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Glassy carbon electrodes modified with mixed covalent monolayers of choline, glycine, and glutamic acid for the determination of phenolic compounds

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Abstract A series of mixed covalent monolayer carbon glassy electrodes (choline/amino acid/GCE) was prepared using choline and amino acids, and the properties were investigated by cyclic voltammetry, electrochemical impedance spectroscopy and X-ray photoelectron spectroscopy. The oxidations of phenolic compounds including dopamine hydrochloride, epinephrine and phenol have been studied. Electrooxidation of phenols, first leads to the formation of phenoxy radical, which reduces to phenols and hydroquinone; the reaction of polyoxyphenylene is effectively restrained at this electrode, unlike only at chlorine or amino acids modified electrode or bare electrode, which rapidly forms polyoxyphenylene and results in electrode passivation. Owing to significant improvement of electrode passivation shaped insulating polymerization, these mixed covalent monolayer carbon glassy electrodes show themselves excellent resistance ability for pollution, could be successfully used as amperometric sensor for phenolic compounds.

Keywords Mixed covalent modified carbon glassy electrode · Choline · Amino acids · Phenolic compounds

Introduction

Phenolic compounds are found not only in animals, but also in various natural compounds. They are also found

in industrial wastewaters due to their wide usage in the field of medicine, in the synthesis of insecticides and dye, etc. Among these, many phenols have a regulatory effect in biological systems, such as dopamine (DA) and epinephrine (EP). Others have a serious effect on wildlife species and human health over a long period even when they are present at very low concentration, for example phenol. For the simple, sensitive and less expensive detection of phenolic compounds, the method based on electroanalysis may be the most useful due to their electrochemical activity. As we know, the accumulation of reaction products, insulating polymerization formed phenoxy radicals attack unreacted substrate can induce the inactivation of electrode with a subsequent instability and decreasing precision in further electroanalysis measurements [1–7]. Although this phenomenon was described a long time ago, there is a little practical solution for the effective inhibition of film formation. For example, cellophane membranes or Nafion polymer films were employed to cover the electrode surface for reducing the loss of electrode activity [5]. But the application of membrane covered electrodes for amperometric analysis resulted in a loss of sensitivity due to slow mass transport of the analyte through the membrane in solution. Recently, Susanne Rath reported a method, which avoided the formation of insulating polymerization by EDTA medium with an improved effect on the electrochemical behavior of phenolic compounds [8].

On the other hand, as we know covalent modified methods on carbon electrodes were used to bond one kind of substance for electrochemical purposes [9–18]. In earlier works, we have reported various amino acids [14, 15] and choline [19–21] covalent monolayer modified carbon glassy electrodes based on –C–O and –C–N covalent bond, respectively, and the preliminary application respectively showed electrocatalytic effect of either amino acids or choline (Ch) modified film to phenolic compounds at first, and then, the electrode passivation gradually brought out in further amperometry detection. Moreover, carboxy functions of

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amino acid modified layer may be complex with metal ion, or absorb cation; the quaternary ammonium ($[-N^+(CH_3)_3]$) functions of Ch film could attract anion. These modified electrodes, thus, could be polluted in practice.

Based on these facts, therefore, it is significant to alter the charge properties of the modified layer for biosensors and restraining pollution purpose. In the present work, we have successfully developed a series of planar mixed-covalent modified electrode based on Ch and amino acids including glutamic acid (Glu), glycine (Gly), and aminobutyric acid (Aba). The mixed modification mainly focused on Ch and Glu, and application on the Ch and Glu or Gly for phenolic compounds biosensor.

Experimental

Materials

Choline chloride (Ch), glutamic acid (Glu), glycine (Gly), aminobutyric acid (Aba) and phenol were purchased from Chemical Reagent Factory of Beijing (Beijing, China). Dopamine hydrochloride (DA) and epinephrine hydrochloride (EP) were obtained from Sigma (USA). All other reagents used were of analytical grade. Solutions of amino acid and Ch were prepared in 0.01 mol/L $LiClO_4$ + acetonitrile (ACN). Solutions of DA, EP and phenol were prepared in water prior to use. Acetonitrile (ACN) was of analytical quality and dried over molecule sieves (3 Å) before use. Phosphate-buffered solutions of 0.1 mol/L PBS was used as buffer. Double distilled water was used.

Apparatus and procedure

Electrochemical techniques including cyclic voltammetry (CV), differential pulse voltammetry (DPV) were performed on CHI 832 electrochemical analyzer (Cheng-Hua, Shanghai, China). Electrochemical impedance spectroscopy (EIS) was carried out at CHI 660 A workstation (Cheng-Hua, Shanghai, China). A conventional three-electrode system was used, including a platinum wire counter electrode, a saturated calomel reference electrode (SCE) and a testing electrode. All potentials reported in this paper are calculated versus the SCE. The basal glassy carbon disk electrodes (GCE) with a geometric area of 0.125 cm^2 were purchased from Tianjin-Lan-Like high chemical electronic technology company (Tianjin, China). XPS was obtained using an ESCALAB MK2 spectrometer (Vg Corporation, UK) with a Mak-Alpha X-ray radiation source. EIS measurements were carried out before and after the surface modification in 0.1 mol/L $Fe(CN)_6^{3-}$ + 0.1 mol/L PBS (pH 7.0) in the range of 100 kHz to 0.05 Hz at 0.215 V, which is the formal potential of the redox couple.

Preparation of modified electrode

The GCE was prepared as follows: first, the GCE was step by step polished to a mirror-like finish with fine wet emery paper (grain size 1,000, 3,000, and 4,000). After sonication cleaning in water for 15 min, it was resurfaced using 1.0 μM alumina slurry. After cleaning, the electrode was electrochemically pre-treated by cyclic scanning in the ACN/10 mmol/L $LiClO_4$ solution including 2.0 mmol/L Ch or 1.0 mmol/L amino acid or the mixture of Ch (2.0 mmol/L) and amino acid (1.0 mmol/L) in the potential range of 0–1.6 V at 20 mV/s for four scans. The electrode was then rinsed with ethanol and water-sonicated for 15 min. The mixed modified electrodes of Ch and Glu, Ch and Gly, Ch and Aba were labeled Ch/Glu/GCE, Ch/Gly/GCE, Ch/Aba/GCE, respectively. The Ch-only, Glu-only, Gly-only, Aba-only modified electrodes were labeled Ch/GCE, Glu/GCE, Gly/GCE, Aba/GCE, respectively, and stored in 0.1 mol/L PBS (pH 7.0) except Ch/GCE which was stored in double distilled water at 4°, and was ready for using. High-purity nitrogen was used for de-aeration. All experiments were carried out at ambient temperature.

Results and discussion

Mixed electrochemical modification of choline and glutamic acid on GCE

Ch/GCE, Glu/GCE and Ch/Glu/GCE were, respectively, fabricated in $LiClO_4/ACN$ solution including Ch, or Glu, or the mixture of Ch and Glu. As shown in Fig. 1, the oxidation peaks of Ch (Fig. 1b), and Glu (Fig. 1a), respectively, appear at 1.12 and 1.16 V, which are reproducible from scan to scan if CVs are run in blank ACN/ $LiClO_4$ solution at these electrodes indicating a successful surface modification [14, 15, 19–21].

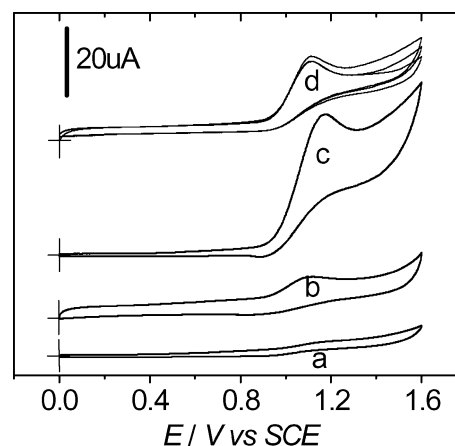


Fig. 1 Cyclic voltammograms (CVs) of glassy carbon disk electrodes (GCE) in ACN + 10 mmol/L $LiClO_4$ containing: 1.0 mmol/L Glu (a), 2.0 mmol/L Ch (b), 2.0 mmol/L Ch + 1.0 mmol/L Glu (c), the CVs of Ch/Glu/GCE (d) at blank ACN + 10 mmol/L $LiClO_4$. Scan rate: 20 mV/s

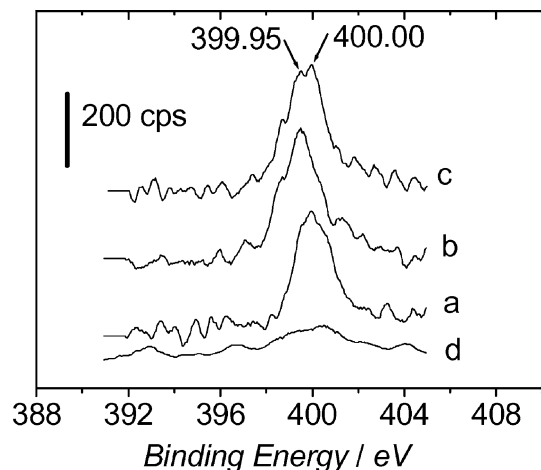


Fig. 2 X-ray photoelectron spectroscopy (XPS) of N(1s) region of the Glu/GCE (a), Ch/GCE (b) and Ch/Glu/GCE (c). Prepared by cycling from 0 to 1.6 V for four scans at 20 mV/s in 1.0 mmol/L Glu or 2.0 mmol/L Ch or 1.0 mmol/L Glu + 2.0 mmol/L, +ACN + 10 mmol/L LiClO₄. d XPS of N(1s) region of the GCE new prepared by cycling in the blank ACN/LiClO₄

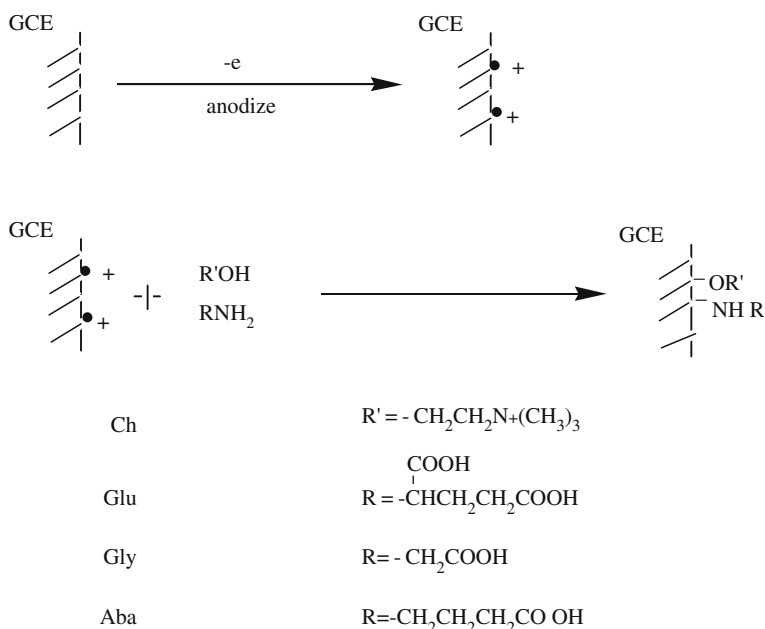
A similar phenomenon was also observed at benzene acetic acid covalent modified carbon electrode [11]. Due to the electrostatic attraction of Ch and Glu with opposite charges, the mixed modification overlaps to a higher irreversible oxidation peak at approximately 1.17 V (Fig. 1c). After the Ch/Glu/GCE was carefully rinsed and transferred to the blank solution, a reproducible peak can be seen at 1.17 V (Fig. 1d) at this electrode.

As the apparent area of the electrode is probably smaller than the real area, the concentrations (Γ) of Ch, Glu and the mixture of Ch and Glu can be calculated from the area of the first CV in blank solution. In the

example studied (Fig. 1) the concentration of Ch, Glu and the mixture of Ch and Glu were 4.8×10^{-10} , 1.4×10^{-10} and 8.2×10^{-10} mol cm⁻², respectively, suggesting monolayer modification [14, 15]. The value of the Ch/Glu/GCE is obviously large. This is probably attributed to the charge compensation of modified Ch and Glu residues on the GCE. However, the question is whether mixed modification is based on covalently modified or simple static absorption. This will be discussed in a later section.

X-ray photoelectron spectroscopy of the elemental nitrogen-to-carbon ratio (N/C) at the modified electrode surface is shown in Fig. 2. The position of the N (1s) peak is a small flat in 400.2 eV at bare GCE (Fig. 2d); However, the positions of the peak maximum N (1s) are in 399.50 and 400.00 eV region at Ch/GCE (Fig. 2b) and Glu/GCE (Fig. 2a), respectively. The mixed XPS of Ch and Glu (Fig. 2c) divaricates two little interval peaks maximum N (1s) in 399.50 and 400.00 eV. Although this little interval peak is not sufficient to illustrate the modification, the tests of stability of immobilization support the formation of a covalent linkage. For example, after the treatment of the GCE as described above, they were sonicated for 15 min in a variety of solutions including water, ethanol, and pH 4.0~7.0 PBS, and then thermodesorbed under 100°C; the position of XPS was still unchanged, but the area showed 10–20% decrease. Accordingly, the similar scheme is a mixed monolayer covalent modification based on C–O and C–N accompanying a little static absorption (Scheme 1). The surface of the GCE is anodized first, generating cation radicals; the surface cation radicals then react with Ch by nucleophilic attack forming ether-bound C–O linkages [14, 15]. Similarly, the surface cation radicals can also react with Glu forming C–N bound linkages [19–21]; Meanwhile, some Ch and Glu residues could be embedded in

Scheme 1



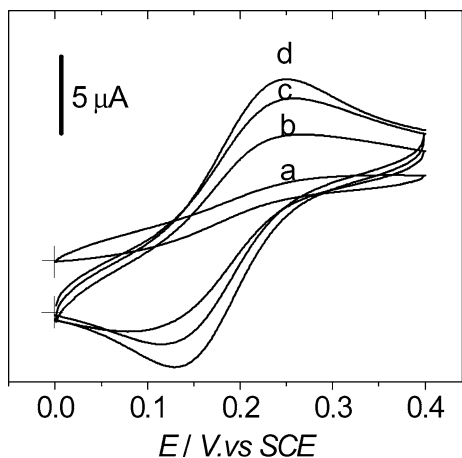


Fig. 3 The CVs of 5 mmol/L $K_3[Fe_3(CN)_6]$ at Glu/GCE (a), Ch/Glu/GCE (b), bare GCE (c) and Ch/GCE (d) in 1.0 mol/L KCl + 0.1 mol/L pH 7.0 PBS. Scan rate: 50 mV/s

the assembled mixture layer due to electrostatic interaction.

CVs for bare GCE, Ch/GCE, Glu/GCE and Ch/Glu/GCE were recorded in Fig. 3 in PBS (pH 7.0) solution including $Fe(CN)_6^{3-}$. In comparison with bare GCE (Fig. 3c), owing to the electrostatic repulsion of Glu film to $Fe(CN)_6^{3-}$ and electrostatic attraction of Ch film to $Fe(CN)_6^{3-}$, a blocked effect at Glu/GCE (Fig. 3a) and an accelerated effect at Ch/GCE (Fig. 3d) are shown for the redox of $Fe(CN)_6^{3-}$, respectively. Figure 3b shows that the electrochemical response of $Fe(CN)_6^{3-}$ is middle at Ch/Glu/GCE due to the interaction of the mixed modified layer (Fig. 3b), indicating a change of charge characteristic. This illustrates a successful surface modification of Ch and Glu.

Electrochemical impedance spectroscopy measurement can be used to value the kinetics of a redox reac-

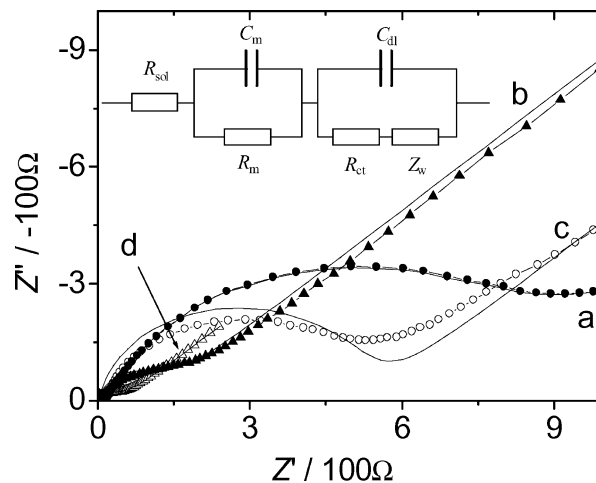


Fig. 4 Electrochemical impedance spectroscopy spectra at Glu/GCE (a), Ch/Glu/GCE (b), bare GCE (c) and Ch/GCE (d) in 5 mmol/L $K_3[Fe_3(CN)_6]$ + 1.0 mol/L KCl. 100 kHz–0.05 Hz, at 0.215 V versus SCE. *Insert* An equivalent circuit for the modified electrodes

tion. The EIS of GCE, Glu/GCE, Ch/GCE and Ch/Glu/GCE are shown in Fig. 4 in $Fe(CN)_6^{3-}$ solution. An equivalent circuit shown in Fig. 4 (inset) comprising the solution resistance (R_{sol}), film resistance (R_m), film capacitor (C_m), charge-transfer resistance (R_{ct}), Warburg impedance (Z_w) and the double layer capacitance (C_{dl}) was used for data simulation. The equivalent circuit used for data fitting was quoted from Refs. [20, 33, 34]. The R_{ct} values were obtained as 590 Ω for GCE (Fig. 4c), 1,150 Ω for Glu/GCE (Fig. 4a), 83 Ω for Ch/GCE (Fig. 4d) and 272 Ω for Ch/Glu/GCE (Fig. 4b). These indicate the existence of block effect on $Fe(CN)_6^{3-}$ redox reaction at the Glu modified layer, acceleration effect at the Ch modified layer; however, these split the

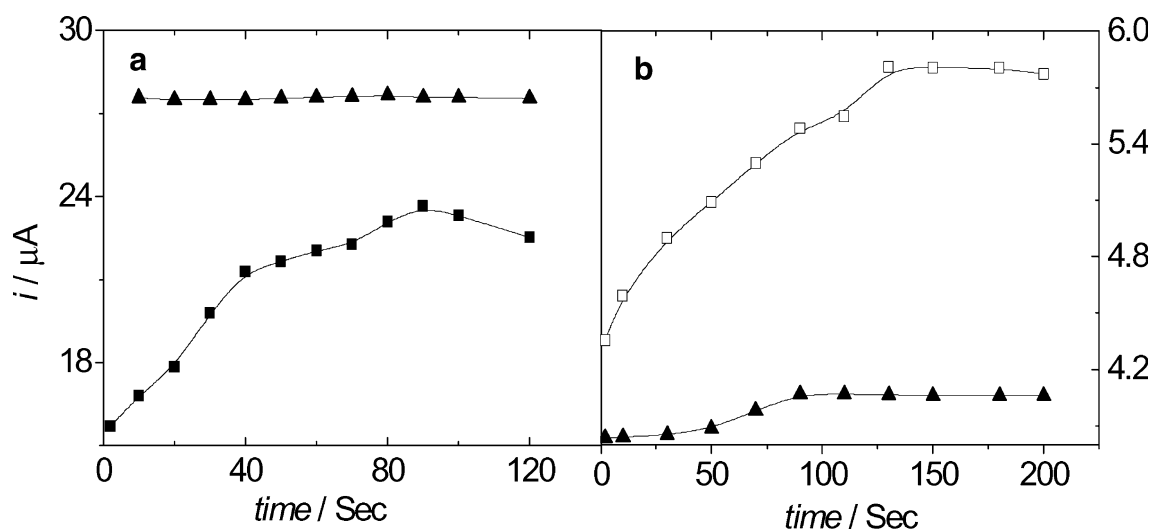


Fig. 5 Dependence of the peak current on the stasis time in 5 mmol/L $Fe(CN)_6^{3-/4-}$ + 0.01 mol/L PBS (pH 7.0) (a); in 0.1 mmol/L $Ru(bpy)_3^{2+}$ + 0.01 mol/L PBS (pH 7.0) (b) at open circuit potential, recorded at Glu/GCE (open square), Ch/GCE (filled square), Ch/Glu/GCE (filled triangle)

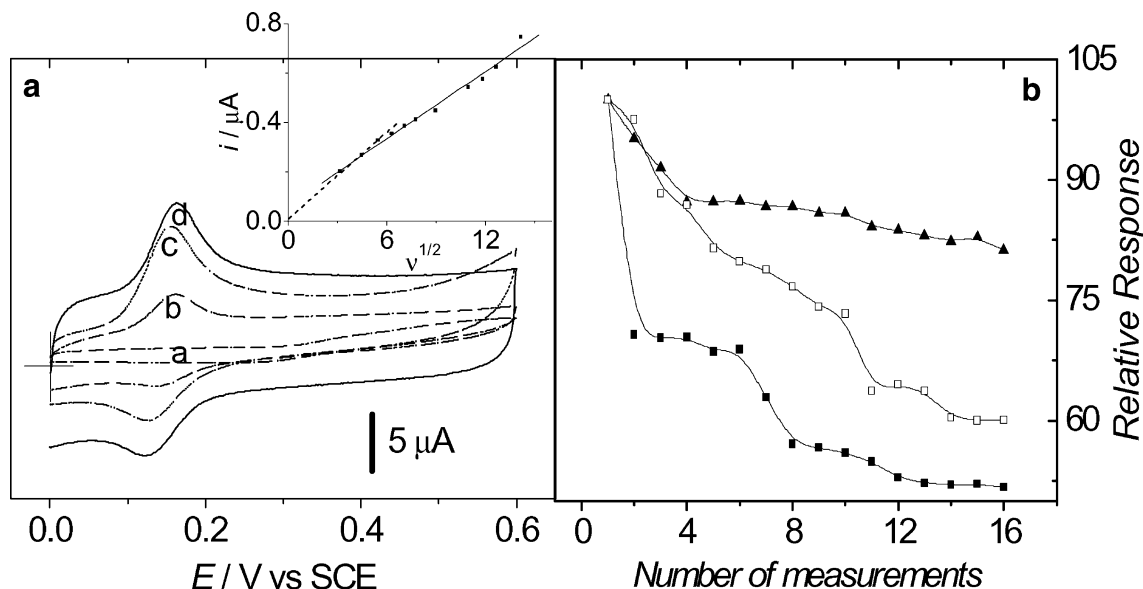


Fig. 6 The CVs (a) of 0.5 mmol/L DA at bare GCE (a), Ch/GCE (b), Glu/GCE (c), Ch/Glu/GCE (d) in 0.1 mol/L PBS (7.0). *Insert* the peak current of DA versus scan rate at Ch/Glu/GCE. The peak current (b) of DA on the number of determinations, recorded at Glu/GCE (open square), Ch/GCE (filled square), Ch/Glu/GCE (filled triangle)

difference effect at the mixed Ch and Glu modified layer. It is probably attributed to counteracts the functions of mixed modified layer to the redox of $\text{Fe}(\text{CN})_6^{3-}$. Accordingly, these impedance data are in good agreement with the results of CVs in Fig. 3.

To compare the resistance ability to pollution, CV experiments were carried out at Ch/GCE (filled square), Glu/GCE (open square) and Ch/Glu/GCE (filled triangle) at open circuit potential in PBS (pH 7.0) solution including $\text{Fe}(\text{CN})_3^{3-}$ or $\text{Ru}(\text{bpy})_3^{2+}$. As shown in Fig. 5a, with increasing stasis time, the current response of $\text{Fe}(\text{CN})_3^{3-}$ slowly increases at Ch/GCE, suggesting an adsorption effect to anion; whereas, the current response is almost unchanged at Ch/Glu/GCE, implying an anti-adsorption effect to anion. On the other hand, as shown in Fig. 5b, with increasing of stasis time, the current response of $\text{Ru}(\text{bpy})_3^{2+}$ slowly increases at Glu/GCE, indicating an absorption effect to cation; but a less increase at the mixed film, shows a resistance absorption effect to cation. From these differences, therefore, it demonstrates that the mixed film has excellent resistance absorption ability to cation or anion. Moreover, the experiments showed the best restraint adsorption ability to anion and cation as modified rate of 2:1 of Ch and Glu.

Electrocatalytic behavior of phenolic compounds at mixed covalent monolayer

Electrocatalytic behavior of DA

The CVs of DA recorded with bare GCE (curve a), Ch/GCE (curve b), Glu/GCE (curve c), Ch/Glu/GCE (curve

d) are shown in Fig. 6a, respectively. The distance of oxidation and reduction peak potential was labeled as ΔE_p , and the rate of oxidation and reduction peak current labeled as i_{pa}/i_{pc} . The ΔE_p and i_{pa}/i_{pc} values were obtained as 0.25 V and 2:1 for GCE, 0.024 V and 1.7:1 for Glu/GCE, 0.025 V and 1.9:1 for Ch/GCE and 0.035 V and 1.3:1 for Ch/Glu/GCE. These indicate improved effect at the three modified electrodes, particularly, the mixed layer shows the strongest electrocatalytic function to the redox of DA with improving reversible behavior ($i_{pa}/i_{pc} = 1.3$) and significantly increasing peak current. On the other hand, the oxidation peak currents of DA are linearly proportional to the scan rate in the range 10–120 mV/s and 10–200 mV/s at Ch/GCE or Glu/GCE, suggesting an accumulation process. Whereas, the oxidation peak currents of DA are linearly proportional to the scan rate in the range of 10–40 mV/s, and to the square root of scan rate in the range 50–200 mV/s at Ch/Glu/GCE, signifying an adsorption process in lower scan rate and a diffusion process in higher scan rate (Fig. 6a, insert). It illustrated a change of transport characteristic for DA at Ch/Glu/GCE.

Figure 6b shows the relative response of the anodic peak current for DA oxidation during successive CV scans with Ch/GCE, Glu/GCE and Ch/Glu/GCE. With 16 continuously CV scans, the decrease of anodic peak current of DA of 52% at Ch/GCE (filled square), and 40% at Glu/GCE (open square) are shown due to the accumulation of insulating polymerization formed phenoxy radicals attacking unreacted substrate with continuous scanning [8, 22]. However, an obvious improved effect can be readily seen at Ch/Glu/GCE with the decrease of 15% (filled triangle). It is contributed to

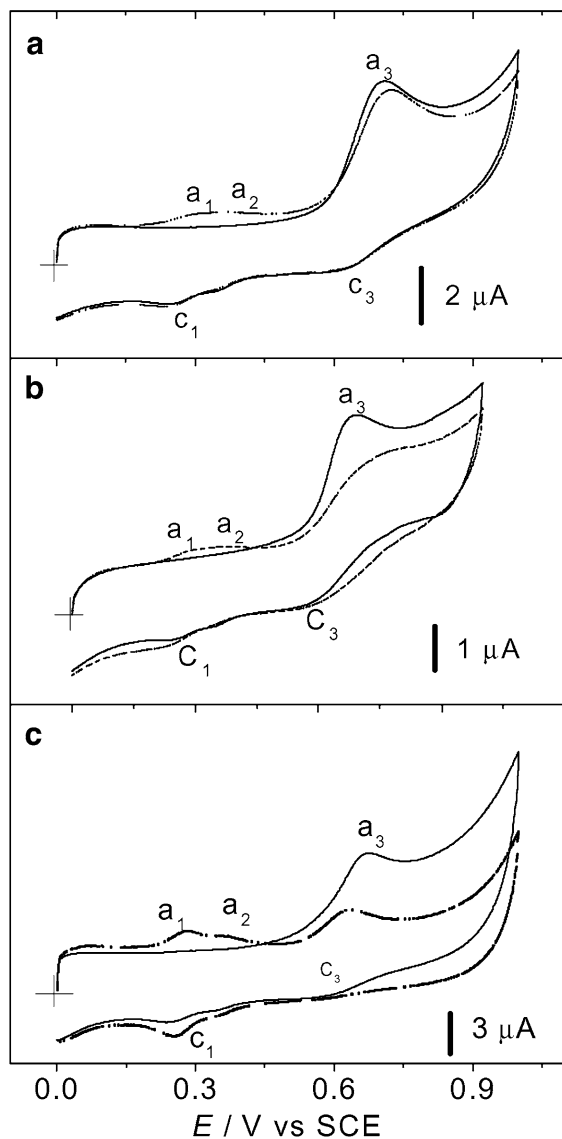


Fig. 7 The CVs of 0.1 mmol/L phenol at Ch/Gly/GCE (a), Gly/GCE (b) and Ch/GCE (c) in 0.1 mol/L pH 7.0 PBS. Scan rate: 50 mV/s, the solid line first scan, the dotted line second scan

the mixed modified layer restraining the formation of insulating polymerization due to improvement in the reversibility of DA redox. Moreover, the mixed layer showed a similar result for EP redox following identical performances.

Electrocatalytic behavior of phenol

The CVs of phenol recorded with Ch/Gly/GCE (Fig. 7a), Gly/GCE (Fig. 7b) and Ch/GCE (Fig. 7c) are shown in Fig. 7. In the first scan (solid line), the oxidation of phenol gave a sluggish and much smaller CV peak response of about 1.0 V at bare GCE. But Fig. 7a, b and c, respectively shows an obviously enhancing peak a_3 about 0.70 V, and two little cathodic counterpart c_3 about 0.60 V and c_1 about 0.25 V at the three electrodes;

among these, the response of a_3 at Ch/Gly/GCE is largest. It is in line with an alternative pathway of phenol electrooxidation leads to the formation of phenoxy radical [22–26], which reduces to phenol at higher potential and hydroquinone at lower potential [27]. In second cycling (dotted line), Fig. 7a, b and c, respectively, shows a new small anodic peak a_2 about 0.35 V at the three electrodes, besides a_1 and c_1 , this result is similar to the CV of the quinone/hydroquinone system [22]. It is noticeable that the response of a_3 sharply decreases at Gly/GCE (Fig. 7b) and Ch/GCE (Fig. 7c), which contributed to the formation of phenoxy radicals [1, 6, 19–21], which can initiate the chain or branch polymerization, depending on the type of substitution in the phenol molecule [22, 24, 28, 29]. However, we can readily see that the response of a_3 slightly decreases at Ch/Gly/GCE (Fig. 7a), implying a resistant effect of the mixed film to the formation of insulating polymerization. This finding is significant for the continual electroanalysis measurements of phenolic compounds. From the difference of phenol reaction at three modified electrodes, the scheme of oxidation for phenol at Ch/Gly/GCE could be suggested as follows (Scheme 2): phenol electrooxidation, first, leads to the formation of phenoxy radical [22–24, 30], which form phenol at higher potential and hydroquinone at lower potential (reaction 1), the formation of polyoxyphenylene could be effectively restrained. In first the phenoxy radical formed in reaction (1) can be oxidized to the phenoxonium cation [31] (reaction 2), which in turn can undergo nucleophilic attack by water to form hydroquinone, i.e. the quinone/hydroquinone redox system should be expected (reaction 3) [23, 25, 32].

To investigate the adsorption behavior of phenol on the electrode surface, the CVs of phenol with various scan rates in the range 10–200 mV/s were measured. The peak currents of a_3 at the three modified electrodes were directly proportional to the square root of scan rate in first scan. It means that the electrode process for phenol is controlled by diffusion. Thus, the decrease of a_3 is because of the electropolymerized film on the electrode surface, and not the interference in the electrode reaction by the adsorption of phenol monomer.

Figure 8a shows the relative response of the anodic peak current of a_3 during successive CVs scans at the three electrodes. Due to the continuous covering of oxide coating at the surface-active sites [22, 24, 28–30], the strong adherence of insulating polymer at the electrode surface, the further redox of phenol could be inhibited [24, 28, 31], and thus, the anodic peak current of a_3 is shown to sharply decrease at Ch/GCE (filled square) or Gly (open square). However, an obvious improved effect for the response of a_3 can be readily seen at Ch/Gly/GCE (filled triangle), the reason may be that the mixed film effectively minimizes the polymerization of the phenoxy radical. This illustrates that the mixed film has better restraint pollution ability for phenol reaction products. This effect is very useful for analytical purposes and is in line with Fig. 6b.

Scheme 2

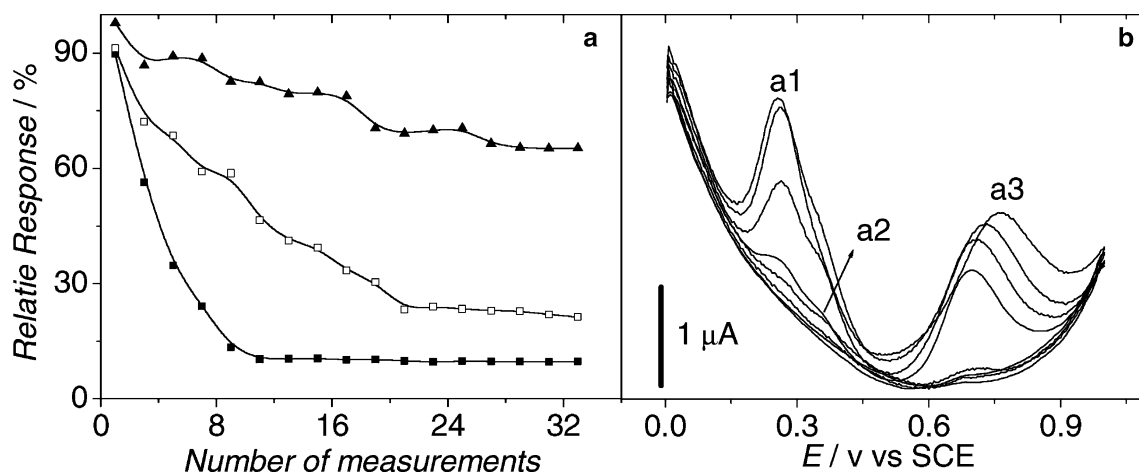
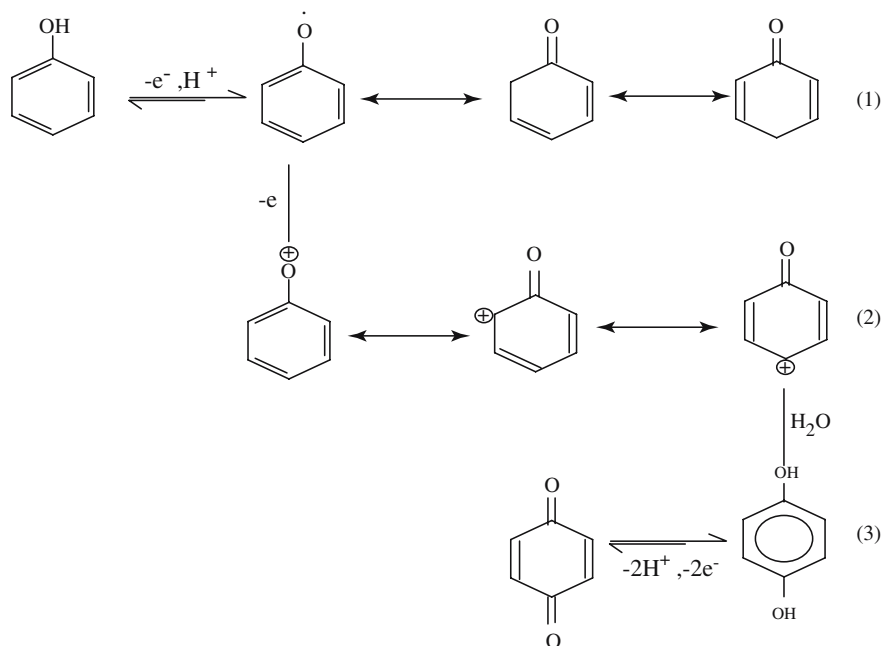


Fig. 8 **a** The peak current of phenol on the number of determinations, recorded at Gly/GCE (*open square*), Ch/GCE (*filled square*), Ch/Gly/GCE (*filled triangle*). **b** DPVs of phenol at Ch/Gly/GCE in 0.1 mol/L PBS (pH 7.0), concentration: 1, 3, 5, 7, 10, 30, 50, 70 and 90 $\mu\text{mol/L}$, DPV condition: 4 mV incre; 50 mV amplitude; 0.05 s pulse width; 0.2 s pulse period

As shown in Fig. 8b, under the same conditions, using the DPV method, the catalytic peak current a_1 and a_3 was linearly related to phenol concentration in the range 7×10^{-6} – 7×10^{-5} mol/L and 7×10^{-6} – 9×10^{-5} mol/L with correlation coefficients of 0.995 and 0.985 and practical detection limit of 1×10^{-6} and 7×10^{-7} mol/L.

Electrode stability

The Ch/Glu/GCE shows high stability. For example, during the first 2 days the signal showed a 5% decrease, in the fifth day the current response decreases by about 9% of its initial response and in the following month by 20%.

Conclusion

The study has demonstrated the ability to create a mixed covalent modification at the glassy carbon electrode with two different compounds including choline and amino acids. Significantly, this develops a facile means for altering the interfacial architecture of glassy carbon electrodes. These modified electrodes could inhibit the formation of insulating polyoxyphenylene. Together with low cost and ease of preparation, especially, good resist are to pollution and catalysis ability for phenolic compounds, these modified electrodes could be used for continuous detections of phenolic compounds.

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