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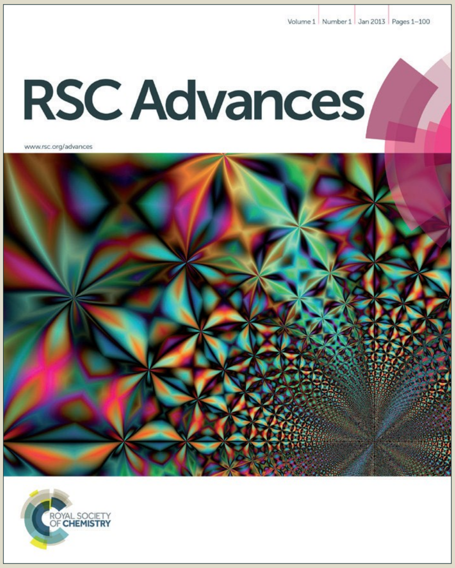

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Chiral electrochemical recognition of tryptophan enantiomers on multi-walled carbon nanotube-chitosan composite modified glassy carbon electrode

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Abstract: Multi-walled carbon nanotube-chitosan composite modified glassy carbon electrode (MWCNT-CS/GCE) was prepared and used for chiral recognition of tryptophan (Trp) enantiomers. Cyclic voltammetry (CV) was employed to characterize the electrical conductivity of the modified electrode. Differential-pulse voltammetry (DPV) was employed to observe the oxidation peak potentials (Ep) of L- and D-Trp on the modified electrode. Different Ep of L- and D-Trp on the modified GCE towards the solutions containing only L- or D-Trp enantiomer were observed. As for a mixed aqueous solution containing both L- and D-Trp, only one Ep peak would appear. However, the Ep of peak was found to shift positively and linearly with an increasing percentage of L-isomer in the racemic Trp mixture solution, making it possible to determine the percentage of L- and D-Trp enantiomers in a racemic Trp mixture.

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Keywords: Electrochemical recognition; Enantioselective; Multi-walled carbon nanotube-chitosan composites; Tryptophan

1. Introduction

Chirality is one of the most common properties in natural systems. More than half of the medicines clinical applied [1], about 30% of the pesticides in use [2] and almost all of the amino acids [3] are chiral. Meanwhile, the different enantiomers of a chiral compound generally behave identically to human beings and other creatures. Therefore, the forensic illegal chiral substances and the chiral pollutants in environmental science need to be monitored. The content of amino acid enantiomers in the foods, and the enantiomeric excess in the drugs should also be determined and quantified to ensure their safe use.

It is difficult to detect the chiral compounds which lack aromatic chromophores by UV spectroscopy, not to mention the identification and quantification of their enantiomers. The determination of the enantiomers of various chiral compounds is really a big challenging up to now. What is unexpected, however, is that electrochemical analysis can achieve this goal [4]. Normally, conventional electrode cannot be used in the analysis of chiral compounds. In order to identify a couple of enantiomers, chiral recognition materials need to be modified on the electrode firstly [5, 6]. Various functional groups of the recognition materials including amino, hydroxyl, carbonyl, phenyl, carboxyl groups and even the hydrophobic cavity are important for the chiral recognition performance of the modified electrode [7, 8]. The noncovalent interactions between the chiral selector and the enantiomers such as electrostatic interactions, π-π stacking, and hydrogen bonding make necessary contributions to the chiral recognitions [9, 10]. Due to the advantages of ease of use, low-energy consumption, continuous operation mode, sensitivity and highly selectivity, electrochemical analysis is becoming increasingly important for the rapid identification of chiral molecules in modern society. And chiral electrochemical recognition has attracted increasingly attention throughout the world over the last two decades. In recent years, the utilization of enantioselective electrodes in enantiomers discrimination has proven to be effective and successful alternatives for enantioselective analysis [11].

To increase the sensitivity of electrochemical measurement, some useful nanomaterials with high surface area, high intrinsic mobility and good electrical conductivity such as Au nanoparticles (AuNPs) [12], carbon nanotubes (CNTs) [12, 13], graphene [14] and so on have been introduced onto the electrodes. The homogeneous dispersion of such nanomaterials have contributed to the sensitivity and the lowest detection limit (LOD) of the modified electrodes.

L-Tryptophan (L-Trp) (**Fig.1**), one of the 22 standard amino acids, acts as a useful building block in protein biosynthesis. However, L-Trp cannot be synthesized by the organism. Therefore, L-Trp is called an “essential” amino acid and must be acquired from the human diet. Up to now, it is still difficult to make a distinction between the L- and D- isomers of Trp due to their very similar physical/chemical properties. It's necessary to develop effective method for identification of the two optical enantiomers of Trp. Recently, L-cysteine was electrochemically polymerized on the surface of multi-walled CNTs (PLC/MWCNTs), and the obtained porous cluster-like nanocomposite films showed an obvious change of oxidation peak currents between D- and L-Trp [15]. Thionine–graphene (positively charged) nanocomposite covered GCE was further modified by ds-DNA (negatively charged), and an obvious difference of oxidation peak currents for D- and L-Trp was observed by the assistance of Cu(II) [16, 17]. However, preparation of such chiral biosensors was time-consuming, complex and costly.

To achieve an efficient chiral electrochemical recognition, the development of novel materials for electrode surface modification is a challenge and always essential. Researchers from all over the world have been devoted to developing chiral composite materials with cheaper and more stable properties through more facile procedures. For example, cysteic acid (CyA) was selected as a chiral selector to directly modify GCE, and the obtained electrode (CyA-GCE) exhibited chiral recognition capacity for tyrosine (Tyr) enantiomers by electrochemical impedance spectroscopy (EIS) [18]. The enantioselective recognition of Tyr enantiomers based on different oxidation potential signals provided a helpful reference for developing better electrochemical sensing system for chiral recognition.

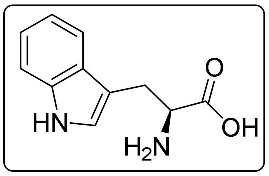

Fig.1 Chemical structure of L-Trp.

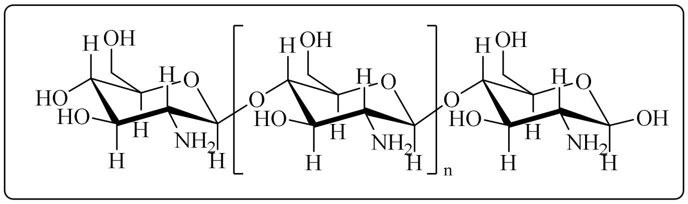

Fig.2 Chemical structure of CS.

Chitosan (CS) (**Fig. 2**), a linear polysaccharide, is composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. CS and its derivatives have shown high chiral recognition abilities for metal acetylacetonate complexes [19], glutamic acid [20], phenylglycine, catechin, tryptophan [21] and so on [22]. Due to the low-price, easy preparation and biological degradability, CS and its derivatives have been widely used as chiral resolving agents. Most recently, Ou *et al.* developed a graphene quantum dots (GQDs)-CS composite film for chiral electrochemical recognition of Trp enantiomers [23]. However, the preparation of GQDs–CS composite film by such an electrochemical deposition method is time consuming, complex and costly although it combine both the advantages of GQDs and CS. Meanwhile, such a composite modified GCE couldn’t recognize D- or L- Trp enantiomers through appreciable difference in the peak potentials (Ep).

Herein, we hope to develop a novel electrochemical sensor for chiral electrochemical recognition of Trp enantiomers based on CS functionalized MWCNT (MWCNT-CS) composites modified GCE. The tiny difference between the two Trp enantiomers and the chiral sensor could be identified directly, and an obvious difference of oxidation potential signals for D- and L- Trp could be observed. To the best of our knowledge, this is the first report describing an electrochemical sensor which could exhibit different Ep difference for D- or L- Trp enantiomers through enantioselective interactions.

2. Experiments

2.1 Reagents and apparatus

L-Trp (98%) was purchased from Shanghai Chemical Co., Ltd. D-Trp (98%) was obtained from Sinopharm Chemical Reagent Co., Ltd. Multi-walled carbon nanotubes (MWCNTs; 95% of purity;) were provided by Shenzhen Nanotech Port Co. Ltd. CS (B.R.; 80.0-95.0% of deacetylation degree) was bought from Sinopharm Chemical Reagent Co., Ltd. CS modified MWCNT (MWCNT-CS) composites were prepared according to the microwave-assisted methods reported by our group [24]: A mixture of oxidized MWCNTs (100 mg) and CS-CH_3COOH solution (0.1 g of CS in 5 mL of 2% CH_3COOH and 5 mL of N, N-dimethylformamide) was reacted in a microwave oven for 20 min, then the mixture was cooled to room temperature, filtered, purified and dried to obtain MWCNT-CS (53 mg). All other chemicals were of analytical grade and used without any further purification. The aqueous solutions were freshly prepared by using double distilled water.

Electrochemical measurements were performed with a CHI650D Electrochemical Workstation (Shanghai Chenhua Instrument Co., Ltd.). A conventional three-electrode system contained a bare glassy carbon electrode (GCE) or the modified electrode as working electrode, and Ag/AgCl (in saturated KCl solution) as reference electrode, a platinum wire as auxiliary electrode. Transmission electron microscope (SEM) measurements were operated on a JEM-2100 high-resolution transmission electron microscope (HT-TEM) at an acceleration voltage of 200 kV. All the experiments were performed at room temperature.

2.2 The preparation of working electrode

Prior to modification, the bare GCE (4 mm of diameter) was polished with 0.05 μm alumina slurry to a mirror finish, and then ultrasonically cleaned in nitric acid (HNO_3), ethanol and distilled water for 2 minutes, respectively. After the electrode was allowed to dry under N_2 gas, it was then immersed in 5 mM of $K_3Fe(CN)_6$ and scanned in the potential range of -0.1 to +0.6 V (vs. Siliver chloride electrode, AgCl) until the peak potential separation (Ep) of CV profiles was less than 80 mV.

The modification of the GCE was prepared according to the following steps. Firstly, the black suspension of MWCNT-CS (8 μL, 2 mg/mL) was dropped onto the surface of the processed GCE; secondly, the electrode was dried with an infrared lamp and then stored at 4 °C before use.

3. Result and discussion

3.1 TEM measurements of MWCNT-CS

The morphologies of pristine MWCNTs and MWCNT-CS have been investigated by HR-TEM. As shown in Fig. 3, the surface of pristine MWCNTs is smooth (**Fig. 3**A), while the surface of MWCNT-CS displays obviously rough (**Fig. 3**B), indicating CS had been grafted on the MWCNT surface. Due to the covalently grafted of CS on the surface of MWCNTs, the diameters of the MWCNT-CS increase a little.

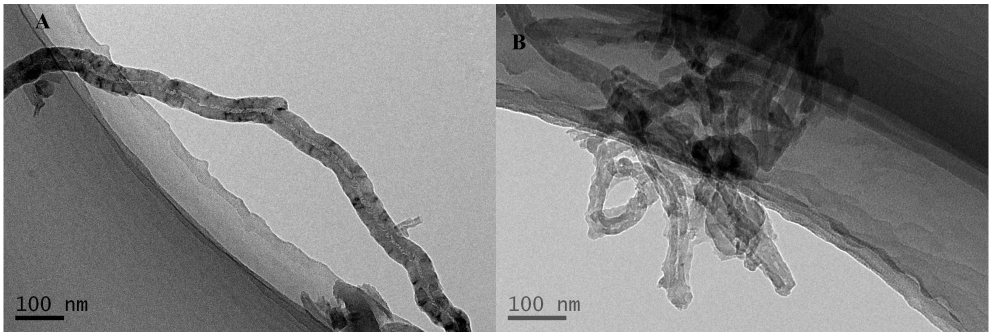

Fig. 3 TEM images of MWCNT samples: (A) pristine MWCNTs; (B) MWCT-CS.

3.2 Characterization of MWCNT-CS modified electrode

As shown in **Fig. 4**, the cyclic voltammograms of different electrodes including bare GCE, MWCNT/GCE and MWCNT-CS/GCE were obtained in 1 mM $K_3Fe(CN)_6$ containing 0.1 M KCl with a scan rate of 50mV/s. A couple of redox peaks could be observed at the MWCNT-CS/GCE with ΔE_p=117 mV, while the oxidation peak current at MWCNT/GCE was improved a lot, and ΔE_p became relatively small (50 mV) in contrast with those of bare GCE or MWCNT/GCE. The result indicated that the introduction of CS would hinder the electron transfer of the electrode.

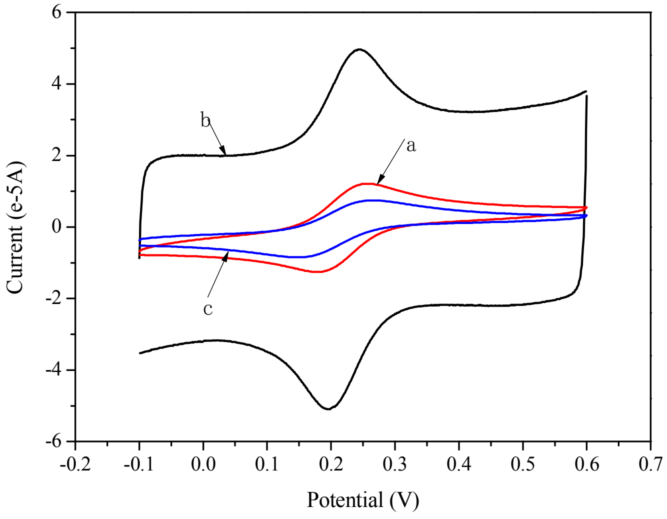

Fig. 4 Cyclic voltammograms of the electrodes in 1 mM $K_3Fe(CN)_6$ (containing 0.1M

KCl, pH= 7.0): (a) bare GCE; (b) MWCNTs/GCE; (c) MWCNT-CS/GCE.

3.3 Chiral selective recognition of Trp enantiomers

In order to obtain the electrochemical behaviors of L-/D-Trp (2.5mM) enantiomers on the modified electrode, the response of Trp enantiomers was investigated by DPV in the solution containing only L-Trp or D-Trp. As shown in **Fig.5** (a) and (b), almost overlapped differential pulse voltammograms (DPV) of D-Trp and L-Trp could be found at the bare GCE, indicating there was almost no difference of Ep between D-Trp and L-Trp, and no stereoselective recognition would occur on the bare GCE. However, the voltammetric responses including the Ep and peak current of MWCNT-CS/GCE to Trp enantiomers [**Fig.5**(c) and **Fig.5**(d)] were changed a lot. Compared to the Ep and peak current of D-Trp [**Fig.5**(d)], the Ep of L-Trp [**Fig.5**(c)] shifted positively, and the peak current of L-Trp increased accordingly. The differences of peak potential and peak current implied that the CS grafted on the MWCNTs would interact with L-Trp and D-Trp in different way, and therefore MWCNT-CS/GCE displayed chiral recognition ability to L-Trp.

The selective capability of MWCNT-CS/GCE can be attributed to the chirality of CS grafted on the surface of MWCNT-CS. Additionally, because the DPV signal was greatly amplified by the MWCNT substrate of MWCNT-CS, the recognition to L- or D-Trp enantiomers could be monitored and qualitatively analyzed. Therefore, MWCNT-CS/GCE would have a different binding affinity towards D- or L-Trp enantiomers, leading to a difference in the free energy and reflecting as different potential shift.

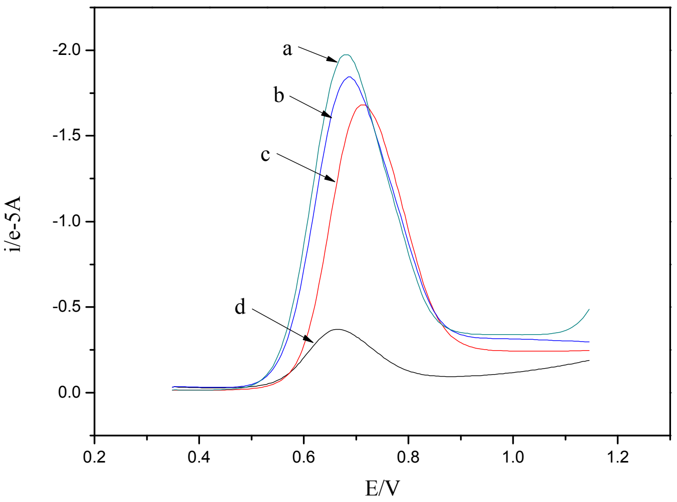

Fig. 5 Potential voltammograms of the D- or L- Trp enantiomers on the electrodes: (a) D-Trp on GCE; (b) L-Trp on GCE; (c) L-Trp on MWCNT-CS/GCE; (d) D-Trp on MWCNT-CS/GCE.

3.4 A proposed recognition mechanism

It has been proposed that the electrochemical oxidation of amine-containing compounds through one-electron oxidation of the amine functional group to its corresponding cation radical, which removes a hydrogen cation to form a carbon-nitrogen linkage on the surface of an inert solid such as GCE [25, 26]. As shown in **Fig. 6(a)**, there might be a carbon-nitrogen linkage formed by the amine functional group of Trp and MWCNT-CS composites. As for the MWCNT-CS composites, the grafted CS was used as a probe molecule for the chiral electrochemical recognition due to the chirality of CS could provide chiral detection sensitivity for D- or L- Trp enantiomers [**Fig. 6(b)**]. The −OH groups of CS are in the double helixes and show chirality properties, the $-NH_2$ groups of Trp are also in the chiral position. The formation of hydrogen bonds between the –OH groups of CS and the −NH_2 groups of D- or L-Trp, different affinities of MWCNT-CS for these two optical isomers of Trp would occur due to the different steric hindrance, therefore the chiral recognition would be found [26].

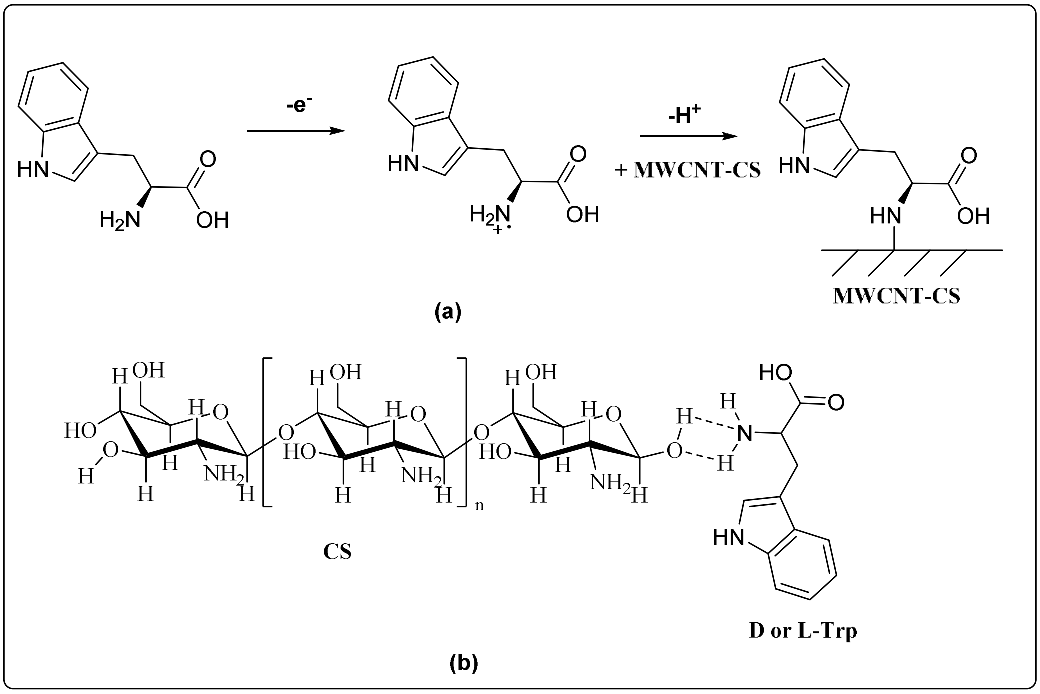

Fig. 6. The proposed mechanism for the chiral electrochemical recognition of Trp enantiomers on MWCNT-CS modified GCE: (a) the electrochemical oxidation of Trp; (b) the possible interactions among MWCNT-CS and D- or L- Trp.

3.5 Application of enantioselective sensor in racemic solutions

In this experiment, the application of chiral surface in practical research was also investigated, thus a series of proportion of L-Trp from 0% to 100% was performed by measuring the change of peak potential (E_p). As shown in **Fig. 7**, the enantiomeric ratios of L- or D-Trp mixtures could be determined from a calibration curve. The chiral sensor could be used to recognize the percentages of D-/L- enantiomers of Trp in the enantiomer mixtures because the calibration curve exhibited good linearity.

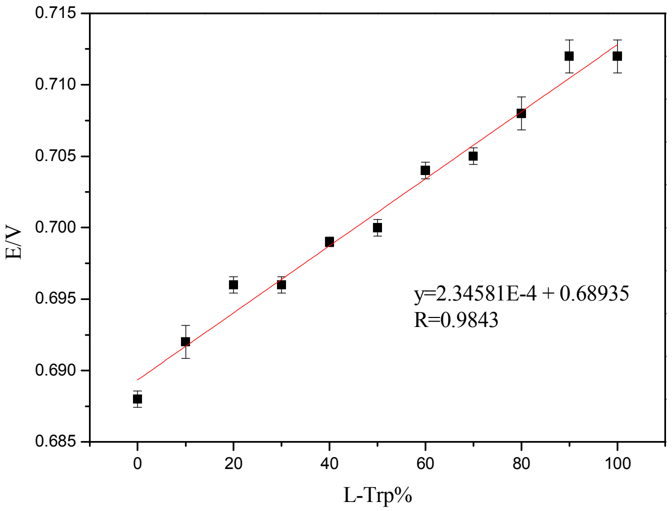

Fig. 7. The relationship between Ep and different percentage of L-Trp (%) of 2.5 mM Trp racemic mixture on MWCNT-CS/GCE.

4. Conclusions

Herein, we have prepared a chiral electrode by depositing MWCNT-CS onto the surface of GCE. The chiral selective recognition of Trp enantiomers was successfully found on the surface of MWCNT-CS/GCE. A significant peak potential difference could be observed for D- or L-Trp enantiomer solutions. Meanwhile, a progressively positive shift could be observed for the racemic solutions containing an increasing percentage of L-Trp enantiomer. The developed method was simple and sensitive, providing a novel way to quickly detect the chirality of racemic Trp enantiomers via the electrochemical responds on the modified GCE. The results could provide some useful guidance for the researchers to develop novel composite materials for chiral electrochemical analysis.

Acknowledgments

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Conflict of Interest None.

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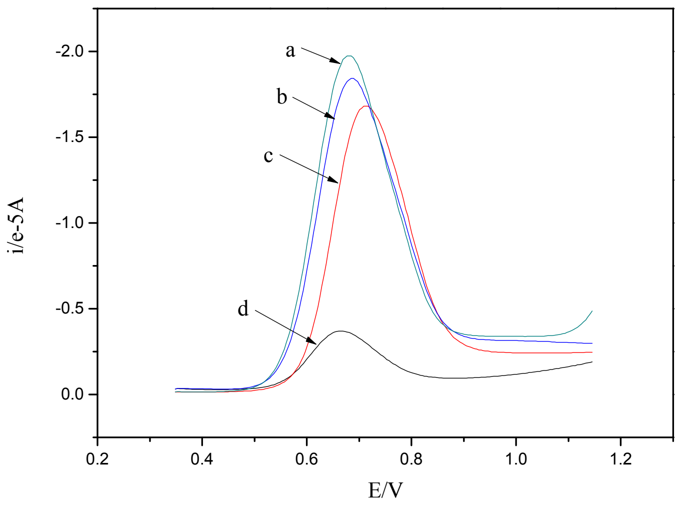

Multi-walled carbon nanotube-chitosan composite modified glassy carbon electrode (MWCNT-CS/GCE) was developed for the chiral recognition of tryptophan (Trp) enantiomers.