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ARTICLE

A novel electrochemical chiral sensor for 3,4-dihydroxyphenylalanine based on the combination of single-walled carbon nanotubes, sulfuric acid and square wave voltammetry

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We demonstrate, for the first time, an electrochemical sensor that provides antipode signals upon application of square wave voltammetry (SWV), for enantioselective recognition of 3,4-dihydroxyphenylalanine based on chiral single-walled carbon nanotubes (SWCNTs) in the presence of sulphuric acid. Interestingly, the enantioselectivity was not observed with the common method of cyclic voltammetry (CV) but their SWV peak currents of enantiomers were found quite different and hence the enantiomers could be successfully recognized. Moreover, the antipodal signals provided by two SWV scan modes offer the possibility for results to be confirmed mutually, showing a great practical value and analytical application prospects.

1. Introduction

Single-walled carbon nanotubes (SWCNTs) that have high structural perfection are widely used as electrode material for electrochemistry. SWCNTs consist of a single graphite sheet seamlessly wrapped into a cylindrical tube. The sheet direction and nanotube diameter are obtained from a pair of integers (n,m) which denote the tube type.[1] When the value of indices $n \neq m \neq 0$, the SWCNTs is a chiral one. And each pair (n,m) defines a different way of rolling the grapheme sheet to form a carbon nanotube of certain chirality.[2,3] Chiral SWCNTs have a distinct optical absorption, with each chirality demonstrating its own characteristic absorption spectrum, making it an ideal choice for applications such as displays and solar cells.[4] The chirality-dependent electronic and vibrational states of SWCNTs provide laboratory for novel 1D physics and its catalyst interface will be critical to advances in chirality-selective synthesis.[5,6] To our best knowledge, the property of chiroptical activity[7] owing to kink sites has also been observed but the field of using this property of chiral SWCNTs for the enantiomeric recognition still remains unexplored.

L-3,4-dihydroxyphenylalanine (*L*-DOPA) has been widely used in the treatment of Parkinsons disease for more than forty years and it plays a very crucial role in clinic and neurochemistry.[8] Nevertheless, *D*-3,4-dihydroxyphenylalanine (*D*-DOPA) has toxic properties.[9] Owing to the different metabolisms of the active and inactive components, using racemic mixtures containing *D*- and *L*-DOPA may cause serious side effects.[10]

Therefore, the study of chiral recognition of DOPA enantiomers is necessary in both of chemical biology and pharmacology. Several methods for the enantioseparation and determination of DOPA have been reported in the past years, by high-performance liquid chromatography,[11-13] capillary electrophoresis,[14-16] tandem mass spectrometry,[17] and electrochemical method,[18-20] etc. With the advantages of low cost, high speed and high sensitivity, electrochemical methods are always regarded as the highest cost-effective for the discrimination of DOPA.

In this paper, chiral SWCNTs were used to recognize the enantiomers of DOPA. Enantioselectivity was not observed with the method of cyclic voltammetry (CV). But the peak current for the enantiomers was found different from each other when *D*- and *L*-DOPA was detected by square wave voltammetry (SWV). Due to the less processes of separation on the electrode surface, the fabrication of an effective selection of sensing interface on the electrode represents a major challenge. Herein, the combination of SWV with the use of chiral SWCNTs and sulfuric acid shows chiral discrimination for DOPA, and the three are indispensable.

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2. Experimental

2.1. Apparatus

Electrochemical measurements were performed with a CHI 660C electrochemical workstation (Shanghai, China) and an Autolab PGSTAT 302 (ECOChemie, The Netherlands). The three-electrode electrochemical cell contained a glassy carbon electrode (GCE) or GCE modified with SWCNTs, a platinum wire as auxiliary electrode and a saturated calomel reference electrode (SCE). All measurements were conducted at room temperature (25±2℃).

2.2. Reagents

Enantiomerically pure DOPA (99%), chiral (*6,5*) SWCNTs and chiral (*7,6*) SWCNTs were purchased from Sigma Aldrich. The diameter of chiral (*6,5*) SWCNTs or (*7,6*) SWCNTs is 0.7-0.9 nm and the purity is more than 90%. Achiral SWCNTs (JCST1 and JCST2) were obtained from Nanjing Jcnano technology Co., LTD (Nanjing, China). The diameter of JCST1and JCST2 is less than 2 nm and 0.8-1.2 nm, respectively. All other chemicals were analytical reagent grade and used without further purification. Triply distilled water was used for aqueous solutions preparation.

2.3. Procedure

2.0 mg SWCNTs was sonicated in 2 ml dimethyl formamide (DMF) for 10 min,[21] then the chiral modified solution could be obtained. Prior to use, GCE (3 mm diameter) was first polished with 1 μm and 0.05 μm alumina slurry, and then washed ultrasonically in distilled water and ethanol for 2 minutes, respectively. After that, the electrochemical chiral sensor was fabricated by casting modified solution on the well-polished GCE and being dried under an infrared lamp.

3. Results and discussion

3.1. Establishment of chiral discrimination method

The electrochemical behaviours of the enantiomeric pairs (*D*- and *L*-DOPA) were studied by CV and SWV, respectively. There is no apparent Faradic response of DOPA at bare glassy carbon electrode (GCE) in this system. When the GCE was modified by chiral (*6,5*) SWCNTs, a pair of significant redox peaks were observed on the CV curves owing to two-electron-two-proton oxidation and reduction of DOPA/dopaquinone.[22] As shown in Fig. 1, the differences of CV peaks were too small to be distinguished between the enantiomers of DOPA, and thus *D*- and *L*-DOPA cannot be recognized with CV technique if the concentration of the enantiomers is the same. But a different story emerged with SWV technique, the SWV peak current of *D*- and *L*-DOPA is 15.5 and 35.2 μA, respectively when the scanning potential of SWV was changed from low to high values (forward scan). In contrast, the peak current is -33.7 and -15.7 μA corresponding to *D*- and *L*-DOPA, respectively with a reverse scan. The peak current value of one enantiomer is two times larger than the other one either in the forward or reverse scanning. So the difference in SWV peak currents may be sufficient to enable an accurate determination of the enantiomeric purity and composition of the DOPA analyte.

The influence of scan rate on the redox of each enantiomer of DOPA was studied by CV. Nernstian behaviour of surface-confined species is manifested by symmetrical cyclic voltammetric peaks. With the increase of scan rate, the redox peak current was linearly related to the scan rate over the range of 5-500 mV s^{-1}. These results confirm that the electron transfer reaction of DOPA has the characteristic of surface wave. Corresponding to different scan rate, the CV peak currents of *D*- and *L*-DOPA were similar with each other, suggesting the electrode surface coverage for enantiomers were almost the same.
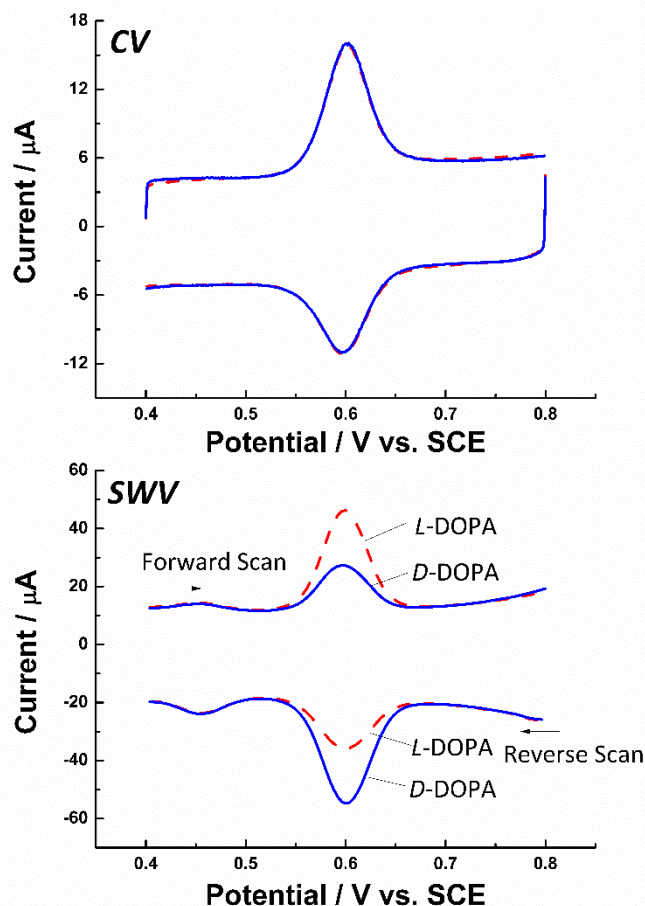

Fig. 1 CVs and SWVs of chiral (*6,5*) SWCNTs modified electrode in 0.25 mol L^{-1} H$_2$SO$_4$ containing 50 μmol L^{-1} *L*-DOPA (dash line) or *D*-DOPA (solid line). CV: scan rate, 50 mV s^{-1}; SWV: step height, 0.004 V; frequency, 15 Hz.

3.2. Mechanism of chiral discrimination

In this system, the DOPA enantiomers show different SWV behaviours and thus enable the sensor to convert the enantioselective recognition event into current changes. Basically, DOPA in the solution should exchange electrons with GC electrode after passing through the recognition layer. Chen et al. aligned the SWCNTs on graphite surface with chirality-dependent orientations and took the graphite trenches with ZZ edges as references. Then they summarized detailed formulas which are suitable to determine the handness and chiral angle of grown SWCNTs on graphite.[23] According to his conclusion, chiral (*6,5*) SWCNTs are a right-handed form. When enantiomers of DOPA were close to surface of electrode, the left-handed DOPA is easier to pass through the SWCNTs while right-handed DOPA is hindered. In spite of the presence of right-handed SWCNTs and hence their retention to

enantiomers, the electrochemical activity of DOPA is so good that the enantiomers cannot be distinguished by using common linear sweep voltammetry due to similar peak currents. Consequently, extending the interaction time between DOPA enantiomer and chiral SWNTs is a key point to success. The waveform of SWV is a staircase scan, each tread of which is superimposed by a symmetrical double pulse, one in the forward direction and the other in the reverse. When SWV is applied in this case, the DOPA enantiomers maybe pass through chiral selectivity zone in a manner of oscillation, which would effectively extend the retention time. Each waveform scan makes such an interaction time being increased a little. After many cycles, the enantioseparation is finally amplified and thus the enantiomers are discriminated. In other words, SWV makes limited recognition sites reused, which greatly increases the efficiency of chiral recognition. Meanwhile, the chiral (*6,5*) SWCNTs also act as a bidirectional switch, so the peak currents of *D*- and *L*-DOPA are different with the different potential scanning direction.
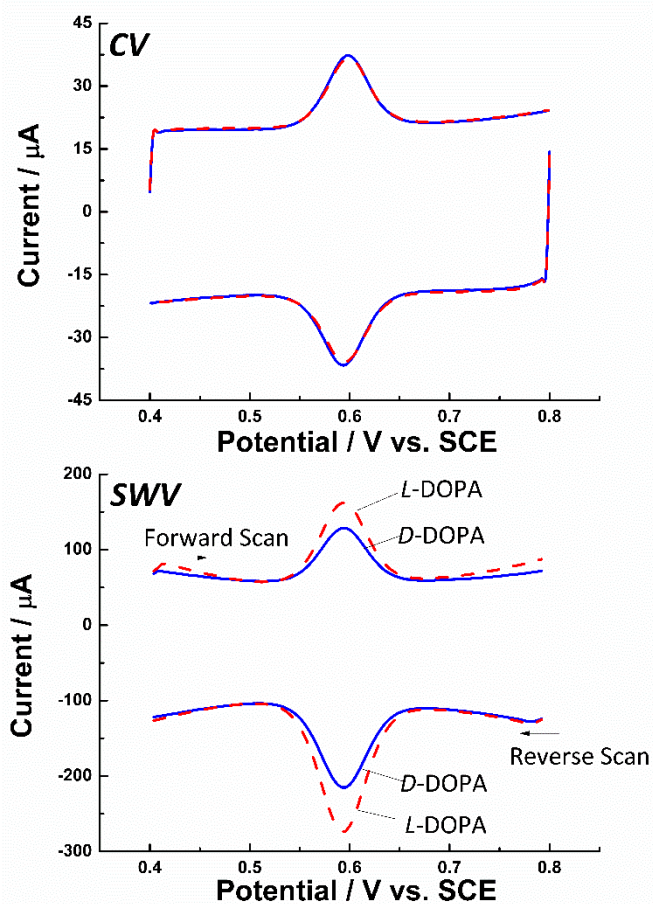

Fig. 3 CVs (a, c) and SWVs (b, d) of JCST1 (a, b) and JCST2 (c, d) modified electrode in 0.25 mol L^{-1} H_2SO_4 containing 50 μmol L^{-1} *L*-DOPA (dash line) or *D*-DOPA (solid line). Other conditions the same as in Fig. 1.

Fig. 2 CVs and SWVs of chiral (*7,6*) SWCNTs modified electrode in 0.25 mol L^{-1} H_2SO_4 containing 50 μmol L^{-1} *L*-DOPA (dash line) or *D*-DOPA (solid line). Other conditions the same as in Fig. 1.

Interestingly, if the chiral (*6,5*) SWCNTs being replaced by another kind of right-handed type SWCNTs, such as chiral (*7,6*) SWCNTs, there would be no bidirectional effect (Fig. 2). Regardless of scan direction, *L*-DOPA shows a larger current response than *D*-DOPA, indicating that the integers in SWNTs should have contribution to the bidirectional effect.
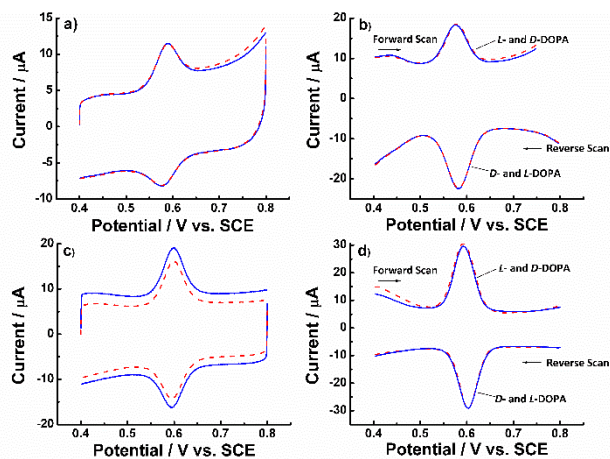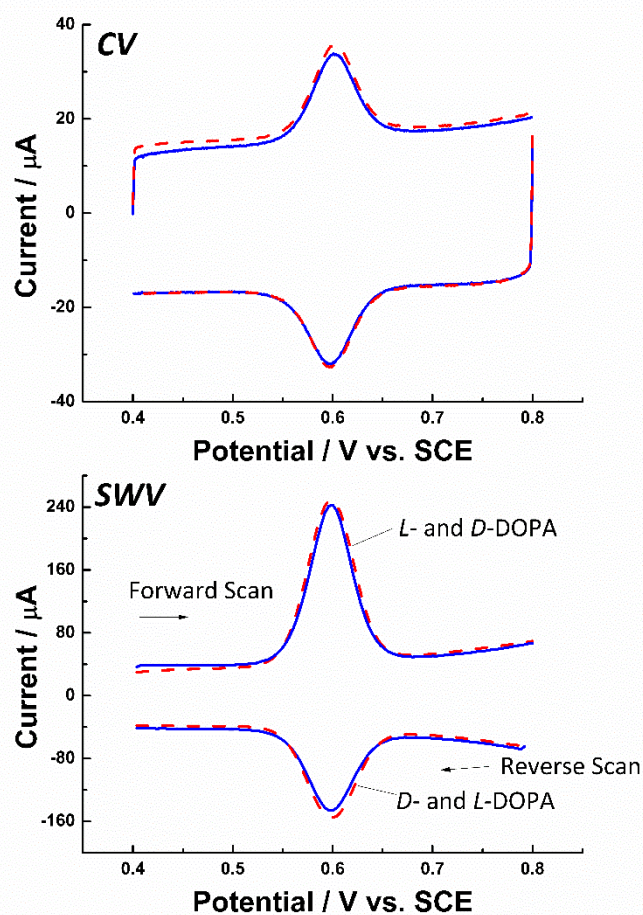

Fig. 4 CVs and SWVs of chiral (*6,5*) SWCNTs modified electrode in 0.50 mol L^{-1} $HClO_4$ containing 50 μmol L^{-1} *L*-DOPA (dash line) or *D*-DOPA (solid line). Other conditions the same as in Fig. 1.

To gain a deep understanding of the mechanism of chiral discrimination in this system, achiral SWNTs (JCST1 and JCST2) that have the similar diameter to chiral (*6,5*) SWCNTs were used to detect DOPA enantiomers. As shown in Fig. 3, both of *D*- and *L*-DOPA demonstrate almost the same

electrochemical responses on either CV or SWV curves. This means that the enantiomers could not be discriminated without chiral environment, and thus the constructing of a chiral environment is critical important for an electrochemical chiral sensor.
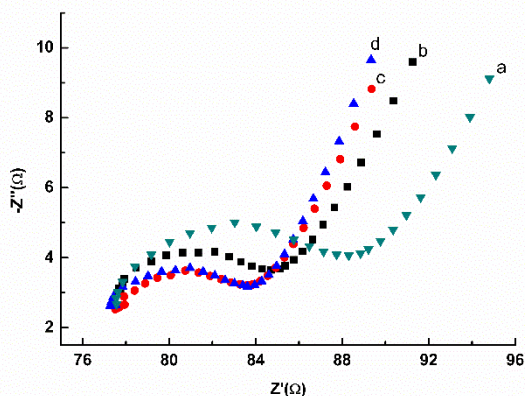

Fig. 5 Impedance plots (-z'' *vs* z') for chiral (*6,5*) SWCNTs (a), chiral (*6,5*) SWCNTs/H_2SO_4 (b), chiral (*6,5*) SWCNTs/*L*-DOPA/H_2SO_4 (c) and chiral (*6,5*) SWCNTs/*D*-DOPA/H_2SO_4 (d). The EIS solution consists of 0.1 mol L^{-1} KCl-0.01 mol L^{-1} phosphate buffer solution (pH =7.4) containing 5 mmol L^{-1} $K_3Fe(CN)_6$-$K_4Fe(CN)_6$ (1 : 1), respectively.
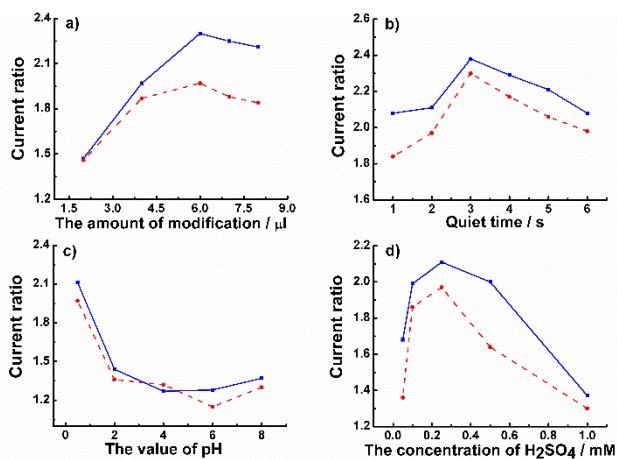

Fig. 6 Effect of some parameters on enantioselectivity and stability of electrochemical chiral sensor for 50 μmol L^{-1} *L*-DOPA or *D*-DOPA: (a) the amount of modified solution; (b) quiet time before scanning; (c) the pH of the solution; (d) the concentration of electrolyte solution. The solid and dash line represent the current ratio $I_{D\text{-DOPA}/L\text{-DOPA}}$ and $I_{L\text{-DOPA}/D\text{-DOPA}}$, respectively.

Based on Attard's result, the adsorption of specifically adsorbed anions like bisulfate/sulfate influences strongly the chiral discriminatory behaviour of all surfaces of platinum single crystal.[24] A similar phenomenon has occurred in our system, as evidenced by that the sensor would lose the ability to discriminate if the sulphuric acid being replaced with perchloric acid having very low strength of adsorption (Fig. 4). The properties of chiral (*6,5*) SWCNTs modified electrodes which had been used to detect H_2SO_4, *L*-DOPA/H_2SO_4 and *D*-DOPA/H_2SO_4, respectively, were measured by electrochemical impedance spectroscopy (EIS) (Fig. 5). The result of EIS also illustrates that bisulfate/sulfate ions adsorbed on the chiral SWCNTs, leading to an increase of surface roughness. As a result, the more efficient quasi-chiral nano-spaces would be

formed on the surface of the chiral SWCNTs, which biases towards the right-handed DOPA in this system. Then the intrinsic chirality of SWCNTs is magnified. In conclusion, the enantioselective discrimination in this case can be achieved only with the combination of chiral SWNTs, sulfuric acid and SWV technique.
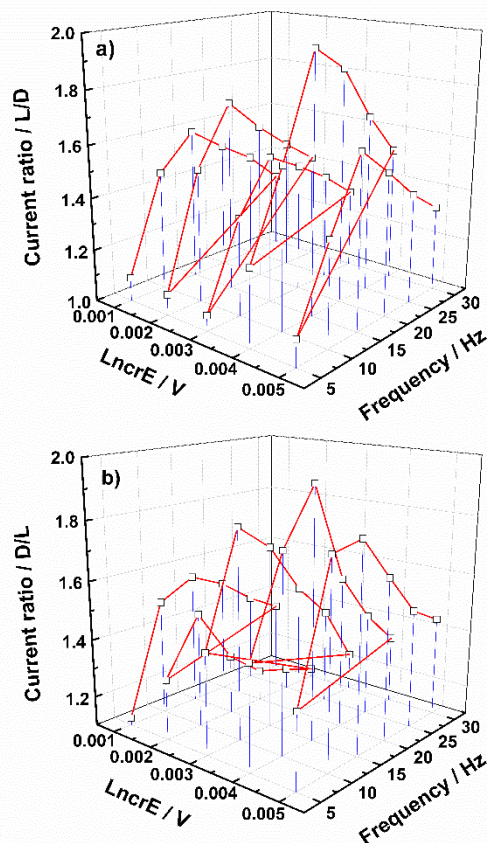

Fig. 7 Effect of the different step height and frequency for detecting DOPA with forward and reverse scan. The lines represent the SWV peak current ratio $I_{L\text{-DOPA}/D\text{-DOPA}}$ (a) and $I_{D\text{-DOPA}/L\text{-DOPA}}$ (b), respectively.

Besides the selection of supporting electrolyte, significant enhancement of enantioselectivity was achieved by the optimization of other experimental parameters. Their dependences on the peak current ratio for the enantiomers are shown in Fig. 6. The number of chiral cites on the surface of the electrode should play a key role to recognize the chiral DOPA. In order to get a proper modification of chiral (*6,5*) SWCNTs, the amount of the modified solution was changed and it showed a dual character. Theoretically, more modification favours the enantioselective effect. But they may decrease the electrochemical response and had bad effect on the sensor stability. 6 μL was chosen as the optimal (Fig.6a). Other parameters including quiet time, scan rate, open potential and so on have also been optimized (Fig.6b-d and Fig7).

3.3. Analytical Application

Different concentrations of DOPA were detected by CV and SWV, respectively. Both of *D*- and *L*-DOPA showed the similar current responses on CV curves and the linear range for the detection of the DOPA was 2.5-200 μmol L^{-1} (Fig. 8a and b).

As far as SWV was concerned, corresponding to different concentration, the peak current value of *L*-DOPA was larger than *D*-DOPA with forward scan for SWV, while in the case of reverse scan, it's the opposite. The platform shown in Fig. 8c is particularly useful for practical application of such an electrochemical chiral sensor, because that means the sensor would have similar and optimal performance corresponding to a wide response range from 30 to 100 μmol L⁻¹.
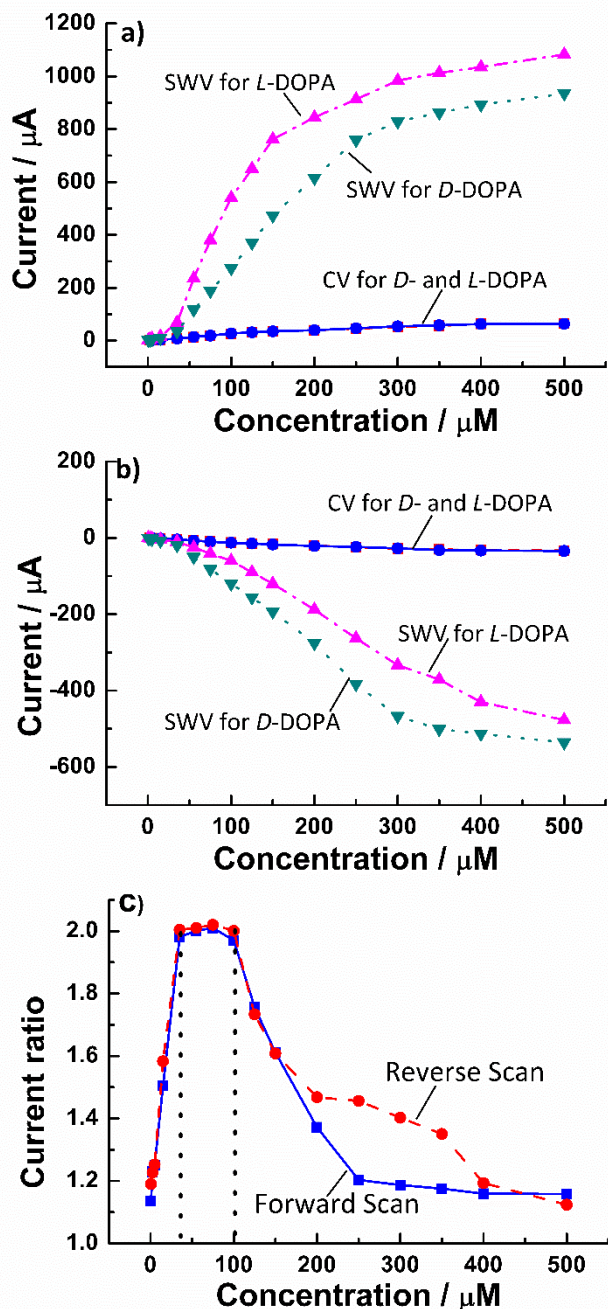

Fig. 8 (a), (b) and (c) represent the dependence of different concentrations of *L*-DOPA or *D*-DOPA on oxidation peak current, reduction peak current and current ratio, respectively. Other conditions the same as in Fig. 1.
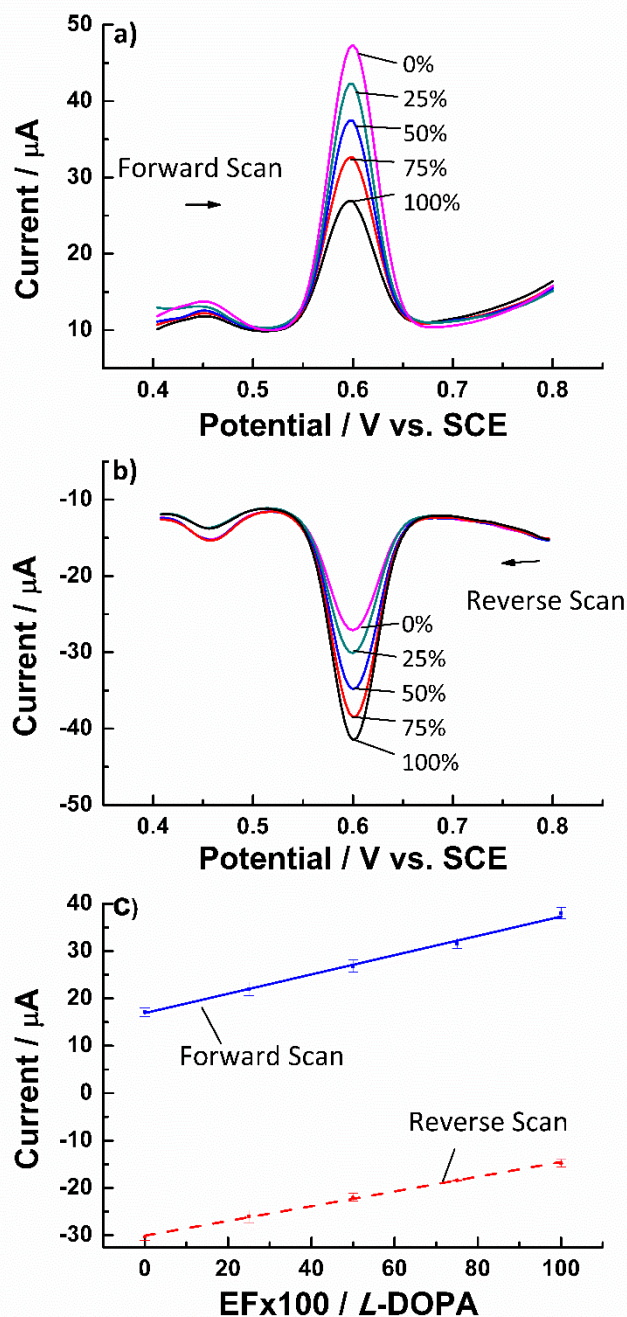

Fig. 9 (a) and (b) represent SWVs for different EF values of *L*-DOPA with the total concentration of enantiomers of 50 μmol L⁻¹. (c) represents linear relationship between peak current and EF of L-DOPA. Other conditions the same as in Fig. 1.

To further investigate the effect of the chiral electrochemical sensor, the total concentration of 50 μmol L⁻¹ DOPA with different EF value (enantiomeric fraction) of *L*-DOPA was detected. Under the optimized conditions, a series of SWV curves corresponding different EF value of *L*-DOPA in enantiomers mixture were obtained (Fig. 9a and b). The peak current reveals a linear dependence for EF value for both forward and reverse scan with the correlation coefficient of 0.995 and 0.998, respectively (Fig. 9c). To our knowledge, so far very few electrochemical approaches to chiral sensing have

been developed which can determine EF values in a practically useful range of 90-100%. Clearly, in this sensing system, the antipodal signals provided by two SWV scan modes offer the possibility for results to be confirmed mutually, showing a great practical value and analytical application prospects.

4. Conclusions

In summary, we have demonstrated the discrimination of DOPA enantiomers based on chiral SWCNTs modified electrode with the help of sulphuric acid, and SWV has played a significant role in this sensing strategy. The features of this new approach are simplicity, rapidity and sensitivity. This methodology gives a new concept for using different electrochemical method toward the chiral recognition of various biomolecules.

Acknowledgements

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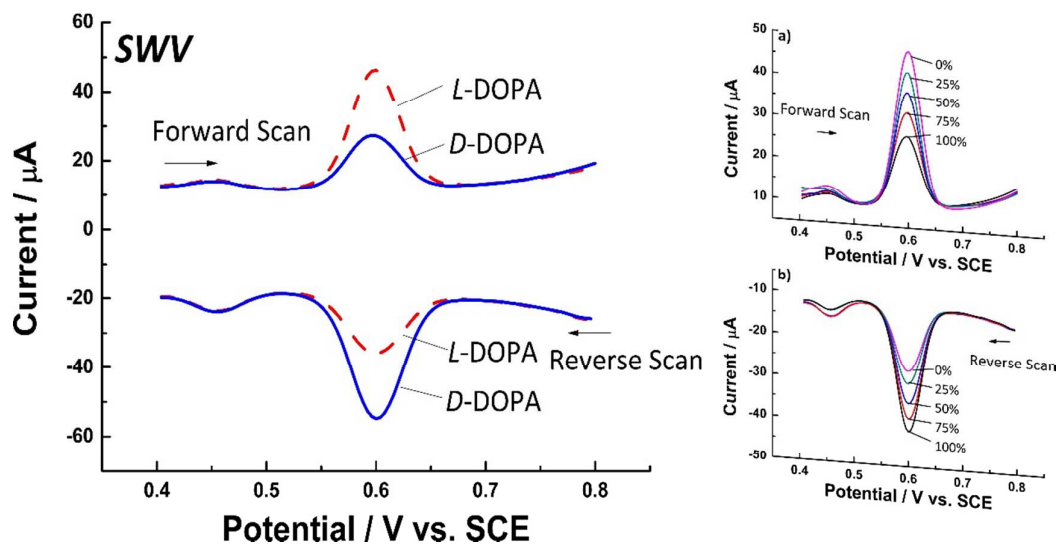
The combination of SWV with chiral SWCNTs and H_2SO_4 shows chiral discrimination for 3,4-dihydroxyphenylalanine, and the three are indispensable.