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Electrochemical Behaviors of Horseradish Peroxidase on MoS_2 Nanosheets Modified Electrode

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In this paper molybdenum disulfide (MoS_2) nanosheets and horseradish peroxidase (HRP) modified electrode was prepared with ionic liquid 1-hexylpyridinium hexafluorophosphate based carbon ionic liquid electrode (CILE). UV-Vis and FT-IR spectra showed that the native framework of HRP was sustained after mixed with MoS_2 nanosheet. As a 2D layered nanosheet MoS_2 acted as the electron transfer bridge for HRP. A pair of well-defined redox peaks of HRP appeared on MoS_2/CILE, implying the realization of direct electron transfer. Electrochemical parameters of HRP were calculated according to the cyclic voltammetric data with the electron transfer coefficient and electron transfer rate constant as 0.51 and 1.65 s^{-1}. The HRP modified electrode exhibited a prominent electrocatalytic behavior towards the reduction of trichloroacetic acid with wider dynamic range and lower detection limit of 0.67 mmol L^{-1} (3σ).

Keywords: Carbon ionic liquid electrode; Molybdenum disulfide; Horseradish peroxidase; Direct electrochemistry; Electrocatalysis.

1. INTRODUCTION

Direct electrochemistry of redox proteins has been a novel topic for mechanistic investigation on the electron transfer in biological systems, with also can provide a basis for bioreactors/biosensors without using mediators [1, 2]. However, the accomplishment of direct electrochemistry of redox proteins on the traditional electrodes is difficult to be realized due to the active center dearly buried inside the proteins [3]. To increase the electron transfer rate, various materials including polymer ionic

liquid, polymer, nanoparticles and surfactant-containing composite film have been selected as the efficient electron transfer approach [4]. Nanomaterials with particular properties such as tunable porosity, large surface area, biocompatibility and catalytic activities [5] had been applied to prepare modified electrodes, which can enhance the electron transfer rate of redox proteins.

As a two-dimensional layer-structured transition-metal dichalcogenides, molybdenum disulfide (MoS_2) has attacked lots of attentions in the fields of electrochemical equipments, capacitors, catalysis, hydrogen storage, solid lubricant and intercalation host due to the especial chemical and physical capabilities [6, 7]. MoS_2 has a similar structure to graphite, which is normally consisted of three atomic layers including a Mo layer sandwiched between two S layers. There are weak Van der Waals interactions between three layer stacks [8, 9], and different types of atoms and molecules can be embedded into layers by intercalation [10]. Horseradish peroxidase (HRP) is an iron heme enzyme and has been generally used in electrochemical biosensors [11, 12]. Due to its well-document structure, HRP based electrodes have been widely reported with electrocatalytic behaviors investigated.

In this paper MoS_2 nanosheet was used for the preparation of an electrochemical HRP biosensor. By using carbon ionic liquid electrode (CILE) as the substrate electrode, direct electron transfer of HRP was realized on MoS_2/CILE. CILE is a new kind of working electrode with ionic liquid used as modifier in carbon paste electrode, which exhibits the advantages such as wider electrochemical windows, higher sensitivity and increased reversibility [13]. The bioactivity of HRP was remained with MoS_2 nanosheet based on the spectroscopic results and the modified electrode shown good electrocatalytic activity.

2. EXPERIMENTAL

2.1. Reagents

Graphite powder (particle size 30 μm, Shanghai Colloid Chem. Co., China), 1-hexylpyridinium hexafluorophosphate ($HPPF_6$, Lanzhou Yulu Fine Chem. Co., China), HRP (MW 40000, Sigma-Aldrich Co., USA), MoS_2 nanosheet (Nanjing Jcnano Tech. Ltd. Co., China), Nafion (5% ethanol solution, Sigma) and trichloroacetic acid (TCA, Tianjin Kemiou Chem. Ltd. Co., China) were used as received without further purification. 0.1 mol L^{-1} phosphate buffer solutions (PBS) were used as supporting electrolyte, which were deoxygenated by pure nitrogen thoroughly for 30 min before electrochemical measurements. All the chemicals were of analytical grade and doubly distilled water was used throughout.

2.2. Apparatus

Voltammetric experiments and electrochemical impedance spectroscopy (EIS) were performed on CHI 1210A and CHI 660D electrochemical workstation (Shanghai CH Instrument, China). A three-electrode system was used with a saturated calomel electrode (SCE) as the reference electrode, a platinum wire electrode as the auxiliary electrode and a Nafion/HRP/MoS_2/CILE as the working

electrode. Ultraviolet-visible (UV-Vis) absorption spectra and Fourier transform infrared (FT-IR) spectra were recorded on TU-1901 double beam UV-Visible spectrophotometer (Beijing General Instrument Ltd. Co., China) and Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific Inc., USA). The morphology of MoS_2 nanosheet was characterized by JSM-7100F scanning electron microscope (SEM, Japan Electron Co., Japan).

2.3. Preparation of Nafion/HRP/MoS_2/CILE /CILE

Based on the reference [14] CILE was fabricated by using $HPPF_6$ as the binder and the modifier. Then 6.0 μL of 2.0 mg mL^{-1} MoS_2 solution was directly casted on the CILE surface and dried at the room temperature for 2 h to get MoS_2/CILE. 6.0 μL of 15.0 mg mL^{-1} HRP solution was further casted onto the electrode and stayed silent to allow the water evaporated gradually. At last 8.0 μL of 0.5% Nafion solution was spread onto the surface of HRP/MoS_2/CILE and dried to get the biosensor (Nafion/HRP/MoS_2/CILE). During these steps a small bottle was fit over the electrode to evaporate the solvent slowly.

2.4. Electrochemical measurements

Electrochemical measurements were carried out in the 10 mL supporting electrolyte containing 0.1 mol L^{-1} pH 3.0 PBS at room temperature, which was remained a nitrogen atmosphere environment during the experiments. EIS experiments were implemented in a 0.1 mol L^{-1} KCl solution containing 10.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) with the frequencies sweep range from 10^4 to 0.1 Hz. UV-Vis absorption spectroscopic experiments were implemented with a mixture solution containing HRP and MoS_2 with the wavelength scanned from 300 to 500 nm. The HRP-MoS_2 film assembled on a glass slide was used for FT-IR experiments.

3. RESULTS AND DISCUSSION

3.1. Characteristics of materials used

SEM image of the MoS_2 nanosheets on the electrode surface were recorded with the result shown in Fig. 1A. A lamellar structure could be observed with the nanosheets aggregated on the electrode surface. The roughness of the interface was increased greatly due to the presence of MoS_2, which is suitable for HRP immobilization.

In UV-Vis absorption spectrum the Soret absorption band from the four iron heme groups may provide the information on the conformational integrity or the possible denaturation of the proteins [15]. As shown in Fig. 1B, native HRP in water displayed the Soret band at 403.0 nm (curve a). The mixture solution of MoS_2 and HRP gave the same absorption at 403.0 nm (curve b), indicating that HRP remained its native structure.

FT-IR spectroscopy is further used to examine the structural integrity of the heme proteins. The typical infrared adsorption bands of amide I (1700-1600 cm^{-1}) and amide II (1600-1500 cm^{-1}) can provide specific information on the secondary structure of polypeptide chain [16]. The strong broad band of HRP at 1651 cm^{-1} and 1543 cm^{-1} was similar to that of HRP-MoS_2 at 1648 cm^{-1} and 1532 cm^{-1}. These results also exhibited that HRP kept up the fundamental features of its original structure after mixed with MoS_2 nanosheets.

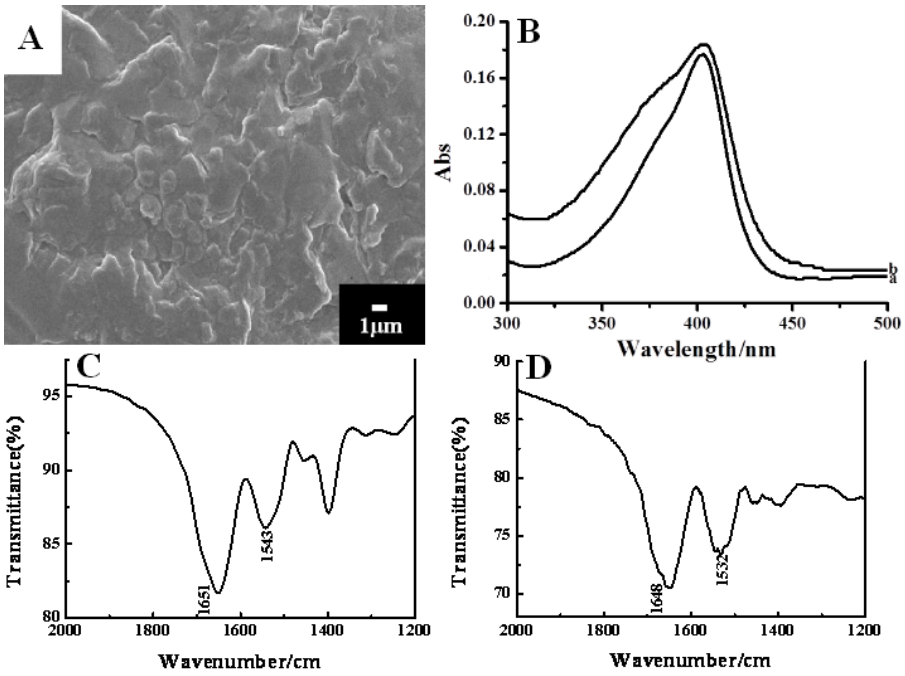

Figure 1. (A) SEM image of MoS_2 nanosheets; (B) UV-Vis absorption spectra of (a) HRP and (b) HRP-MoS_2 in water; FT-IR spectra of (C) HRP and (D) HRP-MoS_2 film.

3.2. EIS of the modified electrodes

The interface capabilities of the modified electrodes can be characterized by EIS, and the diameter of the semicircle corresponds to the interfacial electron transfer resistance (Ret) [17]. Fig. 2 showed that EIS results of different modified electrodes in a 10.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol L^{-1} KCl across the frequency from 10^4 to 0.1 Hz. The Ret value of CILE was 78.28 Ω (curve c) and that of Nafion/CILE was 99.26 Ω (curve d), indicating that nonconductive Nafion film hindered electron transfer of the electrochemical probe. On Nafion/HRP/CILE a substantial increase of Ret value appeared with the value as 113.04 Ω (curve e), indicating that the existence of HRP molecules on the electrode surface further obstructed the diffusion of ferricyanide and acted as the blocking layer. On Nafion/MoS_2/CILE (curve a) the diameter of semicircle decreased with the Ret value as 40.13 Ω, which was smaller than that of Nafion/CILE, indicating that MoS_2 nanosheet decreased the electron transfer resistance and boosted electron transfer of the electrochemical probe. The further addition of HRP resulted in the increase of the Ret value to 58.78 Ω (curve b), indicating the successful immobilization of HRP on the electrode surface.

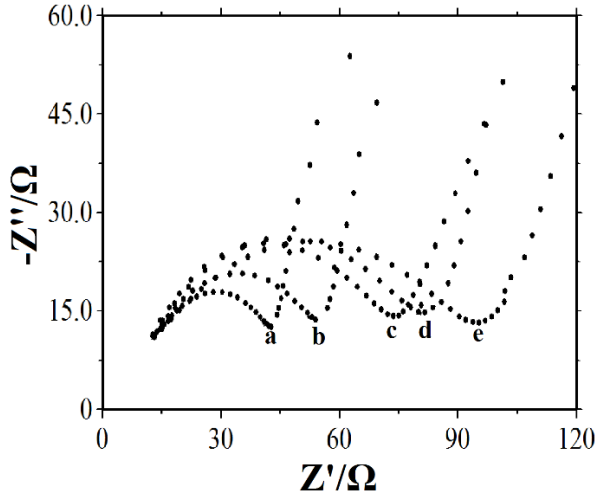

Figure 2. EIS of (a) Nafion/MoS_2/CILE, (b) Nafion/HRP/MoS_2/CILE, (c) CILE, (d) Nafion/CILE and (e) Nafion/HRP/CILE in the presence of 1.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KCl, frequency range: 0.1~10^4 Hz.

3.3. Direct electrochemistry of HRP modified electrode

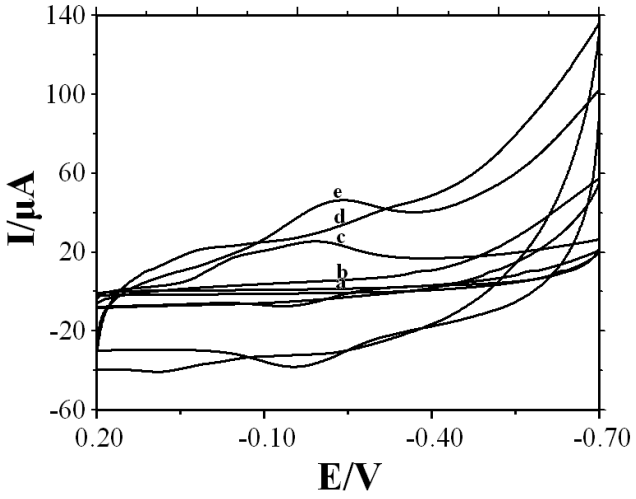

Figure 3. Cyclic voltammograms of (a) CILE, (b) Nafion/CILE, (c) Nafion/HRP/CILE, (d) Nafion/MoS_2/CILE and (e) Nafion/HRP/MoS_2/CILE in pH 3.0 PBS at the scan rate of 100 mV s^{-1}.

Fig. 3 showed cyclic voltammogram of different electrodes in 0.1 mol L^{-1} PBS (pH 3.0) at a scan rate of 100 mV s^{-1}. No electrochemical responses could be observed on CILE (curve a), Nafion/CILE (curve b) and Nafion/MoS_2/CILE (curve d), implying no electrochemical reaction took place. On Nafion/HRP/CILE a pair of unsymmetric redox peaks appeared with small value (curve c), indicating a slow electron transfer rate between HRP and CILE. CILE has shown high conductivity with biocompatible surface, which is suitable for redox proteins to transfer electrons. However a pair of well-defined quasi-reversible cyclic voltammetric peaks was found for Nafion/HRP/MoS_2/CILE (curve a), indicated that MoS_2 nanosheets on the electrode surface could accelerate the electron

transfer. The peak potentials were -0.162 V (Epa) and -0.227 V (Epc) with peak-to-peak separation (ΔEp) as 0.065 V and the formal peak potential ($E^{0'}$) as -0.195 V, which was similar to the reported values for the Fe(III)/Fe(II) redox center of the heme [18]. Additionally, the anodic and the cathodic peak currents were equal nearly. Therefore the presence of MoS_2 nanosheets can provide a favorable microenvironment to enhance the electron transfer efficiency between the electrodes and enzymes.

3.4. Electrochemical investigations

The effect of scan rate on the response of the immobilized HRP was exhibited in Fig. 4A. It was known that both peak currents increased obviously with the increase of the scan rates. The anodic and cathodic peak currents were linearly correlated to the square root of scan rate in the range from 0.04 to 1.00 V s^{-1} with the linear regression equations as Ipc(μA)=121.32υ (V s^{-1})+2.31 (n=10, γ=0.999) and Ipa(μA)=-122.21υ (V s^{-1})-3.46 (n=10, γ=0.998) (as shown in Fig. 4B), which implied a representative adsorption-controlled electrode procedure. The average surface coverage (Γ^*) of electroactive HRP can be calculated to be 1.14×10^{-9} mol cm^{-2} from integration of the cathodic peak according to formula [19], $\Gamma^*=Q/nAF$, where Q is the charge passing through the electrode with full reduction of electroactive HRP in the film, n is the number of electrons involved in the direct electron transfer reaction, F the Faraday constant, and A is the electrode area. This surface concentration is higher than that of the theoretical monolayer coverage (1.89×10^{-11} mol cm^{-2}). The entire amount of HRP cast on the electrode surface was 1.79×10^{-8} mol cm^{-2}, so 6.37% of HRP molecules on the electrode surface participated in the electrochemical reaction.

Along with the increase of scan rate, the redox peak potentials were also shifted slowly with the enlargement of ΔEp. From cyclic voltammetric data in Fig. 4C, two linear regression equations between Ep and lnv were got with the results of Epc(V)=-0.018lnυ (V s^{-1})-0.23 (n=10, γ=0.998) and Epa(V) = 0.018lnυ (V s^{-1})-0.12 (n=10, γ=0.995), respectively. According to the Laviron's equations [20], the values of the electron transfer number (n), the electron transfer coefficient (α) and the heterogeneous electron transfer rate constant (k_s) can be estimated as 1.09, 0.51 and 1.65 s^{-1}, respectively. The obtained value of k_s was obviously higher than that of HRP based on a graphite electrode (0.66 s^{-1}) [21], HRP coated on a hexagonal mesoporous silica matrix (0.92 s^{-1}) [22], HRP immobilized on nano-Ni-SnO_2 composite (1.10 s^{-1}) [23] and HRP immobilized in DNA film (1.13 s^{-1}) [24]. Therefore MoS_2 nanosheets facilitated the interfacial electron transfer between heme center of HRP and the electrode surface.

It is well-known that the electrochemical behaviors of redox protein are dependent on the solution pH, which was investigated on Nafion/HRP/MoS_2/CILE by cyclic voltammetry (Fig. 4D). Along with pH increasing in the range from 3.0 to 7.0, both the cathodic and anodic peaks shifted toward negative direction. The formal peak potential ($E^{0'}$) had a linear relationship with the buffer pH and the regression equation was calculated as $E^{0'}$(mV)=-42.8 pH-32.6 (n=5, γ=0.998). The slope value of -42.8 mV pH^{-1} was reasonably a little smaller than the expected theoretical value of -59.0 mV pH^{-1} at 25 ℃ for a single-proton coupled to the reversible one-electron transfer, which confirmed that HRP

molecules underwent a characteristic single protonation process accompanying with one electron transfer to electrode.

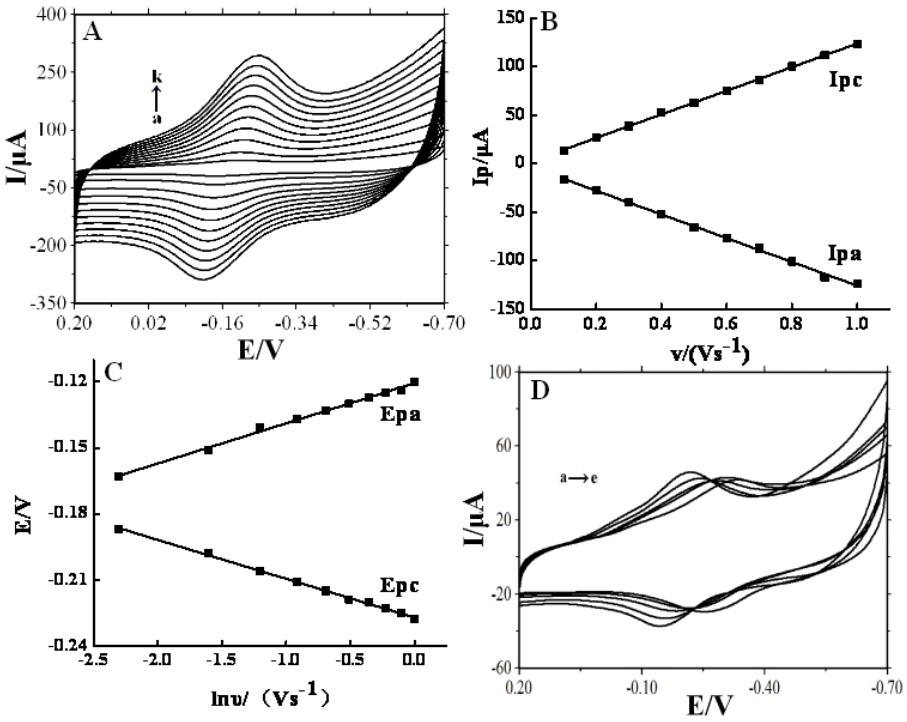

Figure 4. (A) Influence of scan rate on electrochemical responses of Nafion/HRP/MoS_2/CILE in pH 3.0 PBS with scan rate from a to k as 40, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mV s^{-1}, respectively; (B) Linear relationship of the redox peak currents versus scan rate (υ); (C) Linear relationship of the redox peak potentials versus lnυ. (D) Cyclic voltammograms of Nafion/HRP/MoS_2/CILE in different pH PBS (from a to e as 3, 4, 5, 6, 7) with the scan rate of 100 mV s^{-1}.

3.5. Electrocatalytic activity of the HRP modified electrode

The bioelectrocatalytic behavior of Nafion/HRP/MoS_2/CILE towards TCA reduction was further explored by cyclic voltammetry in optimized conditions. With the successive increments of TCA concentration in 0.1 mol L^{-1} PBS (pH 3.0), cyclic voltammogram of Nafion/HRP/MoS_2/CILE was recorded and the irreversible reduction peak appeared at -0.526 V (curves a-l) with current increased remarkably (Fig. 5), which demonstrated a characteristic of electrochemical catalytic reduction of TCA by the heme proteins. The linear range of this biosensor to TCA detection was in the range from 10.0 to 63.0 mmol L^{-1} with a linear regression equation of Iss (μA) =1.766C (mmol L^{-1})+29.68 (n=11, γ=0.997) and the detection limit of 0.67 mmol L^{-1} (3σ). When the concentration of TCA was higher than 63.0 mmol L^{-1}, a response plateau of peak current was observed, representing the feature of the Michaelis–Menten kinetic process. The apparent Michaelis–Menten constant (K_M^{app}), which can provide an indication of the enzyme–substrate kinetics, can be calculated according to Lineweaver–Burk equation [25] with the value as 0.819 mmol L^{-1}. This value was obviously lower

than that of some previous reported values such as 0.915 mmol L^{-1} for De-IL-V_2O_5-HRP film [26], 5.66 mmol L^{-1} for HRP–PVA–C_{12}–C_{12}–C_{12}/GCE [27] and 52.0 mmol L^{-1} for HRP immobilized in agarose hydrogel films in [Bmim][PF_6] on GCE [28]. The low K_M^{app} value revealed that HRP immobilized in MoS_2/CILE retained its prominent bioelectrocatalytic properties and kept a high biological affinity for TCA reduction.

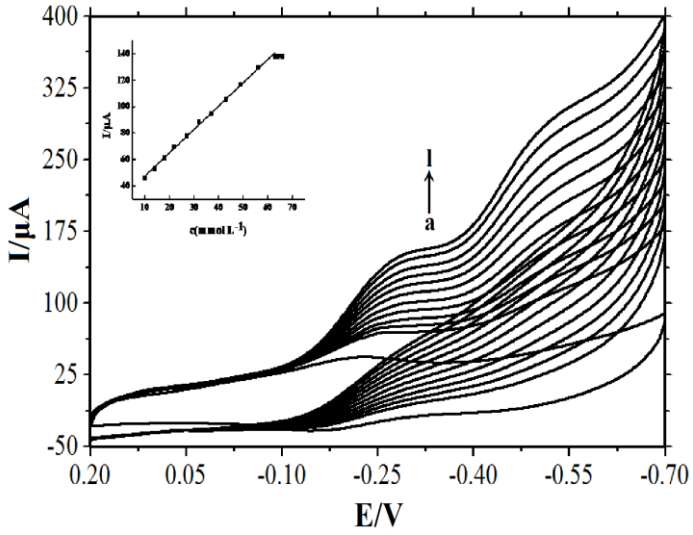

Figure 5. Cyclic voltammograms of Nafion/HRP/MoS_2/CILE in the presence of different concentrations of TCA (curves a to l as 0.0, 10.0, 14.0, 18.0, 22.0, 27.0, 32.0, 37.0, 43.0, 49.0, 56.0, 63.0 mmol L^{-1}) with the scan rate as 100 mV s^{-1}; Inset was the linear relationship of catalytic reduction peak currents and the TCA concentration.

3.6. Analytical application

To evaluate the application of Nafion/HRP/MoS_2/CILE, determination of TCA content in lab water samples was performed and the date were listed in Table 1. No TCA residues could be found in lab water samples and the recovery was in the range from 94.68% to 103.45% by the standard addition method. The results suggested Nafion/HRP/MoS_2/CILE could be used to determine TCA in real samples.

Table 1. Detection results of TCA in the water sample (n=3).

Water Sample	Found (mmol L^{-1})	Added (mmol L^{-1})	Found (mmol L^{-1})	Recovery (%)	RSD (%)
1	0	8.00	7.65	95.68	2.85
2	0	10.00	9.47	94.68	2.20
3	0	12.00	12.41	103.45	1.67

3.7. Reproducibility and stability of Nafion/HRP/MoS_2/CILE

The long-term stability of Nafion/HRP/MoS_2/CILE was examined during storage in a 4 °C refrigerator when not in use, which kept about 96.3% of its initial value after being stored for 1 week and 92.5% after 1 month, indicating that the modified electrode showed excellent storage stability. As shown in Fig. 6, cyclic voltammetric responses of Nafion/HRP/MoS_2/CILE showed no observable changes after 20 cycles in PBS (pH=3.0). Then the redox peak currents decreases slowly for 7.9% with the cycles up to 200, which revealed that great stability of the bioelectrode. Moreover six Nafion/HRP/MoS_2/CILE were prepared independently and utilized to detect 20.0 mmol L^{-1} TCA with the relative standard deviation (RSD) as 2.1%, indicating a good reproducibility of the analysis of TCA.

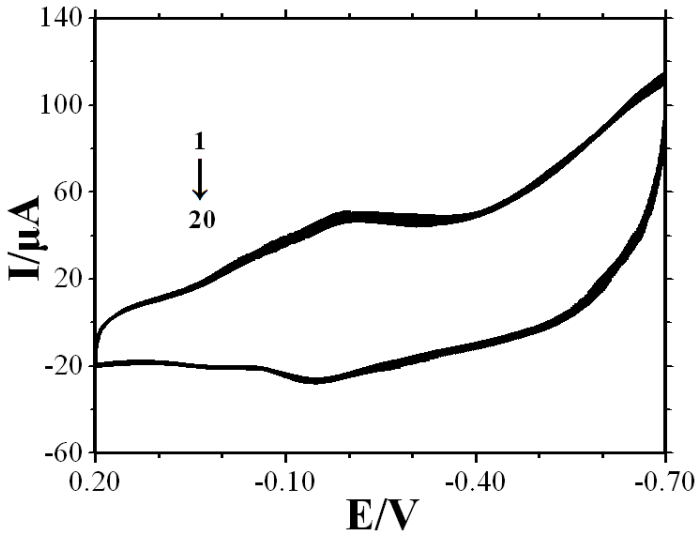

Figure 6. Multi-scan cyclic voltammograms of Nafion/HRP/MoS_2/CILE in pH 3.0 PBS with 20 cycles.

4. CONCLUSION

In this research MoS_2 nanosheets modified electrode was used for encapsulation of HRP. UV–Vis and FT-IR spectra indicated that HRP retained its original structure. Cyclic voltammograms showed that HRP accomplished the direct electron transfer on the modified electrode. Moreover, the modified electrode had an excellent electrocatalytic activity toward TCA with a lower detection limit and smaller K_M^{app} value. This work extended the application of MoS_2 nanosheets in the field of electrochemical biosensor.

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