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Electrochemical Detection of Levo-Tetrahydropalmatine Based on DNA Modified Electrode

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DNA was employed in a novel way to contribute to the electroanalysis. Electrochemical oxidized DNA modified glassy carbon electrode (DNA/GCE) by potentiostatic method for Levo-tetrahydropalmatine (L-THP) determination was prepared. For the first time, L-THP was investigated by electrochemical sensor, and the results indicated that this voltammetric sensor exhibited a special recognition capacity towards L-THP determination. Under the optimize condition, the response peak currents had a linear relationship with the L-THP concentrations in range from 5.6×10^{-5} mol·L^{-1} to 2.8×10^{-3} mol·L^{-1} with detection limit of 2.4×10^{-6} mol·L^{-1}.

Keywords: Levo-tetrahydropalmatine; DNA; modified electrode; electrochemical detection

1. INTRODUCTION

Levo-tetrahydropalmatine (L-THP), alternate name Rotundine, IUPAC name 5,8,13,13a-Tetrahydro-2,3,9,10-tetramethoxy-6H-dibenzo[a,g]quinolizine, is an active constituent derived from the herbal plant species Stephania and Corydalis. It clinically has good analgesic effect of calm. It has also been found that L-THP can reduce the effect of diseases such as resisting arrhythmia, dilating coronary arterial blood pressure. For its cheap market price, L-THP has been wildly used by patients suffering from the pain. However, L-THP overdose will result in severe side effects. Therefore, the accurate determination of L-THP is an important and significant work in clinical. Some of the analytical methods reported including double wavelength ultraviolet spectrophotometry [1], HPLC [2], RP-HPLC [3], HPLC-MS [4], GC-MS [5], Liquid Chromatography-Tandem Mass Spectrometry [6]

were applied for the determination of L-THP. Nevertheless, these methods either required complicated sample preparation, or suffered from low sensitivities and specificities. Thereby, to develop a highly sensitive and simple detection method for L-THP is one of most important analytical challenges.

However, electrochemical methods are the most favorable techniques for the determination of several medicinal molecules because of its low cost, high sensitivity, easy operation and the ability for carry out speciation analysis. In recent years, chemically modified electrodes (CMEs) have received increasing attentions which enhance the sensitivity and selectivity of electrochemical analysis techniques in the past decades [7]. As a unique one, DNA based electrochemical sensor has attracted considerable attention in the electrochemical and electroanalytical field. For instance, several drugs and biological molecules were detected based on DNA modified electrodes, such as West Nile Virus [8], dopamine and uric acid under coexistence of ascorbic acid [9], aromatic amine [10], cyclophosphamide [11], Neisseria meningitidis [12], Neisseria gonorrhoeae [13]. It also have been used for the detection and investigation of organic and inorganic molecules, such as photodegradation products of benzo(a)pyrene [14], proton [15], Pb^{2+} [16], H_2O_2 [17], dioxygen [18], hydrogen [19], et al. Although several methods were employed to obtain different DNA based sensors used in specific analysis, but during most of the analysis procedures, the properties of DNA molecules were still kept. Herein, DNA was employed in a novel way to contribute to the electroanalysis. Through potentiostatic method, DNA molecules were oxidized and adsorped onto the surface of working electrode with an applied positive potential. Although the original structure of DNA molecules were changed in the modified procedure, a simple and stable DNA modified electrode was got with a better activity. Thus, it may offer other useful conditions for the interaction between DNA and drug molecules. By employing the same method, Wang and co-workers [20] use the DNA modified biosensor to investigate the electrochemical behavior of interaction between salvianolic acid B and bovine serum album.

As we know, there is a redox-active group in the molecular structure of L-THP but few reports were retrieved about the determination of L-THP with electrochemical technique. In this approach, a glassy carbon electrode (GCE) modified with oxidized DNA is designed for the determination of L-THP. Compared with the previous one, this DNA/GCE can use the oxidizing current of L-THP as quantitative signal to detection L-THP in phosphate buffered solution (PBS) with lower background currents. Special attention was paid to the influence of experimental conditions on the adsorptive behavior and further electro-oxidation of L-THP. Under the optimal conditions, a novel electrochemical method for L-THP detection was further established.

2. EXPERIMENTAL

2.1. Apparatus and reagents

All electrochemical techniques were carried out on the CHI660D Electrochemical workstation (CHI Instrument Company, Shanghai, China). A traditional three-electrode system consisting of an

Ag/AgCl as a reference electrode, a platinum wire as an auxiliary electrode, and modified GCE as a working electrode (diameter 3 mm) were employed.

All reagents were analytical grade and were used without any further purification. L-THP and DNA were purchased from Aladdin Chemistry Co. Ltd. Stock solution of L-THP was prepared with sulfuric acid (0.1M) and stored at 4 ^{o}C darkly.

2.2 Preparation of DNA/GCE

Prior to modification, a bare GCE was first mechanically polished to a mirror-like surface stepwise using finer emery-paper and 0.3 μm alumina slurry, and was then washed ultrasonically with 1:1 nitric acid, alcohol and double-distilled water successively. The DNA modified GCE (DNA/GCE) was established by amperometric i-t curve through which indeed the DNA could be oxidized at the high potential such as 10V. In addition, two electrodes system was employed, the reference electrode and the auxiliary electrode were combined as the auxiliary electrode. The modified electrode was used directly in electroanalysis of L-THP after washed by double-distilled water.

2.3 Analytical procedure

Unless otherwise statement, 0.1 mol L^{-1} PBS buffer solution was used as blank supporting electrolyte for determination of L-THP. All potential scans were performed at between 0.2 and 1.2 V and carried out at ambient temperature.

3. RESULTS AND DISCUSSION

3.1 Electrochemical characterization of DNA/GCE

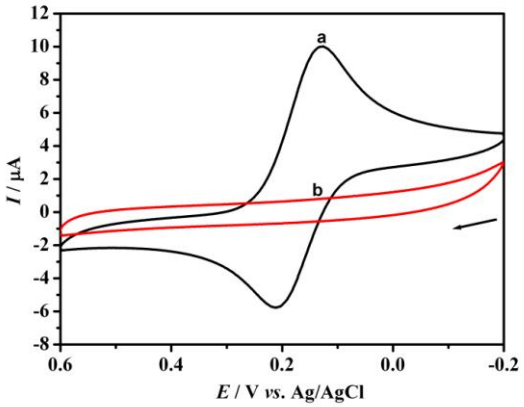

Figure 1. Cyclic voltammograms of 1×10^{-3}mol·L^{-1} $Fe(CN)_6^{3-}$ on different electrodes. a bare GCE; b DNA/GCE. Scan rate 100 mV·s^{-1}.

Cyclic voltammetry (CV) using $[Fe(CN)_6]^{3-}$ as the electrochemical probe which is an effective tool to characterize the interface properties of different film-modified electrodes. This exploration was performed in a 1.0×10^{-3}mol $L^{-1}[Fe(CN)_6]^{3-}$ (containing 0.1 mol·L^{-1} KCl) solution by using the GCE and DNA/GCE respectively; the corresponding voltammograms are displayed in Fig.1 At the GCE, a typical reversible redox voltammogram was obtained with i_{pa} = 14.06 μA, i_{pc}= 14.43 μA and a peak-to-peak separation of 86 mV. However, no obvious redox signals could be found at the DNA/GCE (Fig. 1b), which indicated that the negatively charged DNA molecules were successful modified on the GCE surface and repel the same negatively charged $[Fe(CN)_6]^{3-}$ to the DNA/GCE surface.

3.2 Electrochemical behavior of L-THP on the DNA/GCE electrode

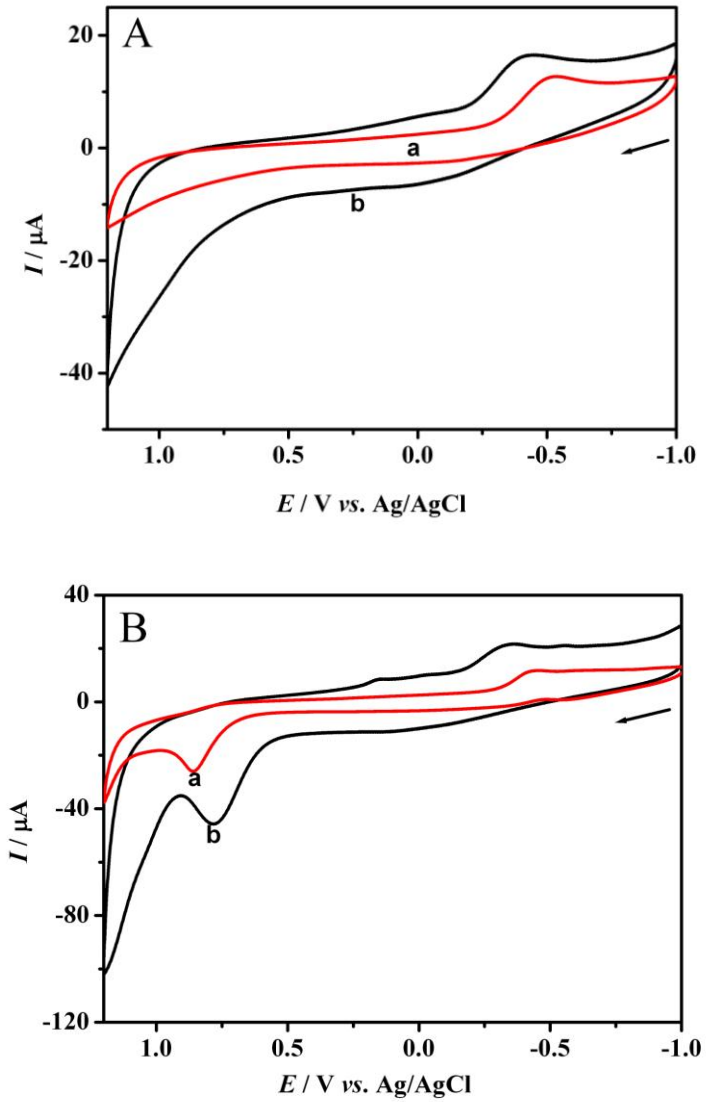

Figure 2. Cyclic voltammograms. (a) GCE, (b) DNA/GCE in (A) 0.1 mol·L^{-1} PBS and (B)5.0×10^{-4} mol·L^{-1} L-THP and 0.1 mol·L^{-1} PBS. Scan rate, 100 mV·s^{-1}.

The electrochemical response of L-THP (5.0×10^{-4} mol·L^{-1}) was investigated at different electrodes, and compared in Fig. 2. There was a reduction peak observed in a blank solution (0.1 mol·L^{-1} PBS) at both GCE and DNA/GCE, which was suggested as the reduction of H^+. Except this, there was no redox peak observed in the blank solution which suggested that DNA was non-electro active within the potential window. In the presence of L-THP, it could be seen that the electrochemical processes of L-THP at different electrodes were all irreversible, due to no reduction peak in the reverse scan. Although well-defined anodic peaks could be seen at both electrodes at 0.78 V in the presence of L-THP, the peak current of L-THP at the DNA/GCE increased comparing with at the GCE. It indicated that DNA and L-THP had a strong interaction and formed a more electroactive L-THP-DNA complex, which resulted in the increase of peak current. Thus, DNA/GCE had a more sensitive response for L-THP (Fig.2).

3.3 Effect of scan rate

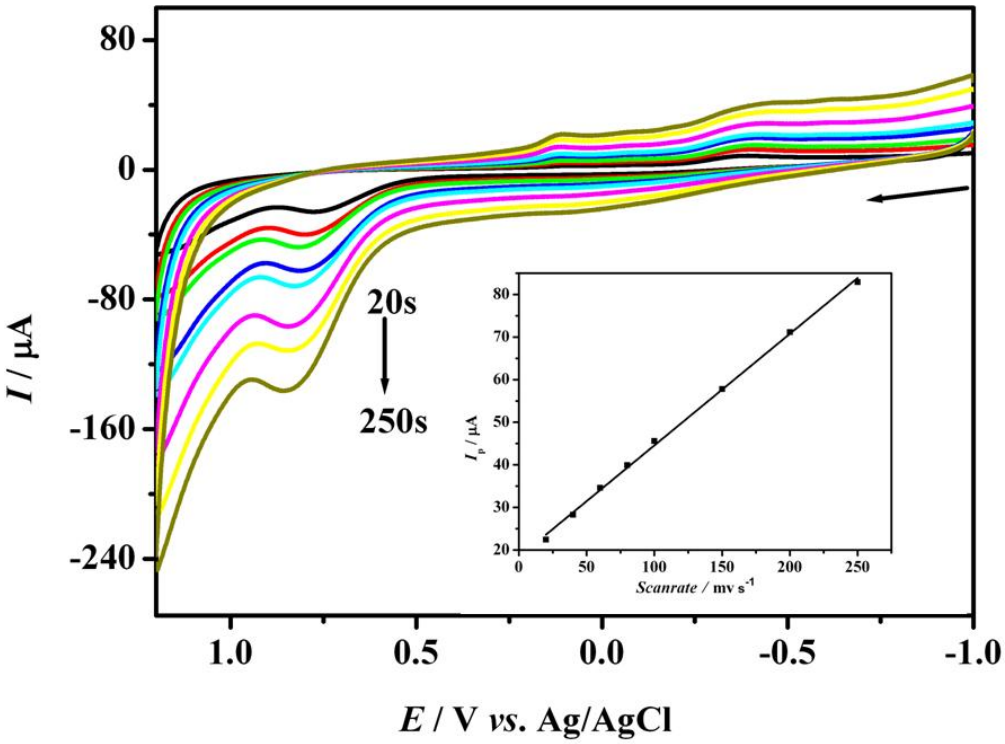

Figure 3. CV curves of 5.0×10^{-4} mol·L^{-1} L-THP with different v. Scan rates (from inner to outer): 20, 40, 60, 80, 100, 150, 200 and 250 mV/s. Supporting electrolyte, 0.1 mol·L^{-1}PBS; Scan rate, 100 mV·s^{-1}.

The scan rate is the most important experimental parameter for evaluating the character of electrode reaction [21]. The influence of scan rate (v) on redox peak currents (i_p) was researched by CV in the range from 20 to 250 mV/s. As shown in Fig. 3, with the scan rates increasing, the i_p increased. Further, the E_{pa} shifted to a positive direction. Extracting the data from Fig. 3, the anodic

peak currents (i_{pa}) was proportional to the scan rates, with the linear correlation equations and coefficients of i_{pa} (μA)=18.388+0.2618 v(R=0.998). These data demonstrated that the electro-reaction of L-THP was an adsorption-controlled quasi-reversible process.

3.4 Effect of solution pH

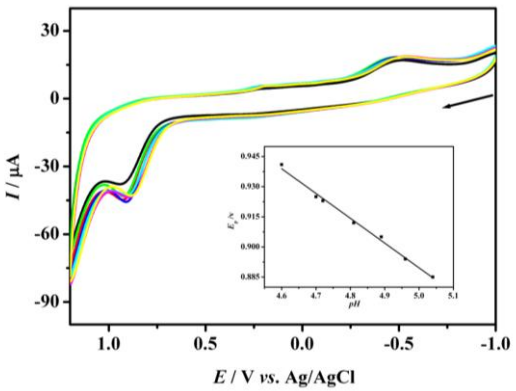

Figure 4. CV curves of 5.0×10^{-4} mol·L^{-1} L-THP with different pH (4.60, 4.70, 4.72, 4.81, 4.89, 4.96, 5.04). The inset shows the relationship of E_p-pH. Scan rate, 100 mV·s^{-1}.

The peak currents of L-THP (5.0×10^{-5} mol·L^{-1}) were not sensitively affected by the pH of PBS buffer solution. The investigation was examined in the pH range of 4.60-5.04. As shown in Fig. 4, the CV peak currents of L-THP changed slightly with the increase of solution pH. However, extracting the data from Fig. 4, the relationship between peak potentials and solution pH were described as E_{pa}= 1.5066-0.12342 pH (R=0.993), which indicated that the peak potentials of L-THP were sensitively affected by the pH of PBS buffer solution.

3.5 Optimization of the parameter for Amperometric i-t curve

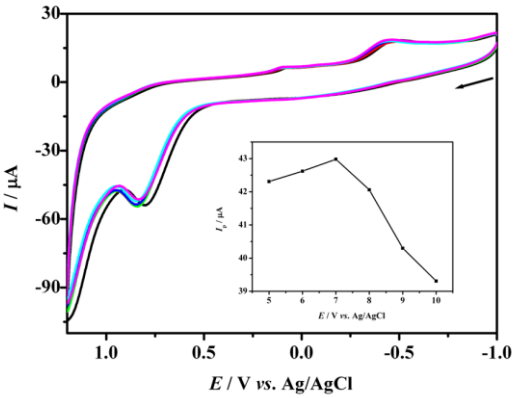

Figure 5. CV curves of 5.0×10^{-4} mol·L^{-1} L-THP with different potential (5V, 6V, 7V, 8V, 9V, 10V) in the method of amperometric i-t curve. The inset shows the relationship of I_p-E. Scan rate, 100 mV·s^{-1}.

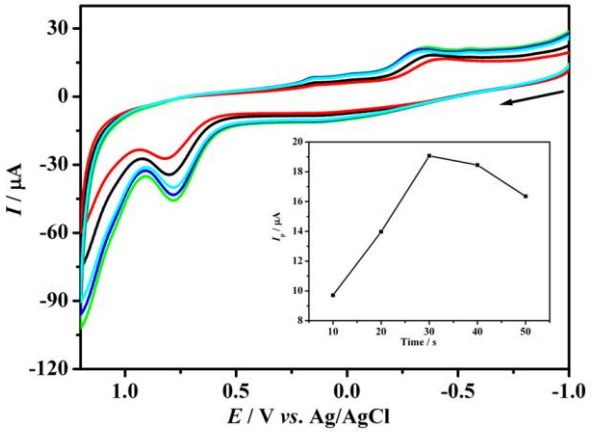

Figure 6. CV curves of 5.0×10^{-4} mol·L^{-1} L-THP with different run time (10s, 20s, 30s, 40s, 50s) in the method of amperometric i-t curve. The inset shows the relationship of I_p-Time. Scan rate, 100 mV·s^{-1}.

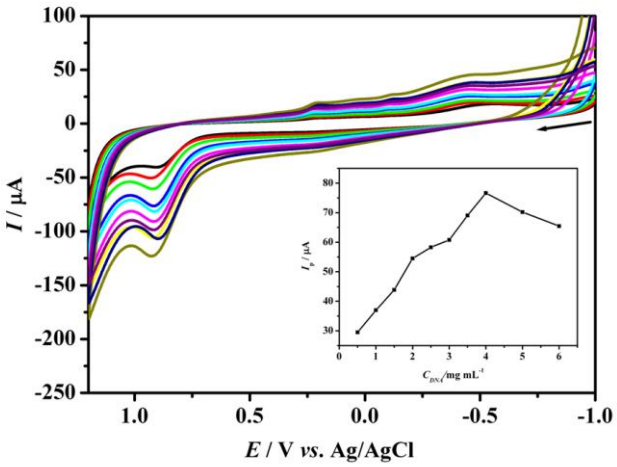

Figure 7. CV curves of 5.0×10^{-4} mol·L^{-1} L-THP with different concentration of DNA (0.5 mg·mL^{-1}, 1.0 mg·mL^{-1}, 1.5 mg·mL^{-1}, 2.0 mg·mL^{-1}, 2.5 mg·mL^{-1}, 3.0 mg·mL^{-1}, 3.5 mg·mL^{-1}, 4.0 mg·mL^{-1}, 5.0 mg·mL^{-1}, 6.0 mg·mL^{-1}) in the method of amperometric i-t curve. The inset shows the relationship of I_p-C_{DNA}. Scan rate, 100 mV·s^{-1}.

To optimize the experimental conditions for Amperometric i-t curve, in the present study, there were three conditions being investigated. Firstly, we discussed the influence of potential and the run time for Amperometric i-t curve. The result was presented in Fig. 5 and Fig. 6, which were respectively got through the discussing of six different potential value from 5V to 10V and different run time from 10s to 50s .Changing the potential in the method of amperometric i-t curve brought little range ability to the curve. But the relationship of I_p-E show us that the biggest I_p was reached at the potential of 7V, and based on that 7V was selected as the optimum potential. In the similar way, 30s was chose as the better one.

And then the concentration of DNA was investigated using CV. As shown in Fig. 7, the line presented an obvious rising trend could be seen before the concentration of 4.0 mg·mL^{-1}, while it began come down from then on. Therefore we chose the concentration of 4.0 mg·mL^{-1} as the optimal one for DNA through our research.

3.6 The relationship between peak currents and L-THP concentrations

Series concentrations of standard solutions of L-THP were detected under the optimized working conditions described above. Fig. 8 showed that the peak currents increased linearly with increasing concentration of L-THP. The inserted calibration plot in Fig. 8 highlights a linear relationship between peak currents and L-THP concentrations with a regression coefficient of 0.999. The peak currents responded were linearly relationship with L-THP concentrations in the range of 5.6×10^{-5} to 2.8×10^{-3} mol·L^{-1}. The linear equation was I_p (μA)=0.57334+ 1.48001c (mM) (R=0.999).

Based on the signal-to-noise ratio of 3 (S/N), the detection limit was obtained as 2.4×10^{-6} mol·L^{-1}. These values confirmed the sensitivity of the proposed method for the determination of L-THP. To estimate the repeatability of the proposed method, the RSD of five times successful measurement of peak current of 1×10^{-4} mol·L^{-1} L-THP on DNA/GCE was calculated to be 3.9%, which demonstrates the good repeatability of the method.

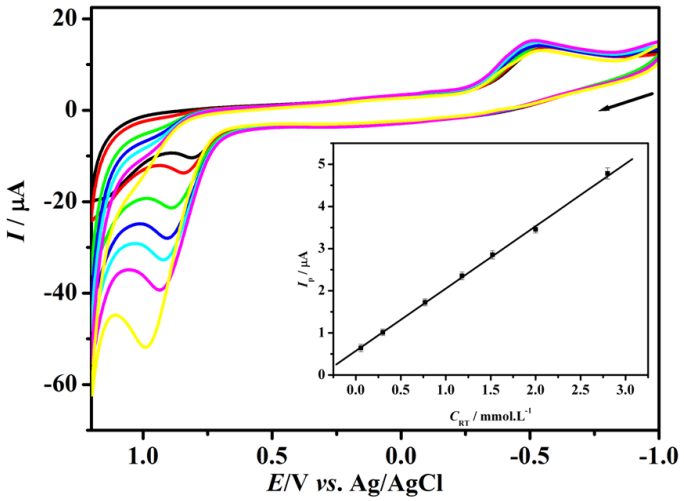

Figure 8. CVs and their associated calibration plot for increasing concentrations of L-THP at DNA/GCE electrode under optimal conditions; L-THP concentration from 5.6×10^{-5} mol·L^{-1} to 2.8×10^{-3} mol·L^{-1}.

3.7 Determination of glucose in real samples

In order to evaluate the applicability of the proposed method to the determination of L-THP in commercial samples, the utility of the developed method was tested by determining L-THP in tablets samples. The results are summarized in Table 1. The good recoveries of the mixture samples indicate the successful applicability of the proposed method to determination of L-THP.

Table 1. Detection results of L-THP in tablets samples.

NO.	L-THP Spiked (mM)	L-THP Found (mM)	Recovery (%)
1	-	0.42	-
2	0.5	0.94	104
3	1.0	1.39	97
4	2.0	2.34	96

4. CONCLUSIONS

In the present work, a new electrochemical sensor for the detection of L-THP based on a new method, Amperometric i-t curve, was designed. By using this oxidized DNA modified electrode, trace amounts of L-THP could be detected with LOD of 2.4×10^{-6} mol·L^{-1} by cyclic voltammetry. These researches suggest also that oxidized DNA might be a very promising material for analytical sensing.

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