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Design and evaluation of a highly sensitive nanostructure-based surface modification of glassy carbon electrode for electrochemical studies of hydroxychloroquine in the presence of acetaminophen

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ABSTRACT

N,N′-bis[(E)-(1-pyridyl) methylidene]-1,3-propanediamine (PMPDA) self-assembled monolayer (SAM) was covalently prepared on a glassy carbon electrode (GCE). The electrode surface modification was characterized by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques. Then GC-PMPDA SAM modified electrode was used to investigate the electrochemical behavior of hydroxychloroquine (HQ) using CV, double potential step chronocoulometry and linear sweep voltammetry (LSV) techniques. Using these techniques, the diffusion coefficient (D), electron transfer coefficient (α) and exchanging current density (j_0) for HQ were calculated. Furthermore the modified electrode was applied as a high sensitive biosensor for determination of HQ in the presence of acetaminophen (AC). The GC-PMPDA SAM modified electrode provides two linear responses for HQ in the presence of AC in the concentration ranges from 0.09 to 10.21 μM and 10.21 to 98.29 μM by differential pulse voltammetry (DPV). The detection limit (three times the signal blank/slope) was 4.65 nM. Finally the modified electrode was satisfactorily used for determining of HQ in human body fluids.

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1. Introduction

Modification of electrode surfaces with thin films has become a well-established field of interest, notably for applications in electroanalysis and biosensors [1,2]. Various materials have been used for that purpose, including clays [3], zeolites [4], silica [5], sol–gel materials [6] and, more recently, self-assembled monolayers (SAMs) [7–9]. Among them, SAMs offer the simplest way to generate ultrafine, reproducible and well structured monolayers. Further modification by SAMs may allow fabrication of highly sensitive and more sophisticated sensors and biosensors for trace analysis.

Schiff bases are compounds containing the azomethine group (R═C—N) and are usually formed by condensation of a primary amine with an active carbonyl compound under specific conditions. They are used in biological systems, electrochemical and optical sensors and various chromatographic methods, to enable detection of enhance sensitivity and selectivity [10,11].

Hydroxychloroquine, 2-[N-4-{(7-chloroquinolin-4-yl)amino} pentyl-N-ethyl]amino ethanol, (HQ) that is a hydroxylated form of chloroquine, was first synthesized in the 1940s. Shortly after that, it was discovered to be as effective as, but less toxic than, chloroquine and became a mainstay of malaria prevention and treatment strategies. Over the years, HQ has also shown favorable effects for systemic and cutaneous lupus erythematosus conditions, rheumatoid arthritis, and several skin disorders and is now routinely used in the management of these diseases [12,13]. It is official in United States Pharmacopeia [14] and British Pharmacopeia [15] in which no related substance or impurity is mentioned in its monographs. HQ is most commonly administered orally, either as 200 or 400 mg of the racemic sulfate salt. A single drug regimen may not be optimal for all patients, however, as several studies report marked pharmacokinetic variability for HQ [16,17]. Also an additional challenge of HQ treatment is distinguishing non-compliance from refractory disease. Patient self-reports, clinician's assessments, electronic monitoring devices, and other strategies are often used to discriminate between these two possibilities but are not always

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effective or accurate. Quantitative HQ blood levels may offer an additional and more reliable means of distinguishing and resolving these two types of clinical scenarios [18].

Acetaminophen (N-acetyl-P-aminophenol or Paracetamol, AC) is a widely used antipyretic and analgesic drug. It is well tolerated and lacks many side effects of aspirin, so it is commonly used for the relief of headaches, fever, and minor aches and pains also for the management of more severe pain, where it allows lower dosages of additional nonsteroidal antiinflammatory drugs to be used, thereby minimizing overall side effects [19,20].

Although many articles on individual determination of the two analytes, especially AC, have been published, but to the best of authors' knowledge no method using voltammetry has been reported for determination of HQ in the presence of AC. So, in the current work, N,N′-bis[(E)-(1-pyridyl) methylidene]-1,3-propanediamine (PMPDA) as a synthetic organic mediator, which is a biomimetic analog, was chosen to form a self-assembled monolayer (SAM) on a glassy carbon electrode (GCE). The structural integrity and compactness of the GC-PMPDA SAM modified electrode were investigated by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) using reversible redox reaction probe. Then the investigations were aimed at evaluating the antifouling performance of this film as a suitable sensor material for the voltammetric studies and analysis of HQ in the presence of AC. Finally, the proposed modified electrode was well-suited for the efficient analysis of serum HQ levels.

2. Experimental

2.1. Chemicals

The PMPDA was synthesized in inorganic laboratory of the University of Kashan and characterized by physical and spectroscopic data. HQ, AC and other materials used in this work were of analytical grade (Merck®) and used without further purification. Phosphate buffer (PB) and Britton–Robinson (B-R) (0.2 M) of different pH values were prepared from stock solutions of 0.2 M phosphoric, acetic, boric acid and sodium hydroxide. Deionized water was used throughout the experiments.

2.2. Apparatus

EIS experiments were carried out using an Autolab potentiostat-galvanostat PGSTAT 35 (Eco chemie Utrecht, Netherlands), equipped with the General Purpose Electrochemical System (GPES 4.9,006 software) and the NOVA 1.8 software (Metrohm Autolab). All voltammetric measurements were carried out using a Sama 500 potentiostat (Isfahan, Iran). A three-electrode cell system composed of a silver/silver chloride ($Ag/AgCl/KCl_{sat}$) electrode (Metrohm, Switzerland) as reference electrode, a platinum wire (Metrohm, Switzerland) as auxiliary electrode and GC-PMPDA SAM modified electrode as working electrode (Metrohm, Switzerland) was employed for the electrochemical studies. A personal computer (Pentium IV) was applied for data storage and processing. A digital pH meter (Metrohm model 691) was used for preparing buffer solutions that served as the supporting electrolyte in the electrochemical experiments. The films morphology was investigated by SEM and micrographs were recorded with a KYKY-EM3200 apparatus. All experiments were conducted at room temperature.

2.3. Preparation of the PMPDA film on GCE

Before electrode modification, the GCE (1 mm in diameter) was polished to a mirror-like surface with 0.05 μm α-Al_2O_3 and then rinsed ultrasonically with a mixture of ethanol/water. Finally, cyclic voltammetry (CV) was carried out in 0.1 M H_2SO_4 solution with scan potential window between −0.1 V and 1.3 V at scan rate 100.0 mV s^{-1} until a stable CV profile was got. GCE were afterwards coated with a PMPDA film, which was prepared by immersing into a 1.0 mM PMPDA ethanol solution for 13 h to form GC-PMPDA SAM modified electrode.

3. Results and discussion

3.1. Characterization of the self-assembled monolayer formation

3.1.1. Scanning electron microscopy analysis

SEM was used to evaluate the physical appearance and surface characteristics of the modified biosensor. The SEM micrographs of the bare GC and GC-PMPDA SAM modified electrodes are shown in Fig. 1A and B [21], respectively. Fig. 1B shows a very uniform structure without aggregation on the electrode surface, which indicated the PMPDA film, was immobilized on the GCE surface. Also as observed in the SEM micrographs, GCE modified with PMPDA film resulted in enhancement of effective surface area which increases the area of GC-PMPDA surface and finally, response sensitivities in

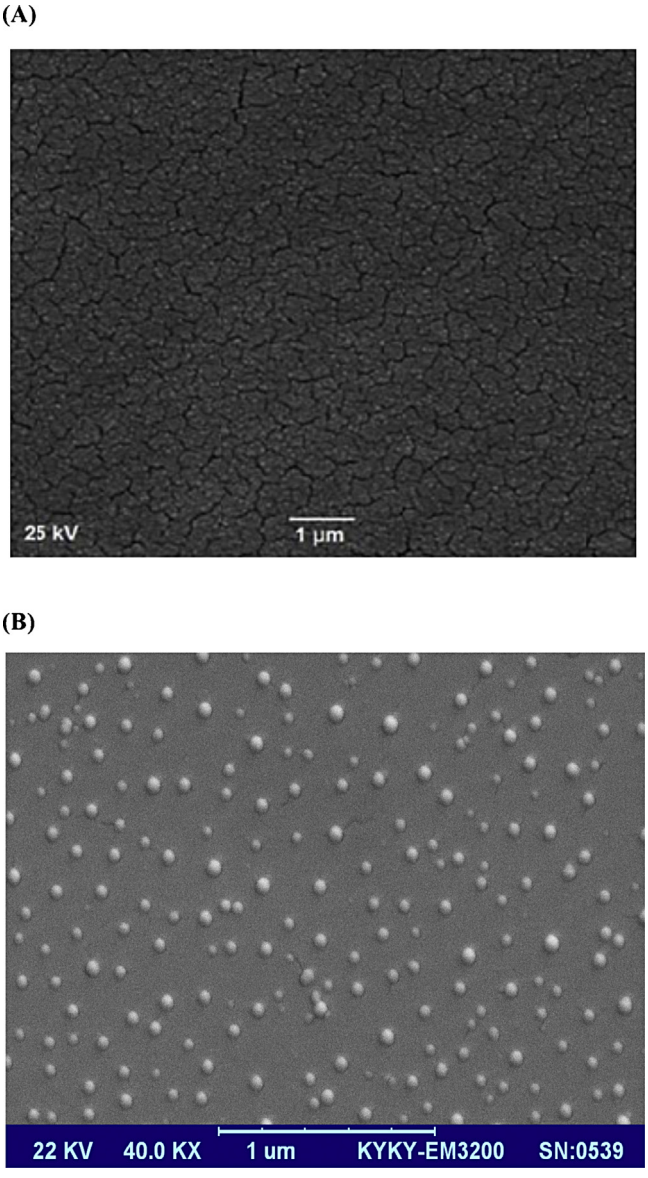

Fig. 1. SEM micrographs of (A) bare GCE and (B) GC-PMPDA SAM modified electrode.

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voltammetric determinations. These results confirm the assembly of PMPDA film on the GCE surface.

3.1.2. Electrochemical impedance spectroscopy analysis

EIS is an informative and powerful technique for probing the interface features of the modified electrodes. In this research, the EIS was performed for recognizing the GC nanosensor using $[Fe(CN)_6]^{3-/4-}$ as negatively charged redox probe. Fig. 2A shows the results of EIS plots ($-Z''$ vs. Z'), presented for the bare GCE (curve a) and GC-PMPDA SAM modified electrode (curves b–d) in the presence of 5.0 mM $[Fe(CN)_6]^{3-/4}$ redox probe, in phosphate buffer solution of pH 3.0, 6.0 and 8.0, respectively. As can be observed, significant changes in the impedance results were occurred during modification process. The charge-transfer resistance (R_{ct}) of the GC-PMPDA SAM was much larger and along with more irreversible behavior in $[Fe(CN)_6]^{3-/4-}$ redox probe than the

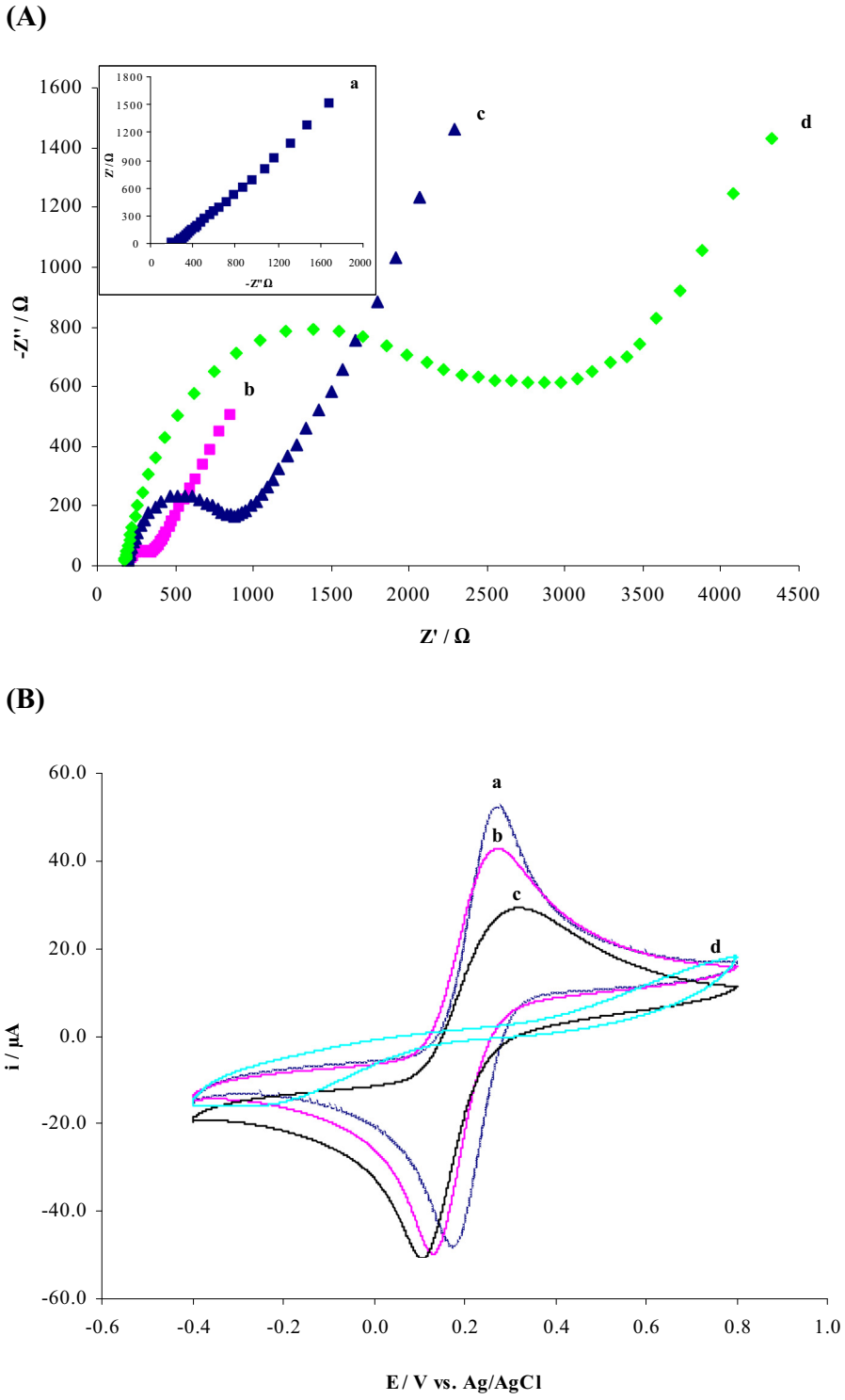

Fig. 2. (A) Complex plane plots obtained on (a) bare GC at pH 3.0, (b) at pH 3.0 (c) at pH 6.0 and (d) at pH 8.0, on GC-PMPDA SAM electrode in 0.2 M PB solution, 5.0 mM $[Fe(CN)_6]^{3-/4-}$. (B) Cyclic voltammograms obtained on (a) bare GC at pH 3.0, (b) at pH 3.0 (c) at pH 6.0 and (d) at pH 8.0, on GC-PMPDA SAM electrode in 0.2 M PB solution, 5.0 mM $[Fe(CN)_6]^{3-/4-}$. Scan rate 100.0 mV s^{-1}.

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bare GCE, suggesting blocking the access of the redox probe to the electrode surface by PMPDA SAM. At pH 3.0 and 6.0 (Fig. 2A curves b and c, respectively) high electrostatic attraction between positively charged amine groups at the modified surface and negatively charged probe in solution is responsible for the decreasing the interfacial charge transfer resistance. At pH 8.0 (Fig. 2A curve d), the interfacial resistance increased highly due loss of electrostatic attraction that was previously available between PMPDA SAM and the probe. Reasonably, hydrogen bonds have more chance to form between amine groups, and π–π stocking are more effectively formed in these conditions. In fact, GC-PMPDA SAM shows opening and closing behaviors in acidic and basic solutions, respectively, for against electron transfer process [22]. Therefore, from the EIS data it can be concluded that attachment of PMPDA SAM to the GCE surface was successfully done. By EIS a value may be obtained for surface coverage (θ) by Eq. (1) [23]:

$$\%\theta = \left[1 - \frac{R_{ct\,bare}^{\circ}}{R_{ct\,modified}}\right] \times 100 \quad (1)$$

where $R_{ct\,bare}^{\circ}$ and $R_{ct\,modified}$ are the charge-transfer resistance of redox probe at the surface of bare GCE and GC-PMPDA SAM modified electrode, respectively. The values calculated for $R_{ct\,bare}^{\circ}$ and $R_{ct\,modified}$ were 36.0 and 111.7 Ω at pH 3.0, 45.5 and 550.4 Ω at pH 6.0 and 53.5 and 1836.8 Ω, at pH 8.0, respectively. Using these data, values of 67.7, 90.3 and 97.1 were obtained for θ in pH 3.0, 6.0 and 8.0, respectively.

3.1.3. Cyclic voltammetry analysis

The surface of the GC-PMPDA SAM modified electrode was also probed using CV technique. The cyclic voltammograms of $[Fe(CN)_6]^{3-/4-}$ was used for monitoring surface changes of GCE after modification by PMPDA SAM. Fig. 2B shows the cyclic voltammograms of the bare GCE (curve a) and GC-PMPDA SAM modified electrode in the presence of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ redox probe, in phosphate buffer solution of pH 3.0 (curve b), 6.0 (curve c) and 8.0 (curve d). Based on Fig. 2a, voltammogram $[Fe(CN)_6]^{-3/-4}$ is shown a couple of well-defined redox waves at bare GC electrode. After film formatting PMPDA on the surface of GCE, cyclic voltammograms changed and the peak current of probe decreased significantly. These results suggest that the surface of the GC-PMPDA SAM modified electrode is fully blocked by PMPDA. The redox probe at the surface of bare GCE at pH 3.0 (Fig. 3B curve a) showed more reversible behavior in comparison with GC-PMPDA SAM modified electrode (Fig. 3B curves b, c and d). Also, the redox reaction current of probe in this pH was more than two other pH. This behavior can be resultant: (a) Electrostatic attraction between positively charged PMPDA monolayer and negatively charged probe. (b) Electrostatic repulsion between positively charged PMPDA molecules, causing an open channel in the structure of SAM that facilitates the electron transfer [24]. (c) The insulation effect of monolayer that retards the electron transfer process. At pH 8.0 (Fig. 3B curve d), the GC-PMPDA SAM can expose a neutrally charged insulting monolayer that caused the faradaic currents for the probe redox reaction decrees. Reasonably, hydrogen bonds have more chance to form between amine groups, and π–π stocking are more effectively formed in these conditions. The values for θ may be obtained using Eq. (2) [23]:

$$\%\theta = \left[1 - \frac{i_{p\,modified}}{i_{p\,bare}^{\circ}}\right] \times 100 \quad (2)$$

where $i_{p\,bare}^{\circ}$ and $i_{p\,modified}$ are peak currents of redox probe at bare GC and GC-PMPDA SAM electrodes under the same conditions. The values determined for i_p° and i_p were 51.1 and 41.8, 52.5 and 31.4 and 51.7 and 4.6 at pH 3.0, 6.0 and 8.0 μA, respectively. Using this data the values of 18.2, 40.2 and 91.0 were obtained for θ at pH 3.0, 6.0 and 8.0, respectively. It is noticeable that the θ values obtained from EIS (Section 3.1.1) and CV (Section 3.1.2) are different. The differences may be attributed to the contribution of tunneling effect [24]. Since the EIS measurements were carried out fairly near the equilibrium, i.e. at +0.2 V, the tunneling effects might have less contribution in the EIS measurements (the observed R_{ct} is large), and therefore, surface seems to be totally blocked.

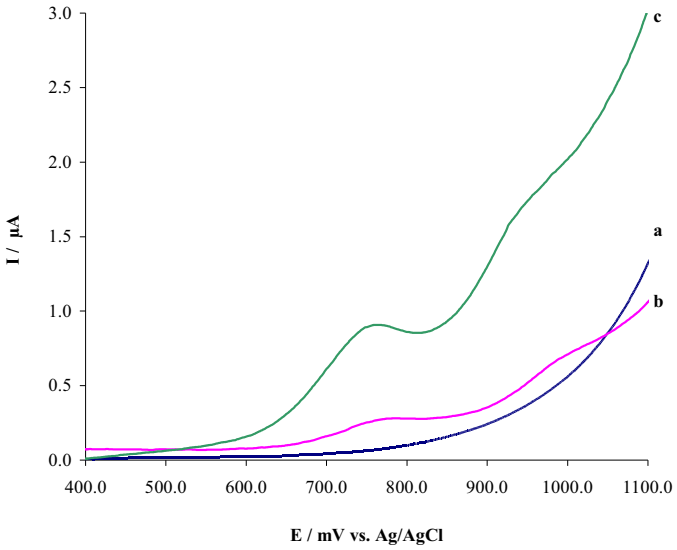

Fig. 3. Differential pulse voltammograms (a) GC-PMPDA SAM modified electrode in 0.2 M B-R buffer solution (pH = 8.0), (b) unmodified GCE in the presence of 0.5 μM HQ, (c) as (a) +0.5 μM HQ.

3.2. Application of modified electrode as a biosensor

3.2.1. Electrochemical behavior of HQ at the GC nanosensor

One of the objectives of this work was preparation of a new modified electrode as an electrochemical nanosensor for the electrocatalytic oxidation of HQ. For reaching the purpose, differential pulse voltammograms for the electrochemical oxidation of 0.5 μM HQ at the surfaces of unmodified GC and GC-PMPDA SAM electrodes in 0.2 M B-R buffer solution of pH 8.0 recorded at a scan rate of 100.0 mV s^{-1}. Fig. 3 showed two oxidation peaks for HQ. But the first anodic peak did not show enough sensitivity in next studies and therefore, second anodic peak is used for all studies. As can be seen, while the anodic peak potential for HQ oxidation at the unmodified GC is 988.0 mV (Fig. 3 curve b), the corresponding potential at the GC-PMPDA SAM electrode is 942.0 mV (Fig. 3 curve c). Furthermore, the anodic peak current increased at the surface of the modified electrode, with a magnification of 2.4 times than unmodified GCE. These results show the presence of PMPDA at the surface of the modified electrode that causes an effective catalytic role in the electro-oxidation of HQ. Also, raising in anodic current can be depended to π–π interaction between the phenyl structure of HQ and the aromatic structure of PMPDA on the surface of the GC electrode may accelerate the electron transfer of HQ in comparison with the unmodified electrode.

3.2.2. pH dependence studies

The current responses of HQ at the surface of GC-PMPDA SAM electrode were investigated under different pH values using differential pulse voltammetry (DPV) technique. As shown in Fig. 4A, for a solution containing 30.0 μM of HQ in 0.2 M B-R buffer solution with pH values ranging from 2.5 to 9.5 the current signal increases as pH value increases up to pH 8.0. Then the current signal declines when the pH value is higher than 8.0. It indicates that the optimum pH 8.0 can be used for the detection of HQ (Fig. 4B).

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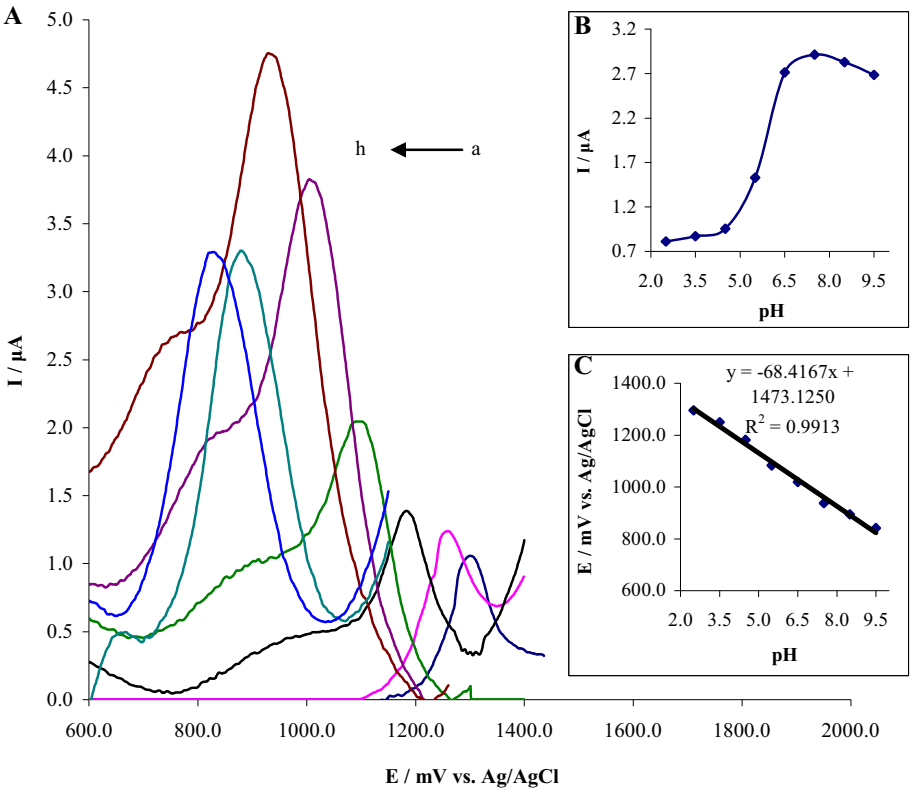

Fig. 4. (A) Differential pulse voltammograms of a solution of 30.0 μM HQ at various B-R buffered pHs, performed with a scan rate of 100.0 mV s^{-1}. The letters a–h correspond to pH values of 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5, respectively. Insets (B) intensity, I (μA) vs. pH and (C) electrochemical potential, E_{pa} (V) vs. pH.

Also, the electrochemical behavior of HQ shows that the anodic peak potential is shifted to more negative values as the pH increases (Fig. 4C), which is thought to be because of that deprotonation favors the oxidation process. According to Nernst equation (Eq. (3)) [25], the variations of oxidation potential of HQ in pH 2.5–9.5 have indicated a two-electron two-proton process.

$$E_p = E^\circ + \left(\frac{59.1}{n}\right) \log\left[\frac{(\mathrm{Ox})^a}{(R)^b}\right] - \left(\frac{59.1m}{n}\right) \mathrm{pH} \quad (3)$$

In which a and b are coefficients of oxidant and reducer in the reaction equation, m is the number of transferred protons and n the number of transferred electrons. The corresponding linear regression equation for HQ was obtained: E_{pa} (mV vs. Ag/AgCl) = 1473.10 − 68.417 pH, with the correlation coefficient of 0.9913.

3.2.3. Scan rate study

The effect of scan rate on the peak current of HQ at the GC-PMPDA SAM electrode was investigated in the range of 20.0–130.0 mV s^{-1}. As it is shown in Fig. 5, the anodic peak (I_p) currents a solution containing 0.5 μM HQ were linearly proportional to $\nu^{1/2}$ (inset of Fig. 5). Relationship between I_p and $\nu^{1/2}$ for a totally irreversible diffusion-controlled process is explained according to Eq. (4) [26]:

$$I_p = 3.01 \times 10^5\, n[(1-\alpha)n_\alpha]^{1/2} A D^{1/2} C \nu^{1/2} \quad (4)$$

where n, α, n_α, A, D, C and ν are the number of electrons involved in the thorough oxidation process, transfer coefficient, the number of electrons involving in the rate determining step, surface area, diffusion coefficient, concentration of reagent and scan rate, respectively. Therefore, it is concluded that oxidation peak current of HQ is a diffusion-controlled process at the surface of modified electrode.

3.2.4. Double potential step chronocoulometry measurements

Electro-oxidation of HQ was characterized by employing chronocoulometry technique for determining diffusion coefficient D (cm^2 s^{-1}) at the surface of GC-PMPDA SAM electrode (Fig. 6A). Using double potential step chronocoulometry, for HQ solutions in concentrations ranging from 1.0 to 33.3 μM, the plot of charge (Q) vs. the square root of time ($t^{1/2}$) showed a linear relationship using a potential step of 1000.0 mV (at the first potential step) and 0.0 mV (at the second potential step) (Fig. 6). According to the integrated Cottrell equation (Eq. (5)) [27], the diffusion coefficient of HQ could then be estimated from the slope.

$$Q = 2nFAD^{1/2}\, t^{1/2} C_b \pi^{-1/2} \quad (5)$$

where D (cm^2 s^{-1}) and C_b (mol cm^{-3}) are diffusion coefficient and bulk concentration, respectively. Fig. 6B shows the fitted experimental plots for different concentrations of HQ. Then the slopes of the resulting straight lines were plotted versus the HQ concentration (Fig. 6C), and the mean value of D was found to be 8.8×10^{-5} cm^2 s^{-1}.

3.2.5. Linear sweep voltammetry study

To obtain the information of the kinetic process of PMPDA adsorption on a GC substrate in determination of HQ, the process of PMPDA SAM formation was investigated by linear sweep voltammetry technique (LSV). Fig. 7A shows the linear sweep voltammograms of a GC-PMPDA SAM modified electrode in 0.2 M B-R buffer solution (pH = 8.0) containing different concentrations of HQ, with a sweep rate of 100.0 mV s^{-1}. Also Tafel plots that are drawn from the data of the rising part of the current–voltage curves

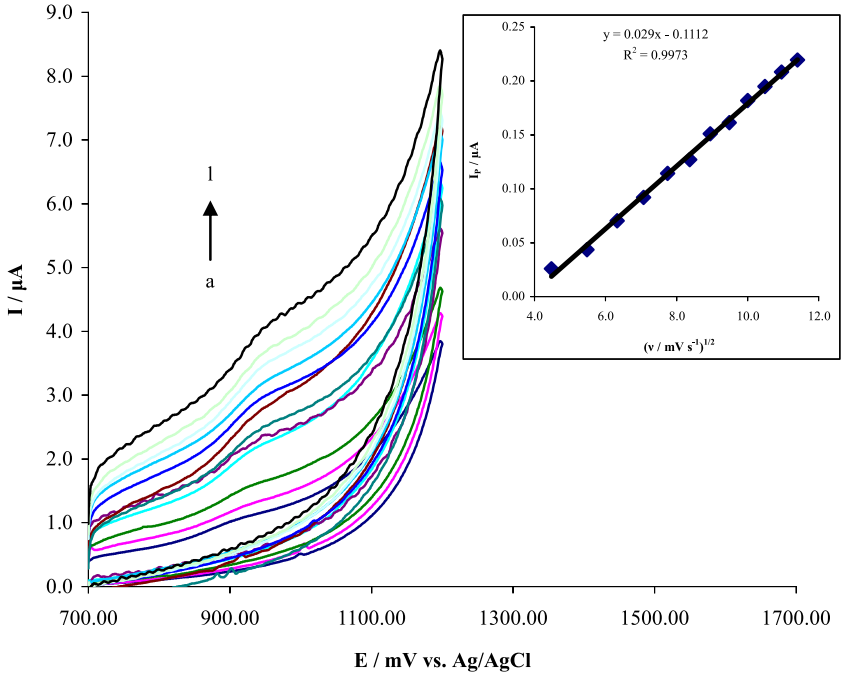

Fig. 5. Cyclic voltammograms of HQ at GC-PMPDA SAM modified electrode at different scan rates of (from inner to outer): 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0 and 130.0 mV s^{-1}. The inset shows the linear relationship between the peak currents and the scan rate.

are shown in Fig. 7B. These voltammogram is affected by the electron transfer kinetics between the substrate (HQ) and GC-PMPDA SAM. Using these data with assuming the number of electrons involved in the rate determining step of $n_\alpha = 1$, charge transfer coefficient (a) and ionic exchanging current density (j_0) can be estimated by averaging the slopes and intercepts of the Tafel plot, respectively [27]. Thus, we obtained the values of α and j_0 equal to 0.9 and 1.75×10^{-9} µA cm^{-2}.

3.2.6. The linear range of HQ at PMPDA film modified GC electrode

DPV technique, which has lower charging current contribution to the background current and a much higher current sensitivity than cyclic voltammetry [27], was applied to estimate the linear range and the limit of detection (LOD) of HQ at the surface of GC-PMPDA SAM modified electrode. Base on these studies, the plot of peak currents vs. HQ concentrations in 0.2 M B-R R buffer solution (pH = 8.0), showed two linear ranges of 0.05–12.28 and

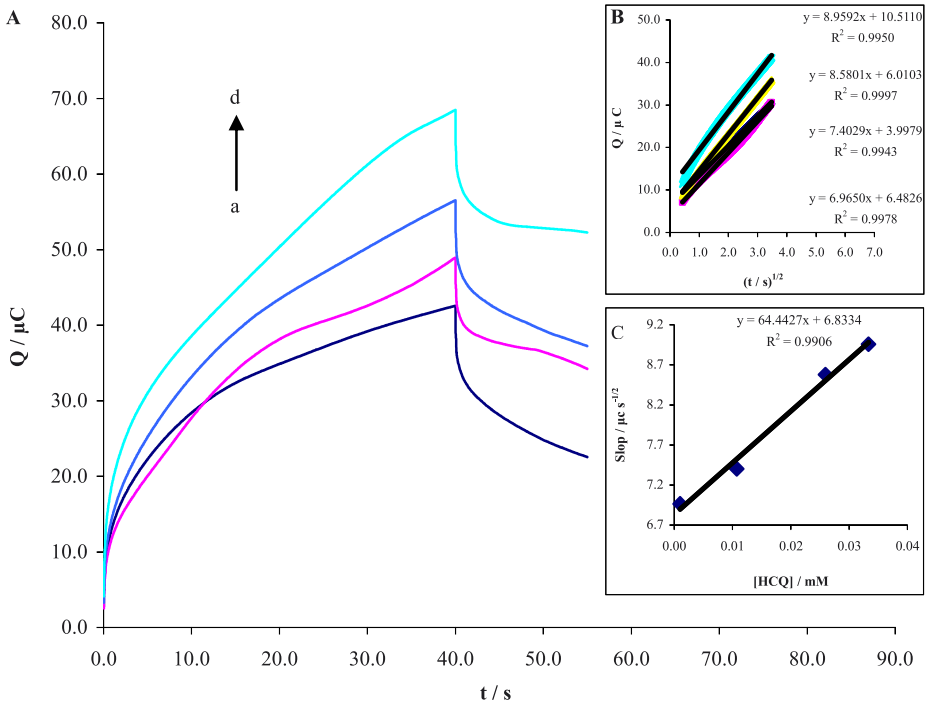

Fig. 6. (A) Double potential step chronocoulograms obtained at GC-PMPDA SAM modified electrode in 0.2 M B-R buffer solution (pH = 8.0) for various concentrations of HQ, with first and second potential steps at 1000.0 and 0.0 mV. a–d correspond to 1.0, 10.7, 25.9 and 33.3 µM of HQ, (B) plots of I vs. $t^{-/2}$ ($s^{-1/2}$), (C) a plot of the slope of the straight lines vs. the concentration of HQ.

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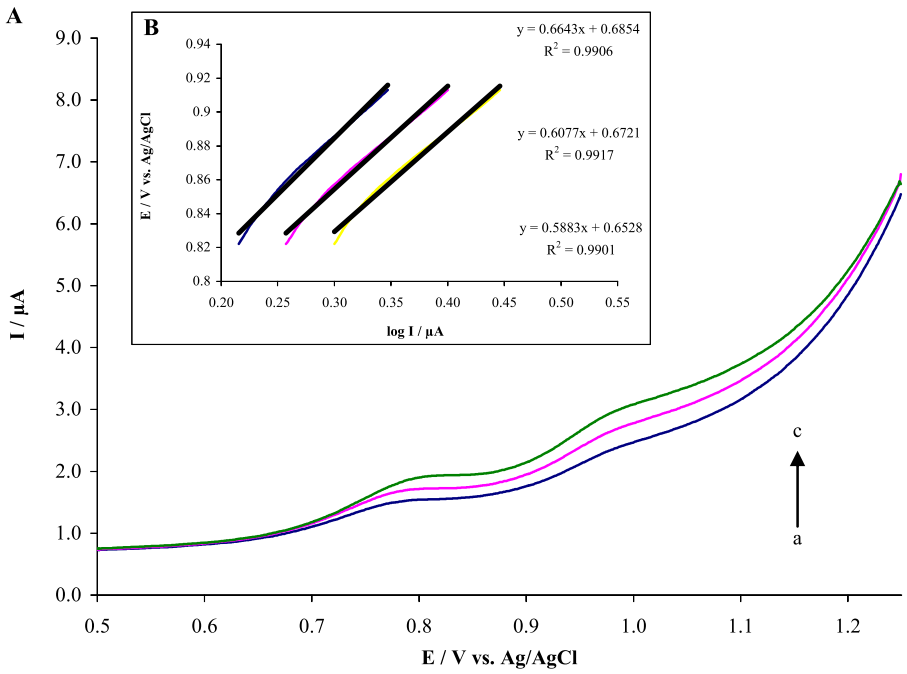

Fig. 7. (A) Linear sweep voltammograms of GC-PMPDA SAM modified electrode in B-R buffer (pH = 8.0) containing 0.15, 0.20 and 0.29 μM HQ at a sweep rate of 100.0 mV s^{-1} and (B) Tafel plot derived from linear sweep voltammograms.

12.28–111.11 μM (inset of Fig. 8A). The equations for the first and second linear dynamic ranges were:

$$i_{pa}(\mu A) = 0.2079C(\mu M) + 0.1130 \quad (R^2 = 0.9961)$$

$$i_{pa}(\mu A) = 0.0087C(\mu M) + 2.5041 \quad (R^2 = 0.9969)$$

Using these results, the LOD (3σ) for determination of HQ was found to be 4.51 nM.

3.2.7. Determination of HQ in the presence of AC at PMPDA film modified GC electrode

To the best our knowledge, there is no report on determination of HQ in the presence of AC using GC-PMPDA SAM modified electrode. Therefore, the main aim of this study was to detect HQ and AC simultaneously at the surface of modified electrode using DPV technique. This was performed by alternatively changing the concentrations of HQ in the presence of 100.0 μM of AC, and recording their DPV signals (Fig. 8). Based on these voltammograms, two well-distinguished anodic peaks at the surface of GC-PMPDA SAM modified electrode corresponding to the oxidation of HQ and AC are observed. Therefore, determination of HQ in the presence of AC was possible at the surface of modified electrode.

Insets in Fig. 8A and B, show the dependence of DPV peak currents on the concentration of HQ. As shown in the two Figures, the electrochemical response of HQ in the presence of a constant concentration of 100.0 μM AC increased linearly with the increase of the HQ concentration, while the response of AC remained almost unchanged. Voltammograms clearly show that the plot of peak current of HQ (in the presence of AC) vs. HQ concentration is constituted of two linear segments with different slopes (slope: 0.2020 μA μM^{-1} for first linear segment and 0.0069 μA μM^{-1} for second linear segment), corresponding to two different ranges of substrate concentration, 0.09–10.21 μM for first linear segment and 10.21–98.29 μM for second linear segment. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation. From the analysis of this data, we estimate that the calculated LOD of HQ in the presence of AC is 4.65 nM.

Results are indicating that even the presence of high concentration of AC did not strongly interfere with the determination of a low concentration of HQ. It is interesting to note that the sensitivity of the modified electrode toward the oxidation of HQ was obtained to be 0.2079 μA μM^{-1}, whereas the sensitivity toward HQ in the presence of AC was obtained to be 0.2020 μA μM^{-1}. This result shows that the sensitivity of the modified electrode toward HQ in the absence and presence of AC are approximately the same. Therefore, the oxidation processes of HQ and AC at the GC-PMPDA SAM modified electrode are independent and simultaneous or independent measurements of the both analysts are possible without any interference. But if the HQ signal was affected by the presence of AC, the above-mentioned slopes would be different.

3.2.8. Determination of HQ in real samples

In order to demonstrate the electrocatalytic oxidation of HQ in real samples, we examined this ability in the voltammetric determination of HQ in human blood serum samples. HQ at the GC-PMPDA SAM modified electrode was not detected in human blood serum. However, when the HQ standard solution was spiked, the presence of AC and some other interfering substances did not interfere with determining HQ. The diluted serum sample was spiked with various concentrations of HQ and its differential pulse voltammograms was recorded at the surface of modified electrode. The results are shown in Table 1. These results demonstrated the ability of GC-PMPDA SAM modified electrode for voltammetric determination of HQ with high sensitivity and good selectivity.

Table 1
Application of the GC-PMPDA SAM modified electrode for determining HQ in spiked human blood serum.

Sample	Added (μM)	Found (μM)	Recovery (%)
Serum	0.00	Not detected	–
	0.30	0.32	106.67
	0.70	0.68	97.14

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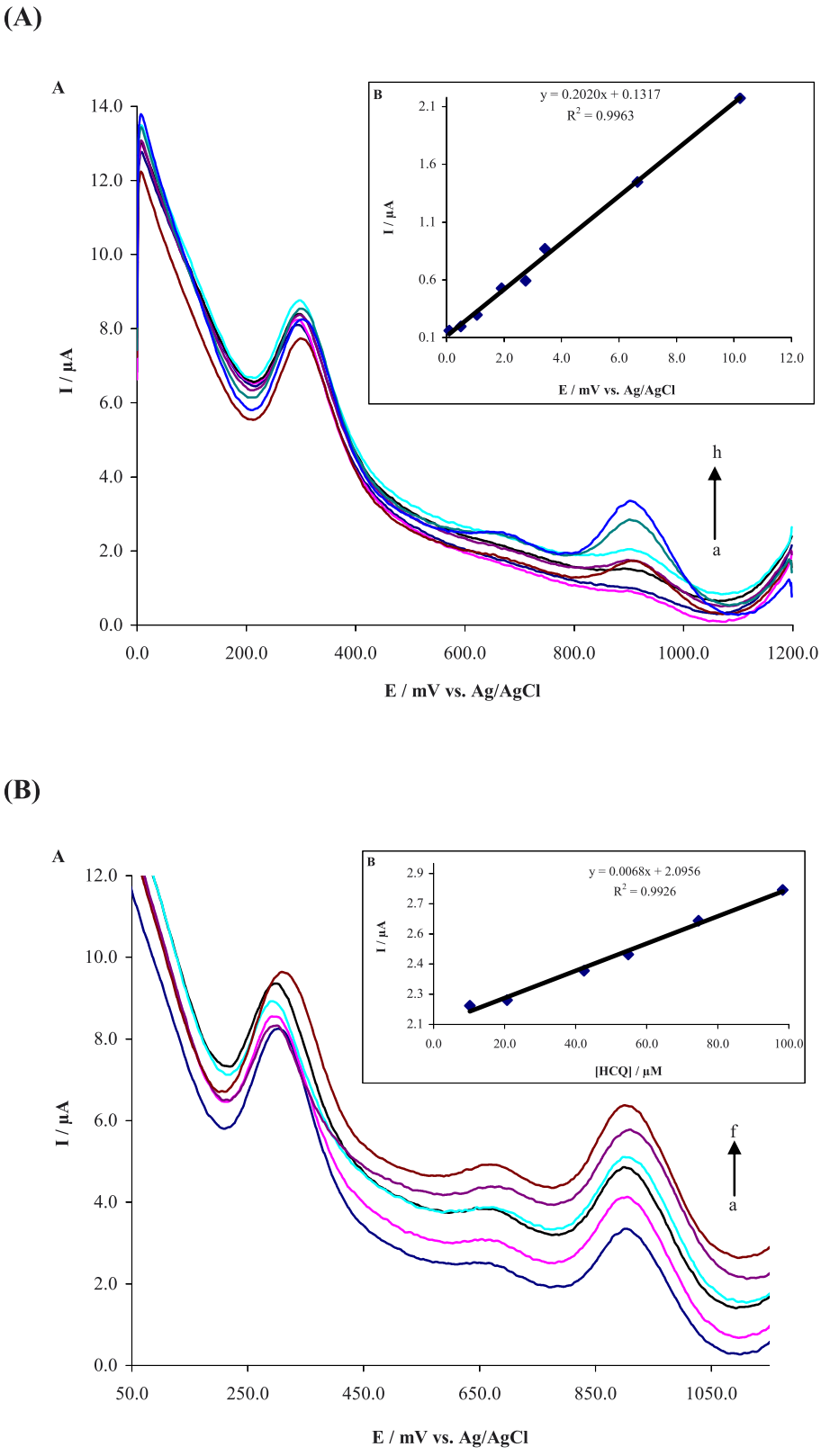

Fig. 8. (A) DPV of GC-PMPDA SAM modified electrode in 0.2 M B-R buffer solution (pH = 8.0) containing 100.0 μM AC and various concentrations of HQ containing a–h corresponding to: 0.09, 0.49, 1.06, 1.92, 2.75, 3.43, 6.64 and 10.21 μM, Inset: *I* (μA) as a function of HQ concentration (μM) (first linear segment). (B) DPV of GC-PMPDA SAM modified electrode in 0.2 M B-R buffer solution (pH = 8.0) containing 100.0 μM AC and various concentrations of HQ containing a–f corresponding to: 10.21, 20.67, 42.34, 54.84, 74.56 and 98.29 μM, Inset: *I* (μA) as a function of HQ concentration (μM) (second linear segment).

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4. Conclusion

Fabricating a novel self-assembly of GC modified electrode is reported here. After film formation of PMPDA on the surface of GCE, the electrode was used successfully for sensitive and selective detection of HQ in the presence of high concentration of AC. EIS and CV techniques were used for characterization of the modified electrode. Using double potential step chronocoulometry, the diffusion coefficient (D) of HQ at GC-PMPDA SAM was estimated. Also, LSV experiments were applied to determine kinetic parameters of HQ. The modified electrode showed a well electrocatalytic response for the oxidation of HQ. The coexisting AC has no interference to detection of HQ, and nano-GC-PMPDA SAM modified electrode could be used to determine HQ in the presence of AC. The proposed method was successfully applied to the determination of HQ in real samples.

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