

Electrochemical oxidation of morin and interaction with DNA

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Abstract

A poly (tetrafluoroethylene)-deoxyribonucleate acid (PTFE-DNA) film-modified glassy carbon electrode (GCE) has been fabricated. The electrochemical oxidation behaviors of morin as well as its interaction with DNA have been studied at PTFE-DNA film-modified GCE and bare GCE by electrochemical methods. This modified electrode shows an enhanced effectiveness towards the oxidation of morin. Importantly, as to the interaction between morin and DNA in solution, characteristic parameters such as the binding stoichiometry and association equilibrium constant according to the Hill model for cooperative binding have been determined on the basis of linear sweep voltammetry and chronocoulometry.

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1. Introduction

Over the past decade, the field of life science has witnessed an explosion of interest in using deoxyribonucleic acid (DNA) detection for all kinds of applications, such as epidemiology, oncology and genetics [1,2]. Because DNA contains all of the genetic information related to cellular function including DNA replication and gene expression, it plays a very important role in life processes. However, DNA molecules can be easily damaged under some conditions [3–6]. As far as it goes, recent trends in nucleic acid damage mainly focus on the interaction of DNA with other molecules, especially certain classes of drugs that may have potential therapeutic applications [7]. The interaction of DNA with drugs has been studied by various techniques including fluorescence [8], UV [9], luminescence [10], electrophoresis [11], NMR [12], quartz crystal microgravimetry [13,14] and electroanalytical methods [6,15–18].

Morin (2', 3, 4', 5, 7-pentahydroxyflavone) is one kind of flavonoids widely distributed in tea, coffee, cereal grains and a variety of fruits and vegetables [19] (Scheme 1). It has aroused

considerable interest due to their broad pharmacological activity. In fact, flavonoids have been found to have antiviral, antiallergic, anticancer and antitumor activities, and possibly even protective effects against chronic diseases [20,21]. As far as morin is concerned, it is endowed with anti-oxidant properties shown to protect cells against the oxygen radical damage [22]. It cannot only scavenge free radicals, but also inhibit xanthine oxidase moderately [23]. The literatures on the electrochemistry of flavonoids are limited, but interaction of morin or its complexes with DNA has been reported recently using fluorescence and electrochemical methods [24,25]. However, the electrochemical behaviors of morin and its interaction with DNA by electrochemical methods have not been investigated in detail. Therefore, in our work, a novel poly (tetrafluoroethylene)-deoxyribonucleic acid (PTFE-DNA) modified GCE was used to study the electrochemical behaviors of morin. PTFE is a well-known hydrophobic material [26–28] and frequently used as an electrode modifier. Here, PTFE was used to immobilize DNA molecules on the electrode surface and the results are satisfying. The electrochemical parameters of morin, binding number, association constant and the possible binding mode with DNA have been obtained using various electrochemical methods. Interacting with DNA morin forms one kind of supramolecular complex which has no electrochemical activity and cannot be subsequently oxidized at the electrode surface.

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2. Experimental

2.1. Apparatus and reagents

All the electrochemical measurements were performed on a CHI 830 electrochemical analyzer (Shanghai Chenhua Co., China) in a three-electrode system. The working electrode was a PTFE-DNA film coated GCE. A Pt wire and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively.

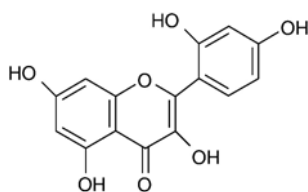
Morin (obtained from Sigma, USA) stock solution was prepared by dissolving it in ethanol–water solution at a concentration of $[\text{morin}] = 1.00 \times 10^{-2}$ mol/L. The solution was stored at 4 °C in dark to avoid any decomposition. Calf thymus DNA (CT DNA, obtained from Sigma, USA) was dissolved in water to form 1.0 mg/mL stock solution and stored at 4 °C. This solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein. The concentration of DNA solution, expressed in moles of base pair ($[\text{BP}] = (3.20 \pm 0.01) \times 10^{-3}$ mol/L), was determined by UV absorbance at 260 nm using the molar extinction coefficient (ϵ) of $13200 \text{ M}^{-1} \text{ cm}^{-1}$. Other chemicals used were analytical reagents. $\text{Co}(\text{phen})_3(\text{ClO}_4)_3 \cdot 3\text{H}_2\text{O}$ was synthesized according to the scientific literature [29] and dissolved in water to form 50.0 $\mu\text{mol/L}$ standard solution containing 5.0 mmol/L NaCl. All the chemicals were used without further purification and all the solutions were prepared with doubly distilled water.

2.2. Preparation of the PTFE-DNA film coated GCE

0.1 mL 1.2% PTFE suspension and 0.1 mL DNA were mixed together. Prior to modification, the GCE ($A = 0.071 \pm 0.001 \text{ cm}^2$) was mechanically polished with polishing microcloth containing 0.05 μm Al_2O_3 slurry to a mirror finish, and then carefully cleaned in 1:1 HNO_3 – H_2O (v/v) and ethanol, water in turn via ultra-sonication each for 2 min. At last, 5 μL of the PTFE-DNA suspension was cast on the GCE surface and dried. Then a stable and uniform PTFE-DNA film was formed. The modified electrode was dipped in water for several hours and then dried. In the same way, DNA film without PTFE or PTFE film without DNA coated GCE was obtained.

2.3. Electroanalytical procedure

A certain volume of phosphate buffer solution ($\text{pH} = 7.28$) was used as the supporting electrolyte in a conventional electrochemical cell. At the beginning of experiment, 5 μL of



Scheme 1. The structure of morin.

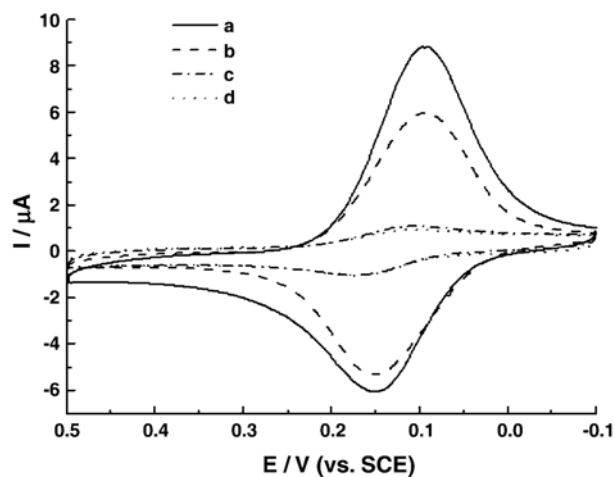


Fig. 1. Cyclic voltammograms of the $[\text{Co}(\text{phen})_3]^{3+}$ at PTFE-DNA film-coated GCE (curve a), DNA film-modified (curve b), bare GCE (curve c), and PTFE film-modified GCE (curve d). Scan rate, 100 mV/s.

1.00×10^{-2} mol/L stock solution of morin were placed into the cell to make up 5 mL mixture solution at a morin concentration of $[\text{morin}] = (1.00 \pm 0.01) \times 10^{-5}$ mol/L. The voltammograms were recorded with cyclic potential scan between -0.10 V and 0.50 V .

3. Results and discussion

3.1. Characterization of the PTFE-DNA film coated GCE

The electrochemical behaviors of the $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ were used as an electrochemical probe to characterize different electrodes including bare GCE, PTFE film modified GCE, DNA film modified GCE and PTFE-DNA film coated GCE. A couple of reversible redox peaks are observed at 0.24 V and 0.16 V at bare GCE due to the electrochemical responses of $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$. When a certain volume of PTFE suspension or DNA solution was cast on the surface of GCE, the peak currents of $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ decrease apparently and the potential difference between cathode and anode increase accordingly. Firstly, this result may be attributed to the negatively charged DNA molecules and PTFE molecules with long hydrophobic chains. Secondly, as unconductive molecule DNA self can block redox reaction so that the peak currents decrease consequentially. Because of the cooperative effects of DNA and PTFE the reversible electrochemical responses of $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ at PTFE-DNA film coated GCE almost disappear and the separation between the oxidation and the reduction is enlarged greatly. On the contrary, the other electrochemical probe $[\text{Co}(\text{phen})_3]^{3+}$ has totally different electrochemical responses at these electrodes mentioned above. The corresponding data are displayed in Fig. 1. Curve c of Fig. 1 represents the reversible electrochemical responses of $[\text{Co}(\text{phen})_3]^{3+}$ at bare GCE. A couple of redox peaks appear at 0.17 V and 0.11 V. Similar results are obtained at PTFE modified GCE (curve d), but because of the repulsive effect of long chain of PTFE molecules the peak currents reduce to a smaller extent. Compared the peak currents of $[\text{Co}(\text{phen})_3]^{3+}$ at bare GCE with those at the DNA film modified GCE (curve b) and PTFE-DNA

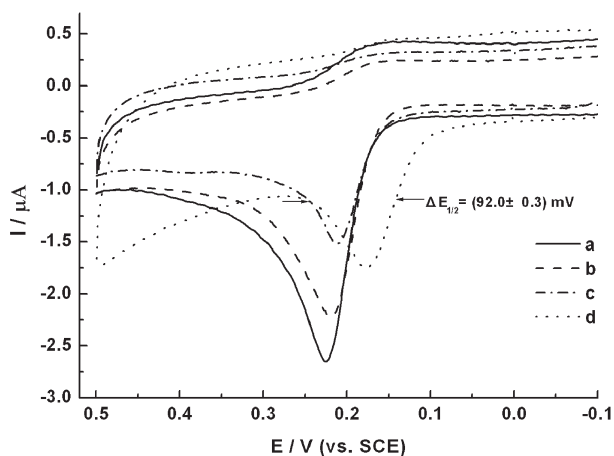


Fig. 2. Electrochemical responses of 1.0×10^{-5} mol/L morin at PTFE-DNA film-coated GCE (curve a), DNA film-modified (curve b), PTFE film-modified GCE (curve c) and bare GCE (curve d). Scan rate, 100 mV/s.

film coated GCE (curve a), the peak currents at bare electrode (see curve c of Fig. 1) decrease markedly. Moreover, there is a fact that the peak potentials shift negatively. It may be mainly caused by the electrostatic attraction of negatively charged DNA to positively charged $[\text{Co}(\text{phen})_3]^{3+}$ in solution with small ion intensity referring to appropriate references [15,30,31]. Furthermore, the current of cathodic peak is larger than that of anodic peak, which indicates that some intercalation effect exists between DNA and PTFE. It is consistent with other workers' reports [32,33]. At PTFE-DNA film coated GCE the peak currents are higher than those at DNA film coated GCE. This is in good agreement with the recent electron transfer studies through mixed monolayers at the electrode surface modified by polymer [34]. Maybe because the double helix of DNA molecules dispersed by PTFE molecules in the PTFE-DNA film are exposed mostly to $[\text{Co}(\text{phen})_3]^{3+}$ compared with that in DNA film adsorbed to the surface of GCE. All results show that DNA molecules have been well immobilized with PTFE molecules on the surface of GCE.

3.2. Studies on the electrochemical behaviors of morin

The electrochemical behaviors of morin were investigated on a bare GCE (curve d), PTFE film modified GCE (curve c), DNA film modified (curve b) GCE and PTFE-DNA film coated GCE (curve a) by cyclic voltammetry (CV). The cyclic voltammograms are illustrated in Fig. 2. It is not difficult to believe that the oxidation of morin at these electrodes is totally irreversible under these experimental conditions. PTFE can block the electron transfer between morin and the electrode surface, and then decrease the oxidation peak current of morin. So the peak current in curve c of Fig. 2 is smaller than that in curve d and the peak potential shifts positively. However, it is the interaction of morin with DNA immobilized on the GCE surface that make the peak currents in curves a and b higher than those in curves c and d. The positive shift of the peak potential indicates that the binding of morin to DNA is via electrostatic interaction. As for the difference between a and b, it may be explained as being due

to the fact that DNA molecules dispersed in the PTFE-DNA film are easier to interact with morin than DNA film directly adsorbed to the surface of GCE.

3.2.1. The electrochemical behaviors of morin at PTFE-DNA film-coated GCE

Fig. 2 demonstrates that the electrode reaction of morin is an irreversible process at PTFE-DNA film-modified GCE. In order to get more information about its reaction mechanism the effects of buffer pH were tested by linear sweep voltammetry (LSV). The positive oxidation peak potential decreases with increasing solution pH and obeys the following equation in the range of pH from 4.0 to 9.0 in phosphate buffer: $E_p = 0.579 \text{ V} - (0.049 \text{ pH}) \text{ V}$.

In all the cases for straight lines in this work the correlation coefficients are >0.99 . With the increase of solution pH the oxidation peak current decreases firstly as shown in Fig. 3 and then keeps stable, finally depresses (inset in Fig. 3). Since E_p can be expressed as (at 25°C):

$$E_p = E^0 - (m/n) 0.059 \text{ V} \quad (1)$$

where E^0 is the standard potential, n is the number of electrons transferred and m is the number of protons transferred in the electrooxidation process of morin. Thus the value of $m/n = (0.9 \pm 0.1)$ was obtained based on the linear section. This value can be accepted as 1.0 and suggests that the number of the electrons transferred in the oxidation of morin at PTFE-DNA film-modified GCE is equal to that of protons.

The slopes derived from the relationships of the peak potential or peak current and the scan rates exhibit different characteristics in Fig. 4. The oxidation peak current is linear with the square root of the scan rate ν (V/s), following equation below: $I_p = -3.597 \mu\text{A} + \{16.82 [\nu(\text{V/s})]^{1/2}\} \mu\text{A}$, suggesting that the electrode process is controlled by the diffusion step. Corresponding fitting lines for the relationship between the peak potential and scan rate (V/s) can be obtained as $E_p = 0.249 \text{ V} + \{0.015 \ln [\nu(\text{V/s})]\} \text{ V}$.

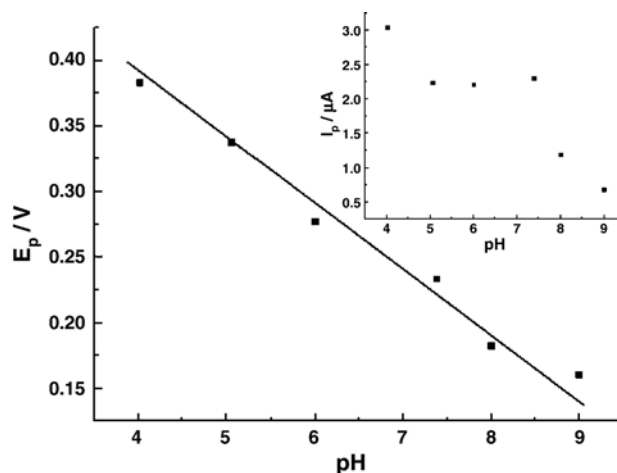


Fig. 3. Effects of solution pH on the peak potential E_p for the oxidation of 1.0×10^{-5} mol/L morin at PTFE-DNA film-modified GCE in phosphate buffer. Inset: variation of the peak current I_p , with different pH.

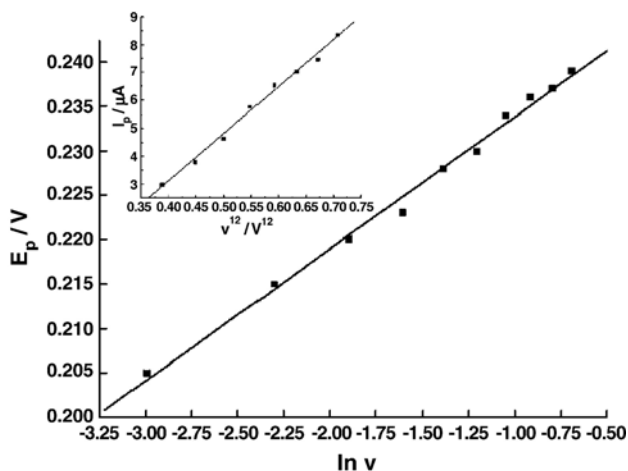


Fig. 4. The dependence of oxidation peak current of 1.0×10^{-5} mol/L morin on scan rate at PTFE-DNA film-coated GCE in phosphate buffer solution (pH=7.28). Inset: variation of the peak current I_p , with different scan rates.

Consequently, based on the slope of $RT/2\alpha n_a F$, the value of αn_a for electrochemical oxidation of morin at the concentration of $[\text{morin}] = (1.00 \pm 0.01) \times 10^{-5}$ mol/L at PTFE-DNA film coated GCE in phosphate buffer solution (pH=7.28) can be calculated as 0.9 ± 0.1 .

According to Laviron [35], as for a totally irreversible peak the width at mid-height of the anodic peak, it can be parametrized as $\Delta E_{1/2} = [62.5/(1-\alpha)n]$ mV. From the curve d in Fig. 2 the width of anodic peak of morin is (92.0 ± 0.3) mV, then considering the above result the value of n is obtained as 1.6 ± 0.1 .

3.2.2. The electrochemical behaviors of morin at bare GCE

Influences of scan rate on the oxidation current and peak potential of morin at bare GCE were estimated. The peak currents increase linearly with increasing the scan rate that ranged from 0.10 V/s to 0.50 V/s at bare GCE in phosphate buffer solution (pH=7.28) containing $(2.00 \pm 0.02) \times 10^{-5}$ mol/L morin in the absence of DNA and can be expressed as follows: $I_p = 1.323 \mu\text{A} + \{7.215 [v/(\text{V/s})]\} \mu\text{A}$.

Thus, the electrode process is controlled by the adsorption step. With increase of scan rate, the relationship between the reduction peak potential and scan rate (V/s) is described by the following equation: $E_p = 0.238 \text{ V} + \{0.021 \ln [v/(\text{V/s})]\} \text{ V}$.

Based on Nernst equation:

$$E = E^0 + (RT/nF) \ln [C_O(0, t)/C_R(0, t)] \quad (2)$$

C_O and C_R being the concentration of the electroactive species (oxidized and reduced forms) at the electrode surface, and Laviron's theory for diffusionless electrochemical systems:

$$E_p = E^j - (RT/\alpha n_a F) \ln [\alpha/|m|]$$

$$m = (RT/F)(k_s/n_a v) \quad (3)$$

E^j being equal to E'^o ,

$$E^b = E^o - (RT/nF) \ln (b_O/b_R) \quad (4)$$

b_O and b_R being the adsorption coefficients of oxidized and reduced forms for the irreversible surface electrochemical reaction involving adsorption of oxidized and reduced forms, and then the relationship between scan rate v and peak potential E_p can be expressed by the following equation [35]:

$$E_p = E^b + (RT/\alpha n_a F) \ln [(RTk_s/\alpha n_a F)/(V/s)] - (RT/\alpha n_a F) \ln [v/(V/s)] \quad (5)$$

where R is the universal gas constant ($8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), T is Kelvin temperature, F is the Faraday constant ($96,487 \text{ C/mol}$), α is the transfer coefficient, k_s , the rate constant of the electrochemical reaction, n_a and n are the numbers of electrons transferred in the rate-limiting step and whole reaction, respectively. E^o is the standard potential for the redox reaction, and E'^o is the formal potential which can be obtained from the intercept of the E_p vs. v curve by extrapolation to the vertical axis at $v=0.00$ (V/s) [36]. Thus from the slope and intercept of the E_p vs. $\ln v$ plot, the values of αn_a and k_s , can be calculated.

The value of αn_a for electrochemical oxidation of morin at bare GCE in phosphate buffer solution (pH=7.28) without DNA is obtained as $\alpha n_a = 1.2 \pm 0.1$. On the assumption that the value of α for an irreversible surface reaction, the numbers of electron involved in the oxidation of morin in phosphate buffer solution (pH=7.28) is 2.0 ± 0.4 .

According to the Eq. (5), the value of k_s for morin oxidation in phosphate buffer solution (pH=7.28) is $k_s(\text{GCE}) = 260 \text{ s}^{-1}$.

The effects of solution pH on the oxidation peak current of morin at bare GCE were researched, too. The fitting line is obtained as $E_p = 0.581 \text{ V} - (0.053 \text{ pH}) \text{ V}$ from pH=4.0 to pH=9.0 in phosphate buffer. With the increase of pH the oxidation peak current of morin firstly increases slowly up to pH=5 and then decreases rapidly.

According to the Eq. (1), $m/n = 0.9 \pm 0.1$ is obtained for the linear section, which can be accepted as 1.0, suggesting that the number of the electrons transferred in the oxidation of morin at bare GCE is equal to that of protons.

3.2.3. Adsorption of morin measured by chronocoulometry

The method of chronocoulometry was applied to characterize the adsorption of morin at bare GCE in phosphate buffer solution (pH=7.28) and determine the diffusion coefficient D and Q_{ads} containing $(1.00 \pm 0.01) \times 10^{-5}$ mol/L morin, according to the formula given by Anson [37].

$$Q(C) = 2nFACD^{1/2}\pi^{-1/2}t^{1/2} + Q_{\text{dl}} + Q_{\text{ads}} \quad (6)$$

where n is the number of electrons transferred in reaction, F is the Faraday constant ($96,487 \text{ C/mol}$), A is the surface area of the working electrode, c is the concentration of morin, D is the diffusion coefficient of morin in phosphate buffer, t is time, Q_{dl} is double-layer charge and Q_{ads} is the faradaic charge due to the oxidation of adsorbed morin. In our experiment, the effects of double-layer charge Q_{dl} can be eliminated via subtraction of the background charge and the plots of Q (μC) against t (s) in Fig. 5 are converted into the plots of Q against $t^{1/2}$, which is depicted in

Fig. 5. It is clear that the charges (Q) have linear relationships with the square roots of time ($t^{1/2}$) for the reduction reaction (inset in Fig. 5). According to the Eq. (6), the diffusion coefficient of morin in phosphate buffer solution (pH=7.28) can be estimated from the slope of the plot of Q vs. $t^{1/2}$. In present work, [morin] = $(1.00 \pm 0.01) \times 10^{-5}$ mol/L, and slope is $(0.18 \pm 0.01) \mu\text{C/s}^{1/2}$. Based on the above conclusion that the value of n is 2.0 ± 0.0 , it is calculated that $D(\text{morin}) = (1.36 \pm 0.01) \times 10^{-6} \text{ cm}^2/\text{s}$. Q_{ads} can be obtained by the difference of the intercepts of the plot of Q vs. $t^{1/2}$ in the presence and absence of morin. Here Q_{ads} is $(0.13 \pm 0.01) \mu\text{C}$, considering the equation

$$Q_{\text{ads}} = nFA\Gamma \quad (7)$$

the value of surface concentration corresponding to a monolayer at the electrode surface, Γ , can be obtained as $(9.49 \pm 0.01) \times 10^{-12} \text{ mol/cm}^2$.

3.3. Interaction of morin with DNA in solution

3.3.1. Electrochemical oxidation of morin in the presence of DNA

Fig. 6 displays linear sweep voltammograms of morin at [morin] = $(2.00 \pm 0.02) \times 10^{-5}$ mol/L in the absence (curve a) and presence (curve b) of $(1.14 \pm 0.01) \times 10^{-4}$ mol/L BP and (curve c) $(2.28 \pm 0.02) \times 10^{-4}$ mol/L BP at bare GCE in phosphate buffer solution (pH=7.28). It is clear that the oxidation peak current of morin decreases obviously after the addition of DNA, and the oxidation potential has not apparently change and no new peaks are found when the potential initially sweeps from -0.20 V to 0.50 V.

The peak currents increase linearly with increase of the scan rate ranging between 0.10 V to 0.50 V at bare GCE in phosphate buffer solution (pH=7.28) containing $(2.00 \pm 0.02) \times 10^{-5}$ mol/L morin in the presence of $(3.42 \pm 0.01) \times 10^{-4}$ mol/L BP (Fig. 7), and can be expressed as follows: $I_p = 1.705 \mu\text{A} + \{7.771[v/(\text{V/s})]\} \mu\text{A}$.

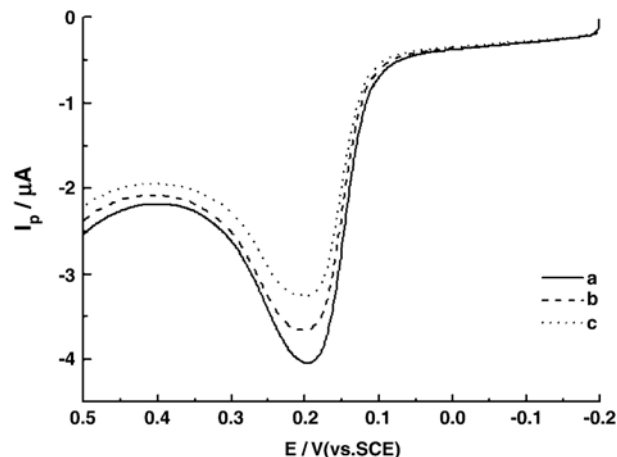


Fig. 6. Linear sweep voltammograms for 2.0×10^{-5} mol/L morin in the absence (curve a) and presence of 1.14×10^{-4} mol/L BP (curve b) and 2.28×10^{-4} mol/L BP (curve c) at bare GCE in phosphate buffer solution (pH=7.28).

Thus, the electrode process is controlled by the adsorption step. With increase of scan rate, the relationship between the oxidation peak potential and scan rate is described by the following equations: $E_p = 0.246 \text{ V} + \{0.024 \ln[v/(\text{V/s})]\} \text{ V}$.

The value of αn_α for electrochemical oxidation of morin at bare GCE in phosphate buffer solution (pH=7.28) in the presence of DNA is obtained as 1.1 ± 0.1 . On the assumption that the value of α for an irreversible surface reaction, then $n = 2.0 \pm 0.1$.

According to the Eq. (5), the value of k_s for morin oxidation in phosphate buffer solution (pH=7.28) in the presence of DNA is $k_s(\text{DNA}) = 251 \text{ s}^{-1}$. As the peak currents decreased the k_s decreased lightly [38,39], but it is all the same close to the value of $k_s(\text{GCE})$. These results suggest that the electrochemical parameters of morin oxidation have no obvious change in the presence or absence of DNA. It is concluded that it is morin but not DNA–morin complex taking part in the electrode process. The coherence of the calculated values of k_s and αn_α for morin oxidation in absence and

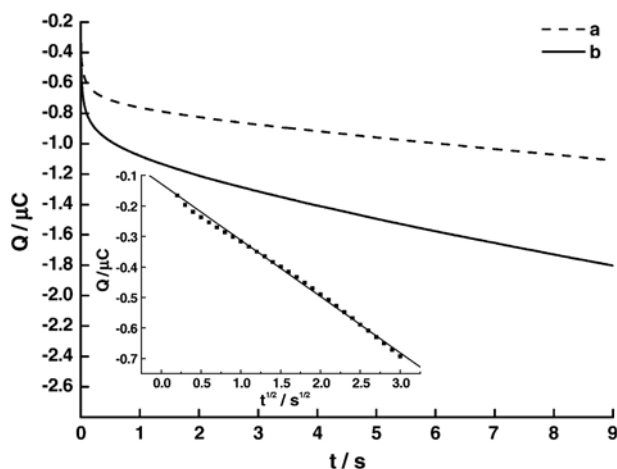


Fig. 5. Chronocoulometry at bare GCE in the absence (curve a) and presence of 1.0×10^{-5} mol/L morin (curve b). The inset shows the linear relationship between the charges (Q) and the square roots of times ($t^{1/2}$) for the oxidation of morin (background subtracted). Initial potential 0.0 V, final potential 0.4 V, and pulse width 9 s.

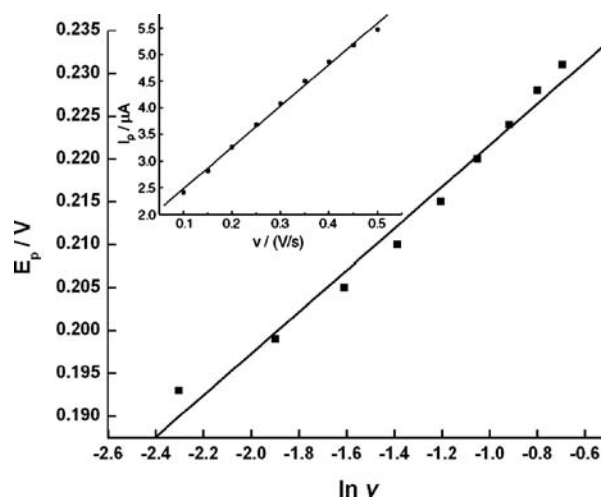
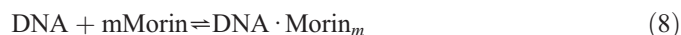


Fig. 7. The dependence of oxidation peak current on scan rate at bare GCE in phosphate buffer solution (pH=7.28) containing 2.0×10^{-5} mol/L morin and 3.42×10^{-4} mol/L BP. Inset: variation of the peak current I_p , with different scan rates.

presence of DNA suggests that no electroactive complex forms between morin and DNA. It is difficult for morin in the complex to make contact with the electrode surface and subsequently be oxidized at that surface. Furthermore, the formation of this complex reduces the free concentration of morin in solution, and thereby the electrochemical oxidation peak current of morin decreases as a result of partitioning behavior of the electrochemical probe in the DNA solution or aggregates [39–47].

3.3.2. Determination of the association constant and binding number between morin and DNA

According to the method of Qu et al. [48], it is assumed that DNA and morin only produce a single complex $\text{DNA} \cdot \text{Morin}_m$. The stoichiometric coefficient, m , and association constant, K_a , between morin and DNA refer to the reaction scheme (8) for all-or-none (Hill) cooperativity of multiple ligand binding:



The condition of association constant is as follows:

$$K_a \cdot [\text{Morin}]^m = \frac{[\text{DNA} \cdot \text{Morin}_m]}{[\text{DNA}]} = \frac{f}{1-f} \quad (8.1)$$

where $f = [\text{DNA} \cdot \text{Morin}_m] / [\text{DNA}]_0$ is the fraction of DNA to which morin is bound as $\text{DNA} \cdot \text{Morin}_m$, relative to the total DNA concentration in the supporting electrolyte $[\text{DNA}]_0 = [\text{DNA} \cdot \text{Morin}_m]_{\text{max}}$. Mass conservation dictates that:

$$[\text{DNA}] = [\text{DNA}]_0 - [\text{DNA} \cdot \text{Morin}_m]$$

$$[\text{Morin}] = [\text{Morin}]_0 - m[\text{DNA} \cdot \text{Morin}_m] \quad (8.2)$$

and

$$I = k \cdot [\text{Morin}], \quad (8.3)$$

$$\Delta I = I(\text{Morin}_0) - I(\text{Morin}) \quad (8.4)$$

where $[\text{Morin}]$ is the free concentration of morin and $I(\text{Morin})$ is the peak current of morin in the presence of DNA. Insertion of Eqs. (8.2) and (8.3) into (8.4) yields:

$$\Delta I = k([\text{Morin}]_0 - [\text{Morin}]) = k \cdot m \cdot [\text{DNA} \cdot \text{Morin}_m] \quad (8.5)$$

and

$$\Delta I_{\text{max}} = k \cdot m \cdot [\text{DNA}]_0 \quad (8.6)$$

where ΔI_{max} is the maximum peak current change, obviously, $[\text{DNA} \cdot \text{Morin}_m]_{\text{max}} = [\text{DNA}]_0$ holds true. Based on the equations above, the following equations can be deduced:

$$\log(\Delta I / \Delta I_{\text{max}} - \Delta I) = \log K_a + m \log\{[\text{Morin}] / (\text{mol/L})\} \quad (8.7)$$

$$1 / \Delta I = 1 / \Delta I_{\text{max}} + (1 / K_a \cdot \Delta I_{\text{max}}) \times (1 / [\text{Morin}]^m) \quad (8.8)$$

The corresponding experimental data are shown in Fig. 8. The $\log \{\Delta I / \Delta I_{\text{max}} - \Delta I\}$ vs. $\log \{[\text{Morin}] / (\text{mol/L})\}$ becomes linear with the slope of m . The results of $m=3$ and $\log K_a=14.8$ were obtained from Fig. 8, which means that only one compound is

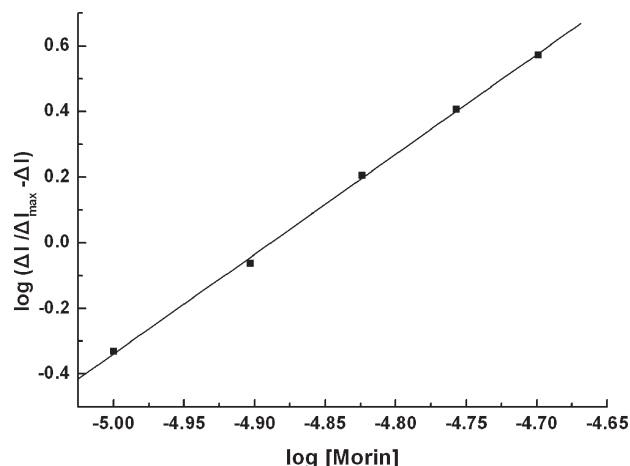


Fig. 8. Dependence of $\log [\Delta I / \Delta I_{\text{max}} - \Delta I]$ on $\log [\text{Morin}]$.

formed. Or according to $K_a = K_H^m$ and $K_H \approx 10^5 \text{ L/mol}$, $K_a = 10^{15} (\text{L/mol})^3$ can be yielded. So, at $K_H = [\text{Morin}]_{0.5} \approx 10^5 \text{ mol/L}$ (Morin), half of the binding sites on DNA are occupied by morin.

4. Conclusion

Morin is an important flavonoid. In our paper, the electrochemical behaviors of morin at bare and PTFE-DNA film modified GCE were investigated. Based on the experimental results, the electrode reaction mechanisms of morin at bare GCE and PTFE-DNA film modified GCE in the absence and presence of DNA were proposed. Electrochemical oxidation of morin exhibits a one-step mechanism involving the loss of two electrons and two protons in both two cases. In sequence, corresponding electrochemical parameters were calculated including diffusion coefficient of morin, transfer coefficient and standard rate constant. Most important of all, the interaction of morin with DNA was studied and the binding number and association constant were obtained. It is helpful for us to understand pharmacokinetics of morin.

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