Electrochemical Oxidation of Flavonoids and Interaction with DNA on the Surface of Supramolecular Ionic Liquid Grafted on Graphene Modified Glassy Carbon Electrode

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ABSTRACT: *The study of the interaction between DNA and small molecules such as drugs is one of the current general interest and importance. In this paper, the electrochemical investigation of the interaction between some flavonoids such as rutin, quercetin, and hesperidin with dsDNA on the surface of Supramolecular Ionic Liquid grafted on the Graphene Oxide Modified Glassy Carbon Electrode (SIL-GO/GCE) is reported for the first time. The apparent binding constant (K) of the interaction between flavonoids and dsDNA was calculated using the current titrations. The apparent binding constants (K) of rutin, quercetin and hesperidin were calculated to be* 4.3×10^5, 2.1×10^5 *and* 9.2×1^{-6} M^{-1}, *respectively. Furthermore, the electrochemical behavior of rutin on the surface of SIL-GO/GCE was studied in details using cyclic voltammetry and linear sweep voltammetry. The mechanism of the electrochemical redox reaction of rutin was proposed. When DNA was added into flavonoid solutions, their cathodic peak currents were decreased with few changes in the peak potentials. Furthermore, the interaction between rutin and bovine serum albumin was studied using differential pulse voltammetry. In conclusion, the SIL-GO/GCE provides a promising platform for the study of the interaction between DNA and small molecules.*

KEYWORDS *Graphene oxide; Supramolecular ionic liquids; Electrochemical techniques; Flavonoids-DNA interactions.*

INTRODUCTION

The study of the interaction of DNA with small molecules such as drugs is one of the current general interest and importance [1], especially for the designing of new DNA-targeted drugs and the screening of these

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in vitro. The recognition of DNA binders includes a complex interplay of difference interactive forces, which involves hydrophobic interaction along the minor groove of DNA, strong electrostatic interaction arising from the exterior sugar-phosphate backbone, and intercalative interaction between the stacked bases pairs of native DNA from the major groove [2, 3]. Electro-analytical methods are well suited for study the electrochemical behavior of organic molecules including drugs and related molecules and their interaction with DNA, because these methods are simpler, less expensive and less time-consuming as compared with other methods and offer an analytical platform with high sensitivity and specificity [4-8]. In addition, there has been a growing interest in the electrochemical study of the interaction between drugs and DNA [9–11]. One of the powerful practical applications of electrochemistry is the investigations of electrode redox process. Because of the existing resemblance between electrochemical and biological reactions, it can be supposed that the oxidation mechanisms which accomplished at the electrode and in the body share similar principles [12, 13]. By observing the electrochemical signal related to DNA–drug interactions, it is possible to study the nature of the formed complex and to evaluate binding constant. Flavonoids are one of the largest nutrient families known to scientists that have recently aroused considerable interest due to their broad pharmacological activity. In fact, flavonoids have been reported to have antiallergic, antiplatelet, antiviral, anti-inflammatory and antitumor activities, and possibly even protective effects against chronic diseases [14, 15]. As flavonoids are electro-active, the electrochemical characters of most active components in flavonoids were investigated and reported in the literature and the study of their electrochemical behavior is very important. As well as, the interactions of flavonoids with dsDNA have attracted considerable interest in the past few years due to their various applications including clinical diagnosis, environmental control, and forensic analysis [16-18].

Graphene, the two-dimensional sp2-hybridized carbon, is a novel material that has emerged as a rapidly rising star in the field of electrochemistry because of its remarkable physicochemical properties [19, 20]. GO-based materials consist of a 2D layered structure with a large surface area and also possess a large number of oxygen-containing functional groups, such as carboxyl, hydroxyl, and epoxy groups, which make it possible to functionalize such GO-based materials using either covalent or noncovalent chemistry in order to modulate the electrode's structural architecture and intrinsic properties [19, 20]. This modification in the field of electrochemistry is very important, First of all, GO itself have excellent electrocatalytic activities toward some important species, Furthermore, the introduction of an organic compound and electrocatalytic centers to GO may offer GO-based electrodes with novel electrocatalytic properties and exhibit novel, interesting properties and hold promise for design and prepare GO-based electrodes for applications in the field of electrochemistry. In this case, the modification of graphene with polymers (graphene nano-composite) has attracted extensive research interest [19-27]. Graphene nano-composites functionalized with polyionic liquid have several advantages such as high dispersity, functionality, high specific surface area, and high surface charge density. As a result, this system can cause highly dispersive and long term stable graphene sheets in reaction media [19-27].

Based on the told preface, Graphene nano-composite functionalized with polyionic liquid can act as a good electrode material to fabrication of new electrochemical systems with unique properties for the detection of biomolecules. Hence, in this work, Graphene nano-composite functionalized with polyionic liquid was used successfully as electrode materials for the study of electrochemical behavior and interaction of some flavonoids such as rutin, quercetin, and hesperidin with dsDNA. To the best of our knowledge, this is the first report for the use of these materials for the study of electrochemical behavior and interactions of flavonoids with dsDNA.

EXPERIMENTAL SECTION

Apparatus and reagents.

All chemicals were purchased from Aldrich and used without further purification. Ultrasonic bath (EUROSONIC® 4D ultrasound cleaner with a frequency of 50 kHz and an output power of 350 W) was used to disperse materials in solvent. Voltammetric experiments were performed using a μAutolab Type III electrochemical system. A conventional three-electrode cell consisting of a glassy carbon working electrode (modified and

unmodified), a platinum wire counter electrode and a saturated Ag/AgCl reference electrode were used for voltammetric experiments. A digital pH-meter (Ion Analyzer 827, Metrohm) with the precision of ±0.001 was used for pH measurements. All electrochemical experiments were done at room temperature (25.0±1 °C).

Double-stranded DNA (dsDNA) was purchased from Sigma. The dsDNA was dissolved in water and stored at 4 0C. Stock solutions of 10-3 M rutin and other flavonoids were prepared by dissolving it with ethanol. Standard working solutions were prepared by diluting the stock solution with the selected supporting electrolyte. All the solutions were prepared in Millipore water.

Preparation of the modified glassy carbon electrodes

Before the electrode modification process, a Glassy Carbon Electrode (GCE) (2mm diameter) was polished with 0.05 µm alumina slurry on a polishing cloth, then, it was washed with deionized water and ethanol. Typically, a stable suspension of SIL-GO containing 5.0 mg/mL in DMF using 20 min ultrasonic agitation was prepared. After the electrode surface was air dried, 5.0 µL of this suspension was cast onto the surface of the pretreated GC electrode with a microsyringe and then it was dried in the air [7]. Furthermore, the suspension of GO was prepared and used with the same procedure. Before the electrochemical measurements, the prepared SIL-GO and GO were reduced at a constant potential of -1.5 V for 30 min.

Analytical procedure

After fabricating each electrode in order to obtain reproducible current-potential curves, cyclic voltammetry was performed at a scan rate of 50.0 mV s-1 between -0.8 and 1.0 V for 10.0 times in 0.1 M in phosphate buffer solution. In addition, the buffer solution was purged with high-purity nitrogen for at least 10.0 min prior to each electrochemical measurement.

For binding studies, differential pulse voltammetry of flavonoids (fixed concentration) was recorded in presence of increasing concentrations of DNA in phosphate buffer of pH 7.0. After each addition of DNA to flavonoids, the interaction times were maintained and DPV was recorded.

Materials preparation

The procedures for preparation of GO and functionalized GO were explained in supplementary data.

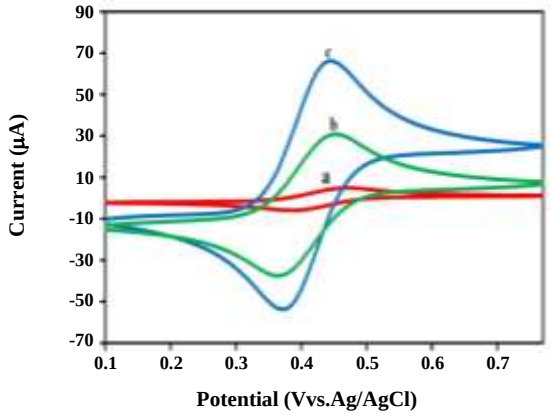


Fig. 1: Cyclic voltammograms of 1.0 mM rutin at (a) GCE, (b) GO/GCE, and (c) SIL-GO/GCE in 0.1 M phosphate buffer (pH=7.0) at the 50.0 mV/s scan rate.

RESULTS AND DISCUSSION

The potential application of GCE, GO/GCE, and SIL-GO/GCE was evaluated for electrooxidation of rutin. Cyclic voltammograms of 1.0 mM rutin on different electrodes were recorded with the results shown in Fig. 1.

It can be seen that a pair of well-defined redox peaks observe on the different electrodes for rutin solution, suggesting that the redox reaction of rutin can take place on the surface of different electrodes. The results were in agreement with the electrochemical reaction mechanism of rutin on the surface of carbon electrodes [28]. The redox peak currents on GO/GCE (curve b) were larger than that on GCE (curve a), indicating the high surface area of GO/GCE than GCE. When the surface of the electrode was modified with the SIL-GO films (curve c), the highest electrochemical responses were observed with the redox peak potentials at 0.43 V (E_{pa}) and 0.38 mV (E_{pc}), confirming an electro-catalytic effect and a good electrochemical response to rutin. This improvement in the electrochemical response of rutin clearly demonstrated that SIL-GO/GCE have advantages such as good conductivity, high-surface area, and inherent electrocatalytic ability. Due to this fact that useful information involving electrochemical reaction mechanism usually can be obtained from the potential scan rate studies, the electrochemical behavior of rutin at scan rate ranges from 10 to 150 mV/s was also investigated at pH 7.0 using cyclic voltammetry in 5 µM solution of rutin (Fig. 2A).

With the increasing of scan rate, the E_{pa} shifted to a more positive value and the peak-to-peak separation also

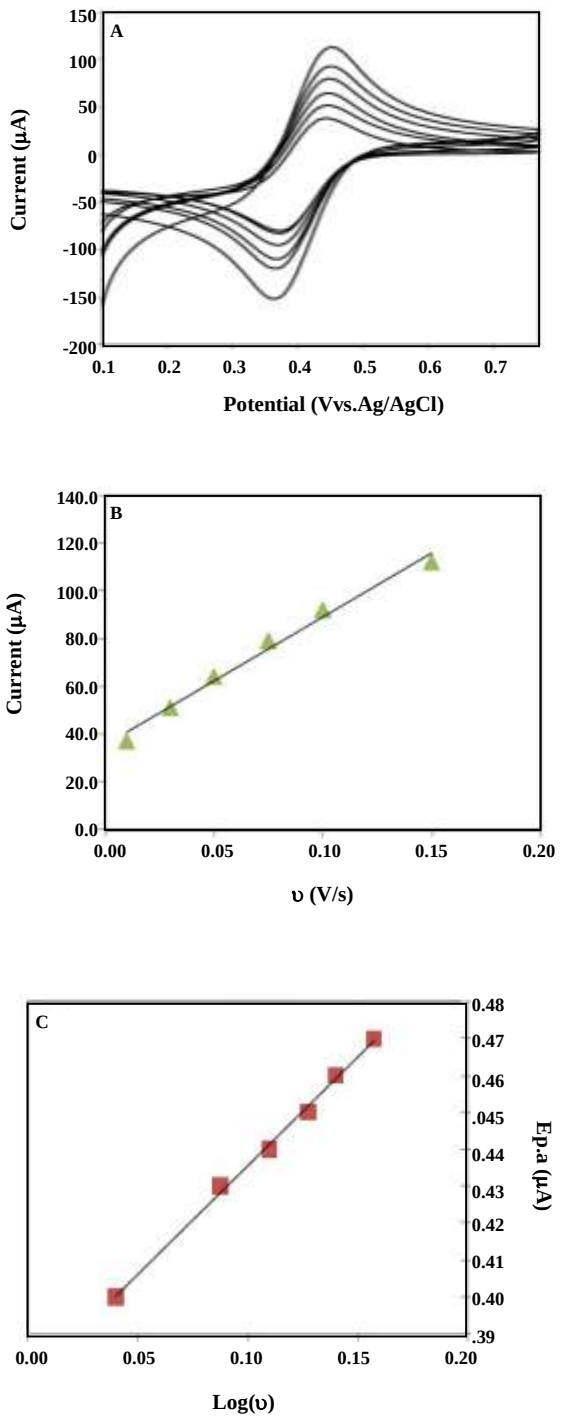


Fig. 2: (A) Cyclic voltammograms of 1.0 mM rutin at SIL-GO/GCE in 0.1 M phosphate buffer solution (pH 7.0) at a different scan rates; (from bottom to top) 10.0, 30.0, 50.0, 75.0, 100.0, and 150.0 mV/s. (B): Plot of variation of oxidation peak current with scan rate. (C): Plot of variation of oxidation peak potential with the logarithm of scan rate.

increased, suggesting the electron transfer was quasi-reversible. When the scan rate was increased, a linear relationship between the peak current and the scan rate in the range of 10–150 mV/s was found (Fig. 2B), suggesting an adsorption behavior [29]. The equation relating to anodic current peak can be represented as: $I_{pa}(\mu A) = 536.92\upsilon\ (V/s) + 35.363$, $R^2=0.98$

For an adsorption-controlled and quasi-reversible interfacial reaction, according to the Laviron's equation [29], the relationship between E_{pa} and scan rate is defined by the following:

$$E_{Pa} = k + \frac{2.33RT}{(1-\alpha)nF}\log \nu \quad (1)$$

$$\log k_s = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nFv} - (1-\alpha)\frac{nF\Delta E_p}{2.3RT} \quad (2)$$

Where α is the transfer coefficient, n is the number of electrons transferred and ν is the scan rate. From the slope of plot E_{pa} vs. log ν (Fig. 2C), the value of (1- α) n was found to be 0.11. Thus, the value of α was found to be 0.53. Furthermore, the value of apparent rate constant K_s can be obtained from the intercept of ΔE_p vs. log ν plot (Eq. (2)). Under different scan rates of 10 to 150 mV/s, the k_s was calculated to be 2.3 s^{-1}.

The pH is an important factor in the electrochemical behavior of organic compound because the proton is always involved in the electrochemical reaction and exerts a significant impact on the reaction speed. Hence, for pH effect studying of the phosphate buffer solution on current response and peak potential of rutin, the linear sweep voltammograms of 5 µM rutin were recorded from pH 5.0–9.0 at a scan rate of 50 m V/s (Fig. 3).

With the increase of pH of the solution, the anodic peak potentials linearly shifted to less positive values. This indicates that protons participate in the oxidation of rutin [6].

$$(\text{Rutin})_{red} \leftrightarrow (\text{Rutin})_{ox} + mH^+ + ne^- \quad (3)$$

Where m is the number of protons involved in the reaction. The Nernstian equation is given by [6]:

$$E_P^a = E_{P(pH=0.0)} - \frac{2.303mRT}{nF}pH \quad (4)$$

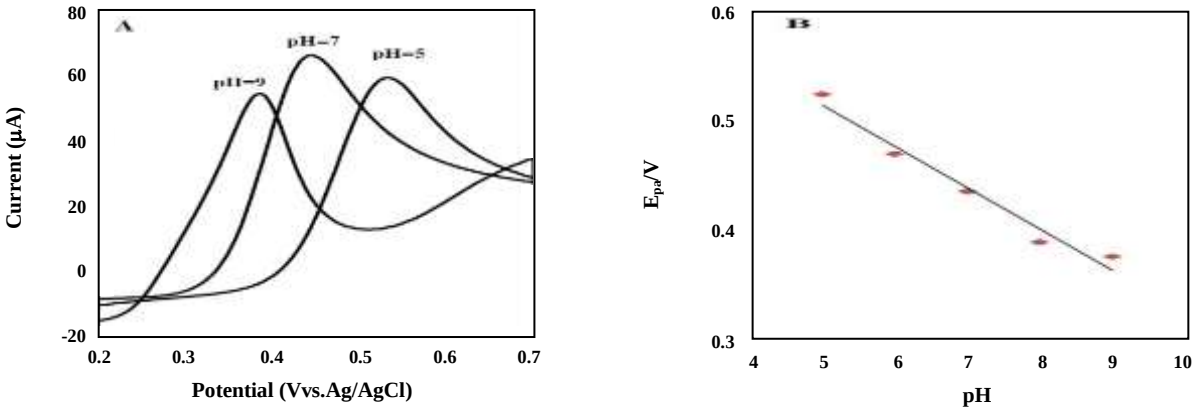
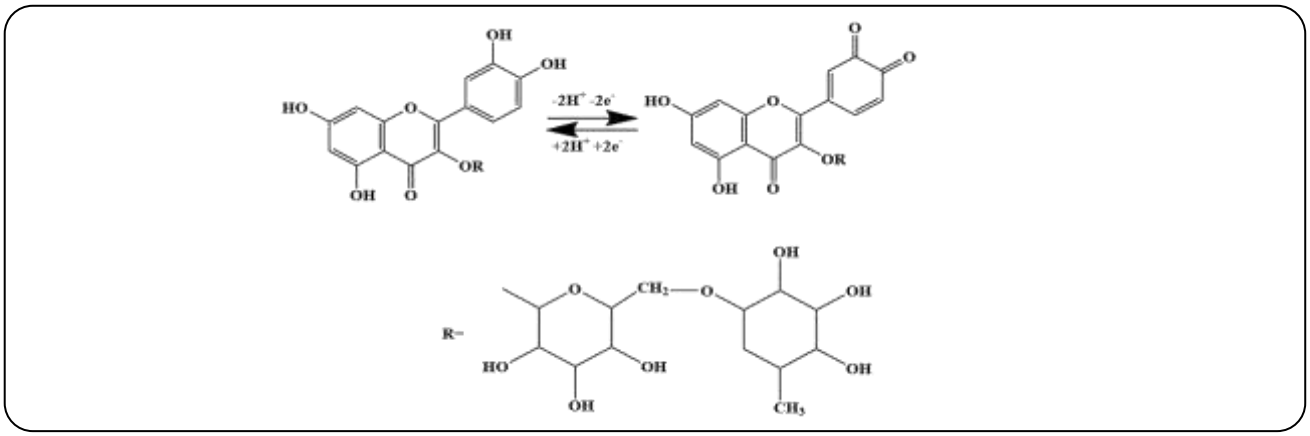


Fig. 3: (A): Linear sweep voltammograms of 1.0 mM rutin at SIL-GO/GCE in 0.1 M phosphate buffer solution at a scan rate of 50.0 mV/s under different pHs: 5.0, 7.0, and 9.0. (B): Plot of variation of oxidation peak potential with pH.



Scheme 1. A proposed redox mechanism of rutin on the SIL-GO/GCE.

Where E_p (pH =0.0) is the peak potential at pH 0.0, R, T, and F have their usual meanings. The E_p–pH diagrams (Fig. 4) are shown that the dependence of E_{pc} on pH can be expressed by the following relations:

$$E_{pa}\,(\mathrm{Va}) = 0.7 - 0.06\mathrm{pH}, \qquad R^2 = 0.984$$

The slope of E_{pc} vs. pH corresponds to 60 mV/pH for the anodic peak. According to Eq. (2), this result revealed that the number of proton in the process is equal to the number of the transferred electrons.

To propose the redox process, the number of electrons involved in the redox reaction was obtained by electrolysis of a 1.0 mM rutin solution using controlled potential coulommetry at +0.43 V (oxidation peak potential of rutin). The coulommetric n was calculated using the equation, Q=nFN, where Q is the charge in coulombs, F is Faraday's constant, and N is the number of moles of the substrate. In our systems, the number of the electrons involved in the redox process, n, was 2.0. Thus, a proposed redox mechanism of rutin is described in Scheme 1.

Because of the good electrochemical response of rutin on the surface of SIL-GO/GCE, this modified electrode was chosen for analytical application and better study of the interaction of rutin and others flavonoids with the dsDNA. Fig. 4A showed the DPV responses of 5.0 μM rutin in the present and absent of different DNA concentrations after 6 min interaction time, when dsDNA is added to a rutin solution, significant changes in peak current and potential were observed for the reduction of rutin. The cathodic peak current decreased to about 69 % of initial value and the peak potential shifted to the more-negative potential in the presence of dsDNA, confirming the interaction of rutin with dsDNA may be intercalation [30, 31].

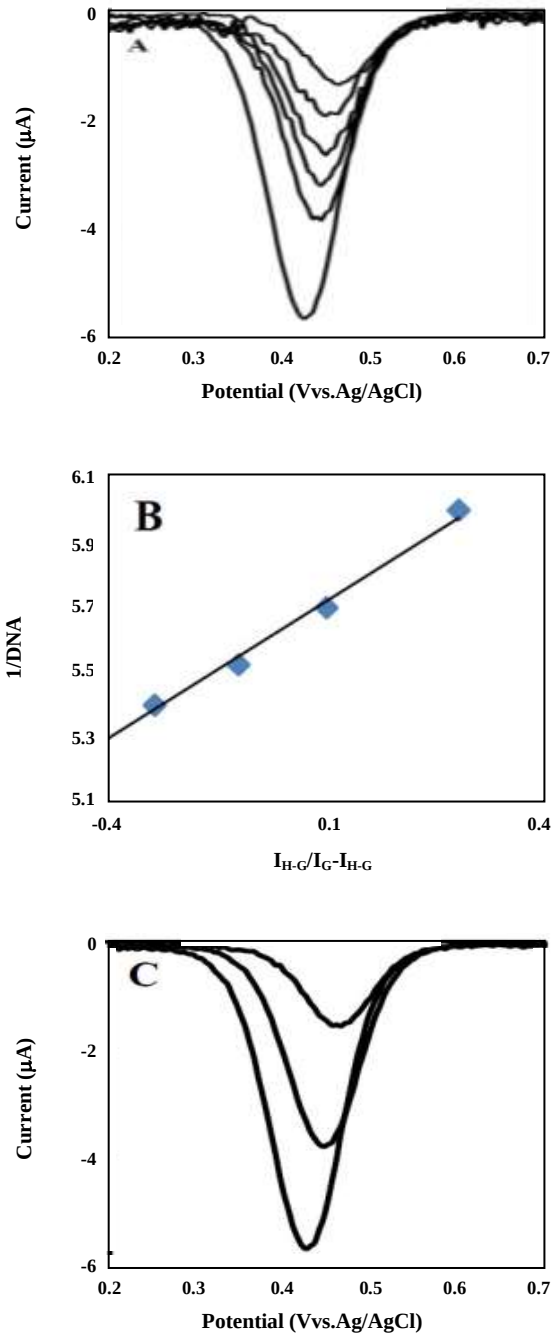


Fig. 4: (A): Differential pulse voltammograms of 5µM rutin at SIL-GO/GCE in 0.1 M phosphate buffer solution (pH 7.0) in the present and absent of different dsDNA concentrations. dsDNA concentrations (from top to bottom): 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 µM. (B): Plot of log (1/[DNA]) vs. log (I_{H-G}/I_G-I_{H-G}). (c): Differential pulse voltammograms of 5 µM rutin at SIL-GO/GCE in 0.1 M phosphate buffer solution (pH 7.0) in the present and absent of different BSA concentrations. BSA concentrations (from top to bottom): 0.0, 2.0, and 4.0 µM.

These results suggested that the intercalation of dsDNA with rutin caused a decrease in the peak current of rutin. This is due to the considerable decrease in the apparent diffusion coefficient of the rutin-dsDNA. Moreover, the apparent binding constant (K) could be calculated by the current titrations [32] using the DPV responses of 5.0 µM rutin solution at different concentration of dsDNA (Fig. 4B):

$$\mathrm{Log}\left(\frac{1}{\mathrm{DNA}}\right)=\log \mathrm{K}+\log\left(\frac{\mathrm{I_{H-C}}}{\mathrm{I_C}-\mathrm{I_H}-\mathrm{C}}\right) \quad (5)$$

Where *K* refers to the apparent binding constant, *[DNA]* is the concentration of dsDNA bound to rutin, I_G and I_{H-G} are the peak current values of the free guest (G; here free rutin) and the host-guest complex (H–G; rutin intercalated into dsDNA), respectively. The plot of log (1/[DNA]) vs. log (I_{H-G}/I_G-I_{H-G}) becomes linear with the intercept of log K. The average value of the binding constant for rutin-dsDNA was calculated to be 4.3×10^5 M^{-1}, which this value suggested that strong binding of rutin to dsDNA occurred. The results indicate the large binding constant for rutin-dsDNA adduct. The voltammetric changes unequivocally suggest that rutin is intercalated into the base-stacking domain of the dsDNA double helix.

Due to the intercalation of rutin into dsDNA, the cathodic peak current of rutin decreased with increasing concentrations of dsDNA, thus, the concentration of dsDNA can be determined using the peak current of the rutin reduction at 0.33 V in the DPV. Under optimum experimental conditions, the change of peak current is linearly dependent on the dsDNA concentration ranging from 0.1 µM to 10 µM when the rutin concentration was fixed at 10 µM. and the detection limit was calculated to be 30 nM. The reproducibility and repeatability of the developed electrode were studied. A relative standard deviation of 3.2% was achieved towards 2 µM dsDNA for 10 electrodes prepared in the same way, indicating the reliability of the method. Moreover, the Relative Standard Deviation (RSD) of 2.7% was obtained for a set of 10 different DPV measurements of 2 µM dsDNA with a single modified electrode.

For more study, the interaction of rutin with Bovine Serum Albumin (BSA) was considered (Fig. 4C). In the presence of BSA, similar to dsDNA, significant changes in peak current and potential were observed for the

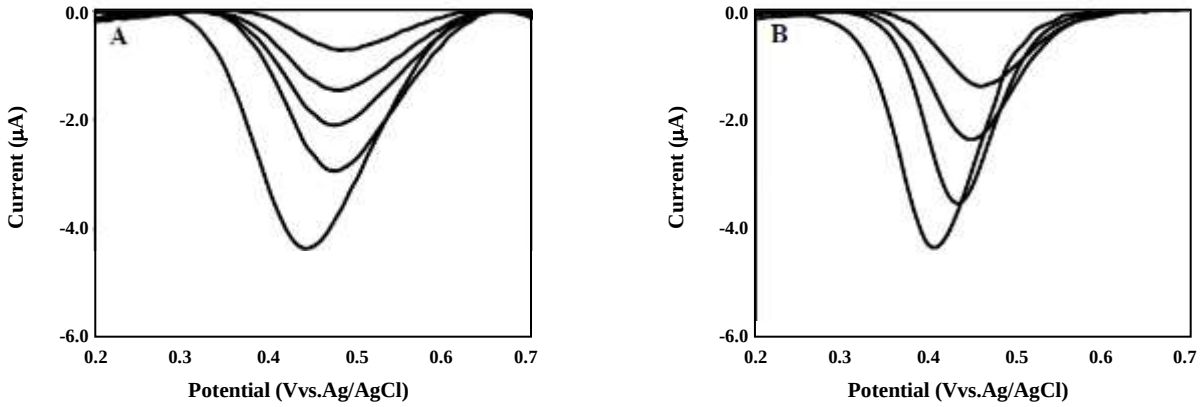


Fig. 5: (A) Differential pulse voltammograms of 5µM quercetin at SIL-GO/GCE in 0.1 M phosphate buffer solution (pH 7.0) in the present and absent of different dsDNA concentrations. dsDNA concentrations (from top to bottom): 0.0, 1.0, 2.0, 3.0, and 5.0 µM. (B) Differential pulse voltammograms of 5µM hesperidin at SIL-GO/GCE in 0.1 M phosphate buffer solution (pH 7.0) in the present and absent of different dsDNA concentrations. dsDNA concentrations (from top to bottom): 0.0, 1.0, 3.0, and 5.0 µM.

oxidation of rutin. In addition, the cathodic peak current decreased to about 71 % of initial value and the peak potential shifted to more-positive potential, suggesting the BSA intercalation of rutin caused a considerable decrease in the apparent diffusion coefficient of the rutin-BSA. Moreover, the apparent binding constant (K) was calculated to be 1.02×10^5 M^{-1}, which this value confirmed a strong binding of rutin with BSA. The results indicate the large binding constant for rutin-dsDNA adduct. The voltammetric changes unequivocally suggest that rutin is intercalated into the base-stacking domain of the dsDNA double helix.

In the next section, the ability of SIL-GO/GCE was evaluated for the study of the interaction of some others flavonoids such as quercetin and hesperidin flavonoids with dsDNA. DPV technique provides higher sensitivity and better peak resolution compared to cyclic voltammetry for studying the electrochemical behavior of biological systems. Thus, DPV technique was employed in the present study for quercetin and hesperidin in the presence and absence of DNA in phosphate buffer of pH 7.0 which shown in Fig. 5. Addition of DNA resulted in a decrease in peak current of flavonoids. In addition, the interaction of flavonoids with DNA depends on time. In order to find the interaction time, we recorded the DPV of quercetin and hesperidin in presence of DNA at different time intervals. We observed a significant decrease in peak current of quercetin and hesperidin up to 7 and 10 min, respectively. After these interaction times, the peak current of these flavonoids became constant. Therefore, an interaction time of 7 and 10 min was maintained throughout, after each addition of DNA. Moreover, the apparent binding constant (K) of quercetin and hesperidin was calculated to be 2.1×10^5 and 9.2×10^6 M^{-1}, respectively, which this value suggested that the large binding constant for flavonoids-dsDNA adduct.

CONCLUSIONS

In conclusion, a glassy carbon electrode was successfully modified with supramolecular ionic liquid grafted on the graphene oxide (SIL-GO/GCE). The studies showed that this novel electrode was a suitable electrochemical platform for study of the interaction between some flavonoids such as rutin, quercetin, and hesperidin with dsDNA. According to the current titrations experiments, the apparent binding constant (K) of the interaction for rutin, quercetin and hesperidin with dsDNA was obtained to be 4.3×10^5, 2.1×10^5 and 9.2×1^{-6} M^{-1}, respectively. The electrochemical studied showed that the redox process of rutin occurred through a quasi-reversible two electrons-two protons reaction on the surface of SIL-GO/GCE. Through this redox reaction for rutin and the ability for interaction with dsDNA, a wide linear range of concentration change (0.1 µM to 10 µM), low detection limit (30 nM), and reproducibility were obtained for detection of dsDNA on the surface of the SIL-GO/GCE. Moreover, the study of the interaction between rutin and BSA confirmed the ability of our system for further investigation of other biomolecules in the futures.

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