_4Fe(CN)_6$, and 0.1 M KCl.

3.3. Electrochemical properties of EE on the surface of the modified electrodes

Figure 4 shows the cyclic voltammogram of the GCE, SBA-MET/GCE, and SBA-MET/MWCNT/GCE in PBS (pH 7) and 100 μ M EE at a scan rate 0.1 Vs^{-1} . According to this **Figure 4**, it is perceived that the maximum peak current of EE oxidation occurs on the SBA-MET/MWCNT/GCE. In addition, these results demonstrate that the novel modified electrode can oxidize EE remarkably.

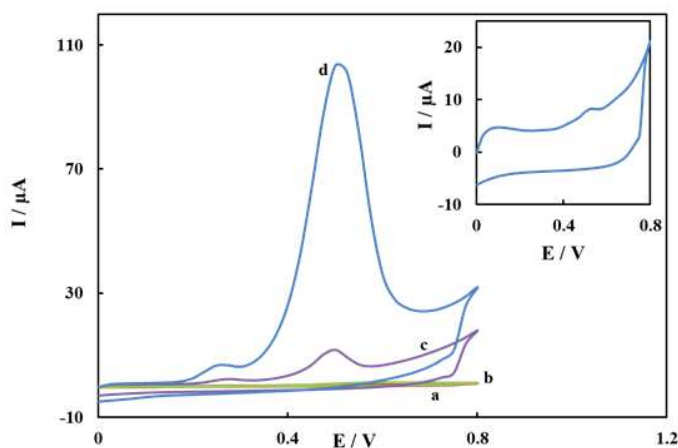


Figure 4. Cyclic voltammograms of (a) GCE, (b) SBA-MET/GCE, (c) MWCNT/GCE, and (d) SBA-MET/MWCNT/GCE electrodes at 10 ml PBS (pH =7, 0.1 M), 100 μ M EE, potential ranges 0 to 0.8 v and 0.1 Vs^{-1} scan rate, Inset; cyclic voltammogram of SBA-MET/MWCNT/GCE at 10 ml PBS (pH =7, 0.1 M) without EE.

3.4. Evaluation of scan rate

Using cyclic voltammetry, the effects of different potential scan rates (10-300 mV/s) were investigated on the electrochemical response of 100 μM EE at the modified electrode (SBA-MET/MWCNT/GCE). Furthermore, as shown in **Bold**, the anodic peak current related to the oxidation of EE has increased linearly with the scan rate increment. Also, the correlation coefficient was 0.9917. Thus, the adsorption process is possible on the electrode surface.

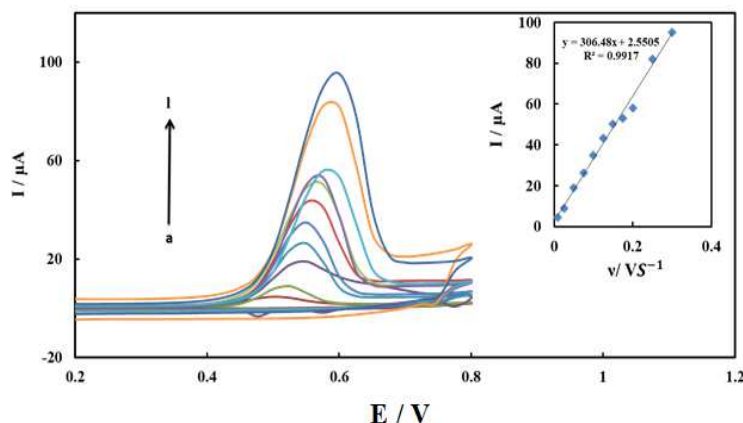


Figure 5. Cyclic voltammograms of SBA-MET/ MWCNT/GCE at different scan rates: (a) 10, (b) 25, (c) 50, (d) 75, (e) 100, (f) 125, (g) 150, (h) 175, (j) 200, (k) 250, (l) 300 $\text{mV} / \text{s}^{-1}$ in 10 ml BPS (pH = 7, 0.1 M) and 100 μM EE. Inset; oxidation peak currents of EE versus scan rate.

3.5. Optimization of the performance of the SBA-MET/MWCNT/GCE biosensor

In this study, experimental conditions were optimized for the determination of EE on the modified electrode surface. The experimental factors such as accumulation time and accumulation potential of EE and pH were investigated. Considering the EE electrochemical reaction that occurs through adsorption on the modified electrode surface adequate time should be allowed for interaction time with the stabilized material on the electrode surface. Therefore, before beginning any test, the electrode was immersed in a certain concentration of EE solution for some time to ensure that the EE has accumulated well on the surface of the electrode.

Hence, it is necessary to evaluate and optimize the time required for the effective interaction of EE with the surface. Consequently, the modified GCE was placed in 10 ml of the buffer solution (0.1 M PBS, pH = 7) at a concentration of 50 μM EE under constant convection conditions via a magnetic stirrer. Then, the resulting cyclic voltammograms were recorded at different times (0, 3, 5, 7, and 15 minutes) with a potential scan rate of 0.1 Vs^{-1} . **Figure 6(I)** shows the cyclic voltammograms of a modified electrode at different accumulation times. According to **Figure 6(I)**, the interaction of EE with the electrode surface modifier was approximately incomplete in 3 minutes, it was concluded that the best time for analytes to accumulate is 3 minutes on the electrode surface. At the above times of 3 min., the surface of the electrode was contaminated due to excessive accumulation of EE on the surface, and as a result, its oxidation current was reduced.

The voltammetric behavior of SBA-MET/MWCNT/GCE has been studied in 100 μM EE with the scan rate of 0.1 Vs^{-1} at different accumulation potentials (-0.5, -0.1, 0, +0.1, +0.3 V), along with 10 ml of the PBS (pH=7, 0.1 M). As it is evident from **Figure 6(II)**, the maximum anodic peak current value has been obtained at zero potential Vs Ag/AgCl ref electrode. Therefore, zero potential was selected as the optimal potential for measuring EE.

The voltammetric behavior of SBA-MET/MWCNT/GCE was investigated with a scan rate of 0.1 Vs^{-1} and at pH values ranging between 4-10. It was deduced from **Figure 6(III)**, the maximum anodic peak current has obtained at pH = 7. So, pH = 7 was selected as the optimal pH. Moreover, in the potential diagram versus pH (**Figure 6(IV)**), the EE oxidation peak potential has changed with the pH. It indicates that the proton was involved in the EE oxidation process. Furthermore, the Nernstian slope value shows that the ratio of electrons and protons is equal in the electrochemical process of EE oxidation [6].

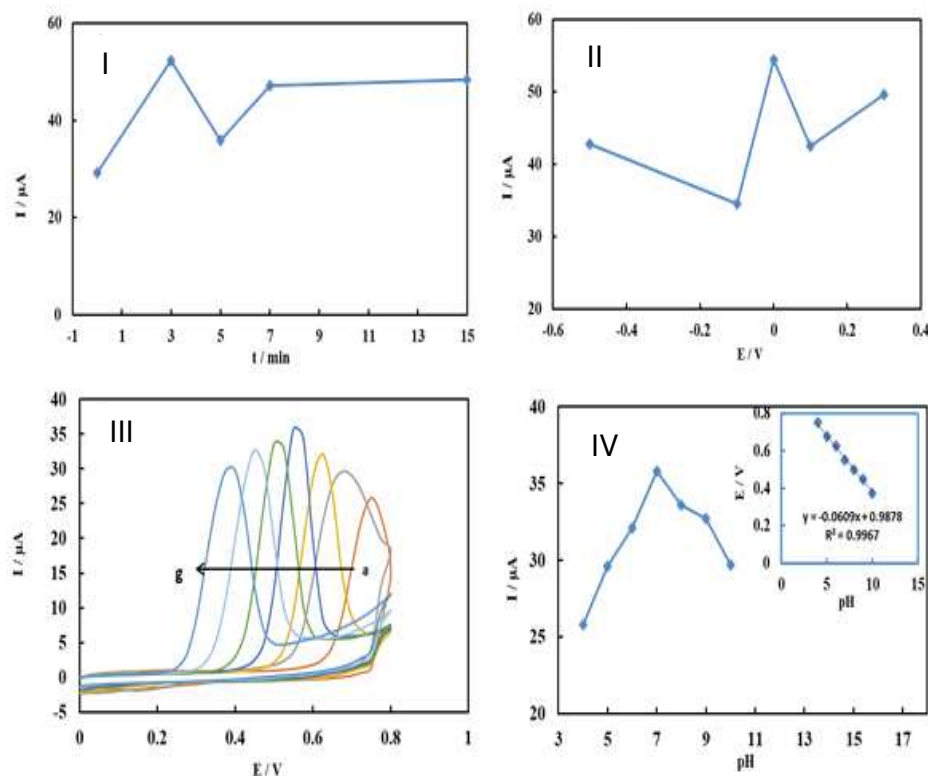


Figure 6. (I) Variation of the peak current versus accumulation time, (II) variation of the peak currents versus accumulation potentials, (III) Cyclic voltammograms of SBA-MET/MWCNT/GCE at different pHs: a) 4.0, b) 5.0, c) 6.0, d) 7.0, e) 8.0, f) 9.0, g) 10.0 at 0.1 V/s in 10 ml BPS (0.1 M) and $100 \mu\text{M}$ EE and D) recorded peak current versus different pH of PBS at the SBA-MET/MWCNT/GCE electrode.

3.6. Analytical performance of the electrochemical sensor

The differential pulse voltammetry method was used under the optimum conditions ($\text{pH} = 7.0$ and 0.1 M PBS) and in different concentrations of the EE ranges between 0.005 – $200 \mu\text{M}$ to record a specific current of EE oxidation. The differential pulse voltammetry method was utilized to determine the different concentrations of EE oxidation and increase the determination sensitivity of the EE at low concentrations. **Figure 7(I)** displays the differential pulse voltammogram. As can be observed, by increasing the concentration of EE, the oxidation of the peak current has also increased. Likewise, the peak response of EE oxidation was plotted versus the EE concentration. The peak current response follows a linear trend versus the different concentrations of EE. The results demonstrate that the linear measurement range of EE was 0.005 – $200 \mu\text{M}$. Also, the LOD method was calculated near 1.68×10^{-9} ($S/N=3$). As can be observed from **Figures 7(II)** and **7(III)**, the obtained plot has two parts. This occurs as a result of sensitivity alternation at higher concentrations. The reason can be related to the EE concentration restrictions on the surface of the modified electrode.

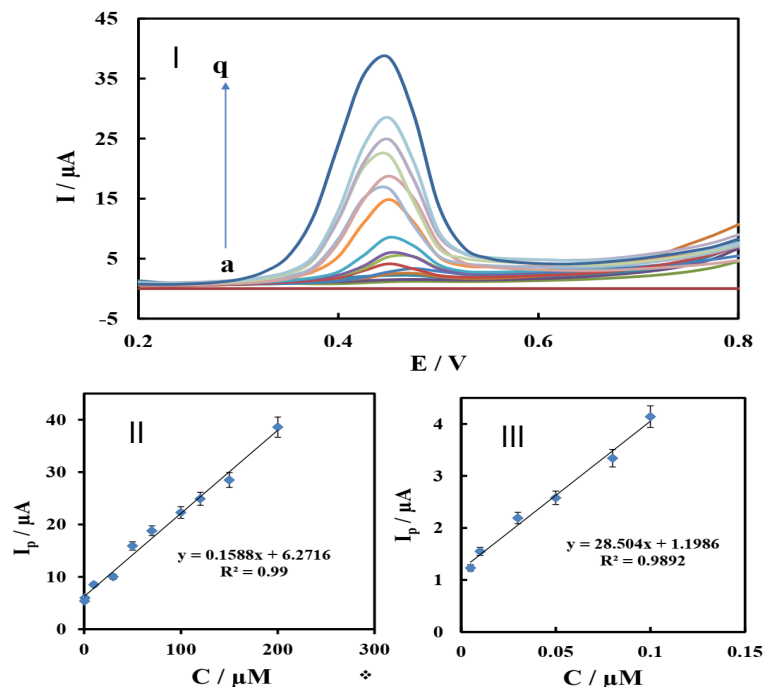


Figure 7. (I) Differential pulse voltammograms obtained at the SBA-MET / MWCNT / GCE electrode in 0.1 M PBS (pH=7) and different concentrations of EE: a) 0.005, b) 0.01, c) 0.03, d) 0.05, e) 0.08, f) 1/0, g) 0.5, h) 1, j) 10, k) 30, l) 50, m) 70, n) 100, o) 120, p) 150, q) 200 μM at 0.1 V s^{-1} scan rate. (II) Peak current plot versus EE concentrations changes (0.005 to 0.1 μM) at the SBA-MET /MWCNT /GCE. (III) Peak current plot versus EE concentrations changes (0.5 to 200 μM) at the electrode SBA-MET /MWCNT /GCE.

Other methods' proficiency was shown in **Table 1** in the case of EE measurements. Likewise, due to the slight oxidation current, various extremely sensitive and expensive methods, containing high-performance liquid chromatography and fluorescence method, were used to test EE after pre-concentration. As a result, it's critical to design electrochemical sensors that can detect small levels of this substance without pre-concentration. In comparison to existing approaches, the suggested EE electrochemical sensor performs admirably (**Table 1**). The extraordinary guiding of MET and their interactions with the MWCNT and SBA are most likely responsible for the superior sensor function.

Table 1. Comparison between various methods for electrochemical detection of EE.

Modifier	Method	LOD (μM)	LDR (μM)	Real sample	Ref
Chi/CNTs/FTO	SWV	0.09	0.05-20	Synthetic urine	[25]
MWCNTs/GCE	SWV	0.034	0.12-236.16	-River water -Tap water	[26]
CPE/CPB	LSV	0.03	0.05-20	-Levonorgestrel -Ethinylestradiol tablets	[27]
BDD	SWV	0.24-0.75	0.99-5.2 ^e 0.79-5.2 ^f	- Pure water - Natural water	[28]
GCE/Rgo/RuO ₂	DPV	0.00204(EE2) ^h	0.055-1.2	Urine sample	[29]
GCE/RGO/ CuTthP	DPV	0.0053	0.1-1	River water	[30]
CBGCE	DPV	0.092	0.15-3.5	Pharmaceutical formulations	[31]
RGO/DHP/GCE	CV	0.077	0.4-20	Synthetic urine	[32]
SBA-Met- MWCNTs/GCE	DPV	0.00168	0.005-200	Urine Serum Contraceptive LD	This work

Chi/CNTs: Chitosan/multi-walled carbon nanotubes; FTO, Fluorine doped tin oxide; CPE, Carbon paste electrode; CPB, Cetyl pyridine bromide; BDD, Boron-doped diamond electrode; RGO, Reduced graphene oxide; CuTthP, Cu(II)-*meso*-tetrathien-2-ylporphyrin; CBGC, Carbon black modified GCE; DHP, Dihexadecylphosphate

3.7. Selectivity evaluation

The results confirm the effects of various species containing magnesium, chlorine, iron (II) and iron (III) ions, urea, ascorbic acid, glucose, and copper up to 37.5 M concentration along with a 10% error. However, other chemical compounds can imitate the behavior of EE hormones, including phenolic compounds such as BPA, BPAF, and BPB. These substances have harmful influences on human health. **Table 2** lists the compound as well.

Table 2. Influence of interference on EE

Species	$C_{\text{species}}/C_{\text{EE}}$	Relative error %
Cu ²⁺	250	11
NO ₃ ⁻	500	1.7
Urea	250	0
Ascorbic acid	250	4.9
Glucose	250	7.3
Mg ²⁺	250	7.7
Cl ⁻	250	3
Fe ²⁺	250	5
Fe ³⁺	250	11
EE	50	-
Bisphenol A	50	-
Bisphenol Af	50	-
Bisphenol B	50	-

3.8. Real sample analysis

Indicating the electrochemical effects of the SBA-MET/MWCNT/GCE in real samples, the SBA-MET/MWCNT/GCE electrode was utilized for EE evaluation in human urine, serum, and LD drug. Thus, it was taken 250 mm of the human blood sample and then centrifuged at 10000 rpm for 15 min to separate the serum. Afterward, 97.5 μ l of the serum was relocated to a 50 ml volumetric container. Then, specific values of EE were added and diluted with PBS (pH =7, 0.1M) up to 50 ml. Each time, 10 ml of the sample was determined via the DPV method and the SBA-MET/MWCNT/GCE novel sensor. Moreover, 50 μ l of the urine sample was moved to a volumetric flask without further preparation. Subsequently, a fixed quantity of EE was added and diluted with PBS (pH=7, 0.1 M) up to 10 ml. Consequently, 10 ml of the prepared solution was tested through the DPV method and the SBA-MET/MWCNT/GCE original sensor. In addition, for EE measurement in an LD pill, at first, one of the purchased tablets (0.0865 g along with 0.03 mg of the intended constituent EE, according to the producer (Abu Reihan Pharmaceutical Company, Tehran, Iran)) was powdered in the porcelain mortar. Next, it was transported to the test tube along with 10 ml of PBS (0.1 M) and dissolved without any additional preparation. Then, LD solution was measured using the DPV method and the SBA-MET/MWCNT/GCE new sensor. Besides, the results were summarized in **Table 3**. As it can be seen from table 3, the obtained recoveries are acceptable for EE estimations in various real samples. Moreover, it was indicated that the real solution matrix does not interfere with EE detection.

Table 3. Determination results of EE in real samples (N=5, P=0.5)

Sample	Added (μ M)	Found (μ M)	R.S.D	Recovery %
Urine	0	*	*	*
	20	20.15 \pm 0.78	4.1	100.7
	50	48.85 \pm 1.62	3.5	97.7
	80	80.09 \pm 1.37	1.8	100.1
	0	10.11 \pm 0.34	3.6	101.1
LD pill	10	20.55 \pm 0.26	2.6	104.4
	20	30.43 \pm 0.75	3.9	101.6
	30	29.95 \pm 0.88	3.1	99.5
	0	*	*	*
Serum	10	10.43 \pm 0.47	4.7	104.3
	20	19.88 \pm 0.77	4.1	99.4
	30	30.71 \pm 0.99	3.4	102.4

4. Conclusions

In order to measure EE, a modified GCE was fabricated and introduced based on the interaction of SBA metformin and multi-walled carbon nanotubes. The modified electrode has indicated high capability in detecting and measuring EE at low concentrations. It should be mentioned that EE is a hormonal substance that is classified as an estrogen substitution in the therapeutic classification. It is also used to treat diseases containing insulin-dependent diabetes, prostate cancer, etc. Therefore, its high consumption has several adverse effects on the body. Consequently, EE measurement is critical in the body, and creating an electrode for its measurement can be extremely beneficial to medical science. Using this modified electrode, the oxidation potential of EE was reduced up to 4 mV, and the intensity of its current measurement was significantly improved. So, even in low quantities, the drug composition could be easily determined. The oxidation peak current of EE was linearly linked to its concentration, and the 1.68×10^{-9} M differential pulse voltammetry technique was used to determine its detection limit. As a result, compared to other highly expensive and advanced approaches, the current study has illustrated the design and manufacture of an electrochemical sensor that is selective, sensitive, and inexpensive for EE detection. This electrode was also utilized to measure EE in blood serum, human urine, and LD pills as a real sample. Eventually, the modified electrode has shown a suitable response when it was applied to real samples.

Authors' contributions

All authors contributed to data analysis, drafting and revising of the paper and agreed to be responsible for all the aspects of this work.

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Declaration of competing interest

The authors declare no competing interest.

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Data availability

Data will be made available on request.

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