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Electrochemical Determination of Mesalazine by Modified Graphite Paste Electrode with Poly (Benzoquinone) Chromium(III) Complex

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In this work, for the first time, poly (benzoquinone) chromium(III) complex (PBQC) was synthesized with a one pot, one step, simple and fast method. This novel polymer was used for modification of graphite paste electrode for electrochemical determination of Mesalazine. Moreover, this novel modifier was characterized by Fourier transform infrared spectroscopy, Field emission scanning electron microscopy, energy-dispersive X-ray spectroscopy, electrochemical impedance spectroscopy, and electrochemistry methods such as cyclic voltammetry and differential pulse voltammetry. Various parameters affecting the electroanalytical application of this modified electrode were optimized. Under the optimum condition, the calibration curve was linear in a wide range from 2-600 µM with a detection limit (S/N = 3) of 70 nM. The proposed modified electrode is a good candidate for the determination of Mesalazine with satisfactory results in comparison with the other literature. Moreover, the proposed modified electrode, GPE/PBQC, was successfully used for determination of Mesalazine in pharmaceutical tablets.

Keywords: Poly (benzoquinone) chromium(III) complex, Mesalazine, Modified graphite paste electrode, Pharmaceutical tablets

INTRODUCTION

Mesalazine or mesalamine, chemically known as 5-Amino salicylic acid (5-ASA), is an important anti-oxidant and inflammatory drug used for bowel diseases that locally affects the large intestine with limited systemic side effects. It can be used as a gastrointestinal agent to induce and maintain remission for patients with mild-to-moderate inflammatory bowel disease [1]. Because of the importance of its determination in pharmaceuticals for quality control in pharmacopeia, many routine analytical methods, such HPLC, UV-Vis spectrometry and spectrofluorometry methods were used for this purpose [2,3] . These methods suffer from some disadvantages, such as time consuming, being expensive, and need to labor operator. Among various analytical methods, electroanalytical methods are rapid, inexpensive and easy for operation [4]. There are several reports for determination of 5-ASA by modified electrodes in the literature [5,6].

Stable chromium multiple oxidation states in the environment are Cr(III) and Cr(VI) [7-9]. The latter is highly soluble and toxic to humans, animals, and plants. The former, Cr(III), is an essential trace element in human daily nutrition [7,10]. Cr(VI) as a strong oxidizing agent reacts with molecules that have redox functional groups. For example, by addition of pyrocatechol to the chromium(VI) saturated solution, the color of the solution immediately changes and black sediments are observed [11]. In this reaction, chromium(VI) is reduced to chromium(V), chromium(IV) and finally to chromium(III), and the catechol functional groups is oxidized to semi-quinone [12]. These semi-quinones react with each other to form carbon-carbon bond and polymer production [13,14]. The detailed structure of this polymer is not entirely known, however, there are many evidences indicating the presence of 1,2-benzoquinone chromium(III) moieties in its structure [12]. In this study, we prepared poly (benzoquinone)

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chromium(III) complex. Based on the results, poly benzoquinone chromium(III) has orthoquinone chromium(III) complex functional groups. Therefore, to study the electrocatalytic properties of these functional groups, for the first time, we used this polymeric product for modification of carbon paste electrode. The modified electrode exhibited high electrocatalytic activity toward electro-oxidation of 5-ASA and this electrode was successfully employed for sensitive electrochemical determination of 5-ASA in pharmaceutical tablets.

EXPERIMENTAL

Reagents

Mesalazine (5-amino salicylic acid, 5-ASA) with assay 99% was purchased from Sigma-Aldrich company (Canada). Phosphoric acid, pyrocatechol (PCT), potassium dichromate ($K_2Cr_2O_7$), and graphite fine powder (G) were purchased from Merck company (Germany) and was used as received. 5-ASA tablets were purchased from the local drugstores (Zahedan, Iran).

Instrumentation

Electrochemical measurements were carried out with an SAMA-500 Electroanalyzer (SAMA Research Center, Iran) controlled by a personal computer. All electrochemical experiments were carried out in a conventional three-electrode cell at room temperature. A platinum electrode and a silver/silver chloride electrode (Ag/AgCl) were used as the counter and reference electrodes, respectively. Field emission scanning electron microscopy (FESEM), and energy dispersive X-Ray (EDX) analyses were carried out using MIRA3 TESCAN and SAMX electron microscope, respectively. Fourier transform-infrared spectroscopy (FT-IR) spectra were measured in transmission mode using Valor III (JASCO) equipped with a MCT detector. Electrochemical impedance spectroscopy (ESI) was performed with an Autolab PGSTAT 128N (EcoChemie, Netherlands) potentiostat/galvanostat controlled by NOVA 1.11 software. Electrochemical impedance measurements were performed in 5 mM $[Fe(CN)_6]^{3-/4-}$ prepared in 0.1 M KCl. EIS was performed over a frequency range of 0.1 Hz to 10 kHz with 0.02 V amplitude (rms). A Metrohm pH meter, model 744 was also used for pH measurements.

PREPARATION OF MODIFIERS

Synthesis of PBQC

Pyrocatechol (0.1 g) was added to a 10 ml saturated solution of potassium dichromate in double distilled water (DDW). The color of solution immediately changed to black and the PBQC precipitated. Then, the mixture was filtered and thoroughly washed with 0.1 M HCl and DDW. The resulting black powder dried in oven at 60 °C for 24 h. The resulting powder was denoted as PBQC.

Preparation of Modified Carbon Paste Electrode

6 mg of PBQC and 194 mg graphite were mixed together, and then, 0.4 g paraffin oil was added. This mixture was ground by mortar and pestle for 10 min, and the resulting paste was packed into a glass tube. A copper wire was inserted to glass tube as electrical contact. New surface was obtained by pushing the carbon paste out of the glass tube and polishing by weighing paper.

RESULTS AND DISCUSSION

FESEM, EDX and Fourier Transform Infrared (FTIR) Characterization of PBQC

Figures 1A-B and 1-C show the FESEMs and EDX of the PBQC. Figure 1A and B showed a flake-like structure for PBQC.The EDX spectrum clearly revealed the presence of C, O and Cr elements in PBQC structure. Figure 1D exhibits the FT-IR spectra of PCT and PBQC. Both PCT and PBQC show absorption bands at higher wave numbers attributed to -OH and -CH stretching vibrations. The absorption band at higher wave numbers for PBQC is stronger and broader due to presence of -OH vibrations of the intercalated water molecules [15]. PBQC shows absorption band at about 530 cm^{-1} attributed to Cr-O vibrations, indicating PBQC coordination to Cr^{3+} ions[16].

Cyclic Voltammetry and Electrochemical Impedance Spectroscopy (EIS) Measurements

Figure 2A shows the CVs of bare graphite paste electrode (BGPE) and GPE/ PBQC in 5 mM $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl. GPE/PBQC shows higher peak currents and lower ΔE_P compared to BGPE. Supporting evidence for this modified electrodes was found by EIS. EIS is a powerful

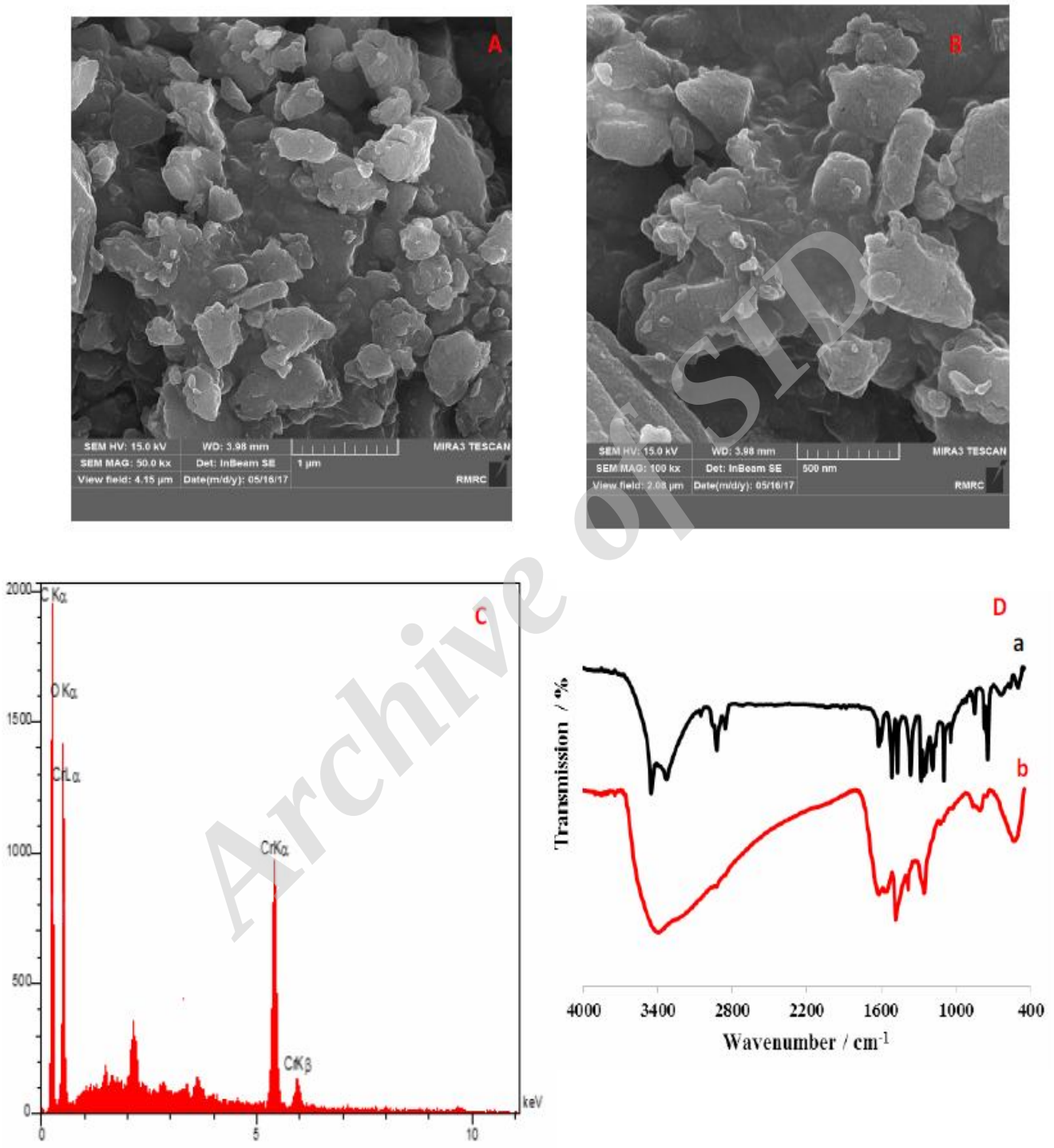


Fig. 1. (A and B) FESEM of PBQC with different magnification, (C) EDX spectrum of PBQC and (D) FT-IR spectra of (a) Pyrocatechol and (b) and PBQC.

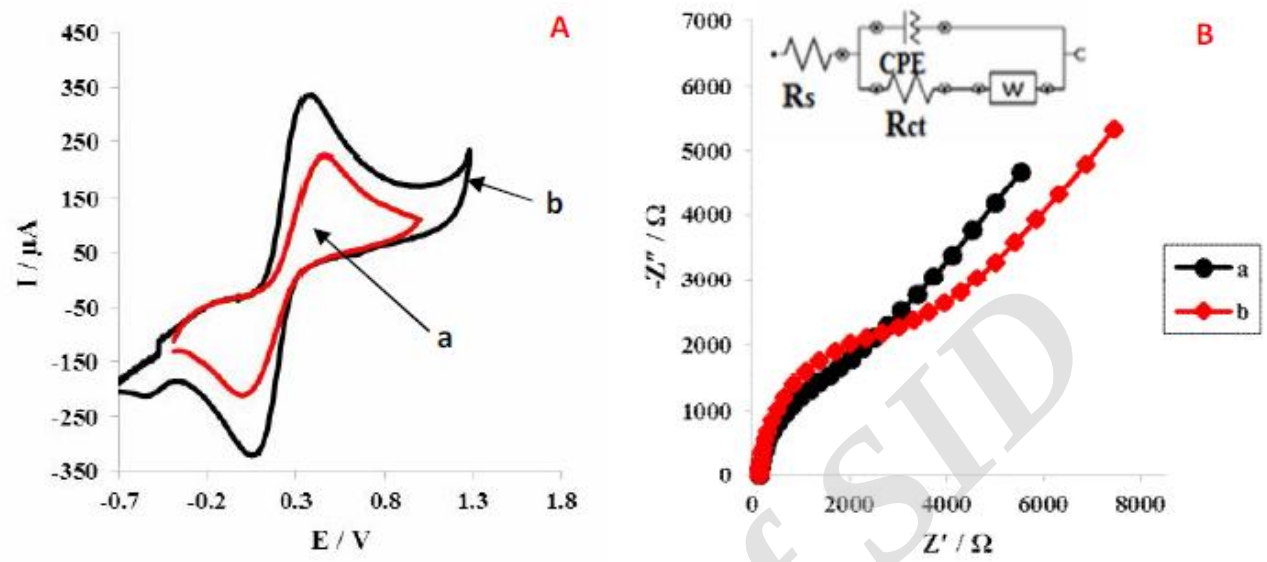


Fig. 2. (A) CVs of BGPE (a) and GPE/PBQC in 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$. Scan rate: 100 mV s^{-1}. (B) Nyquist plots of BGPE (a) and GPE/PBQC (b) in 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$. Inset of Fig. B equivalent circuit. R_s: solution resistance, R_{ct}: charge transfer resistance, W: Warburg element. GPE: constant phase element.

technique to study the electrode-electrolyte interfacial features. As shown in Fig. 2B, the Nyquist plot of BGPE comprises two parts; a semicircle part at higher frequencies that indicates charge transfer limitations and its diameter equals to charge transfer resistance (R_{ct}), and a straight line at lower frequencies that indicates mass transfer limitations [17]. The NOVA software was used for fitting and simulation of EIS data, and also Randles equivalent circuit, illustrated in the inset of Fig. 2B, that was selected as equivalent circuit for fitting and simulation of EIS data. The R_{ct} for GPE/PBQC and BGPE was obtained 1350 and 4800 Ω, respectively. The remarkable decrease in R_{ct} of GPE/PBQC compared to BGPE is indicative of the electro catalytic activity of GPE/PBQC.

Electrochemial Charaterization of Modified GPE for Electrochemical Determination of 5-ASA

As shown in Fig. 3, GPE/PBQC in 0.1 M PBS (pH = 2) shows the redox peaks at 0.37 and 0.27 V that are attributed to *ortho* phenol/*ortho* quinone functional groups. Moreover, this electrode shows weak oxidation peak at lower potentials that is assigned to Cr^{3+} ions [18]. Comparatively, the BGPE shows no peaks in the same condition. The electrocatalytic behavior of GPE/PBQC for electrochemical determination of 5-ASA was investigated. GPE/PBQC exhibits strong oxidation peak at 0.6V and BGPE exhibited weak oxidation peak at 0.63 V. Because of the presence of both Cr^{3+} ions and quinone functional groups at GPE/PBQC surface, this electrode shows high electrocatalytic activity toward electrooxidation of 5-ASA. Therefore, GPE/PBQC is appropriate for sensitive electrochemical determination of 5-ASA.

The Effect of Scan Rate

The effect of scan rate on the electrochemical oxidation of 5-ASA at BGPE and GPE/PBQC was investigated by CV (Figs. 1S-A). Based on this Figure, the anodic peak currents of 5-ASA was increased by increasing the scan rates. Also, the oxidation peak potentials for 5-ASA shifted to more positive values with increasing scan rate, concerning the kinetic limitations of the electrochemical reaction. The plot of peak height (I_p) *vs*. the square root of scan rate ($v^{1/2}$) was linear in the range of 25-500 mV s^{-1}, suggesting that at sufficient over potential, the process is

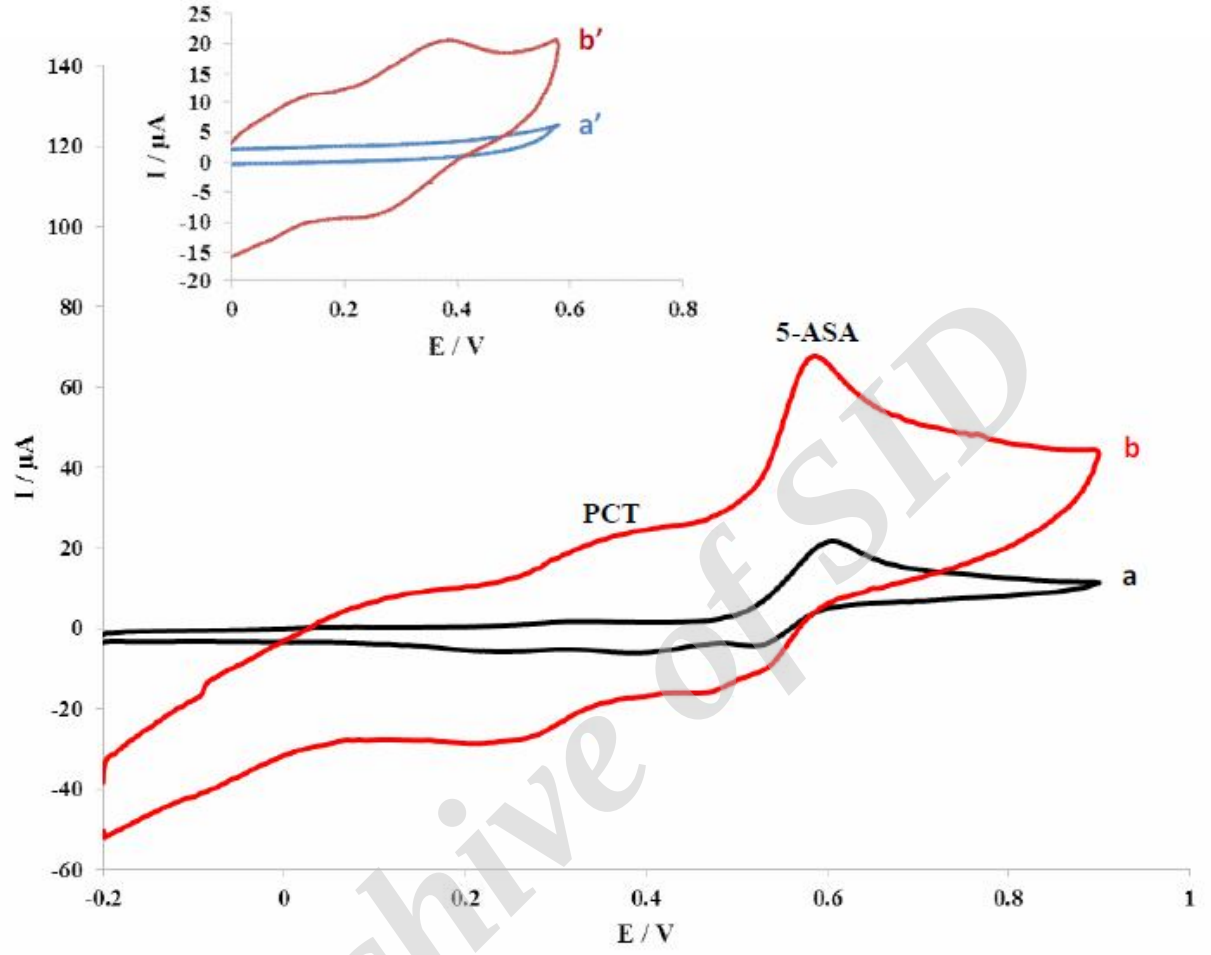


Fig. 3. CVs of BGPE (a) and GPE/PBQC (b) in 0.1 PBS (pH = 2), containing 5-ASA (250 µM); Inset. CVs of BGPE (a') and GPE/PBQC (b') in 0.1 PBS (pH = 2), (scan rate = 100 mV s^{-1}).

diffusion rather than surface controlled (Figs. 1S-B).

Chronoamperometric Studies

As shown in Fig. 2S, diffusion coefficients of 5-ASA were determined by chronoamperometry and using Cottrell equation that explains the variation of current with time for diffusion controlled process [19].

$$I = nFACD^{\frac{1}{2}}\pi^{-\frac{1}{2}}t^{-\frac{1}{2}} \quad (1)$$

Therefore, the linear relationship between current and $t^{-1/2}$ indicates diffusion controlled current. Plots of I *vs* $t^{-1/2}$ were drawn for different concentrations of analytes (Figs. 2S-B). Then, the resulting slope of trend line was plotted against analyte concentrations. From resulting slope and according to Cottrell equation, the values of $D_{5\text{-ASA}}$ was found to be 2×10^{-6} cm^2 s^{-1}. Also, in the same condition the $D_{5\text{-ASA}}$ on the BGPE was found be 6.9×10^{-7} cm^2 s^{-1}.

pH Effect

Figure 4 shows the effect of pH on the GPE/PBQC electrochemical response of 0.1 M phosphate buffer solution (PBS) containing 5-ASA (250 µM) at various pH values. Peak potential for 5-ASA shifted to negative potentials with increasing pH of solution indicating contribution of protons in the electrochemical reaction of 5-ASA. For species in which protons are transferred in their

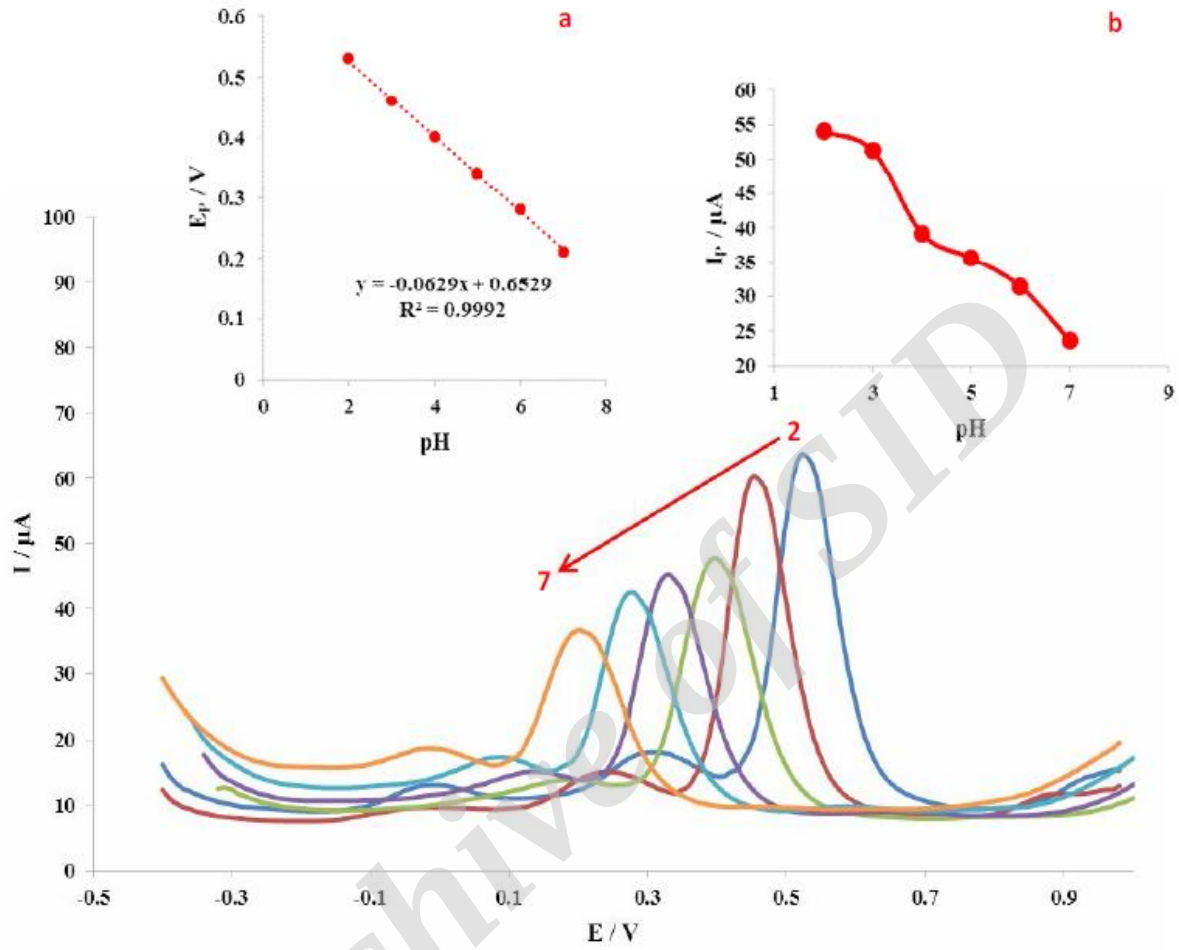


Fig. 4. DPVs of GPE/PBQC in 0.1 M PBS containing 5-ASA (250 µM) at various pH values (2-7) Inset. a. Plots of peak potentials *versus* pH. Inset. b. Plots of peak currents *versus* pH.

oxidation reaction, the following formulas explains the relationship between peak potential and pH [20].

$$E_p(\mathrm{Red}) = E_p(\mathrm{Red}, pH = 0) - 2.303\frac{mRT}{nF}pH \qquad (2)$$

$$\frac{dE_p}{dpH} = -2.303\frac{mRT}{nF} \qquad (3)$$

$$\frac{dE_p}{dpH} = -0.059\frac{m}{n} \qquad at\ 25c \qquad (4)$$

$E_{p(Red,pH\ =\ 0)}$ is the anodic peak potential for analyte at pH = 0, m and n are the number of protons and electrons, respectively. Other parameters such as R (8.314 J mol^{-1} k^{-1}), T (K) and F (96485 c mol^{-1}) have their normal meanings. Figure 4B shows linear relationship between peak potentials and pH values for 5-ASA. According to the slopes of trend line, it was suggested that the oxidation reaction of 5-ASA involves two protons and two electrons. Regarding pK_a value for 5-ASA (pK_{a1} = 2.30 for -COOH and pKa_2 = 5.69 for $-NH_3^+$), the probable electrochemical reaction was given in Eq. (6) [21,22]. Based on the Fig. 4C, pH = 2 exhibited the highest oxidation peak current for 5-ASA. Therefore, pH = 2 was selected as the optimum pH.

$$E_{(pa,\ 5\text{-}ASA)}\,(V) = 0.6529 - 0.0629\ pH \qquad (r^2 = 0.9992) \qquad (5)$$

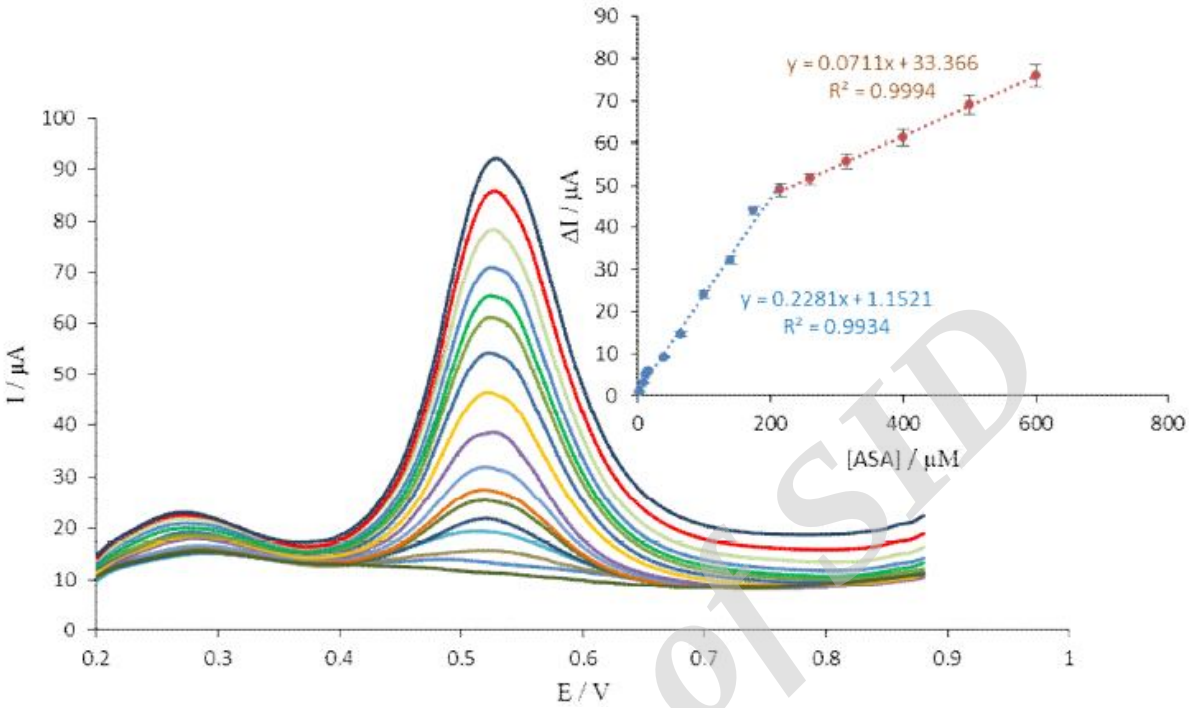
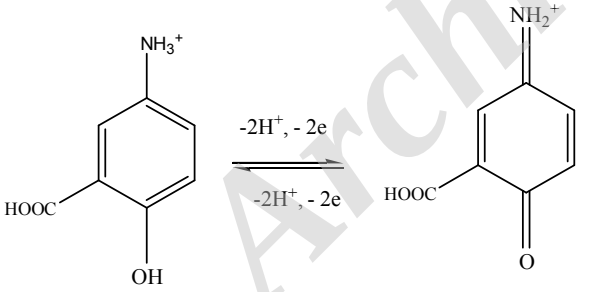


Fig. 5. DPVs of GPE/PBQC in 0.1 M PBS (pH = 2) containing [5-ASA]: 2-600 μM, and Inset; plots of anodic peak current *vs.* concentration of 5-ASA. The error bars represent the standard deviation of three parallel tests (relative standard deviations (%RSD) were found to be < 4% for n = 3).



(6)

Electrochemical Determination of 5-ASA

Under optimum conditions, electrochemical determination of 5-ASA was carried out using differential pulse voltammetry (DPV). The electrocatalytic peak currents of 5-ASA at the surface of GPE/PBQC were linearly dependent on the 5-ASA concentration. Figure 5 shows the DPVs and calibration curves of 5-ASA at GPE/PBQC. The detection limit was calculated based on the relationship of LOD = $3S_{blank}/m$, where S_{blank} is the is the slope of calibration plot. The responses were linear relative standard deviation of blank signals (n = 10), and m with 5-ASA concentration in the two segment ranges 1-215 and 215-600 μM. Also, as shown in Fig. 5, the relative standard deviations (RSD%) of all 5-ASA concentrations analyzed by GPE/PBQC in the linear range were in the range of 1.6-3.5% (n = 3), demonstrating that the electrooxidation current responses of 5-ASA on the GPE/PBQC have a good reproducibility. The detection limit was determined to be 70 nM. A comparison study for determination of 5-ASA with the proposed electrode and literature was shown in Table 1. Based on the Table 1, the advantages of the proposed modified electrode such as linear range and detection limit show satisfactory results in comparison to literature [5,23-27]. So, the proposed modified electrode is a good candidate for the determination of 5-ASA with satisfactory results in comparison with the other literatures.

Real Sample Analysis and Interference Study

To evaluate the practical applicability of the proposed

Table 1. Comparison of the Proposed Sensor with other Electroanalytical Methods for the Electrochemical Determination of 5-ASA

Electrode	Modifier	Method	pH	Linear range (μM)	Detection limit (μM)	Ref.
Boron-doped diamond electrode	-	SWV	7	25-300	0.7	[5]
GCE	Graphene oxide coated with a molecularly imprinted sol-gel	DPV	2	2-150	0.97	[23]
GCE	Functionalized carbon nanotube	DPV	2	0.05-2.5	0.012	[24]
GCE	Polypyrrole doped by 1,5-naphthalenedisulfonic acid	LSV	2	0.01-1	0.003	[25]
GCE	Computationally designed molecularly imprinted polymer	CV/DPV	2	0.05-100	0.015	[26]
GCE	Electropolymerization of nano-composite gold nanoparticles-molecularly imprinted polymer	CV/DPV	3	0.001-2	0.0003	[27]
Carbon Paste	PBQC	DPV	2	2-600	0.07	This work

Table 2. Electrochemical Determination of 5-ASA in Pharmaceutical Tablets with Proposed and Standard Spectroscopy Methods

Sample	Declared (mg/tbl)	Proposed method			Spectroscopy [31]		
		Found (mg/tbl)	Recovery (%)	RSD (%)	Found (mg/tbl)	Recovery (%)	RSD (%)
1	500	510	102	1.7	505	101	1.5
2	500	495	99	1.5	498	99.6	1.7
3	250	255	102	1.3	252	100.8	1.4

modified electrode, the GPE/PBQC was examined for the determination of 5-ASA in pharmaceutical tablets with the proposed and standard methods [28] and the results are shown in Table 2. Twenty of 5-ASA tablets weighted and powdered. Taking into account the labeled values, solutions of samples were prepared by dissolving appropriate amount of powdered tablets in 5 ml PBS (pH = 2). The results listed in Table 2 agreed satisfactorily with the labeled content of 5-ASA. Low values of the relative standard deviation (RSD%) indicated good reproducibility of the results. The relative error (%) for determination of 5-ASA with the proposed method and standard method is lower than ± 1%, indicating the excellent precision of the proposed method for determination of 5-ASA in pharmaceutical tablets. Also, using GPE/PBQC daily and stored under ambient conditions over a period of 30 days, the electrode retained 98.1% of its initial peak current response for a 5-ASA concentration of 100 µM, which shows long-term stability of the modified electrode. Finally, the fabrication repeatability was evaluated by preparing four modified electrodes independently. The RSD% for peak current determinations with four prepared electrodes on 25, 50 and 100 µM of 5-ASA were calculated to be 3.2, 2.9 and 3.5 %, respectively. Accordingly, the modified electrode has a good repeatability and reproducibility in both analytical determinations and preparation procedure with good long-term stability.

The effects of various substances such as glucose, sucrose and some ions such as PO_4^{3-}, SO_4^{2-}, K^+, Na^+ and Cl^- as coexisting ingredients and ions in the quantitative determination of 5-ASA were tested. In a certain range of concentration, one substance has no interference if it causes relative error lower or equal to ± 5 (%). 20 µM 5-ASA was employed in the study and twenty times lower (the concentration ratio was 1:0.05), the same (1:1), and twenty times higher (1:20) amounts of the potentially interfering agent were used. It was found that non of them interfere significantly, and maximum of the obtained changes of 5-ASA response did not exceed 5%. Furthermore, acceptable recovery values indicate that active ingredients in tablets have no interference for electrochemical determination of 5-ASA, confirming the applicability of this modified electrode for the trace amounts of 5-ASA in the pharmaceutical formulations.

CONCLUSIONS

In this work, for the first time, a simple modified GPE was prepared using PBQC and applied to the electrochemical determination of 5-ASA. The PBQC improves the electrochemical catalytic activities towards the oxidation of 5-ASA. The developed methodology of this study was simple, fast, sensitive and cheap in comparison with other techniques such as HPLC. The fabricated sensor identified 5-ASA in the linear range up to 600 µM by DPV technique with a detection limit of 70 nM. The proposed electrochemical sensor was well selective for 5-ASA in the presence of common interferences. Furthermore, the major advantages of the developed GPE are good reproducibility and repeatability, wide linear range, low detection limits and stability. Besides, the GPE/PBQC was successfully applied for the determination of 5-ASA in pharmaceutical samples.

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