ORIGINAL PAPER

Chemically modified glassy carbon electrode for electrochemical sensing paracetamol in acidic solution

Guocheng Yang · Lu Wang · Jianbo Jia · Defeng Zhou · Dongfeng Li

Abstract Different organic molecules were covalently grafted on glassy carbon electrodes (GCEs) by an electrochemical reduction or potentiostatic process of several in situ-generated diazonium cations in acidic aqueous solution containing $NaNO_2$. The cyclic voltammetry implemented in 0.1 M KCl aqueous solution containing 5 mM $Fe(CN)_6^{3-}$ or $Ru(NH_3)_6^{3+}$ confirmed the blocking properties of the modified GCEs. The electrochemical impedance spectroscopy (EIS) performed in 0.1 M KCl aqueous solution containing 5 mM $Fe(CN)_6^{3-/4-}$ was used to measure the surface coverage of the modifiers on GCE; the results showed that the modified layers on GCEs are very compact. The linear sweep voltammetry (LSV) was employed to investigate the electrochemical sensing properties of the bare and modified GCEs toward paracetamol (PCT) in sulfuric acid solution of pH 1.02, and the corresponding calibration plots were obtained, respectively. The results indicated there is an oxidation peak of PCT in the linear sweep voltammograms on the bare and modified GCEs with the active terminal groups such as $-OPO_3H_2$, $-SO_3H$, $-COOH$, and so on, but do not appear on GCEs modified with the inert terminal groups such as $-NO_2$ and $-Br$. These imply that the GCEs modified with the active terminal groups display an electrochemical behavior like bulk GCE; however, those with the inert terminal groups present an electrochemical behavior like microelectrode. The varying electrochemical sensitivity of all the electrodes toward PCT was explained according to electronegativity and pK_a of the terminal groups of the modifiers on the electrodes and hydrogen bond between the modifiers and PCT. Apparent standard rate constants of PCT oxidation reaction on the bare and modified GCEs were obtained from the Laviron's approach.

Keywords Carbon electrode · Organic modifier · Covalent bonding · Paracetamol · Hydrogen bond · Electronegativity

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Introduction

Chemically modified electrode (CME) is one of the most active parts in the field of the electroanalytical and electrocatalytical chemistry. Its emergence broke the traditional electrochemical study, which was limited to the range of the bare electrode–electrolyte interface, and created a new research area, that is the structures and functions of the electrode surfaces can be artificially tailored [1].

After the original electrodes were physically or covalently grafted with different modifiers such as organic molecule, inorganic species, metal nanoparticles, polymer or biomacromolecules to tailor its surfaces forming monofilm, multilayer film, or array, etc., the selectivity, sensitivity, stability, and repeatability of the original electrodes may be promoted greatly [2–4]. These CMEs can be universally utilized to analyze the active ingredients of drug, heavy metal, organic pollutions, antiseptics, food additives, protein, DNA, and so on. Moreover, CMEs also can reveal fully their existing advantages in drug analysis such as pharmacological analysis, clinical pharmacology, efficacy analysis, valid identification, and pharmacokinetics [5–7].

Among the numerous preparation methods of CMEs, the modification of carbon substrate by electrochemical reduction

of in situ-generated diazonium cations attracted a lot of interest in recent years [8–14]. This is a straightforward derivatization method involving widely available amines, which can be covalently grafted on glassy carbon electrode (GCE) [15]. Using this method, the modified GCEs are able to withstand sonicating, the reiterant scanning over a wide electrochemical window and deactivating in air [16–18]. It is commonly accepted as a very versatile and simple way to graft a wide variety of functional groups on carbon substrate for various applications [19–24].

Paracetamol (PCT) is a widely used antipyretic and analgesic drug. It is an effective substitute that is applied to reduce fever, cough, colds, and moderate pain including tension headache, migraine headache, muscular aches, backache, and toothache [25–28]. Generally, PCT shows positive effects when being used limitedly. However, overdose or chronic use of PCT will produce toxic metabolite accumulation that cause kidney and liver damage [29–32]. So, the detection of PCT possesses the extremely vital significance. Among the various detection methods of PCT, the electrochemical one exhibits simple, fast, sensitive, and accurate characteristics and it is widely studied and employed recently [33–38].

In this paper, the electrochemical reduction of in situ-generated diazonium cations was applied to modify GCE surface covalently forming the stable CMEs. The electrochemical sensing properties of the bare and modified GCEs with different terminal groups toward PCT were investigated in sulfuric acid solution of pH 1.02 comparatively. The differences of electrochemical sensitivity of the electrodes toward PCT were reasonably explained according to electronegativity and pK_a of the terminal groups on the electrodes and hydrogen bond between modifiers and PCT.

Experimental

Reagents

4-Aminobenzylphosphonic acid (4-ABPA), 4-nitrophenyl phosphate disodium salt hexahydrate (4-NPP), 4-aminobenzenesulfonic acid (4-ABSA), 4-aminobenzoic acid (4-ABA), 3-aminobenzoic acid (3-ABA), 4-aminophenol (4-AP), 3-aminophenol (3-AP), 4-aminopyridine (4-APD), 4-bromoaniline (4-BA), and 4-nitroaniline (4-NA) were bought from Sigma-Aldrich Corporation. PCT was purchased from Johnson Matthey Company. All other reagents were of analytical reagent grade and used as received without further purification. The solution of each chemical above-mentioned was freshly prepared for each modification. Ultrapure water produced by Millipore Milli-Q purification system was used throughout the experiments. The buffer solution was prepared by 0.1 M H_2SO_4–Na_2SO_4 (pH 1.02).

Apparatus

All the electrochemical experiments were carried out in an electrochemical workstation (CHI 852C, USA) with a self-prepared three-electrode glass-based electrochemical cell, which was consisted of a bare GCE (3 mm diameter, CHI 104, USA) or modified one as the working electrode, a twisted platinum wire as the counter electrode and an Ag/AgCl electrode (KCl saturated) as the reference electrode. The temperature of the electrochemical cell was controlled by a super thermostat with the temperature fluctuation ±0.05 °C (Shanghai Cany Precision Instrument Co., Ltd, China). The EIS was performed with an advanced electrochemical system (PARSTAT 2273, Ametek, USA) at room temperature.

Electrodes modification

Before the electrochemical modification, the bare GCE was orderly polished with 1.0-, 0.3-, and 0.05-μm alpha alumina powder on a polishing cloth, sonicated in ultrapure water, and then rinsed thoroughly with ultrapure water, finally dried with high purity nitrogen stream. The surface modification of a bare GCE was performed through the electrochemical reduction (0.7 to –0.5 V) or potentiostatic process (−0.7 V) in a solution containing 1 mM diazonium salt, which is generated in situ during reaction of the modified mixture composed of 800 μL 4-NPP (5 mM) or an amine solution (5 mM), 80 μL $NaNO_2$ (0.1 M), 170 μL HCl (12 M) and 2.95 mL H_2O for about 5 min at room temperature [8, 15]. During the reaction, 4-ABPA, 4-NPP, 4-ABSA, 4-ABA, 3-ABA, 4-AP, 3-AP, 4-APD, 4-BA, and 4-NA will be converted to following diazonium: 4-ethylphosphonicacidphenyldiazonium, 4-phosphatephenyldiazonium (4-OPO_3H_2-PD), 4-sulfophenyldiazonium, 4-carboxyphenyldiazonium (4-COOH-PD), 3-carboxyphenyldiazonium, 4-hydroxyphenyldiazonium, 3-hydroxyphenyldiazonium, 4-diazoniumpyridine, 4-bromophenyldiazonium, and 4-nitrophenyldiazonium, respectively. Subsequently, the respective 4-ethylphosphonicacidphenyl (4-$CH_2PO_3H_2$-P)-, 4-phosphatephenyl (4-OPO_3H_2-P)-, 4-sulfophenyl (4-SO_3H-P)-, 4-carboxyphenyl (4-COOH-P)-, 3-carboxyphenyl (3-COOH-P)-, 4-hydroxyphenyl (4-OH-P)-, 3-hydroxyphenyl (3-OH-P)-, 4-pyridyl (4-PD)-, 4-nitrophenyl (4-NO_2-P)-, and 4-bromophenyl (4-Br-P)-modified GCEs can be obtained. After the modification, the electrodes were rinsed thoroughly with water and sonicated in water for 30 s. Then 5 mM $K_3Fe(CN)_6$ or $Ru(NH_3)_6Cl_3$ dissolved in 0.1 M KCl aqueous solution as redox probes was used to measure the blocking properties of the bare and modified GCEs. When measuring EIS of the bare and modified GCEs, an AC voltage of 10 mV in amplitude with a frequency range from 0.1 Hz to 100 kHz was superimposed on the DC potential and applied to the studied electrodes. The DC potential was always set up at the formal potential of $Fe(CN)_6^{3-/4-}$. The experimental data of

the electrochemical impedance plot were analyzed by applying the nonlinear least squares fitting to the theoretical model represented by a Randles equivalent electrical circuit attached by PARSTAT 2273.

According to previous reports [8–16], the production of an aryl diazonium cation giving the corresponding aryl radical, which can further react with a carbon atom of the carbon substrate to yield the covalent bonding of this aryl group, can be shown in Scheme 1.

Procedure for electrochemical sensing paracetamol

The bare and modified GCEs were employed to sense PCT comparatively. A sulfuric acid solution (15 mL; pH 1.02) containing a specific amount of PCT was added to an electrochemical cell, the system temperature was controlled at 36.8 °C by a super thermostat. The linear sweep voltammograms were recorded in the potential range of 0.0~1.0 V at scan rate of 10 mV s^{-1} after quiet time of 4 s. The effect of the potential scan rates on the electrochemical behavior of PCT on the electrodes was also studied.

Results and discussion

Electrochemical modification of GCEs

All the cyclic voltammograms of GCEs modified by several in situ-generated diazonium species in acidic aqueous solution containing NaNO$_2$ are very similar; here, that modified

by 4-COOH-PD is provided as a sample as shown in Fig. 1a. From Fig. 1a it can be seen that the first cycle of the cyclic voltammetric curves shows two irreversible reduction peaks at 0.35 and −0.15 V, respectively. These reduction waves disappeared and the cyclic voltammetric curve only present a very small reduction current during the second cycle, which demonstrates the presence of the grafted layer on GCE surface [15]. Regarding the modification of a GCE with 4-OPO$_3$H$_2$-P, the work of Xu et al. is repeated here [8]. Figure 1b exhibits a potentiostatic i–t curve of a GCE at −0.7 V running 300 s for the reduction of 4-NPP. With increase in reaction time, the current gradually decreases and finally maintains steady, this indicates the formation of the grafted 4-OPO$_3$H$_2$-P layer on GCE surface. The theory manifests that either cyclic voltammetry or potentiostatic process can graft diazonium on GCE under the suitable conditions. This is also proved by our abundant experiments. In other words, we can choose any method among them to modify GCE with diazonium species.

The blocking properties of all the electrodes were measured by using redox probes. Here, the cyclic voltammograms on the bare (a), 4-COOH-P (b)-, 4-NO$_2$-P (c)- and 4-PD (d)-modified GCEs in 0.1 M KCl aqueous solution containing 5 mM Fe(CN)$_6^{3-}$ at a scan rate of 100 mV s^{-1} are given as a sample as shown in Fig. 2.

It is well known that the bare GCE can totally implement the electron transfer of Fe(CN)$_6^{3-}$ redox probe as shown in Fig. 2a. It is found that the electron transfer of Fe(CN)$_6^{3-}$ redox probe on the 4-COOH-P-modified GCE is completely blocked as shown in Fig. 2b, this is because

$R_p = CH_2PO_3H_2$, SO_3H, $COOH$, OH, Br, NO_2
$R_m = COOH$, OH
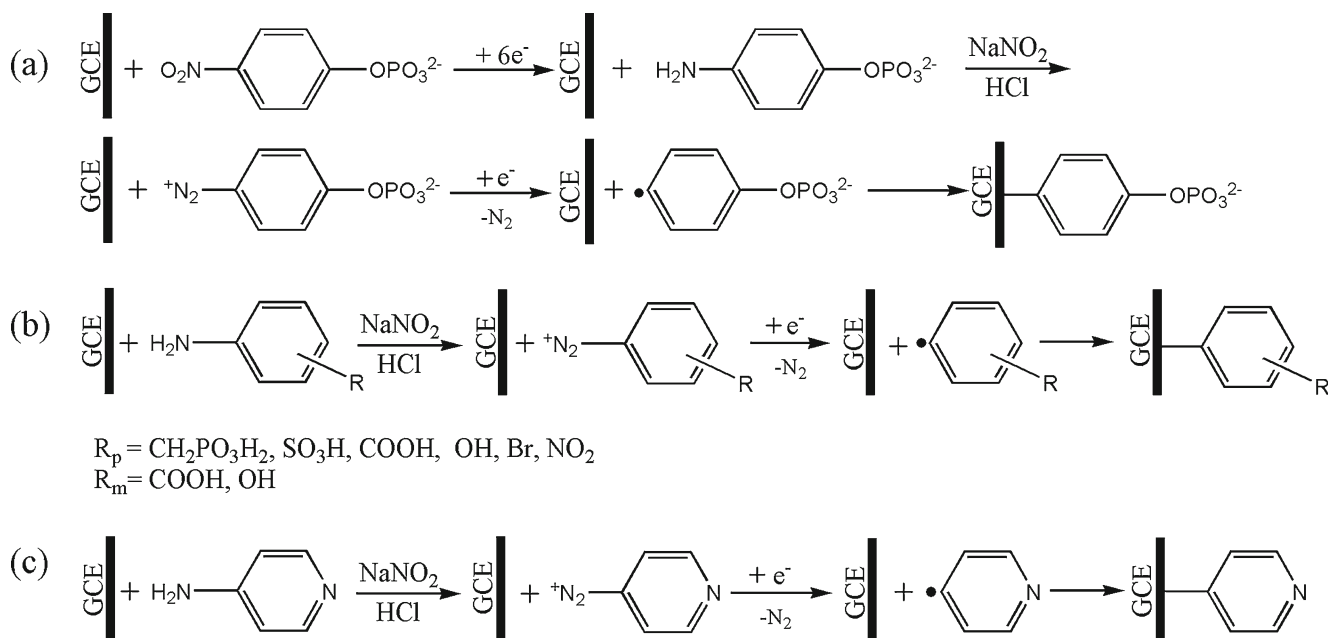
Scheme 1 Reaction route for the derivatization of a GCE by the electrochemical reduction of diazonium salts produced in situ from 4-NPP (**a**), amine excluding 4-APD (**b**) and 4-APD (**c**)
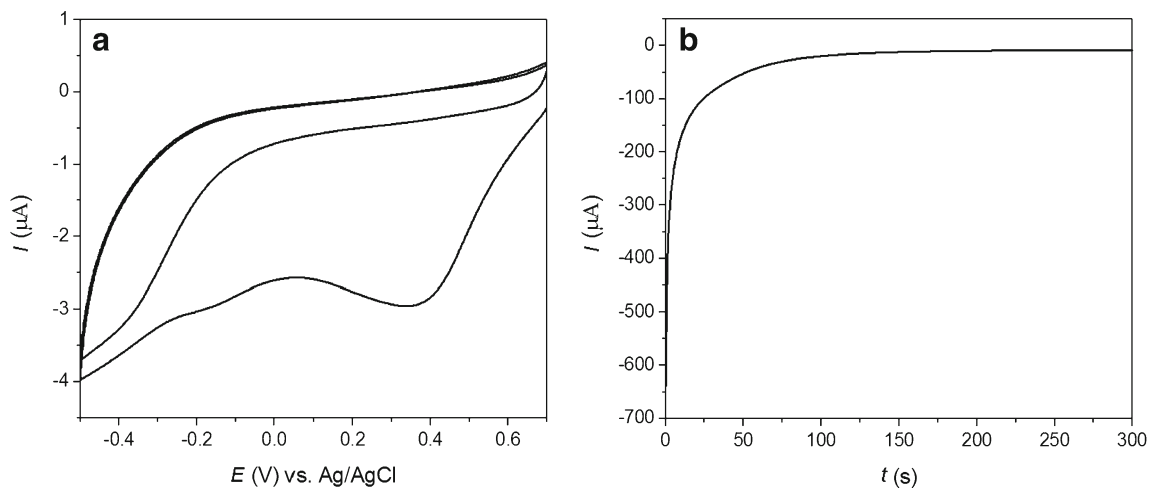
Fig. 1 **a** Cyclic voltammograms on a bare GCE for the reduction of 1 mM 4-COOH-PD at scan rate of 10 mV s^{-1}; **b** Potentiostatic i–t curve at −0.7 V for the reduction of 1 mM 4-OPO$_3$H$_2$-PD

the terminal −COOH on GCE in a neutral solution will dissociate to the −COO$^-$ group, that repulses the charged negatively Fe(CN)$_6^{3-}$ approaching the modified electrode surface [39]. The electrochemical behaviors of the 4-CH$_2$PO$_3$H$_2$-P-, 4-OPO$_3$H$_2$-P-, 4-SO$_3$H-P-, 4-COOH-P-, 3-COOH-P-, 4-OH-P-, and 3-OH-P-modified GCEs toward Fe(CN)$_6^{3-}$ redox probe are similar to that of the 4-COOH-P-modified GCE, because their terminal groups
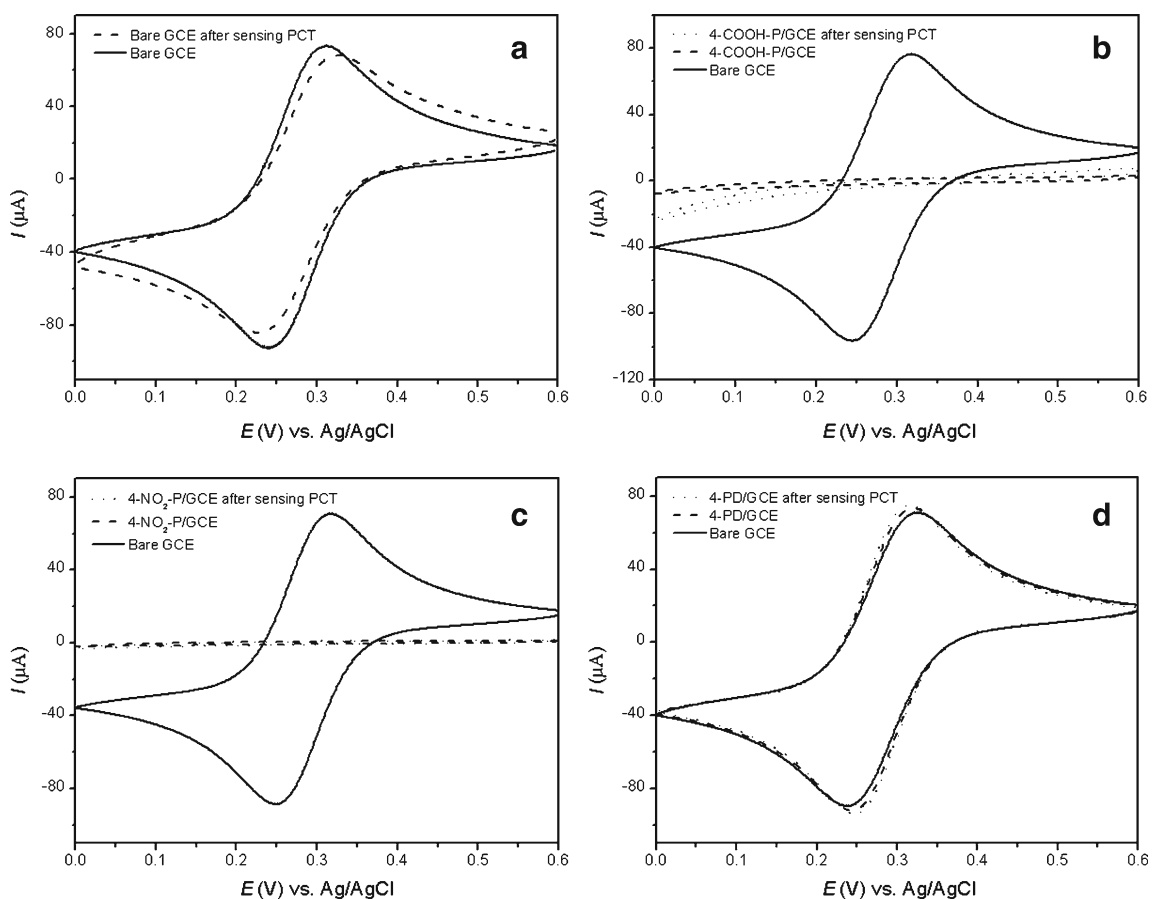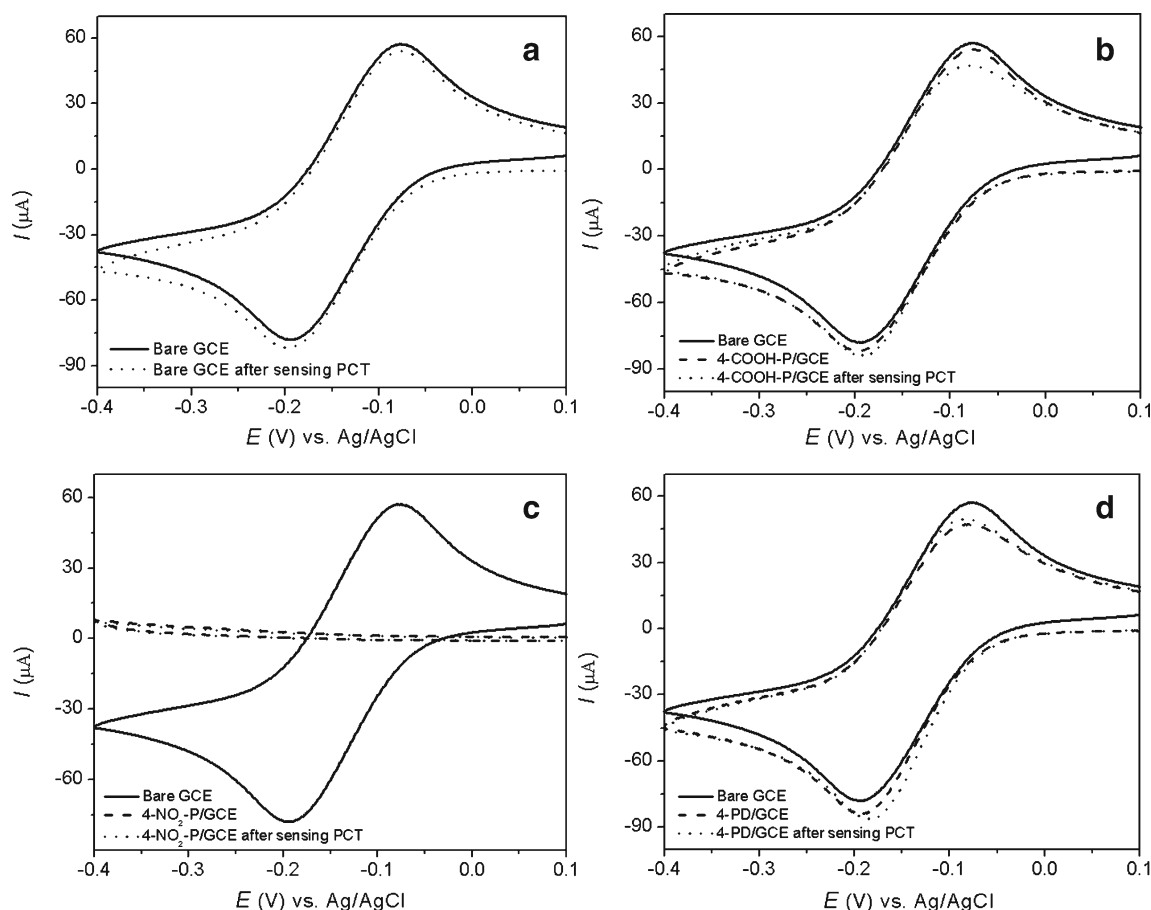

Fig. 2 Cyclic voltammograms on the bare (**a**), 4-COOH-P (**b**)-, 4-NO$_2$-P (**c**)- and 4-PD (**d**)-modified GCEs in 0.1 M KCl aqueous solution containing 5 mM Fe(CN)$_6^{3-}$ at a scan rate of 100 mV s^{-1}

will dissociate and charge negatively in a neutral solution. The cyclic voltammetric results exposed that the 4-NO$_2$-P- and 4-Br-P-modified GCEs also cannot accomplish the electron transfer of Fe(CN)$_6^{3-}$ redox probe (shown in Fig. 2c) because the terminal −NO$_2$ and −Br groups are nondissociated and inert, which cannot be used as a platform of the electron transfer. The 4-PD group on GCE, whether it is to be hydrogenated or not in a neutral solution, it is able to realize the electron transfer of Fe(CN)$_6^{3-}$ redox probe as shown in Fig. 2d.

Figure 3 depicts the cyclic voltammograms on the bare (a), 4-COOH-P- (b), 4-NO$_2$-P- (c), and 4-PD (d)-modified GCEs in 0.1 M KCl aqueous solution containing 5 mM Ru (NH$_3$)$_6^{3+}$ at a scan rate of 100 mV s^{-1}. The 4-CH$_2$PO$_3$H$_2$-P-, 4-OPO$_3$H$_2$-P-, 4-SO$_3$H-P-, 4-COOH-P-, 3-COOH-P-, 4-OH-P-, and 3-OH-P-modified GCEs can realize the electron transfer of Ru(NH$_3$)$_6^{3+}$ redox probe, the resulting cyclic voltammogram is similar to that of the 4-COOH-P-modified GCE as shown in Fig. 3b. The cyclic voltammetric results exposed that the 4-NO$_2$-P- and 4-Br-P-modified GCEs cannot accomplish the electron transfer of Ru(NH$_3$)$_6^{3+}$ redox probe as shown in Fig. 3c, because the terminal −NO$_2$ and

−Br groups are nondissociated and inert. Concerning the 4-PD-modified GCE, it is able to accomplish the electron transfer of Ru(NH$_3$)$_6^{3+}$ redox probe too as shown in Fig. 3d. As the results showed in Figs. 2 and 3, several of the modified molecules were successfully grafted on GCEs, separately.

Figure 4 shows the AC impedance spectroscopic result on the bare and 4-OPO$_3$H$_2$-P-modified GCEs in 0.1 M KCl aqueous solution containing 5 mM Fe(CN)$_6^{3-/4-}$. The profiles are composed of a semicircle part and a linear part. The semicircle part at high frequencies is corresponding to the electron transfer limited process, and the linear part at low frequencies is to the diffusion limited process. For giving more detailed information about the electrode/solution interfaces, the Randles circuit (inset Fig. 4) is chosen to fit the obtained impedance data [39]. CPE represents a constant phase element, which is used instead of a pure capacitor in the equivalent circuit due to microscopic surface roughness and inhomogeneity, R_{ct}, Z_W, and R_s delegate the charge-transfer resistance, Warburg impedance and the solution resistance, respectively. The impedance data were carefully analyzed using this electrical equivalent circuit.

Fig. 3 Cyclic voltammograms on the bare (a), 4-COOH-P (b)-, 4-NO$_2$-P (c)- and 4-PD (d)-modified GCEs in 0.1 M KCl aqueous solution containing 5 mM Ru(NH$_3$)$_6^{3+}$ at a scan rate of 100 mV s^{-1}
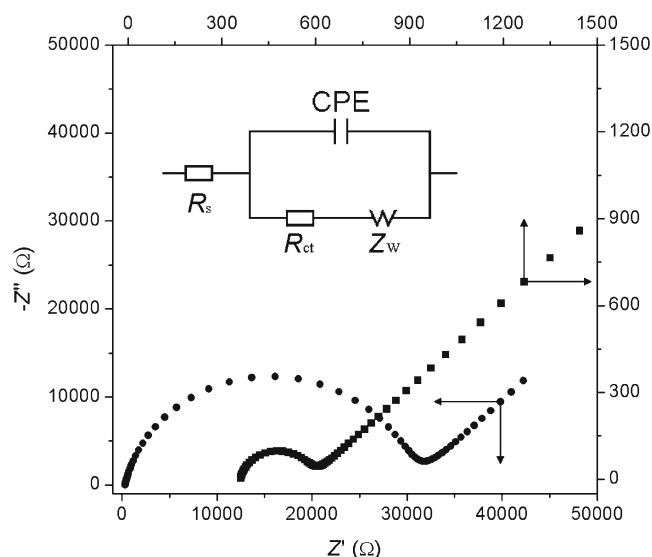
Fig. 4 Impedance plots on the bare (*filled square*) and 4-OPO$_3$H$_2$-P (*filled circle*)-modified GCEs in 0.1 M KCl solution containing 5 mM Fe(CN)$_6^{3-/4-}$. The *top inset* shows the equivalent circuit model for the analysis of the impedance data. *Arrows* direct the corresponding axis

According to the impedance data, the surface coverage of the modified GCEs could be calculated by the equation [40] as follows:

$$1 - \theta = \frac{R_{ct}^0}{R_{ct}} \qquad (1)$$

where θ is the apparent electrode coverage, assuming that all the currents are passed via bare spots on the electrode, and R_{ct}^0 and R_{ct} are on behalf of the charge-transfer resistance measured on a bare GCE and a modified one, respectively. But remarkably, the 4-PD-modified GCE can complete the electron transfer of Fe(CN)$_6^{3-/4-}$ redox probe, so we cannot obtain the surface coverage by using this method. According to calculation, R_{ct}^0 and R_{ct} on the bare and 4-OPO$_3$H$_2$-P-modified GCEs equal to 237 and 30,157 Ω, respectively. Using Eq. (1), the surface coverage of the 4-OPO$_3$H$_2$-P-modified GCE was calculated to be 99.2%. Equally, every surface coverage of the other modified GCEs except the 4-PD one was also estimated to be more than 99.2% (data not shown here). The results indicated that the modified layers on GCE surface were very compact.

Electrochemical sensing PCT

The bare and modified GCEs were employed to sense PCT comparatively. Figure 5, as a sample shown here, reveals the linear sweep voltammograms of PCT on the bare (a), 4-OPO$_3$H$_2$-P- (b), and 4-NO$_2$-P (c)-modified GCEs in sulfuric buffer solution of pH 1.02 at 36.8 °C at a scan rate of 10 mV s^{-1}.
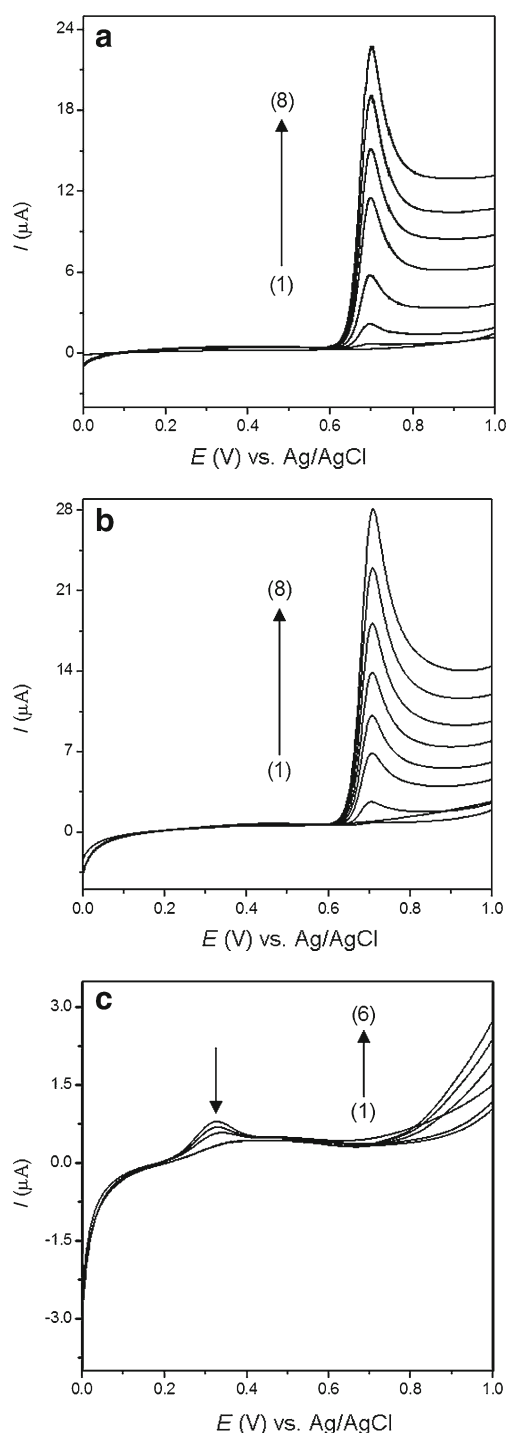
Fig. 5 Linear sweep voltammograms of the different PCT concentration on the bare (**a**), 4-OPO$_3$H$_2$-P (**b**)- and 4-NO$_2$-P (**c**)-modified GCEs in sulfuric buffer solution of pH 1.02 at 36.8 °C at a scan rate of 10 mV s^{-1}

The electrochemical behaviors of PCT on the bare, 4-CH$_2$PO$_3$H$_2$-P-, 4-SO$_3$H-P-, 4-COOH-P-, 3-COOH-P-, 4-OH-P-, 3-OH-P-, and 4-PD-modified GCEs are perfectly
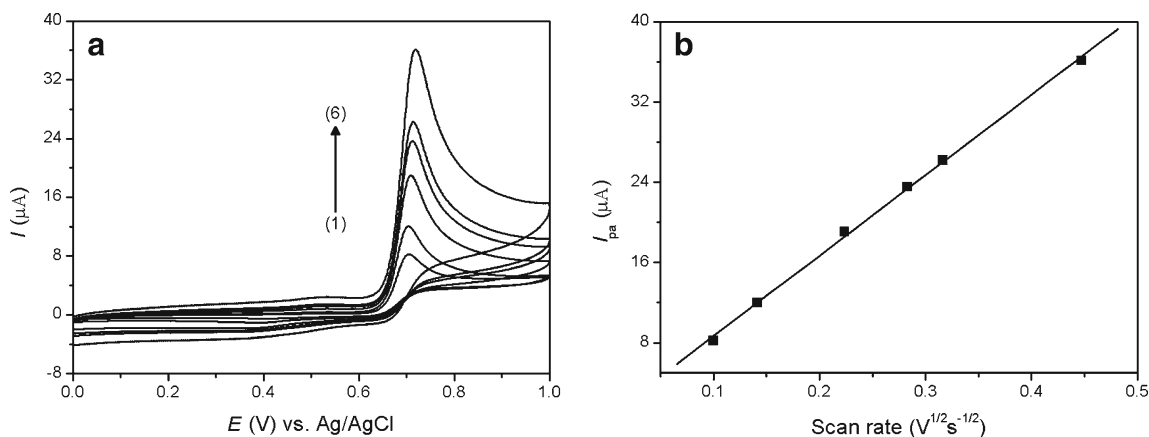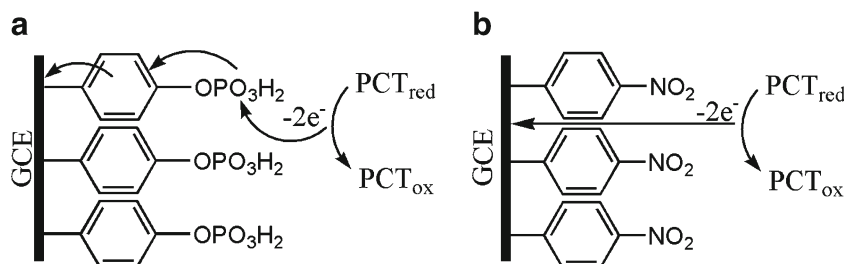
Fig. 6 **a** Cyclic voltammograms of 4-SO$_3$H-P/GCE in pH 1.02 buffer solution containing 5.09×10^{-4} M PCT at 36.8 °C under different scan rates, (*1*)–(*6*): 10, 20, 50, 80, 100, and 200 mV s^{-1}; **b** The linear relationship between the peak currents and the square root of scan rates

similar, namely there are oxidation peaks at ca. 0.72 V in the linear sweep voltammograms of PCT on those electrodes, and the oxidation currents are gradually increased with increase in PCT concentration. This is very similar to that on the bulk GCE. But the distinct light-blue thin film can be obviously observed on the 4-CH$_2$PO$_3$H$_2$-P-modified GCE after sensing PCT, this indicates that the electropolymerization of the 4-CH$_2$PO$_3$H$_2$-P on GCE occurs during sensing, this may be caused by the very strong electrochemical reactivity of the benzyl group in the 4-ABPA molecule [41]. On that account, the 4-CH$_2$PO$_3$H$_2$-P-modified GCE sensing PCT will be discussed a little later. The electrochemical behaviors of PCT on the 4-NO$_2$-P- and 4-Br-P-modified GCEs are similar, but there are no oxidation peaks of PCT in the linear sweep voltammograms on the both electrodes, only the increasing oxidation currents happen with increase in PCT concentration; this means that the 4-NO$_2$-P- and 4-Br-P-modified GCEs show an electrochemical behavior like microelectrode when sensing PCT [42–45]. In addition to this, an oxidation peak at about 0.34 V on the 4-NO$_2$-P- and 4-Br-P-modified GCEs gradually become weaker with increase in PCT concentration, which should be produced by the modified electrodes themselves, namely, that is the background current of the both electrodes.

During the oxidation reaction on the electrodes, PCT will be converted to its oxidation product *N*-acetyl-*p*-quinoneimine via two electrons and two hydrogens [46].

The effect of potential scan rate (ν) on the peak current (I_{pa}) and the peak potential (E_{pa}) of PCT on the bare and modified GCEs was evaluated, Fig. 6a shows cyclic voltammograms of the 4-SO$_3$H-P-modified GCE in pH 1.02 buffer solution (36.8 °C) including 5.09×10^{-4} M PCT at different scan rates, the other molecule modified GCEs obtained the similar results (figures not shown here). From Fig. 6b, we can see that the square root of the scan rate is linear with the peak current between 10 and 200 mV s^{-1}, this indicates that the electrode process is diffusion-controlled [47], the corresponding linear regression equation is

$$I_{pa}(\mu A) = 80.2064\nu^{1/2}\left(Vs^{-1}\right) + 0.6499 \qquad (2)$$

The apparent standard rate constant k_s for the oxidation reaction of the surface confined PCT on the bare and modified GCEs can be calculated by Laviron's approach from the variation of peak potential with scan rate [48–50]

$$E_p = E^0 + \frac{2.303RT}{\alpha nF}\log\frac{RTk_s}{\alpha nF} - \frac{2.303RT}{\alpha nF}\log\nu \qquad (3)$$

where E_p is the anodic peak potential, E^0, α, k_s and ν are the formal potential, electron transfer coefficient, apparent standard rate constant and potential sweep rate, respectively. R, T, F, and n have their usual significance. Since α is assumed to be 0.5 in a totally irre-

Fig. 7 The electron transfer model of PCT on the 4-OPO$_3$H$_2$-P (**a**) and 4-NO$_2$-P (**b**) modified GCEs
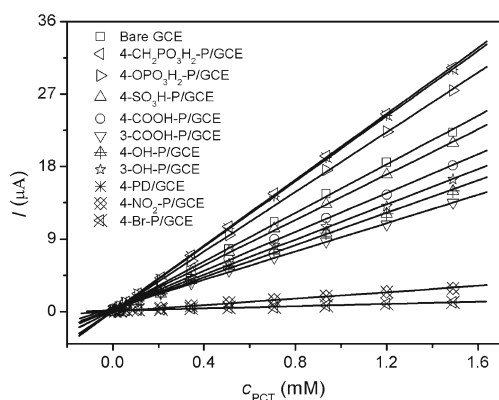
Fig. 8 The calibration curves of PCT on the bare and modified electrodes

versible electrode process, and we have known that two electrons are involved in the oxidation of PCT [46]. An intercept containing k_s can be calculated if the value of E^0 is known. The value of E^0 in Eq. (3) can be obtained from the intercept of the E_p vs. v curve by extrapolation to the vertical axis at $v=0$. After calculations, k_s of PCT on the bare, 4-$CH_2PO_3H_2$-P-, 4-OPO_3H_2-P-, 4-SO_3H-P-, 4-COOH-P-, 3-COOH-P-, 4-OH-P-, 3-OH-P- and 4-PD-modified GCEs are 79.04, 49.95, 132.75, 41.43, 46.12, 43.97, 53.38, 58.26, and 102.87 s^{-1}, respectively.

Here, the electrodes can be divided into two types; one is that with active terminal groups such as $-OPO_3H_2$, $-SO_3H$, $-COOH$, and $-OH$, and the other is that with inert terminal groups such as $-NO_2$ and $-Br$. On the modified GCE with active terminal groups such as $-OPO_3H_2$, the electron that the reduced PCT loses can be transferred to GCE surface through the 4-OPO_3H_2-P molecule itself as shown in Fig. 7a. The electrode process of 4-OPO_3H_2-P/GCE follows the diffusion current theory resulting in its normal electrochemical behavior similarly happened on the bulk GCE. However, the terminal $-NO_2$ group on 4-NO_2-P/GCE is

inert and nondissociated, and it cannot attract any molecule; the electron that the reduced PCT loses cannot be transferred to GCE surface through 4-NO_2-P molecule by itself, it can only be done via the gap among 4-NO_2-P molecules as shown in Fig. 7b. As the electrode surface coverage is very compact and the spare gap is very limited, the electrode process of 4-NO_2-P/GCE does not follow the diffusion current theory, resulting in its exceptional electrochemical behavior, similarly occurring on microelectrode not on the bulk GCE and absolutely low electrochemical sensitivity toward PCT. The 4-Br-P-modified GCE also offers a similar result with the 4-NO_2-P one.

Figure 8 shows the calibration curves of PCT on all the electrodes, and the corresponding parameters are summarized in Table 1. From the data, it can be seen that all the correlations of the linear regression equation are very good indicating all the modified GCEs possess very stable electrochemical sensing properties toward PCT in acidic solution. The linear range from micromolar to millimolar of the linear regression equation is enough to monitor pharmacokinetics of the PCT-based drug system. The limit of detection (LOD) of PCT on the bare and modified electrodes was also calculated according to the 3σ method [51]

$$LOD = \frac{3s_{bl}}{k} = 3\sigma \qquad (4)$$

where s_{bl} is the standard deviation of the peak currents of the blank ($n=5$), and k is the slope of the calibration curve.

When studying the electrochemical sensing properties of CMEs toward any model molecule, it is extremely important to explain the relationship between the slopes of the linear regression equation and the terminal groups of the modified electrodes. As now well known, the concentration of target molecule, that is PCT in current experiment, in the electrode (or modifier)–solution interface double electric layers will

Table 1 The parameter of the linear regression equation for the electrodes

| Electrode	Linear range	k (μA/μM)	b (μA)	r	LOD (μM)
Bare GCE	2.0 μM–1.49 mM	0.01505	0.2318	0.999	1.36
4-$CH_2PO_3H_2$-P/GCE	2.0 μM–1.49 mM	0.02023	−0.0182	0.999	1.47
4-OPO_3H_2-P/GCE	2.0 μM–1.49 mM	0.01852	0.0286	0.999	1.08
4-SO_3H-P/GCE	2.0 μM–1.49 mM	0.01404	0.1364	0.999	1.27
4-COOH-P/GCE	2.0 μM–1.49 mM	0.01205	0.2256	0.999	1.76
3-COOH-P/GCE	2.0 μM–1.49 mM	0.00879	0.0428	0.999	1.82
4-OH-P/GCE	2.0 μM–1.49 mM	0.01088	0.2397	0.999	1.75
3-OH-P/GCE	11.0 μM–1.49 mM	0.01190	0.0619	0.999	2.88
4-PD/GCE	2.0 μM–1.49 mM	0.02043	0.0164	0.999	1.56
4-NO_2-P/GCE	11.0 μM–1.49 mM	0.00198	0.03066	0.998	4.25
4-Br-P/GCE	32.0 μM–1.49 mM	0.00069	0.12981	0.994	8.65
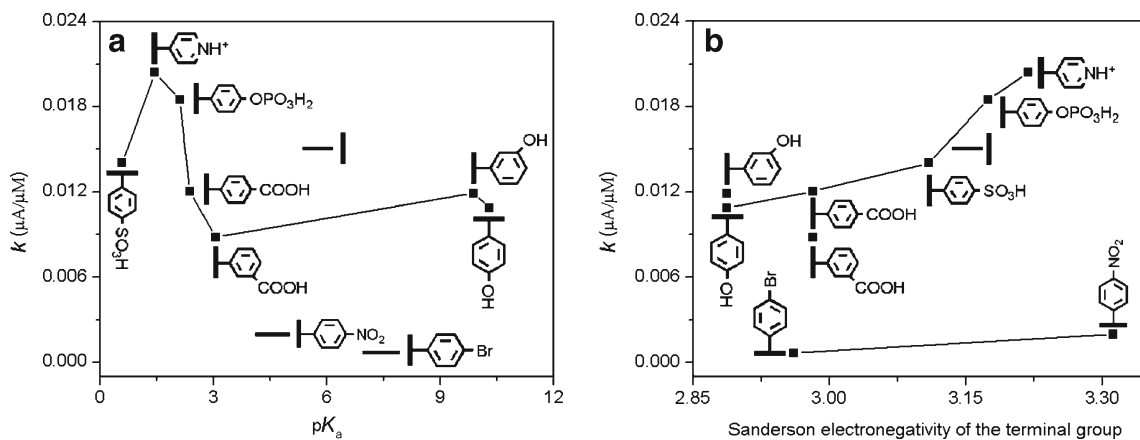
Fig. 9 **a** Plots of k vs. the pK_a of the terminal group, **b** Plots of k vs. the electronegativity of the terminal groups

impact the detection sensitivity, namely the higher the PCT concentration, the bigger the sensitivity. In this point, four essentials including pK_a and electronegativity of the terminal groups, hydrogen bond interactions and steric hindrance between modifier and PCT were suggested to explain the electrochemical sensing properties [52]. We think that the hydrogen bond among them is foundation, others are very important influence factor. Here, the qualitative discuss will be focused on pK_a and electronegativity. Figure 9a outlines the relationship pK_a of the terminal groups and the slopes of the linear regression equation. By the way, the 4-NO$_2$-P, 4-Br-P and bare GCE are inert and nondissociated, they do not have the value of pK_a, we put their k values for comparison. With increase in acidity namely with decrease in pK_a, the k values are increased. Here, only circumstances on the 3-COOH-P-, 4-COOH-P-, 4-OPO$_3$H$_2$-P-, and 4-PD-modified GCEs follow that. The k value of the 3-OH-P-modified GCE is bigger than that of 4-OH-P one under the rule, but they are conflicting with the above-mentioned four groups.

The electronegativity values of every substituent group were calculated according to Sanderson's balance principle

of electronegativity [53–56]. As shown in Fig. 9b, the slope of the linear regression equation namely the sensitivity of PCT on the GCEs modified with 4-OH-P, 4-COOH-P, 4-SO$_3$H-P, 4-OPO$_3$H$_2$-P, and 4-PD increases with increase in electronegativity of the terminal substituent group. From this result we can see the intensity of the electronegativity of the terminal group on GCE can evaluate the sensitivity of the modified electrodes more than that of pK_a.

Normally, the −NO$_2$ group in the 4-NO$_2$-P and the −Br group in the 4-Br-P are inert, and the steric hindrance of the −NO$_2$ group toward PCT is bigger than that of the −Br group, the sensitivity of the 4-NO$_2$-P-modified GCE toward PCT should be less than that of the 4-Br-P-modified one. In fact, they are just the opposite; the sensitivity of the 4-NO$_2$-P-modified GCE toward PCT is bigger than that of the 4-Br-P-modified one. That also can be explained legitimately according to the electronegativity of the groups. The electronegativity of the −NO$_2$ group is bigger than that of the −Br group, the concentration of PCT in the modifier-solution interface double electric layers of 4-NO$_2$-P/GCE is higher than that of 4-Br-P/GCE, so the sensitivity of the 4-
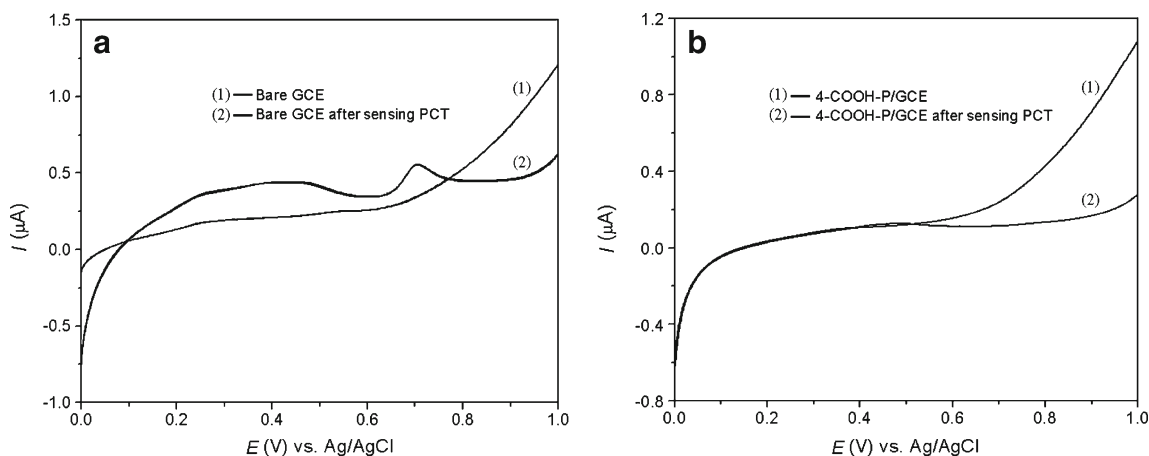
Fig. 10 Linear sweep voltammograms of the bare (**a**) and 4-COOH-P (**b**)-modified GCEs before (*a*) and after (*b*) sensing PCT in a sulfuric buffer solution of pH 1.02 at 36.8 °C at a scan rate of 10 mV s^{-1}

NO_2-P-modified GCE toward PCT is bigger than that of the 4-Br-P-modified one, although the $-NO_2$ group possesses stronger steric hindrance than the $-Br$ group. In addition, the nondissociated and inert 4-NO_2-P and 4-Br-P themselves on GCEs cannot be used as a platform transferring electron that the reduced PCT loses to GCE surface, at this case the electron is transferred to the electrode surface via gap among modified layer [57], namely, the electron that the reduced PCT loses can be transferred to the GCE surface only via the gap among 4-NO_2-P or 4-Br-P, so that the sensitivity of 4-NO_2-P-modified GCE is very small, although the electronegativity of the $-NO_2$ group is biggest in all the substituent groups as shown in Fig. 9.

Whereas, it is not easy to get the intensity of the bare GCE-solution interface double electric layer, as the electronegativity is difficult to be calculated. The slope sequence shows the sensitivity of the bare GCE toward PCT is only less than those of the 4-PD- and 4-OPO_3H_2-P-modified GCEs, higher than those of the 4-SO_3H-P- and other layer-modified GCEs. This signifies that the intensity of the bare GCE-solution interface double electric layer possibly is in between the 4-SO_3H-P- and 4-$O_3PO_3H_2$-P-modified GCEs. In fact, the steric hindrance should not be neglected. In a word, the concentration of PCT on modifier-solution interface is dependent on the electronegativity and pK_a of the terminal groups of the modifiers on the electrodes, and hydrogen bond and steric hindrance between the modifiers and PCT.

Reproducibility of the modified electrode

All the electrodes before and after sensing PCT were separately measured in the blank sulfuric buffer solution of pH 1.02 at 36.8 °C. Figure 10 shows the linear sweep voltammograms of the bare (a) and 4-COOH-P (b)-modified GCEs before (1) and after (2) sensing PCT in a sulfuric buffer solution of pH 1.02 at 36.8 °C at a scan rate of 10 mV s^{-1}. It can be seen that the bare GCE after sensing PCT via sonicating for 30 s in water still shows the strong oxidation peak of PCT, this indicates that PCT stays on the bare GCE surface during sensing. But, this phenomenon does not happen on any other layer modified GCE as shown in Fig. 10b. The electrochemical behavior of the bare GCE before and after sensing PCT in 0.1 M KCl aqueous solution containing 5 mM $Fe(CN)_6^{3-}$ redox probe as shown in Fig. 2 can also provide a evidence. On the bare GCE after sensing PCT, the potential difference of redox peak of $Fe(CN)_6^{3-}$ become bigger and the current of redox peak of $Fe(CN)_6^{3-}$ become smaller than those on the bare GCE before sensing PCT. This is due to the remains of PCT on the bare GCE surface. The results also show the other layers do not peel off from the GCE surface. These are very important for the real repetitive usage of the modified electrodes. On the side, the bare GCE will be partly deactivated in air. In brief, the

greatly stable and reusable GCEs modified by organic molecule with active terminal group and with high sensitivities, namely 4-COOH-P, 4-SO_3H-P, 4-OPO_3H_2-P, and 4-PD, are the preferred choice for sensing PCT.

Conclusion

GCE was covalently modified by electrochemical reduction of several diazonium salts generated in situ from the amino or nitro precursor in acidic aqueous solution containing $NaNO_2$ successfully. The electrochemical sensing effect of the modified electrodes toward PCT was studied respectively by LSV in acidic solution. The results showed that the bare GCE will be deactivated and hold a part of PCT after sensing PCT, however, the modified GCEs can be repeatedly used. Among them, the 4-COOH-P-, 4-SO_3H-P-, 4-OPO_3H_2-P-, and 4-PD-modified GCEs possess better properties than others. GCEs modified with the active terminal groups still show the electrochemical behavior like bulk GCE, but GCEs modified with the inert terminal groups display the electrochemical behavior like microelectrode, those are caused by their different electron transfer mechanism. According to the slopes of the calibration curves, the electrochemical sensitivity of the bare and modified GCEs toward PCT was sequenced. The electronegativity and pK_a of the terminal groups of the modifiers on the electrodes, and hydrogen bond and steric hindrance between the modifiers and PCT were used to explain the sequences of the electrochemical sensitivity. The results proved that hydrogen bond is foundation; others possess important influence on it. But, the electronegativity of the terminal group on GCE has better cogency than pK_a of the terminal groups.

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