

A Comparison of the Electrochemical Behavior of Rutin at an Inactivated, Activated, and Multi Wall Carbon Nanotubes Modified Glassy Carbon Electrode

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Electrochemical characteristics of rutin, as an important biological molecule, have been studied at a glassy carbon electrode using cyclic voltammetry and chronocoulometry methods. The redox response of rutin solution at an inactivated glassy carbon electrode (IGCE) was investigated and E_rC_i mechanism was deduced for rutin oxidation by voltammetric data at various scan rates and in different rutin concentrations. The results show that the subsequent chemical reactions of the E_rC_i mechanism for rutin oxidation at an IGCE are dimerization and intramolecular reactions. Also, it has been shown that the oxidation of rutin at an activated glassy carbon electrode (AGCE) leads to the formation of a deposited layer which shows one pair of peaks with surface confined characteristics. The surface excess of bonded rutin, Γ_R , and diffusion coefficient, D , for free rutin were determined as $5.8 \times 10^{-11} \text{ mol cm}^{-2}$ and $1.9 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ respectively from chronocoulometry experiments. The heterogeneous charge transfer rate constant, k_s , and the transfer coefficient, α , for electron transfer between bonded rutin and the AGCE were calculated as $98.0 \pm 2.0 \text{ s}^{-1}$ and 0.48 respectively. The results indicate that the k_s of bonded rutin at an AGCE is almost twice larger than reported for adsorbed rutin at a multi-wall carbon nanotubes (MWCNT) glassy carbon electrode.

Keywords: Electrochemical behavior, rutin, activated glassy carbon electrode, cyclic voltammetry, E_rC_i mechanism

1. INTRODUCTION

Rutin is one of the bioactive flavonoid compounds that occur naturally in fruit, vegetables, legumes, nuts and seeds of vascular plants [1, 2]. It has been estimated that humans consuming high amount of fruits and vegetables may gain up to 1 g of these compounds everyday [1, 2]. There exist more than 4000 flavonoids. They have been shown to have a wide range of biological activities. These include antimicrobial effect [3], anticarcinogenic effect [4], enzyme-modulating activities [5, 6], and

cytotoxic effects [7]. Also, they may act as antioxidants to inhibit free-radical mediated cytotoxicity and lipid peroxidation [8-10]. Rutin (3',4',5,7-tetrahydroxyflavone-3 β -D-rutinoside) is one of the most bioactive flavonoids, known as vitamin P. It was thought to be an activating factor for vitamin C [11]. Rutin has been applied to treat high blood pressure and vascular fragility. Rutin is also shown to have antioxidative action *in vitro* and *in vivo*. It can act directly by entering the redox reactions, and indirectly by chelation of iron.

There are a few papers which have reported electrochemical behavior of rutin [8, 12-14]. Here, we report some of the electrochemical characteristics of rutin at inactivated and activated glassy carbon electrode surfaces. The electrochemical oxidation mechanism of rutin is demonstrated at an inactivated glassy carbon electrode. Also, the charge transfer rate constant, k_s , between activated glassy carbon electrode surface and bonded rutin is compared with those previously reported for adsorbed rutin on the multi-wall carbon nanotubes modified glassy carbon electrode [15].

2. EXPERIMENTAL PART

All the electrochemical experiments were carried out using potentiostat PGSTAT 30 model from Autolab (Netherlands) equipped with GPES 4.9 software. The geometric area of the glassy carbon-working electrode (Azar electrode, Iran), was 0.031 cm². A platinum electrode and a saturated calomel electrode (SCE) were used as the counter and reference electrodes respectively. All the potentials were reported with respect to this reference electrode.

The chemicals used for buffer solution preparation were prepared from Merck Company and used as received. Rutin was purchased from Fluka Company and used without further purification. All the chemical reagents used were of analytical grades. All the solutions were prepared with doubly distilled water. The rutin solution was prepared just prior to use.

For the preparation of the bonded rutin at the activated glassy carbon electrode (AGCE), the glassy carbon electrode was first polished mechanically with 0.05 μ m alumina in water slurry using a polishing cloth and then rinsed with doubly distilled water. The electrochemical activation of the inactivated glassy carbon electrode (IGCE) was performed by a continuous potential cycling from -1.1 to 1.6 V at a sweep rate of 100 mV s⁻¹ in sodium bicarbonate (0.1 M) solution, until a stable voltammogram was obtained. For the electrodeposition of rutin at an AGCE, the activated electrode was placed in a 0.1 M phosphate buffer (pH 2.0) containing 1.0 mM rutin and was electrodeposited by 6 cycles of potential sweep between 300 and 650 mV at 25 mV s⁻¹.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of rutin solution at an inactivated and activated glassy carbon electrode

The cyclic voltammograms of a 0.1 M phosphate buffer (pH 7.0) containing 0.20 mM of rutin at an inactivated, activated and multi-wall carbon nanotubes modified glassy carbon electrode are

shown in curves (a), (b) and (c) of Fig. 1 respectively. As it can be seen, at an IGCE surface, the voltammogram has the characteristics of a species in solution, while at an AGCE and a multi-wall carbon nanotubes modified glassy carbon electrode (MWCNT-GCE), the voltammetric peaks are typical of a surface-immobilized redox couple. Fig. 2A shows the cyclic voltammetric responses of 0.1 M phosphate buffer (pH 7.0) containing 0.10 mM rutin at an IGCE surface at different sweep rates. For these voltammograms, anodic and cathodic peak potentials are observed at 230 mV and 154 mV respectively. The conditional formal potential, which is approximated by the midpoint potential (E_{mid}) between the anodic and cathodic peaks potentials, is about 192 mV and almost independent of the potential scan rate. As it can be seen in Fig 2B, the anodic and cathodic peak currents increase linearly with the square root of the scan rate, suggesting that the reaction is mass transport or diffusion limited. Fig . 2C shows the peak current ratio ($I_{\text{p,c}}/I_{\text{p,a}}$) increases gradually with increase of the potential scan rate until it reaches a fixed value which is less than a unit. Also, in proportion with the increase in the potential scan rate, the normalized current ($I_{\text{p}}/v^{1/2}$) diminishes gradually (Fig. 2D). These results are characteristics of an $E_{\text{r}}C_{\text{i}}$ mechanism [16]. The subsequent chemical reaction can be considered as a dimerization, hydroxylation, or intramolecular reaction. The effect of a subsequent chemical reaction is observed when the characteristic lifetime of a coupled chemical reaction and the characteristic time (time window) of the chosen electrochemical technique are comparable [17].

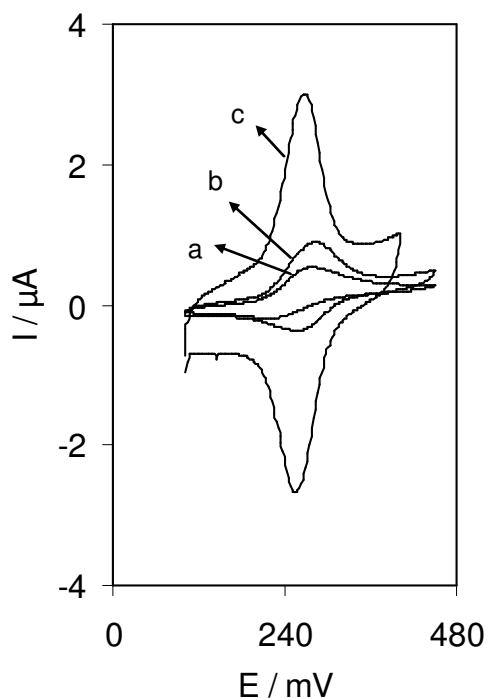


Figure 1. Cyclic voltammetric responses of 0.1 M phosphate buffer (pH 7.0) containing 0.20 mM rutin at an (a) inactivated, (b) activated and (c) MWCNT modified glassy carbon electrode. Scan rate is 25 mV s^{-1} .

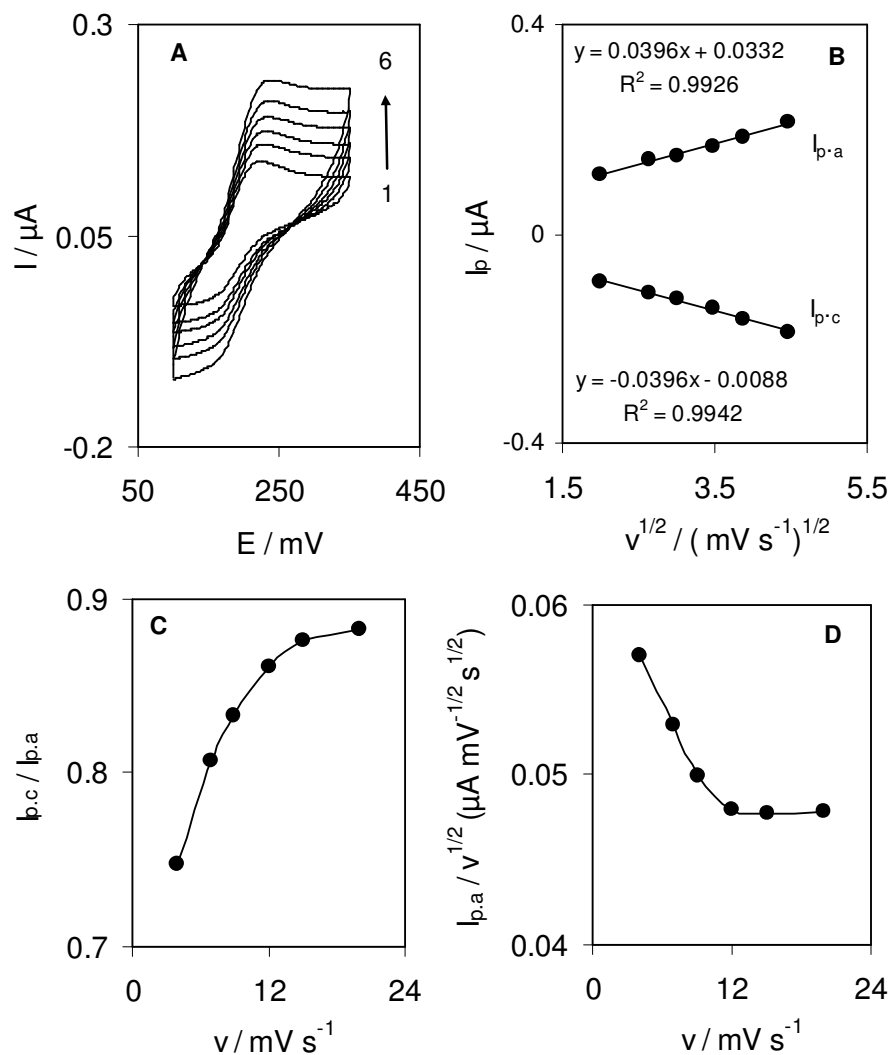


Figure 2. (A) Cyclic voltammetric responses of an inactivated glassy carbon electrode in a 0.1 M phosphate buffer (pH 7.0) containing 0.10 mM rutin at different scan rates. The numbers of 1 to 6 correspond to scan rates of 4, 7, 9, 12, 15, and 20 mV s^{-1} . (B) Variations of anodic and cathodic peak currents versus square root of scan rate. (C) Variation of the peak current ratio, $I_{p,c}/I_{p,a}$ versus scan rate. (D) Variation of scan rate normalized current ($I_p/v^{1/2}$) versus scan rate.

Fig. 3 shows the cyclic voltammograms of 0.1 M phosphate buffer (pH 5.5) containing different concentrations of rutin at an IGCE. As illustrated in the inset of Fig. 3, the peak current ratio ($I_{p,c}/I_{p,a}$) decreases with increase of rutin concentration. The results are shown in a relatively low concentration of hydroxyl ions (pH 5.5), the rate of subsequent chemical reaction (or the ratio of $I_{p,a}/I_{p,c}$) increases with the increase of bulk concentration of rutin. The present results therefore suggest an E_rC_i mechanism in which the subsequent chemical reaction includes dimerization and intramolecular reaction [16]. Also, it is supposed that intramolecular subsequent chemical reaction in E_rC_i mechanism has a fixed effect for the consumption of rutin oxidized form, but the extent of

dimerization coupled chemical reaction is dependent to bulk concentration of rutin and sweep rate of cyclic voltammetry method. This is because the characteristic lifetime of an intramolecular reaction is very short compared with the characteristic time of cyclic voltammetry, while the characteristic lifetime of a subsequent dimerization reaction is comparable with the characteristic time of the electrochemical method.

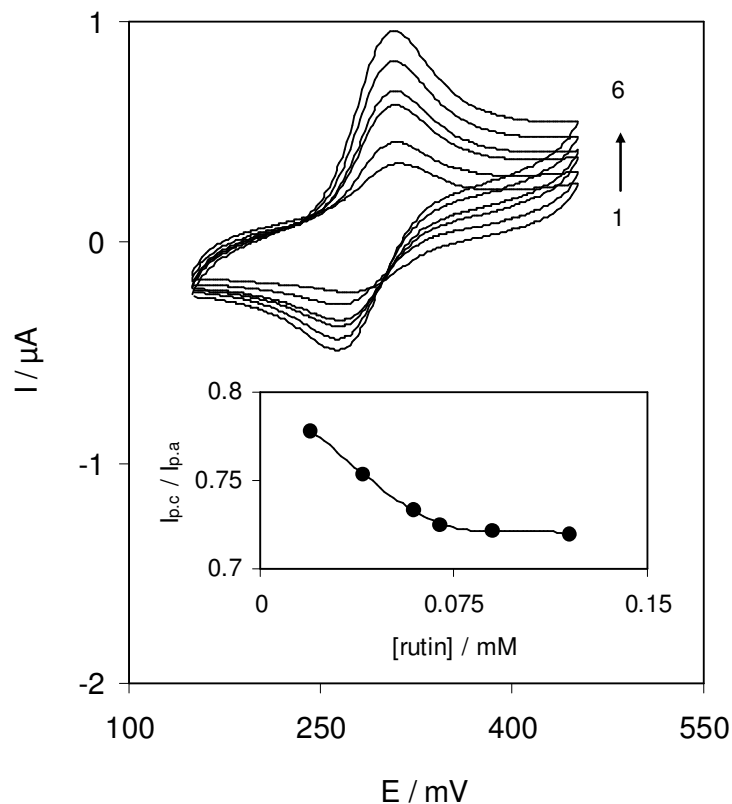


Figure 3. Cyclic voltammetric responses of an inactivated glassy carbon electrode at 25 mV s^{-1} and in a 0.1 M phosphate buffer (pH 5.5) containing different concentrations of rutin. The numbers of 1 to 6 correspond to concentrations 0.02, 0.04, 0.06, 0.07, 0.09 and 0.12 mM rutin, respectively. The inset shows the variations of the ratio $I_{p,c}/I_{p,a}$ versus rutin concentration.

Fig. 4 shows the cyclic voltammograms of the AGCE in 0.1 M phosphate buffer (pH 7.0) containing 0.10 mM rutin at different scan rates of 7 to 25 mV s^{-1} . The anodic and cathodic peak potentials of the voltammograms are observed at 230 mV and 210 mV respectively. The peak-to-peak potentials separation (ΔE_p) is small and also $E^{0'}$ is almost independent of the potential scan rate, suggesting facial charge transfer kinetics over this range of sweep rate. The plot of the anodic and cathodic peak currents versus the scan rate (Fig. 4, inset) yielded straight lines in the worked range of scan rates. This result indicates that the immobilized rutin exhibits electrochemical responses which are characteristic of the redox species confined on the electrode surface.

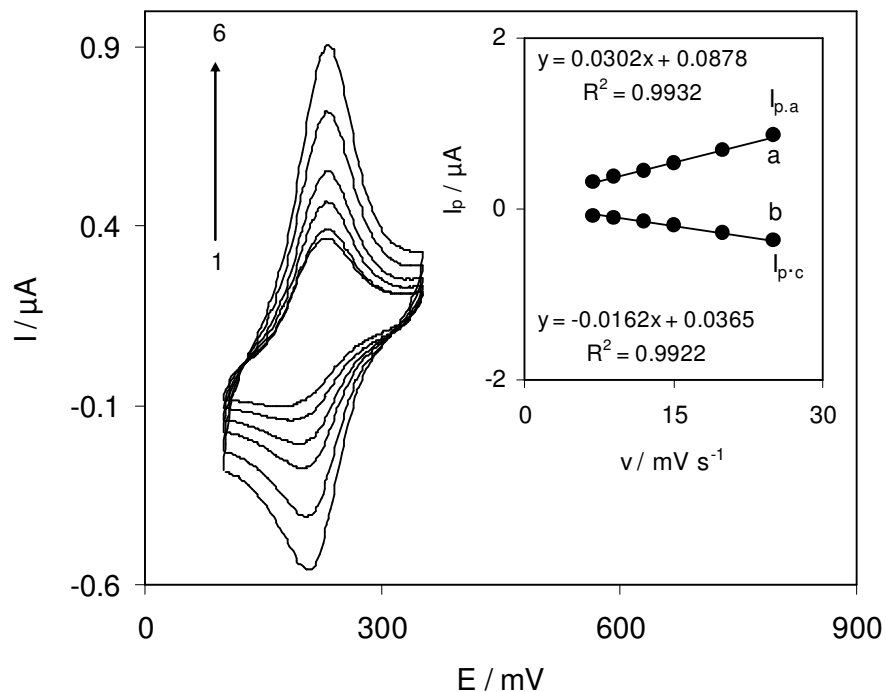


Figure 4. Cyclic voltammetric responses of an activated glassy carbon electrode in a 0.1 M phosphate buffer (pH 7.0) containing 0.10 mM rutin at different scan rates. The numbers of 1 to 6 correspond to scan rates of 7, 9, 12, 15, 20, and 25 mV s^{-1} , respectively. The inset shows the variations of anodic and cathodic peak currents versus potential scan rate.

The oxidation of rutin at AGCE was also studied by the chronocoulometry method. Chronocoulograms were obtained at different concentrations of rutin at the potential step of 240 mV (Fig. 5). Diffusion coefficient, D , and the surface excess of bonded rutin, Γ_R , at AGCE can be obtained by chronocoulometry measurements.

The cumulative charge passed in the oxidation of the reactant (rutin in this case) with a diffusion coefficient of D , is described by the following equation [17]:

$$Q = \frac{2nFAD_R^{1/2}C_R^*t^{1/2}}{\pi^{1/2}} + Q_{dl} + nFA\Gamma_R \quad (1)$$

where D_R is the diffusion coefficient of rutin ($\text{cm}^2 \text{s}^{-1}$), C_R^* is the bulk concentration of rutin (mol cm^{-3}), Q_{dl} is the capacitive charge, and $nFA\Gamma_R$ quantifies the faradic component given to the oxidation of the surface excess, Γ_R (mol cm^{-2}), of the bonded rutin. The inset shows the variation of Q versus $t^{1/2}$ obtained from chronocoulograms of Fig. 5. Based on equation (1), the intercept and slope of Q versus $t^{1/2}$ are $2nFAD_R^{1/2}C_R^*\pi^{-1/2}$ and $Q_{dl} + nFA\Gamma_R$ (interfacial components) respectively. Using the slopes of plots in Fig. 5, an average diffusion coefficient of $1.9 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for rutin was obtained. A usual application of chronocoulometry is to calculate surface excess of electroactive species, Γ_R . Hence, first it is necessary to separate these two interfacial components from each other. The value of $nFA\Gamma_R$ can

be calculated by comparing the intercept of plot Q versus $t^{1/2}$ obtained for a solution containing electroactive species (rutin in here) with the intercept resulted from the same experiment performed with supporting electrolyte only. The latter quantity is Q_{dl} for the background solution, and it can be used for estimating Q_{dl} of the solution containing rutin. We have carried out such studies at various rutin concentrations (inset of Fig. 5, plots 2-7) and one supporting electrolyte (inset of Fig. 5, plot 1) at an AGCE surface. Intercepts of Q versus $t^{1/2}$ (Fig. 5, inset) were used for calculating the average surface excess of the bonded rutin, Γ_R . As obtained, it is $5.8 \times 10^{-11} \text{ mol cm}^{-2}$ for $n=2$.

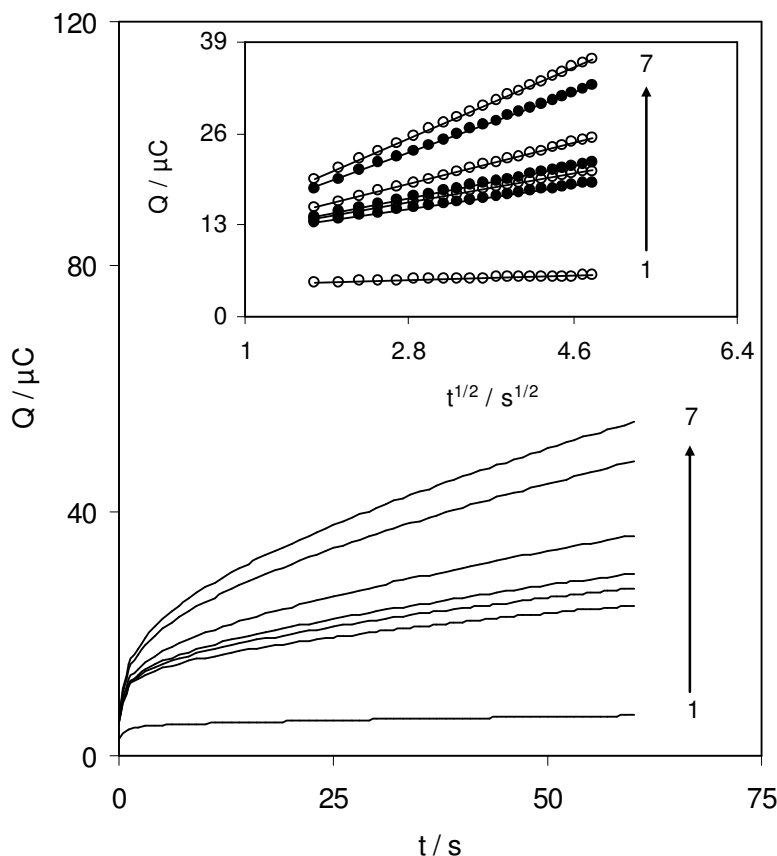


Figure 5. Chronocoulometric responses of an activated glassy carbon electrode in a 0.1 M phosphate buffer (pH 7.0) at potential step 240 mV for different concentrations of rutin. The numbers of 1 to 7 correspond to rutin concentrations of 0.0, 0.05, 0.06, 0.08, 0.13, 0.20, and 0.25 mM rutin, respectively. The inset shows the plots of Q versus $t^{1/2}$ obtained from the chronocoulograms. Lines are the best fits to the data.

3.2. Electrochemistry of bonded rutin at an activated glassy carbon electrode

Cyclic voltammetry responses of bonded rutin at an AGCE (RAGCE) in 0.1 M phosphate buffer solution (pH 7.0) at various scan rates are shown in Fig. 6A. The results show that the voltammetric peaks are typical of a surface-immobilized redox couple with a conditional formal potential, $E^{0'}$, value of 220 mV. The conditional formal potential, which was obtained from the

equation of $E^{0'} = E_{p,a} - \alpha(E_{p,a} - E_{p,c})$ [18] and taking $\alpha = 0.48$ (reported below), is almost independent of the potential scan rates below 1000 mV s^{-1} . Also, the peak currents of the RAGCE are those anticipated for

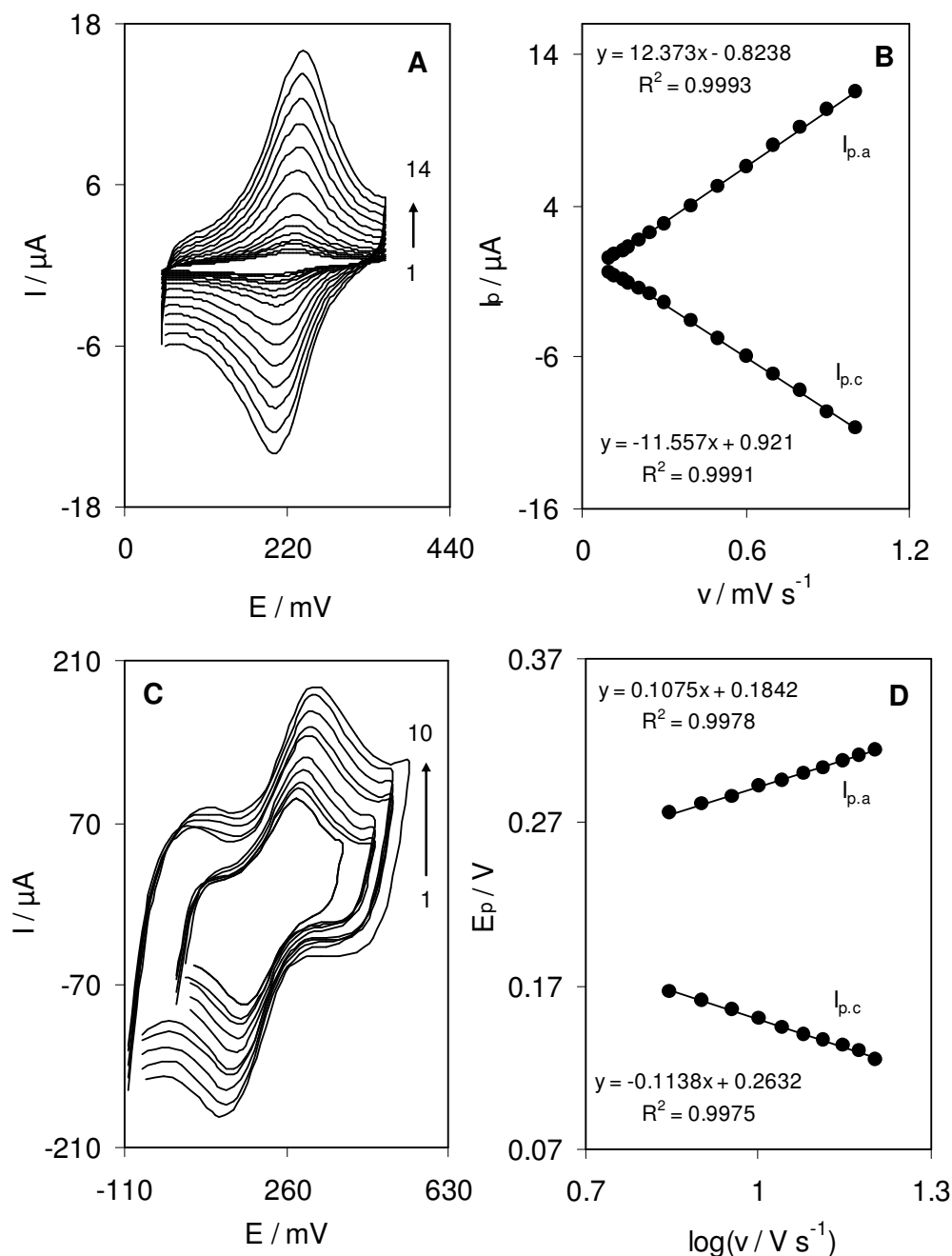


Figure 6. (A) Cyclic voltammetric responses of bonded rutin at an activated glassy carbon electrode in 0.1 M phosphate buffer (pH 7.0) at different scan rates. The numbers of 1 to 14 correspond to scan rates of 100, 120, 150, 170, 210, 250, 300, 400, 500, 600, 700, 800, 900, and 1000 mV s^{-1} , respectively. (B) Variations of the peak currents versus scan rates. (C) Cyclic voltammetric responses same as (A). The numbers of 1 to 10 correspond to scan rates of 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16 V s^{-1} . (D) Variations of peak potentials versus logarithm of the scan rate.

a surface-confined redox couple, because the peak currents were directly proportional to the scan rate (Fig. 6B). The peak-to-peak potential separation ($\Delta E_p = E_{p,a} - E_{p,c}$) is small for scan rates below 1000 mV s^{-1} (Fig. 6A) and something smaller than that was reported for rutin embedded in lipid cast film (DPPC-rutin film) at GCE [19]. These results suggest facile charge transfer kinetics over this range of scan rates. However, for scan rates above 1000 mV s^{-1} , the peak separation begins to increase, indicating the limitation arising from the charge transfer kinetics. Fig. 6C shows the cyclic voltammograms of an RAGCE in 0.1 M phosphate buffer (pH 7.0) and at scan rates ranging from 7 to 16 V s^{-1} . Within this range of the scan rates, as shown in Fig. 6D, the anodic and cathodic peak potentials are proportional to the logarithm of the scan rate and also, $n\Delta E_p = 2(E_{p,a} - E_{p,c})$ is bigger than 200 mV. Under these conditions, by using slopes of the plots of Fig. 6D and the values of ΔE_p corresponding to different scan rates, the charge transfer coefficient, α , and the charge transfer rate constant, k_s , between the electrode surface and the bonded rutin can be calculated according to the procedure of Laviron [20]. The calculated average values of α and k_s for the bonded rutin at the AGCE surface at pH 7.0 were about 0.48 and $98.0 \pm 2.0 \text{ s}^{-1}$ respectively. This value of k_s is almost twice as large as the charge transfer rate constant recently reported for the adsorbed rutin on the multi-wall carbon nanotubes (MWCNT) modified glassy carbon electrode [15]. This difference is probably due to the modification procedure of the working electrode surface. In the present work, during the modification of the AGCE, the hydroxyl groups on the activated surface of electrode [21] behave as nucleophiles against the *o*-quinone formed from rutin oxidation. This process leads to covalent bond formation between rutin and the surface active groups of GCE [22], while the deposition of rutin at the MWCNT modified GCE [15] could be considered as an adsorbed process. In other words, under new circumstances, the hydroxyl groups are partly blocked at an activated GCE surface by MWCNT. This inhibits the formation of the covalent bonds between rutin and the AGCE surface to same extent. Thus, k_s is higher for the bonded rutin than for the adsorbed rutin.

4. CONCLUSIONS

Electrochemical studies of rutin at a glassy carbon electrode have shown that, at an inactivated glassy carbon electrode (IGCE), the cyclic voltammograms have the characteristics of a species in solution while, at an activated glassy carbon electrode (AGCE), the voltammetric responses are typical of a surface-immobilized redox couple. The results of the cyclic voltammetry refer to the fact that the oxidation of rutin at an IGCE is an E_rC_i mechanism. Also, the voltammetric responses demonstrate that the subsequent chemical reactions in E_rC_i mechanism are dimerization and intramolecular reactions and hydroxylation reaction is slow on the characteristic time of cyclic voltammetry method. Some electrochemical parameters such as the diffusion coefficient (D) for rutin in solution, the surface excess of the bonded rutin at an AGCE, Γ_{rutin} , electron transfer coefficient (α), and the heterogeneous charge transfer rate constant (k_s) between an AGCE surface and the bonded rutin were obtained by analyzing the electrochemical data of the chronocoulometry and the cyclic voltammetry measurements. The results of the cyclic voltammetric responses gained through using Laviron theory

for oxidation of bonded rutin show that the heterogeneous charge transfer rate constant (k_s) at an AGCE is almost twice larger than those reported at a multi-wall carbon nanotubes (MWCNT) modified glassy carbon electrode.

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