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A novel electrochemical enzyme biosensor for detection of 17β-estradiol by mediated electron-transfer system

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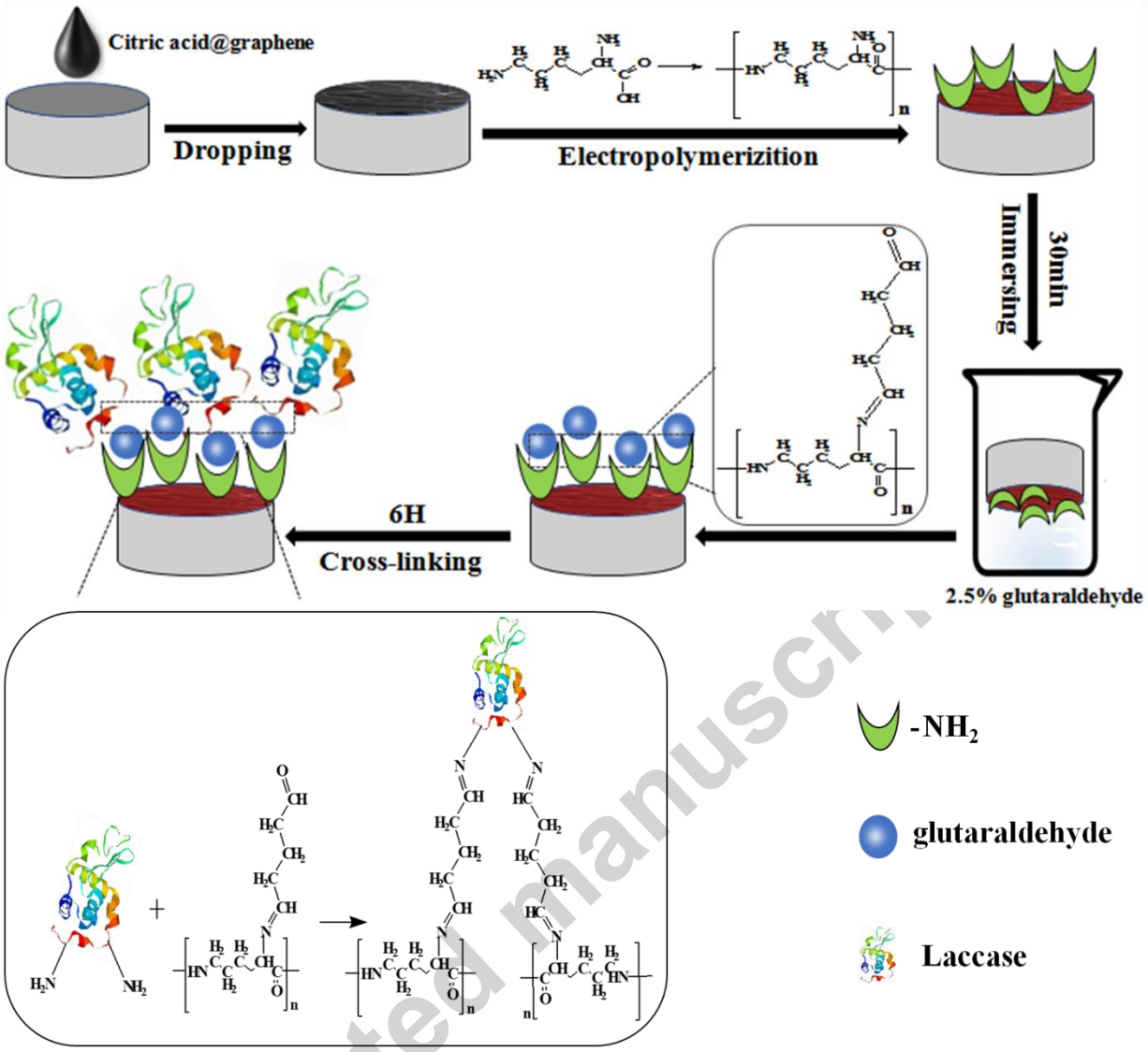
Abstract

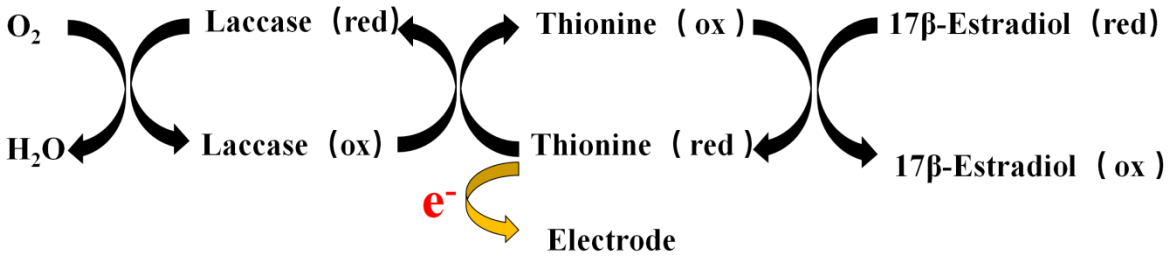
An extremely sensitive enzyme sensor for detection of 17β-estradiol based on electropolymerized L-lysine molecules on a glassy carbon electrode (GCE) modified with critic acid@graphene (CA-GR) and cross-linked with laccase enzyme has been developed in this work. As the laccase immobilization, glutaraldehyde was chosen as cross-linker through the groups reactions. The novel enzyme sensor could recognize and determinate 17β-estradiol effectively. The morphology of the enzyme modified electrode was characterized by transmission electron microscopy (TEM) and electron microscopy (SEM). The amino interaction between cross-linker and enzyme was characterized by Fourier transform infrared spectroscopy (FTIR). Under the optimal experimental conditions, good linear relationships were achieved in the range of 4×10^{-13}-5.7×10^{-11} M and a limit of detection as low as 1.3×10^{-13} M. Moreover, the enzyme sensor exhibited good reproducibility, stability and high selectivity to 17β-estradiol. Excellent performance was showed in the human urine samples analysis, thus confirming great prospect for further application in clinic diagnosis and biological research.

Graphical abstract

1. The preparation of the Lac/Poly L-lysine/CA-GR/GCE



2. The electrocatalytic mechanism proposed for the enzyme biosensor.



Keywords: Enzyme biosensor; Poly L-lysine; Covalent immobilization; 17β-estradiol

1. Introduction

17β-Estradiol (E_2), a natural steroid with estrogenic activity[1], which has been widely used in animal husbandry to promote animal growth[2], increasing the lean meat percentage and milk production[3]. Excessive E_2 remained in animal food like meat and milk will cause women's fertility problem[4], increasing the risk of exposure to ovarian and breast cancer[5]. Therefore, studying 17β-Estradiol is significant for clinical analysis/diagnosis. Conventionally, various of methods, such as HPLC[6], immunological methods[7], gas chromatography[8], and chemiluminescence[9], have been developed for detecting 17β-Estradiol. However, these methods require complex precipitation procedures and it suffer from low sensitivity, time consumption and high cost. Hence, it is extremely urgent to develop a simple, rapid and sensitive method for the determination of E_2.

Enzyme based on electrochemical sensor is one of the most promising sensors for ultra-trace detection in complex environment, due to its high specificity and excellent accurate[10]. Laccase, a typical multicopper oxidase, has been intensively researched as biocathode electrocatalysts for oxygen reduction in detecting system by exploiting direct and mediated electron-transfer reactions[11]. However, because of intricate catalytic mechanism and enzyme molecular immobilized orientation, achieving the direct electron-transfer process of laccase is difficult[12]. Medicated electron-transfer reaction can avoid these problems. Therefore, it is important for enzyme biosensor to construct a medicated electron-transfer system. Developing a novel composite material for immobilization of laccase is the vital step to assemble the biocathode. There are some immobilization techniques such as covalent immobilization[13],

physical adsorption method[14], and embedding method[15]. Compared to other methods, enzyme molecules can be immobilized by the cross-linking method more effectively due to the connection with chemical bond[16]. It is well known that the proper concentration of glutaraldehyde is an effective cross-linking agent through providing aldehyde groups for reaction with enzyme molecules and nanocomposite materials[17].

Graphene, a type of dimensional nanomaterial[18], has good biocompatibility, high charge mobility and great mechanical stability[19]. It has been successfully applied to electrochemical sensors as a signal amplifier and the supporter of subsequent materials decoration[20]. However, the high hydrophobicity and lack of functional group limited its utility in the field of assembling materials[21]. These problems can be resolved by functionalized graphene which has been treated with citric acid. The graphene modified by citric acid (CA-GR) not only has better conductivity, but also possesses more oxygenic groups for the polymerization of intermediate than pure GR. Moreover, the ability of catalytic oxidation of CA-GR was also enhanced effectively[22].

In this work, a novel 17β-Estradiol sensor was fabricated by laccase loading with CA-GR and electropolymerized L-lysine film modified glassy carbon electrode (Lac/PLLY/CA-GR/GCE) as sensing platform, in which L-lysine was chosen to form an electro-polymer film due to its small size, good biocompatibility and much amino and carboxyl[23]. The sensor was applied in sensitive detection of 17β-Estradiol in 0.1 M phosphate buffer solution (pH 6.0) contained 1.2 mM thionine as electron-transfer mediator. The electrochemical behavior of 17β-Estradiol on laccase enzyme sensor were investigated by differential pulse voltammetry (DPV) and cyclic voltammetry (CV). The oxidation peak current of 17β-Estradiol at Lac/PLLY/CA-GR/GCE increased largely, compared with that at bare/GCE. The proposed laccase enzyme sensor showed good stability and low detection limit for the determination of 17β-estradiol.

2. Experimental

2.1. Apparatus

Fourier transform infrared (FTIR) spectra were recorded on the AVATAR 370 Fourier transform infrared spectrometer (Thermo Nicolet corporation). Raman scattering was carried out on the INVIA Raman Microscope using a 545.5 nm laser

source (Renishaw corporation, England). Scanning electron micrographs (SEM) were performed on the JSM-6700F scanning electron microscope (15.0 kV). The JEM-211F Transmission electron microscope (Japan) was employed to obtain Transmission electron micrographs (TEM).

All electrochemical experiments were carried out by a CHI 852C electrochemical workstation (Chenhua corporation, Shanghai, China) with a conventional three-electrode system consisting of a bare or modified GCE (3.0 mm in diameter) as a working electrode, a saturated calomel electrode and a platinum electrode as the reference electrode and the counter electrode respectively. The pHS-3C acidity meter was used to determine pH value.

2.2. Reagents

GR was purchased from XF NANO Advanced Tech Co., Ltd (Nanjing, China). L-lysine was obtained from LANJI Co., Ltd. (Shanghai, China). Glutaraldehyde was provided by Macklin Co., Ltd. (Shanghai, China). Thionine was supplied by Regal Co., Ltd. (Shanghai, China). Laccase from agaricus bisporus (≥10U/mg). 17β-estradiol and critic acid were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and used without further purification.

2.3. Fabrication of CA-GR/PLLY/GCE

The preparation of CA-GR was similar to what has been recorded in the literature[24] and you can find them at supplementary material in detail. Prior to modification, the bare GCE had been polished on chamois leather with 0.05 μM α-alumina powder, rinsed with doubly distilled water and sonicated in 1 : 1 (v/v) HNO_3, absolute alcohol and doubly distilled water for 3 min successively. The CA-GR/GCE was obtained via dropping with 5 μL CA-GR suspension, and an infrared lamp was employed to dry the modified electrode to gain the CA-GR/GCE. Electro-polymerization of L-lysine on the CA-GR/GCE was performed in 10 circles in 0.1 M sodium phosphate buffer (pH 8.0) which contained 1 mM L-lysine. The parameters of this method were set as follows: potential range, -2.0-2.0 V; scan rate, 1mv; sweep segment, 20; quiet time, 2 seconds. Then CA-GR/Poly L-lysine/GCE was fabricated.

2.4. Preparation of the enzyme electrode (Lac/PLLY/CA-GR/GCE)

The CA-GR/Poly L-lysine/GCE immersed in 2.5% glutaraldehyde solution and reacted for half an hour at room temperature. Then the CA-GR/Poly L-lysine/GCE

was rinsed with doubly distilled water and dried at room temperature. Laccase enzyme was further immobilized on the electrode surface which had been modified by nanocomposite material, by casting with 5 μL of 3 mg/mL enzyme solution in 0.1 M PBS (pH 5.6) for cross-linking for 6 hours at 4 ℃. After reaction, the modified GCE was rinsed with PBS (pH 5.6) to remove redundant laccase molecules and kept at 4 ℃ for further apply.

3. Results and discussion

3.1. Preparation and Characterization of Lac/PLLY/CA-GR/GCE

Scheme 1 illustrated the principle of the proposed assembly for enzyme electrode. The process can be summarized as four steps: (1) CA-GR suspension was dripped on the surface of GCE; (2) L-lysine monomers were electro-polymerized on the surface of CA-GR/GCE. Through this process, the amino groups had been generated on nanomaterial planar sheets; (3) The nanocomposite material was immersed in glutaraldehyde solution for reaction about half an hour. In this procedure, part of aldehyde groups of glutaraldehyde was cross-linked with nanomaterial; (4) The remaining aldehyde groups of glutaraldehyde could act as the points for the covalent connection with laccase. Laccase solution was casted on the surface of nanocomposite material and then kept in refrigerator for cross-linking reaction about 6 hours.

It is well known that the drawback of enzyme-based biosensors, which is enzyme immobilization may affect active site of enzyme, it could be buried in their complex protein structure[25]. Therefore, it is necessary to find an effective cross-linker and immobilization method. The method will not decrease catalytic activity and limit electrochemical performances. Comparing to absorbed immobilization in Fig.S1, the larger voltammetric peaks were achieved by cross-linked immobilization. Thus, the cross-linked immobilization method was employed for further experiments. Glutaraldehyde was evaluated as a promising cross-linker because aldehyde group can react with many nucleophiles such as hydroxyl, thiol, and amine groups, and they formed stable cross-linking adducts. Scheme 1 depicts that the groups of glutaraldehyde connect nanocomposite material and enzyme laccase. Thionine was used as electrochemical mediator for determining 17β-estradiol in enzyme electrode.

As shown in Fig.S2, although it is better to avoid using thionine, the differential pulse voltammetry response in presence of thionine toward E_2 was obviously higher than what had been recorded in absence of thionine to 17β-estradiol. Thus, the electrochemical mediator was employed for further experiments.

We empolyed SEM to investigate the morphology of the as-prepared CA-GR, PLLY/CA-GR/GCE and Lac/PLLY/CA-GR composite. As shown in Fig.1(A), the CA-GR film presents the flake-like and crimple shapes structure, which can provide an ideal matrix for electro-polymerization of L-lysine molecules. The increasing surface area of CA-GR electrode is more favorable for anchoring poly L-lysine film. In Fig.1(B), the poly L-lysine film was covered on surface of bare GCE. It can be clearly seen that each monomer granules were uniformly dispersed on the surface of GCE. As shown in Fig.1(C), poly L-lysine nanoparticles loads on crimple shape of CA-GR, because the configuration combine CA-GR with poly L-lysine morphology, and it indicates that the composite film has been successfully modified on the electrode surface. After cross-linking laccase, the surface of GCE was covered with uniform laccase sphere which had been illustrated in Fig.1(D). Owing to laccase had been cross-linked on nanocomposite, the material exhibited excellent electro-catalytic performance to a certain kind of analyte. Some details about the environment of enzyme was showed in Fig.1(E) and (F), indicating that laccase was coated on nanocomposite firmly and greatly integrated with matrix.

Fig.2(A) illustrated the FTIR spectra of the PLLY/CA-GR (a) and Lac/PLLY/CA-GR (b). The stretching bands at 2914 cm^{-1}, 3425 cm^{-1} and 1034 cm^{-1} are the vibration of C-H, N-H and C-O respectively. These peaks are caused by characteristic absorption of PLLY/CA-GR. As shown in curve b, the increasing stretching band for C=N at 1661 cm^{-1} appeared and the peaks in fingerprint region became broader after cross-linking. This was caused by the cross-linking of laccase, indicating that the laccase enzyme had been attached.

Raman spectroscopy was used as a powerful nondestructive tool to distinguish

ordered and disordered crystal structures of carbon [26]. The G-band is a feature of all sp^2-hybridized carbon networks, while the D-band is assigned as an imperfect structure which formed by the attachment of polymerization on the carbon basal plane. Therefore, the intensity ratios of the D and G bands (I_D/I_G) indicate the disorder degree and defects of the graphitized structures[27]. Fig.2 (B) shows the Raman spectra of CA-GR (a) and PLLY/CA-GR (b), which differ from 0.92 of CA-GR to 1.01 of PLLY/CA-GR. The two prominent peaks of the D and G bands also shifted from 1341.1 cm^{-1} and 1588.0 cm^{-1} of CA-GR to 1336.8 cm^{-1} and 1596.6 cm^{-1} of PLLY/CA-GR. Which means an interaction between the polymerization and functional graphene sheets, as previously described for other polymerization-graphene derivatives[28].

The TEM image of the Lac/PLLY/CA-GR is given in Fig. 3 (A), it can be observed that uniformly disperse sphere-like laccase molecules of 250 nm±50 nm were coated on nanocomposite materials. It also can demonstrate that laccase enzyme was successfully cross-linked with nanocomposite materials. There are a lot of nano-holes on the enzyme molecules, which is correspond with the inset (SEM), that is the magnifying SEM of Lac/PLLY/CA-GR. It indicates that electrons can also go through the enzyme molecules.

The electron-transfer kinetics at electrode was probed by Electrochemical impedance spectroscopy (EIS) [29]. The EIS contains a higher frequency semicircle and a lower frequency linear portion, which corresponds to the electron transfer limitation process and diffusion process[28] . The semicircle diameter indicates the charge transfer resistance (Rct) of the $[Fe(CN)_6]^{3-/4-}$ redox probe[30]. As shown in Fig.3 (B), compared to bare electrode, the CA-GR/GCE shows a small semicircular, which represents faster electron-transfer kinetics of $[Fe(CN)_6]^{3-/4-}$. However, Rct (80.42Ω) is amplified after the poly-L-lysine membrane is produced on the surface of the modified electrode. When the laccase anchored on the Poly L-lysine films, the R_{ct} (1064.07 Ω) was obviously enlarged, that because of its high selectivity electrochemical catalysis, what's more, its large size impede electron transferring. Meanwhile, it indicates that laccase electrode was assembled successfully. Fig.3 (C) and Fig.3 (D) show cyclic voltammograms and differential pulse voltammograms

respectively toward 5 pM 17β-estradiol in 0.1 M sodium phosphate buffer pH 6.0 containing 1.2 mM thionine as electrochemical mediator at bare GCE, CA-GR/GCE, PLLY/CA-GR/GCE, Lac/PLLY/CA-GR/GCE. For Lac/PLLY/CA-GR/GCE, the peak current became larger than PLLY/CA-GR/GCE, CA-GR/GCE, and bare GCE obviously. This may be attributed to the combination of CA-GR, Poly L-lysine and laccase which is special for its electrochemical catalysis towards 17β-estradiol. In addition, compared to CA-GR, a new peak around 0.0 V was clearly observed in CV and DPV of modified electrodes, which should be ascribed to the redox reaction of L-lysine polymer.

3.2 Optimization of parameters for Lac/ PLLY/ CA-GR/ GCE

Fig.4 (A) shows the relationship between buffer pH and peak current of 17β-estradiol. The oxidation peak potential of 17β-estradiol shifted negatively with the increase of pH and the current signal of 17β-estradiol peaked at pH 6.0 then decreased when the pH was less than 6.0. The oxidation peak potential shifted negatively, suggesting that the electrochemical oxidation process is actually the proton-transfer process. The linear relationship was observed between potentials and solution pH with the slope of -53 mV pH^{-1} that is corresponded to the ideal state -59 mV pH^{-1} at 25 ℃. The results show that the same amount of electrons and protons are involved in the electrochemical oxidation process of Lac/PLLY/CA-GR/GCE surface [31].

The cyclic voltammograms of 5 pM 17β-estradiol on the Lac/PLLY/CA-GR/GCE at different scan rates (10-200 mV) are illustrated in Fig.4 (B). The relation between the scan rates and oxidation, reduction peak currents is presented in the inset. When the scan rates (ν) was in the range of 10-200 mV s^{-1}, the inset (top) of Fig. 4(B) show that both the oxidation peak currents (I_{pa}) and reduction peak currents (I_{pc}) had a linear relationship with the square root of the scan rates (ν). The regression equations were obtained as follows: I pa (μA) = 0.0761 + 9.84 $\nu^{1/2}$ (mV s^{-1}) $^{1/2}$ (R^2=0.991) and I pc (μA) = 0.9890 – 17.70 $\nu^{1/2}$ (mV s^{-1}) $^{1/2}$ (R^2 = 0.993), respectively. Accordingly, the

electrochemical processes on Lac/PLLY/CA-GR/GCE were diffusion-controlled process. As shown in the inset (bottom), it can observe that the linear relationship between E_{pa}, E_{pc} and logν having a equation of E_{pa}(V)= 0.05121 logν-0.235(R^2=0.981), E_{pc}(V)=-0.0481 logν-0.148(R^2=0.999) respectively. According to Laviron's model[32], the potential showed a linear dependence with the scan rate and the equation can be summarized as followed: $E_{pa} = E_o + 2.3RT/(1-\alpha)nF \log \nu$, $E_{pc} = E_o + 2.3RT/\alpha nF \log \nu$. In general, the electron-transfer coefficient (α) is about 0.4-0.6[33]. Setting that the electron-transfer coefficient (α) of the irreversible electrode process is 0.5. Therefore, the number of electron-transfer (n) can be calculated as 2.3. As is illustrated in Scheme 2, the proposed electrocatalytic mechanism of 17β-estradiol on the enzyme electrode using thionine as an electrochemical electrode intermediary.

The electropolymerization cycles of Poly L-lysine influences the oxidation peak currents of 17β-estradiol and it was recorded by DPV. As it is obvious from Fig.5 (A), the maximum currents were obtained when the parameter of cycles are set to 10 and decreased when the cycles were further increase. This may be owed to the thickness of the Poly L-lysine film. Therefore, the parameter 10 of electropolymerization cycles was selected. As shown in Fig.S3, the peak current of polymerization was increased constantly before the cycles reaching to 10, after that the peak current keep invariant, which also shows that L-lysine is conductive monomer.

The drop-coating volume of CA-GR influences polymerization film production and sensor sensitivity, which further influences enzyme immobilization. In Fig.5 (B), the largest peak value was achieved when the drop-coating volume was 5 μL. The peak current became weak for 17β-estradiol when the volume was larger than 5 μL. when its volume exceeded a certain degree, gravity will cause it to fall off from the surface of the sensors and the over thickness will affect the transfer of electrons. Therefore, the drop-coating is also a key factor for studying.

In this work, the enzyme modified electrode was immersed in 17β-estradiol containing thionine as electron transferring mediator. Fig. 5 (C) shows the influences of thionine on the peak current of enzyme electrode to analyte, the optimum thionine concentration was 1.2 mM. The peak currents became stable gradually after 1.2 mM. The reason why the currents become stable after 1.2 mM approximately is that enzyme catalysis has approach saturation points and the extra mediator is not working. As shown in Fig.5 (D), we also optimized the enzyme concentration, and different

concentration was cross-linked with nanocomposite material, we can observe that the electrode modified by 3 mg/mL laccase possess the highest peak current. The reason why the currents decrease when the laccase concentration was larger than 3 mg/mL approximately is that limited connections caused a part of them cannot immobilize on nanocomposite material, meanwhile, a large amount of enzyme molecules aggregated which result in the bury of active site of enzyme thus they cannot be exposed.

3.3 Analytical application of the laccase enzyme sensor

Lac/PLLY/CA-GR/GCE was detected different concentration of 17β-estradiol PBS (pH 6.0) solution at room temperature (from 0.4 pM to 57 pM). Under the optimized condition, The peak currents of 17β-estradiol on laccase sensor was recorded by DPV curves in a solution containing 1.2 mM electron-transfer mediator and 0.1 M PBS shown in Fig.6. The oxidative peak current (Ipa) has linear relationship with the concentration (c) of 17β-estradiol. In the range from 0.4 to 57 pM, a linear regression equations: Ipa= 4.15509 +0.118c (pM) (R^2=0.995) was obtained. The detected limit was 0.13 pM. Table 1 summarized the analysis results of electrochemical methods that have been reported previously for analyzing 17β-estradiol. The comparison can demonstrate that the proposed method is comparable to others recorded in literature.

3.4 selectively, reproducibility and stability of laccase enzyme modified electrode

The selectivity and anti-interference ability of laccase enzyme sensor was investigated in the presence of some possible interfering substances, such as ascorbic acid, dopamine, hydroquinone, catechol, bisphenol A, progesterone, ethinylestradiol, Testosterone and oestrone. As shown in Fig.5 (E), 100-folds concentration of interfering substances only cause the current deviations below 5%. The results reveal that the Lac/ PLLY/ CA-GR/ GCE presented high selectivity for 17β-estradiol than for other electroactive interferences. This is because the specific catalysis was created by laccase enzyme.

To study the reproducibility of the sensors, the laccase enzyme sensor was appiled to detect 5 pM 17β-estradiol for 9 times within 30 days, and the sensor was stored in the refrigerator when it was not used. As shown in Fig.5 (F), the enzyme sensors maintained 68% of its initial activity. The high stability of enzyme electrode can be explained by the multi-point cross-linking of enzyme molecules.

3.5 Human urine analysis

In order to confirm the validity and feasibility of the enzyme sensor for detecting 17β-estradiol in human urine. 1mL of human urine coming after consummation of meat was diluted to 10mL with PBS (pH 6.0). Different volume of standard 17β–estradiol was added to 10mL human urine samples. The signals were recorded by DPV curves. The standard addition method was performed to measure and the results were concluded in Table.1S. The recoveries were in the range of 98.4~103.7% with the RSD in the range of 1.3~3.4%. The satisfactory results demonstrated that the proposed enzyme sensor can be applied to the practical determination of E2 in real complex samples.

4. Conclusions

In this study, a sensitive, stable, and convenient biosensor to determinate 17β-estradiol was developed, which was based on electropolymerized L-lysine molecules on the surface of CA-GR, then via glutaraldehyde as cross-linker combined with nanocomposite material and laccase enzyme. The linear range was from 4×10^{-13} to 5.7×10^{-11} M and the low detection limit of 17β-estradiol was 1.3×10^{-13} M. In addition to the advantages of high specificity, low detection limit, wide linear range, and great stability, this enzyme sensor was also successfully employed in human urine for determination of 17β-estradiol. From the above, CA-GR and poly L-lysine nanocomposite material could act as a novel matrix, which can prove anchor points for linking with glutaraldehyde then further cross-linking with laccase enzyme molecules effectively. The results indicate that other enzyme biosensors also can be developed by this method.

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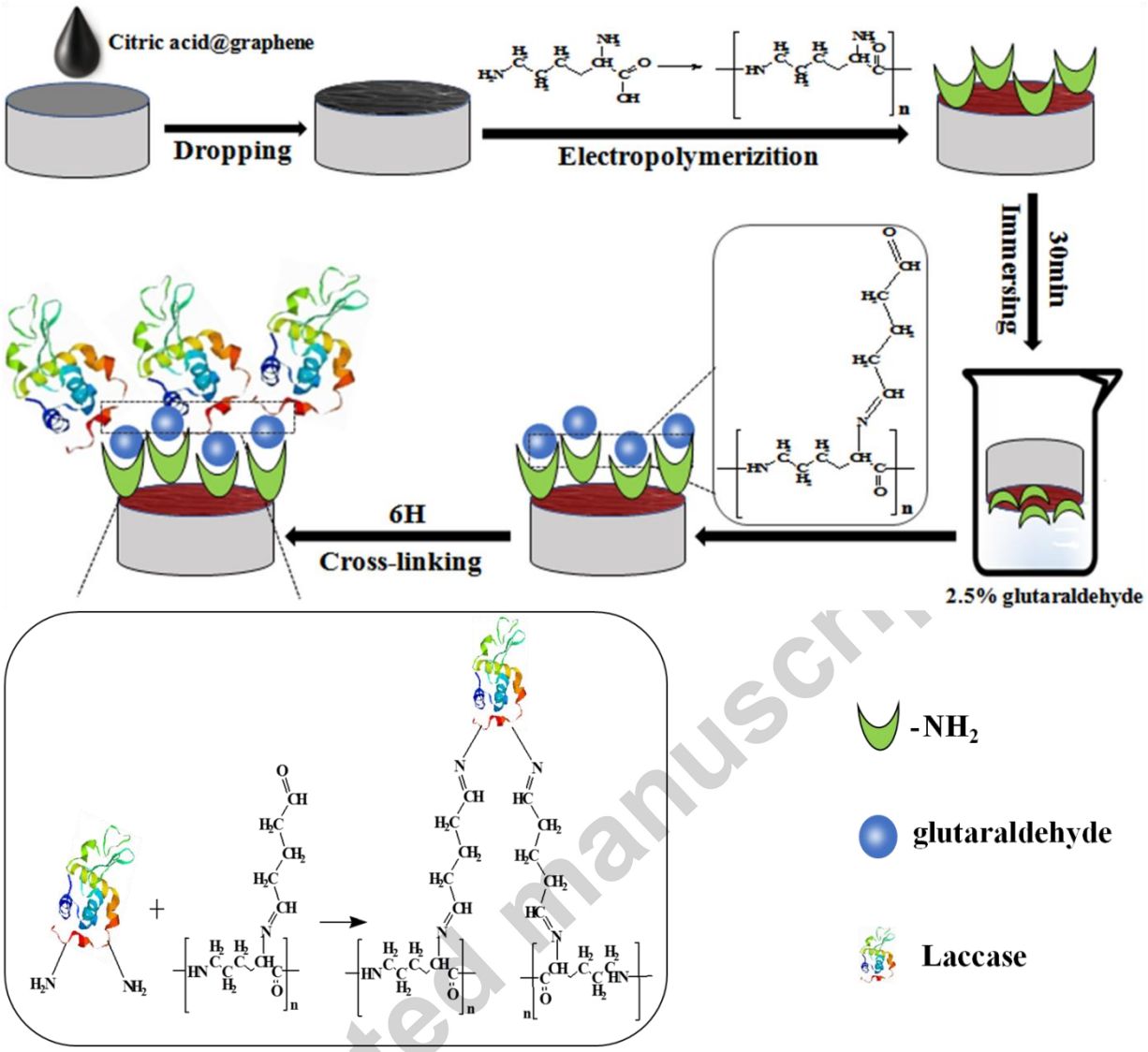
Scheme 1.The preparation of the Lac/Poly L-lysine/CA-GR/GCE



Scheme 2. The electrocatalytic mechanism proposed for the enzyme biosensor.

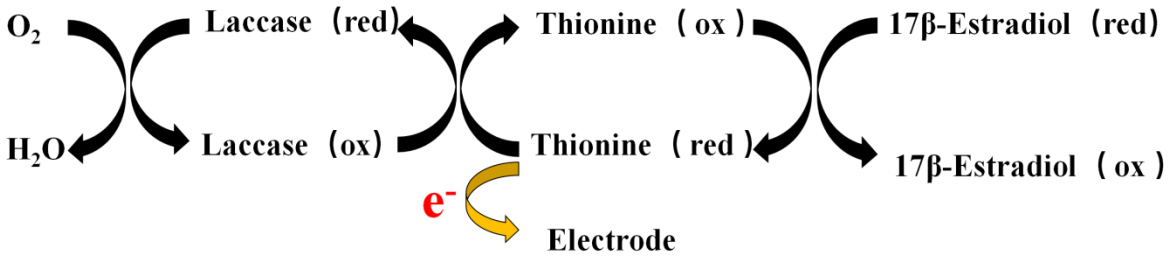


Fig. 1. SEM image of (A) CA-GR/GCE, (B) PLLY/GCE, (C) PLLY/CA-GR/GCE, and (D),(E),(F) Lac/ PLLY/CA-GR/GCE.

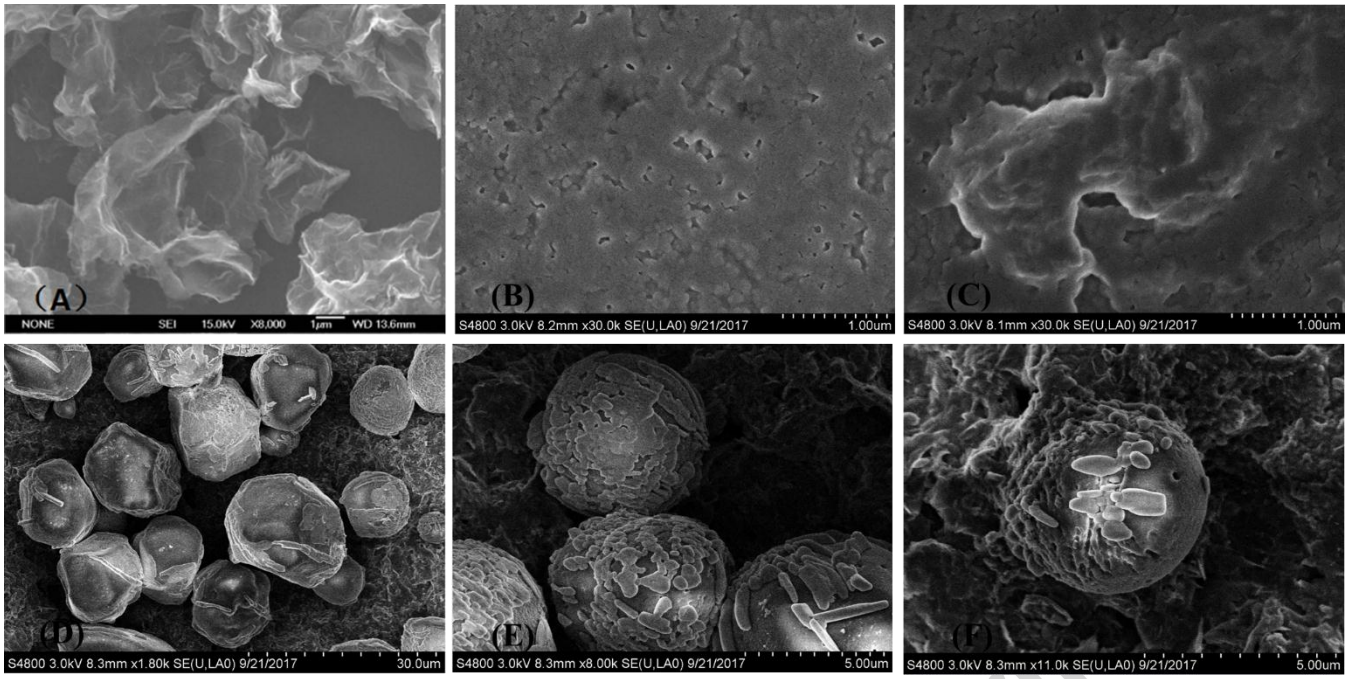
Fig. 2. FT-IR (A) analysis of PLLY/CA-GR (a) and Lac/PLLY/CA-GR (b), and Raman spectroscopy (B) analysis of CA-GR (a) and PLLY/CA-GR (b).

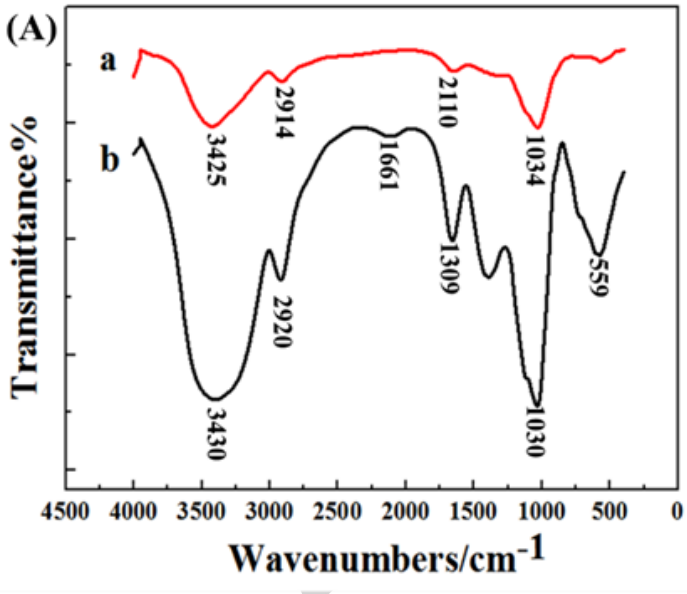
Fig. 3. TEM (A) with the insert images of Lac/PLLY/CA-GR nanocomposite material. EIS (B) of bare GCE, Lac/PLLY/CA-GR/GCE, PLLY/CA-GR/GCE, CA-GR/GCE IN 0.1M KCl containing 5 mM [$Fe(CN)_6$]$^{3-/4-}$ at the scan rate of 0.1 V s^{-1}. CV and DPV for 5 pM 17β-estradiol in 0.1 M PBS (pH 6.0) at bare GCE, CA-GR/GCE, PLLY/CA-GR/GCE, Lac/PLLY/CA-GR/GCE.

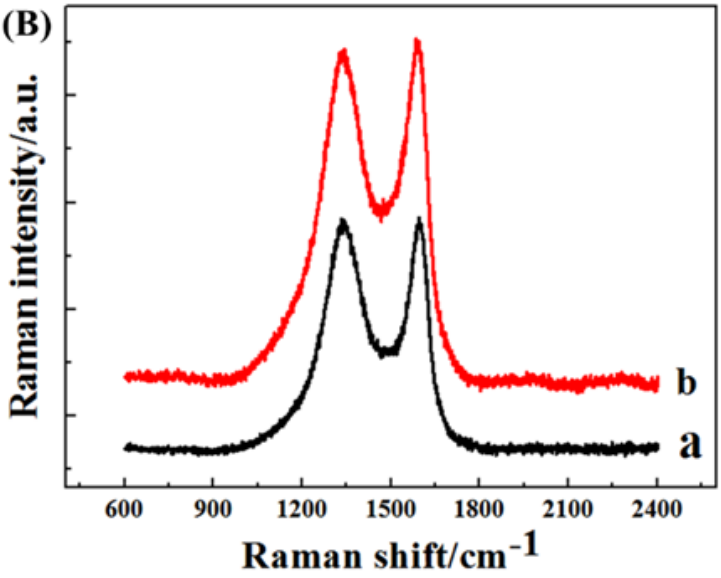
Fig. 4. (A) DPVs of Lac/PLLY/CA-GR/GCE for 5 pM 17β-estradiol in 0.1 M PBS at different pH values (5.0-7.0). Inset (top): Influence of pH on the difference of the peak currents. Inset (bottom): E vs. PH. (B) CVs of Lac/PLLY/CA-GR/GCE for 5 pM 17β -estradiol in 0.1 M PBS (pH 6.0) at different scan rate values(10-200 mV s-1). Inset (top): IP vs. $v^{1/2}$. Inset (bottom): Ep vs. log v.

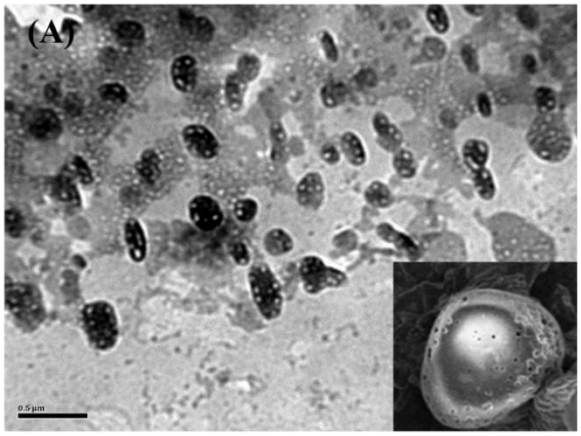
Fig. 5. Effect of (A) electro-polymerization cycles of L-lysine, (B) concentration of laccase enzyme, (C) concertration of electron transferring mediator, (D) drop-coating volume, (E) Specificity of the Lac/PLLY/CA-GR/GCE (F) Effect of time of storage at 4 ºC on the slope of the calibration curve of the enzyme sensor.

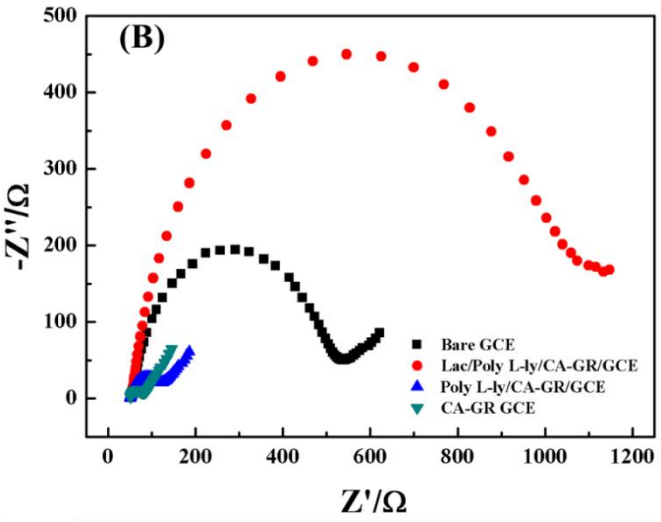
Fig. 6. (A) DPV recorded at the enzyme sensor for 17β -estradiol concentrations form bottom to top 0 pM, 0.4 pM, 1 pM, 5 pM, 9 pM, 15 pM, 21 pM, 27 pM, 33 pM, 41 pM, 48 pM, 57 pM. (B) Calibration curve constructed for 17β -estradiol at the Lac/PLLY/CA-GE/GCE.

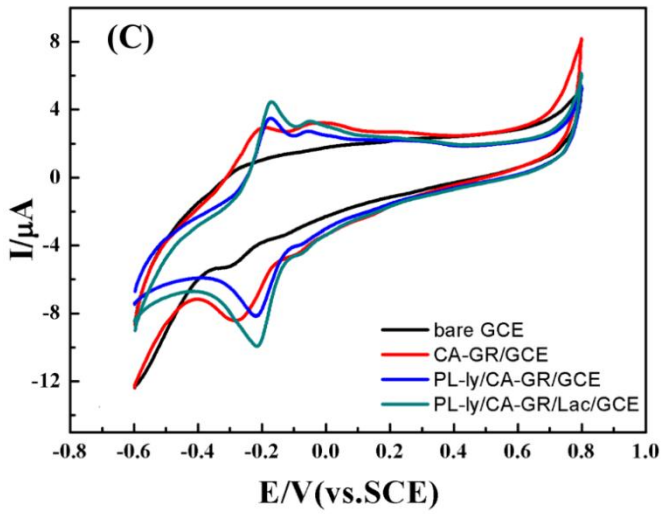


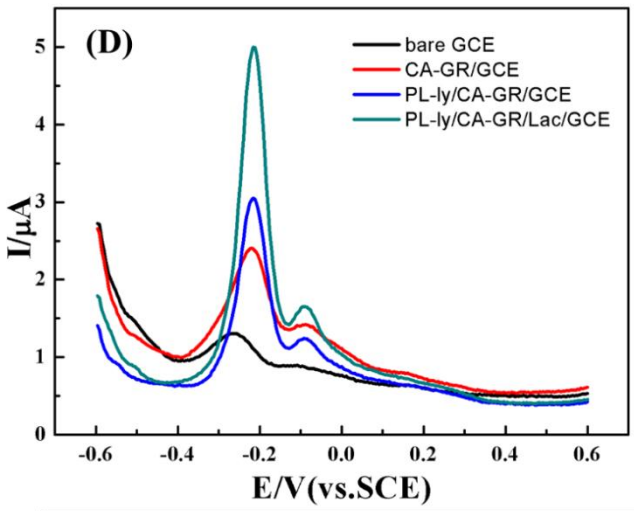


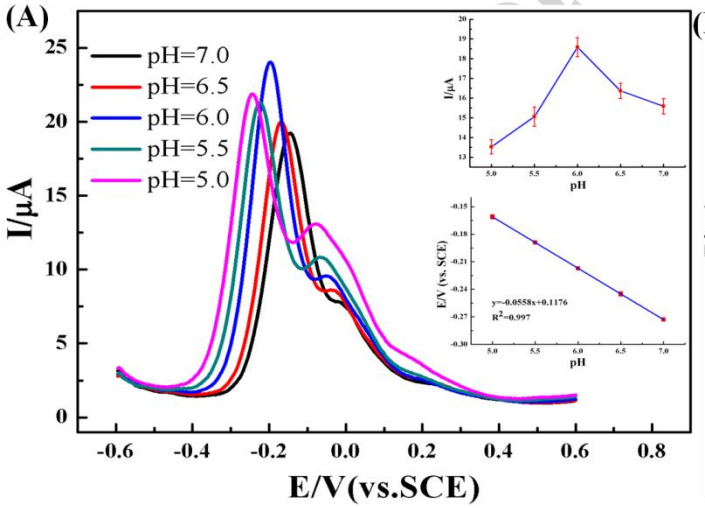


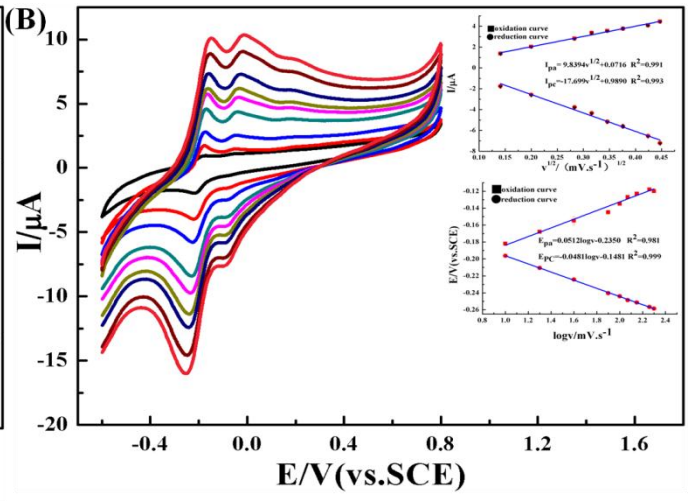


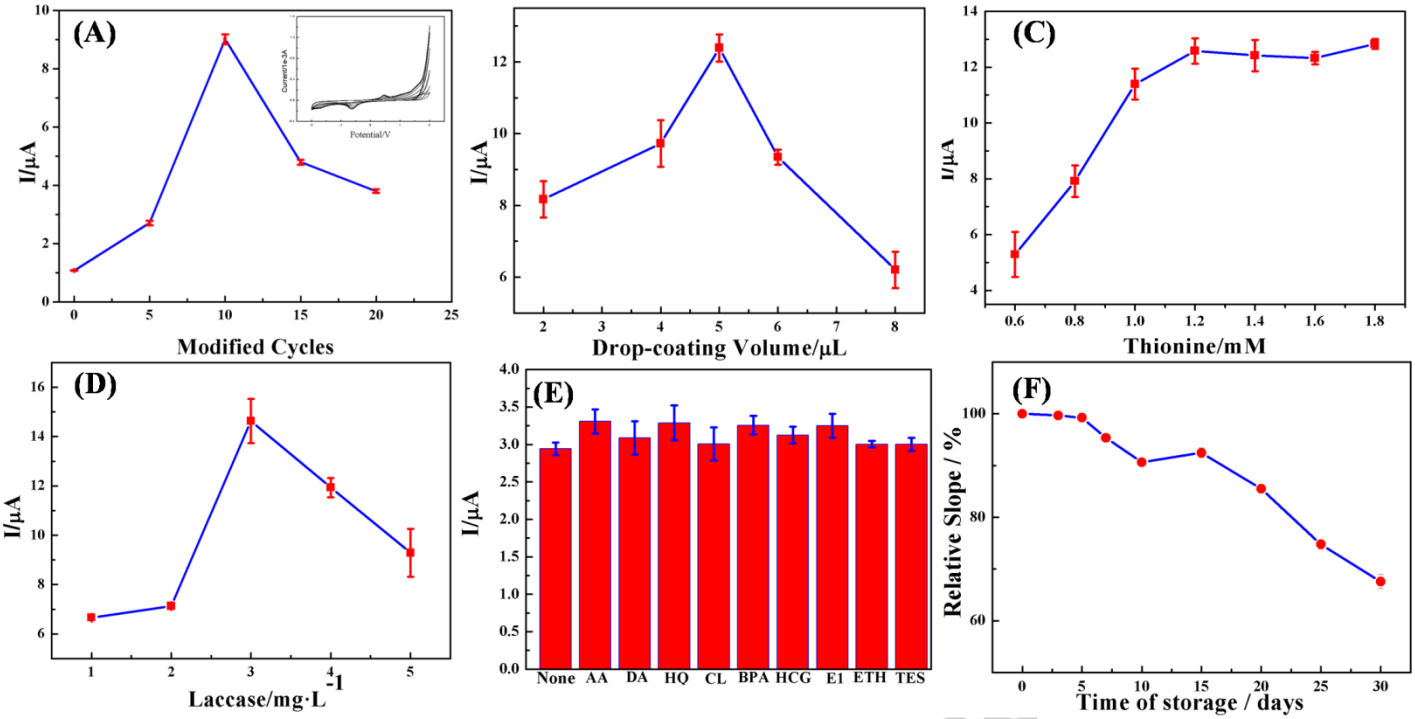












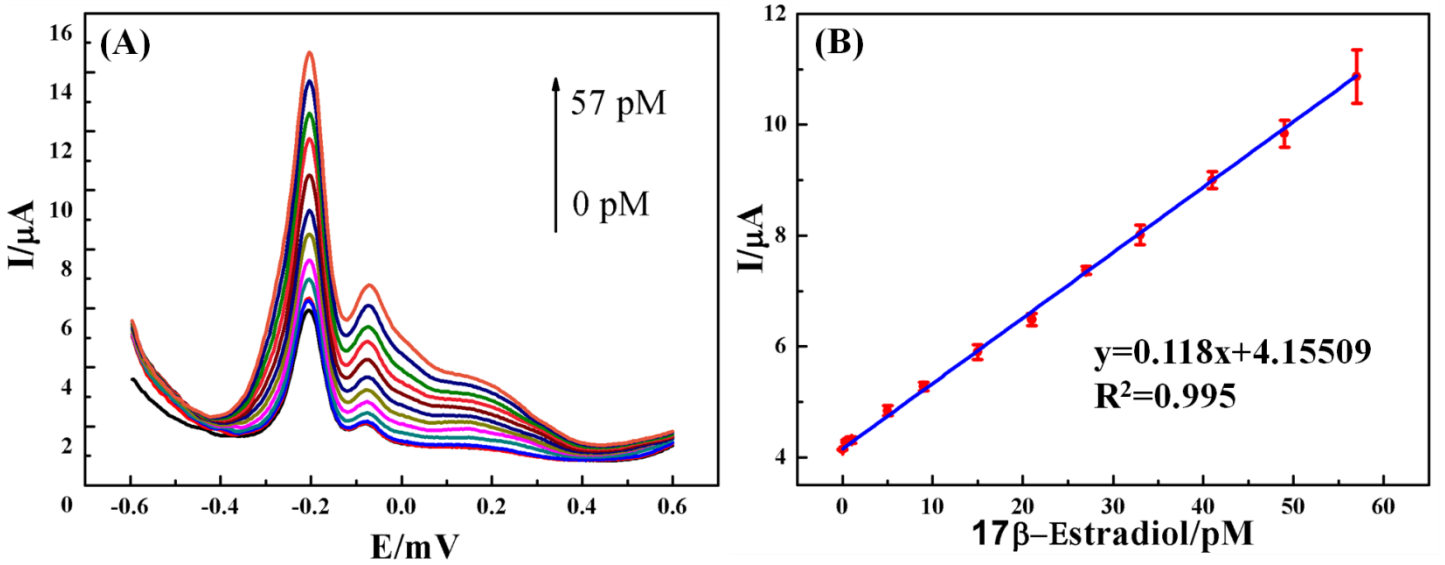


Table 1 Comparison of major characteristics at different modified electrodes for determination of E_2.

Electrodes	Bioreceptor/Method	Linear Range	LOD	Reference
GQDs-PSSA/GO/GCE	Aptamer/DPV	0.001–6.0μM	0.23 nM	[6]
GNR/FS-Au-CA/CPE	Aptamer/DPV	0.1–5.0μM	0.0074 μM	[34]
N-TiO_2 -TP/GCE	Aptamer/DPV	9.9-14μM	0.17 μM	[35]
Lac/rGO-RhNP/GCE	Enzyme/DPV	0.9–11pM	0.54 pM	[25]
E_2-mAb-MNPs	competition/DPV	1.95-2000ng/mL	0.81 ng/mL	[2]
Lac/PLLY/CA-GR/GCE	Enzyme/DPV	0.4–57pM	0.13 pM	This work

Highlights

- A enzyme biosensor was developed for ultra-trace 17β-estradiol detection based on Lac/PLLY/CA-GR/GCE.
- The bio-film Poly L-lysine coated laccase enzyme molecules grew to micro-sized quasi-sphere, generated stable three-dimensional structure and attracted more mediator to catalyze the oxidation of estradiol.
- The biosensor with a mediated electron-transfer system for detection of 17β-estradiol and the linear range was between 4×10^{-13} and 5.7×10^{-11} M.
- The Poly L-lysine films were used to achieve better conductivity and linking with laccase enzyme more solid than self-assembly monolayers.
- L-lysine, amino acids, also can be served as a matrix for developing other enzyme sensors.