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A novel electrocatalyst with highly sensitive to detect glutathione reduced by 2-Hydroxypropyl-β-Cyclodextrin enveloped 10-methylphenothiazine

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This paper reports on an experimental reseach of 2-Hydroxypropyl-β-Cyclodextrin (HP-β-CD) enveloped 10-methylphenothiazine (MPT) catalyst (MPT-HP-β-CD) with highly sensitive and wonderful electrical conductivity for the electrochemical detection of reduced glutathione (GSH) in neutral environment. We obtained a linear response range from 10^{-6} mol/L to $5.8x10^{-4}$ mol/L for GSH with a low detection limit of $2.87x10^{-7}$mol/L, providing a potentially usefuless in the development of sensors for non-enzyme detection of GSH.

1 Introduction

Glutathione is widely distributed in human organs, including reduced glutathione and oxidized glutathione. Among them, the reduced glutathione plays an important role to keep the cells away from the toxic effects of oxidants, free radicals, ionizing agents and so on[1,2,3] , which is composed of three amino acids, such as glutamic acid, cysteine and glycine[4].

The lack of glutathione, may cause many diseases, including human immunodeficiency virus (HIV) infection[5], diabetes[6], acute respiratory distress syndrome[7], cystic fibrous [8] and chronic renal failure [9, 10]. Hence, it is expected to develop a new method for detecting low levels of glutathione.

The phenothiazine is a hydrophobic material and its derivatives contain nitrogen, sulphur atoms on the aromatic heterocyclic, which made it become a rich electronic system, and lead to a good physiological activity and electrochemical activity [11]. So they are easily oxidized and lost electrons to become very active cation radicals [12-19]. Therefore, an interesting work is presented to study the reactions of phenothiazine with some living matter [13, 17, 18].

In recent years, the researchers have developed a lot of methods to quantificat the glutathione in biological samples [20–26]. Most of the methods were reported as follows: spectrophotometry [20,21], electrochemistry [22], spectrofluorimetry [23, 24], and enzymatic method [25, 26] gas chromatography (GC) [27, 28] and high performance liquid chromatography (HPLC) [29-31]. However, the enzymatic determination is very tedious and tanglesome. HPLC and GC methods are very expensive and impractical though providing a high sensitivity and specificity and sensitivity. Therefore, we hope to develop a new simple and fast non-enzyme sensor to detect low concentrations of glutathione.

As we know, 2-hydroxylpropyl-β-cyclodextrin (HP-β-CD) is a kind of a cyclodextrin, consists of hydroxyalkyl derivative and an alternative to β-cyclodextrin, with higher water solubility properties. It also possesses cone-like cavity, and some small molecules can enter into the cavity, form a water-soluble compounds and modify the physical and chemical properties of the small compounds.

The Daniel[32,33] and his co-workers observed that when hydrophobic CoTPP enter into the hydrophobic part of nafion solution, made the CoTPP isolated from the electrode. So，CoTPP cannot obtain electrons from the electrode, then the catalytic reaction cannot be performed. But when we adding with the water soluble $Ru(NH3)_6^{3+}$ enter into the nafion solution, depending on the electrostatic attraction, the

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$Ru(NH3)_6^{3+}$ binding with hydrophilic part of nafion, it transferred the electrons between electrode and CoTPP for the electrocatalytic oxygen reduction.

In this paper, 2-hydroxylpropyl-β-cyclodextrin (HP-βCD) was employed to improve the electrical conductivity between the hydrophobic 10-methyl phenothiazine(MPT) and glassy carbon electrode (GCE). The stable property and highly sensitive of MPT was obtained for detecting low concentrations of GSH in the PBS solution. A novel catalyst was synthesized by host-guest reaction. The MPT and HP-βCD were dissolved in DMF, and then ultrasound for 2 h, making sure that the MPT entered the cavity of HP-βCD. The stable property and sensitivity of the MPT and MPT enveloped in HP-βCD were investigated. The HP-βCD can improve electrical conductivity between the hydrophobic MPT and GCE successfully. The novel catalyst have potential useful in biomedical analysis.

2 Experimental

2.1 Chemicals and reagents

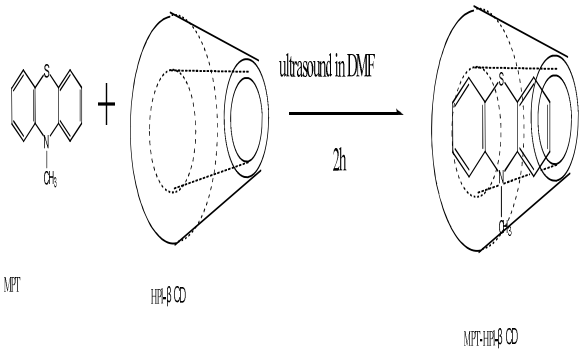
10-methyl phenothiazine (MPT) was obtained from Tokyo Chemical Industry co., Ltd. 2-hydroxylpropyl-β-cyclodextrin (HP-βCD) and L-glutathione reduced were purchased from Aladdin Industrial Corporation. N, N-dimethylformamide (DMF) bought from Xilong Chemical Co., Ltd. All chemicals of analytical reagent grade and used as received. Phosphate buffer solutions were prepared by $NaH_2PO_4 \cdot 2H_2O$, NaCl and $Na_2HPO_4 \cdot 12H_2O$, adjust the pH from 3.0 to 9.0 by HCl and NaOH. Freshly prepared solutions of L-glutathione reduced and double distilled water (DDW) were used in all experiments.

2.2 Instrumentation

The UV absorbance spectra were recorded with spectrophotometer Lambda 35 (PerkinElmer, USA). The FTIR spectra were recorded with a Nicolet 5700 (PerkinElmer, USA).

2.3 Preparation of HP-βCD enveloped MPT catalyst

HP-βCD enveloped MPT catalyst was prepared in an one-pot method. In general, 80 mg HP-βCD and 10 mg MPT were dispersed into 1 mL DMF and ultrasound for 2 h, then dried in the oven. Eventually, a white thin film was obtained. The process is illustrated in Scheme 1.



Scheme1. Schematic illustrates the reaction steps of MPT-HP-βCD

2.4 Fabrication of MPT-HP-βCD modified electrode

A homogeneous aqueous solution containing dispersed MPT-HP-βCD catalyst was prepared by sonication of the white thin film in 1.0 mL DDW for 1 h. Before electrode modification, the 0.1, 0.3 μm Al_2O_3 slurry was used to polish the GCE (3.0 mm diameter) on mirror-like smoothness, and rinsed it by DDW, then sonicated in mixed solution of ethanol and DDW (1:1 v/v). After rinsing with DDW and drying with a stream of nitrogen (N_2). We dropped 10.0 μL aqueous solutions onto the surface of the GCE and dried it under room temperature to obtain the MPT-HP-βCD modified GCE. Finally, 5 μL 0.5% nafion solution was added onto the modified GCE (MPT-HP-βCD/GCE). In the control experiments, 10.0 μL aqueous solution of MPT suspension also cast onto GCE to obtain the MPT/GCE, 5 μL 0.5 % nafion solution was also added.

2.5 Electrochemical measurement

We used the CHI760E electrochemical workstation (Shanghai, China) to do all electrochemical measurements. The conventional three-electrode system, comprising working electrode (GCE electrode), reference electrode (SCE electrode) and counter electrode (a platinum foil), was used. All electrochemical measurements were tested in a single compartment electrochemical cell under the ambient conditions. The high purity N_2 (99.99%) was used to keep oxygen from the solutions. At the beginning of each electrochemical measurement, we used a stream of high purity N_2 (99.99%) to deoxygenate at least 15min, make sure it under atmosphere. We performed CV and other processes in a quiescent solution. The scanning potential was from 0.20V–0.80V and the sweep speed was 50 mV/s.

3 Results and discussion

3.1 Characterization of MPT-HP-βCD

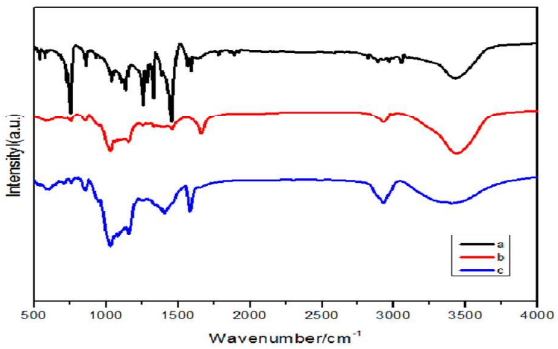


Fig.1. FTIR spectra of (a) MPT, (b) MPT-HP-βCD, (c) HP-βCD.

FTIR spectra of MPT (a), MPT-HP-βCD (b), and HP-βCD (c) are shown in Figure 1. The FTIR spectrum of all catalysts showed a broad absorption band at 3429 cm^{-1}, which is attributed to the O-H groups. As shown in Figure 1(a), the absorption band at 1592, 1458 cm^{-1} is a typical peak of phenyl groups and the C-N vibration in MPT appears at 1133, 1259 and 1327 cm^{-1}. The peaks at 744cm^{-1} and 862 cm^{-1} are assigned to the C-S vibration. As shown in Figure 1(b), the FTIR spectrum of MPT-HP-βCD exhibits typical MPT absorption features of the ring vibrations at 744, 862, 1133, 1259 and 1327 cm^{-1}. But compared (a) with (b), all of the absorption features intensity were weakened, because the MPT entered into the cavity and affects the Stretching vibration of functional groups. This is the phenomenon called FTIR attenuation effect of enveloped by HP-βCD[34]. Moreover, compared (b) and (c), the C-O bond of MPT-CD is occurred a blue shift from 1578 cm^{-1} to 1667cm^{-1}, about 89cm^{-1}. Which indicating that the MPT enter into the cyclodextrin cavity, affected the C-O stretching vibration in the cyclodextrin. All these results clearly confirmed that we prepared HP-βCD enveloped MPT successfully.

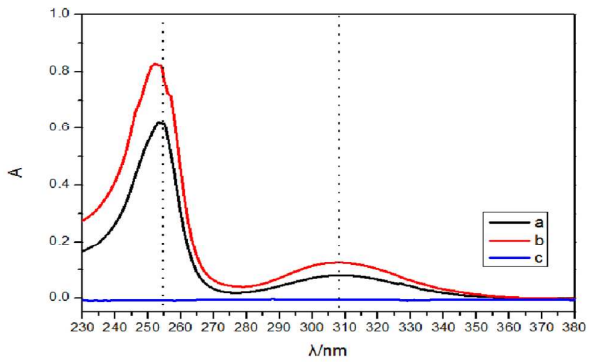


Fig.2. UV -visible absorption spectra recorded of (a) MPT, (b) MPT-HP-βCD, (c)HP- βCD.

UV-Vis absorption spectra of (a) MPT, (b) MPT-HP-βCD and (c) HP-βCD are shown in Figure 2. The experiment was used ethanol as reference solvent. The absorption bands of MPT at 254 nm and 307 nm that were corresponding to the benzene rings conjugated system, respectively. The mentioned absorptions for the MPT-HP-βCD at 251 nm showed a blue shift in comparison to absorption bands of MPT for the π-π^* transition and excitation transition.

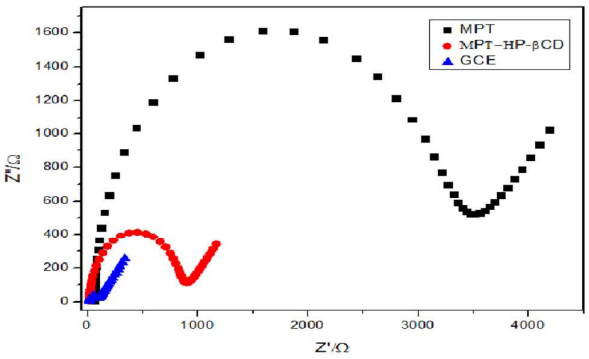
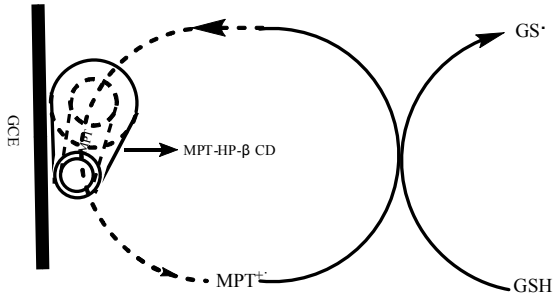


Fig. 3.Nyquist plots of GCE (blue), MPT/GCE (black) and MPT-HP-βCD/GCE (red) electrodes in the presence of 0.1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ with 0.1 M KCl solution. The potential applied to the electrode was the open circuit voltage of this test system (0.2210 V vs. Ag/AgCl).

We can know the changes of the impedance on different modified electrodes according the EIS during the modification process. Fig. 3 shows the results of ac impedance spectroscopy on the GCE (blue), MPT/GCE (black) and MPT-HP-βCD/GCE (red) when the electrodes were applied to open-circuit voltage of this system (0.2210 V vs. Ag/AgCl). The profiles consist of a semicircular part and a linear part. They are correspond to the charge-transfer limited process and diffusion process, respectively [35,36]. Thus, the electron-transfer resistance could be estimated, which equals to 108Ω, 858Ω and 3500Ω for the GCE, MPT-HP-βCD/GCE and MPT/GCE, respectively. This can be explain as the following reasons, when the hydrophobic and water insoluble MPT into the nafion solution, made MPT isolated with the electrode, and cannot obtain electrons from the electrode. Then the catalytic reaction cannot be performed. But when we adding the water soluble HP-βCD into the nafion solution, depending on the electrostatic attraction, the HP-βCD binding with hydrophilic part of nafion, it

transferred the electrons between the electrode and MPT for the electrocatalytic oxygen reduction.

3.2 CV of GSH at MPT-HP-βCD /GCE



Scheme2. Reaction scheme for the electric catalytic oxidation of GSH.

The Scheme 2 showed the possible reaction mechanism of MPT-HP-βCD electriccatalytic oxidized GSH. The MPT entered into the HP-βCD formed MPT-HP-βCD catalyst. At first, MPT loss electrons and obtained $MPT^{+\cdot}$, this reaction is a reversible. When we added GSH which will react with $MPT^{+\cdot}$.The process as follow[37]:

$$MPT \underset{K_2}{\overset{K_1}{\rightleftharpoons}} MPT^{+\cdot} + e$$

$$MPT^{+\cdot} + GSH \xrightarrow{K_3} GS\cdot + MPT + H^{+}$$

And the formation of MPT involved in it goes into the next catalytic cycle.

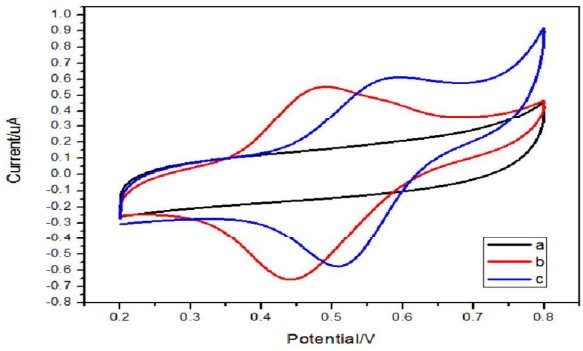


Fig.4. CVs of (a) GCE, (b)MPT/ GCE and (c) MPT-HP-βCD /GCE in 0.1 M PBS solution (pH=7.0)

In this study, MPT was utilized as the guest molecule and the cyclic voltammetry results are shown in Figure 4. There were significant changes in both peak potential and current peak, all of which have a consistent trend with the previous studies [36]. It means that the HP-βCD enveloped MPT successfully, and the MPT can move freely in the HP-βCD.

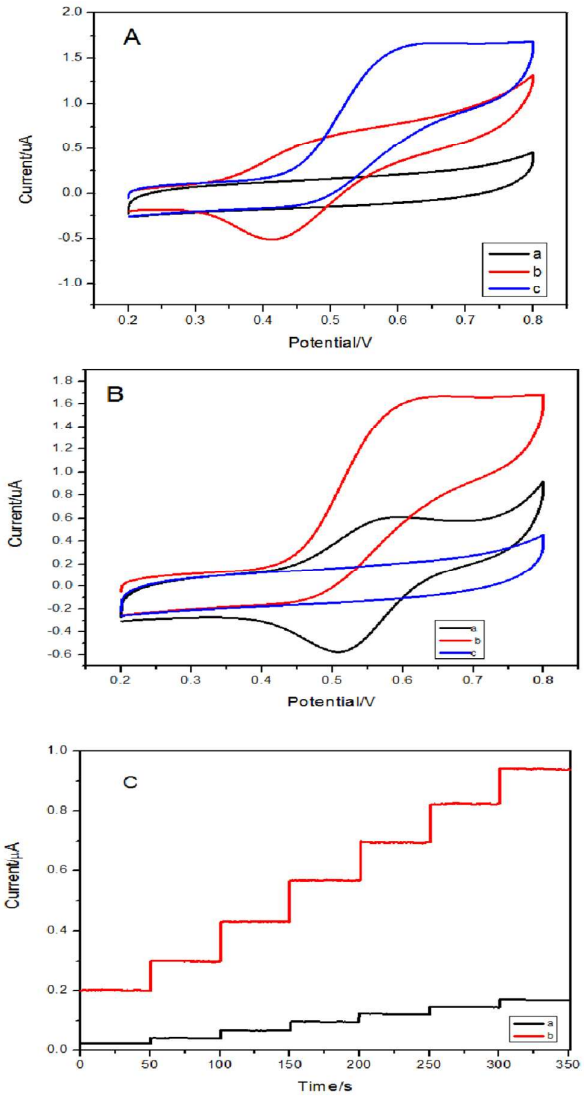


Fig.5. (A) CVs of (a)GCE, (b)MPT/GCE, (c) MPT-HP-βCD / GCE in 9.7 μM GSH + 0.1 M PBS solution (pH=7.0). (B) CVs of (a)MPT-βCD / GCE in 0.1 M PBS solution without GSH, (b) MPT-βCD / GCE in 0.1 M PBS solution with 9.7 μM GSH, (c) GCE in 0.1 M PBS solution with 9.7 μM GSH.(C) Chronoamperometric responses of 16 μM GSH at MPT/GCE(a), MPT-HP-βCD (b) electrode, potential applied: 0.58V (vs. Ag/AgCl).

Fig. 5 shows the CV of GSH at the MPT-HP-βCD /GCE. For comparison, the GCE and MPT/GCE were also studied. For the GCE as the curve (a) (Fig.5A), no oxidation peak at is observed which is derived from the oxidation of GSH, but the MPT/GCE showed in curve (b) (Fig.5A) has a small oxidation peak. It also can be

demonstrated in Fig.5C, the MPT (a) not only has a small the response current for GSH oxidation, but also has a large resistance, compared to MPT- HP-βCD (b). This may be can explain as the followed reasons, when the hydrophobic and water insoluble MPT into the nafion solution, made the MPT isolated from the electrode, and cannot obtain the electrons from the electrode. Then the catalytic reaction cannot easy be performed. Fortunately, the oxidation peak at 0.58 V is observed which is derived from the oxidation of GSH marked as curve (c) (Fig. 5A). In order to observe catalytic activity of MPT-HP-βCD /GCE more clearly, we have compared the performance of MPT-HP-βCD /GCE in 0.1 M PBS solution without GSH and with 9.7 μM GSH (Fig.5B).The oxidized at a positive working potential and a higher current response showed in MPT-HP-βCD /GCE curve (c) than that on MPT/GCE curve(b) (Fig. 5A). MPT/GCE can slightly resolve these analytes, because of its oxidation potential is too low to oxidize the GSH. To improve the catalytic activity of MPT, we used HP-βCD to include MPT (see at Scheme1), so the redox potential of MPT is more positive at 0.55V (Fig.5B) compared to 0.45 V (Fig.5A). We will detect GSH in real sample at the following part.

Effect of scan rates

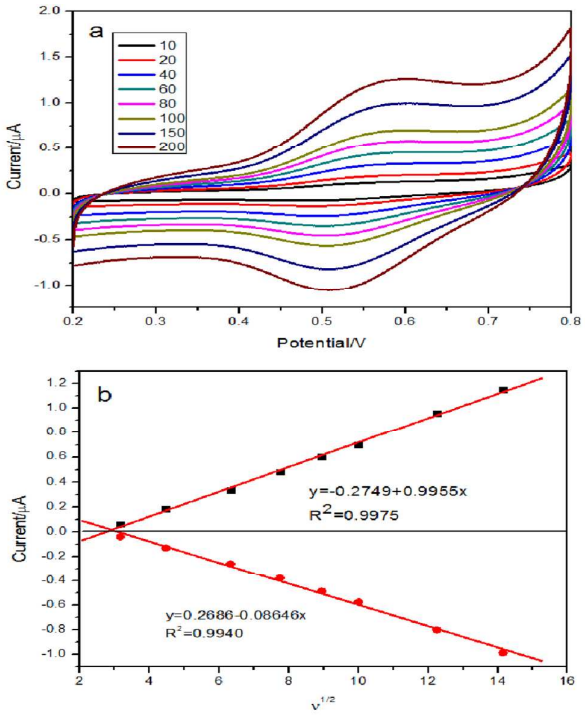


Fig.6. (a) CVs of MPT-HP-βCD / GCE in 0.1 M PBS solution (pH=7.0) at different scan rate (10–200 mV/s). (b) Display the plots of peak current against square root of scan rate.

The CV responses on MPT-HP-βCD/GCE under different scan rates were showed in Fig.6 (a). And the Fig.6 (b) described the plots of the redox peak current as function of the square root of scan rates. We can observe a good linear relationships from the Fig.6 (b), means that all these composite material are electro-active on the MPT-HP-βCD/GCE controlled by diffusion processes. Fig.6 (a) shows that MPT is displays a quasi-reversible response at the MPT-HP-βCD/GCE. The Ep = |Epa−Epc| is 83 mV when the scan rate is 50 mV/s. The electro-oxidation mechanisms of GSH at MPT-HP-βCD / GCE are illustrated in Scheme 2.

Effect of pH

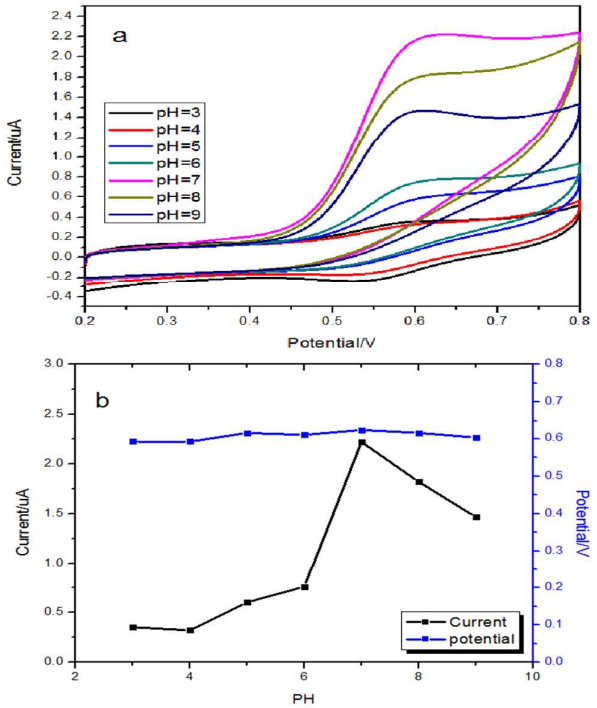


Fig.7. (a) CVs of MPT-HP-βCD/GCE in 9.7 μM GSH + 0.1 M PBS solution at different pHs (3–9). (b) Display the plots of peak current (black) and Potential (blue) against pHs.

In most cases, the electrolyte pH is an important parameter to the electrochemical reaction. Fig.7 (a) displays the effect of pH on the anodic peak potentials (Epa) and current responses of GSH at MPT-HP-βCD/GCE in 9.7 μM GSH + 0.1 M PBS solution. The anodic peak potentials (blue line) for GSH shift negatively with the increase in pH (3.0-6.0 and 8.0-9.0). The peak currents of GSH changed obviously at different pHs (black line). The current increases with the pH from 3.0–7.0 and

then decreases at pH (7.0-9.0). Hence, pH 7.0 was the best condition for catalytic oxidation GSH.

Effect of temperature

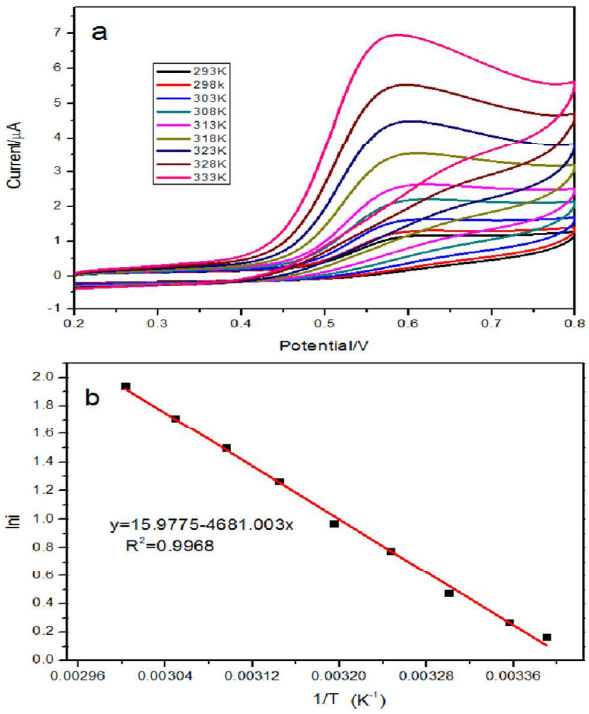


Fig.8. (a) CVs of MPT-HP-βCD/GCE in 9.7 μM GSH + 0.1 M PBS solution under different temperature. (b) Display the logarithm plots of peak current against the reciprocal of temperature.

As we know, the electrolyte temperature is a key factor for an excellent electrochemical reaction. We studied the influence of temperature for the reaction. Fig.8 (a) was the CVs of MPT-HP-βCD / GCE in 9.7 μM GSH + 0.1 M PBS solution under different temperature, and (b) display the logarithm plots of peak current against the reciprocal of temperature. With the temperature going on from 293K to 333K, the peak current increased, but the oxidized peak potential had slightly changed. And based on the Arrhenius equation Ln (i) = -Ea/RT+B, we calculate its activation energy was 38.92KJ/mol. To sum up, the catalytic activity of the catalyst is not lost but increased when rise in temperature, which is difference to the traditional enzyme catalyst. It has a potential application in high temperature condition.

3.3 Amperometric response of GSH at MPT-HP-βCD /GCE

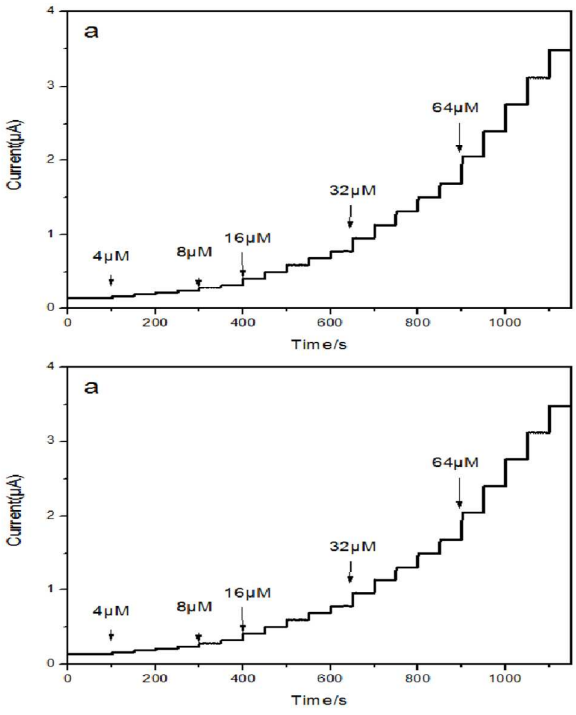


Fig.9. (a) Amperometric current versus time (i–t) curve for the detection of GSH at MPT-HP-βCD/GCE at 0.58 V. GSH was successively injected into the stirred 0.1 M pH 7.0 PBS with 4μM, 8μM, 16μM,32μM, 64μM. (b) The calibration plot of the GSH biosensor.

We used chronoamperometry to study the influence of potentials on GSH oxidation at MPT-HP-βCD/GCE electrode. The electrode was cleaned as previous process. During the applied potential changed from 0.20 V to 0.58 V the steady-state currents correspond to increase, but from 0.58 V to 0.80 V the steady-state currents was decreased. Therefore, we obtained the potential for amperometric detection of GSH on the MPT-HP-βCD/GCE was 0.58 V in pH 7.0 PBS (Fig. 9a). After adding GSH at fixed time intervals, we can see the currents rapid increase. Fig. 9b is the corresponding calibration curve. According the Fig. 9b, we can obtained the following information that the linear range for GSH concentration is 10^{-6}mol/L to $5.8x10^{-4}$mol/L with the linear dependence (R^2=0.999) that is far beyond the physiological level and the detection limit is $2.87x10^{-7}$mol/L at a signal to noise ratio of 3.

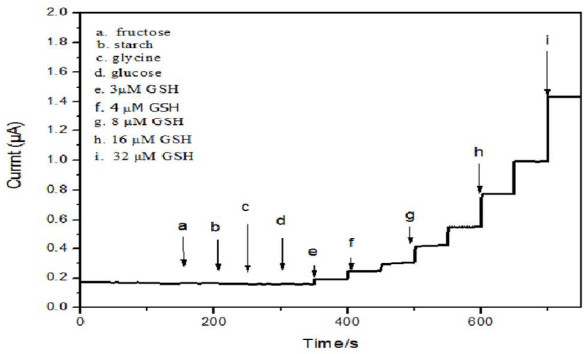


Fig.10. Amperometric response of MPT-HP-βCD/GCE to sequential additions of fructose, starch, glycine, glucose and various concentration of GSH into stirring 0.1 M PBS (pH 7.0) at an applied potential of0.6 V vs. Ag/AgCl.

At first, make sure the MPT-HP-βCD/GCE is immersed in the 0.1 M PBS solution. Then 40 times of fructose, starch, glycine, glucose was added in succession at regular intervals. Thirdly, GSH of 3μM, 4μM, 8μM, 16μM, 32μM were added in the solution subsequently. None of the electroactive species induced any current signal except GSH which shows well-defined current response (Fig. 10).

3.4 Sample detection

In order to study the practical application of MPT-HP-βCD catalyst, we detected the glutathione in reduced glutathione tablets solution by the standard addition method. The results showed in table 1, it's indicating us that the catalyst which we have prepared can be used for the detection of actual samples.

sample	Added (mM)	Found (mM)	Recovery (%)
1	0.004	0.0039	97.5
2	0.02	0.0205	102.5
3	0.04	0.0407	101.75

We studied the storage stability of MPT-HP-βCD catalyst during a period of two weeks, dried under a condition with 4 ℃ in the refrigerator per day, the catalyst exhibiting a high performance over the past two weeks, and maintained 90% of its initial response.

4 Conclusions

A novel modified glassy carbon electrode has been successfully fabricated by using HP-βCD enveloped MPT. After modified by HP-βCD, the redox potential had a positive shift of MPT in 0.1 M PBS solution. The MPT-HP-βCD/GCE exhibits good electro-activity to the oxidation of GSH with a higher oxidation current. The detection limit of GSH is about $2.87x10^{-7}$mol/L. The MPT-HP-βCD/GCE provides good stability and sensitivity to determine GSH in the PBS solution at pH=7.0. The sensitivity of MPT-HP-βCD could be significantly enhanced by HP-βCD, possibly attributing to the HP-βCD. When adding water soluble HP-βCD into the nafion solution, depending on electrostatic attraction, HP-βCD binding with hydrophilic part of nafion solutiom, it transferred the electrons between the electrode and the MPT for the electrocatalytic oxygen reduction. The proposed method is a promising tool for determination of GSH in PBS solution.

Acknowledgments

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