Extraction of the Active Constituents of Maca by O-PD/β-CD/Ag-MWNTs Modified Electrode

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Abstract. The electrochemical behavior of Maca adenine on the modified electrode of o-phenylenediamine/β-cyclodextrin/Ag-carbon nanotube was studied by cyclic voltammetry. The results show that the electrode modified by 7mg of carbon nanotubes is the best when the concentration of silver ions is 0.1mg/ml. The electrochemical behavior of adenine was investigated by the modified electrode, and it is found that in the PBS buffer of pH= 7, the electrochemical response of adenine on the modified electrode is good and the oxidation peak is obvious. The oxidation peak current showed a good linear relationship with adenine in the range of 0.2 mg/ml to 1.0 mg/ml, and the determination method of adenine content in maca was established. The method is sensitive, simple and reproducible and can be used to extract the content of adenine in maca accurately.

Introduction

Maca belongs to the cruciferous Lepi-d ium meyenii Walp. It is an annual herb with the upper part spread flat on the ground and the radish tubers underground, about 10 to 14 cm long and 3 to 5 cm wide. [1]. Maca is a very unique plateau crop with rich nutrients. The methods are commonly used in the determination of adenine include chromatography, spectrophotometry, mass spectrometry, etc. [2-3]. However, these methods cannot meet the testing requirements because of the high price of equipment, complicated operation, long time consuming, or low precision. Electrochemical methods for the detection of substances have the advantages of simple operation, high sensitivity, good selectivity and low cost [4]. Therefore it can provide a new way for the determination of Maca adenine. In order to establish an electrochemical method for determining the adenine content in the maca, the experimental condition was established, which could be used to determine the contents of adenosine in maca, and to provide an important reference for the quality detection and development.

Materials and Methods

Materials and Reagents

Multi-walled carbon nanotubes (MWNT), Chengdu Institute of Organic Chemistry ; β-cyclodextrin (β-CD), o-phenylenediamine (o-PD),Tianjin Kemiou Chemical Reagent Development Center; Adenine, Sinopharm Chemical Reagent Company; phosphate buffer solution (PBS). All reagents were of analytical grade; the experimental water was redistilled water.

Instruments

CS300 electrochemical workstation, Wuhan QUASAR Instruments; QT2060 ultrasonic cleaning, Tianjin Electronics Co. REPro; Centrifugation machine 80-1 type, Shanghai Machinery Plant surgery.

Method

Preparation of Solution

(1) Buffer Solution. The 35.8g Na_2HPO_4 and 15.6g NaH_2PO_4 were dissolved in 1L water, and the 620ml Na_2HPO_4 solution was mixed with the NaH_2PO_4 solution of 380ml. Using NaOH to adjust the pH value of mixed solution to pH=7.0, and get PBS buffer. The concentration of buffer is 0.1mol/l, and the pH value of buffer is 7.0. PH value is determined by Sartorius digital acidity meter. Transfering the buffer to the capacity bottle and refrigerate it.

(2) Reference Standard Solution. Accurately weigh 1 mg adenine standard in a beaker, prepare a 0.01 mg/ml adenine standard solution in PBS with pH=7.0, transfer to a volumetric flask and dilute to 50 ml, and store for refrigeration.

(3) Sample Solution. Take the maca native plant samples, crush and sieve (40 mesh) to obtain maca powder. 5g maca powder was extracted with 150ml acetone under 90 °C condition by soxhlet extraction, and the 30ml extract was obtained. Concentrate the solution to about 10ml by using a rotary evaporator, pass through 0.45um microporous membrane, and prepare 50ml with a PBS buffer solution of pH = 7. Refrigerate the solution for backup.

Activation of Glassy Carbon Electrode.

Grinding GCE on the abrasive paper for metallograph (3000 #), then polishing on chamois with α-alumina powder of 1.0, 0.3 and 0.05um respectively, finally ultrasonic cleaning 3min with anhydrous ethanol and water respectively. The treated GCE were placed in a H_2SO_4 solution with 0.5mol/l concentration, and scan the -0.2 - +0.6v potential interval until stable with the cyclic voltammetry (CV), and the potential change rate was 100mv/s. [5]. After the electrode is taken out and rinsed with water, it is kept in water and set aside. [6-7].

Preparation of o-phenylenediamine/ß-cyclodextrin/Ag-Carbon Nanotube Modified Electrode.

Placing the treated GCE in 10ml iron potassium Cyanide solution (5mmol/L) and scan the -0.2 - +0.6v potential interval until stable with the cyclic voltammetry (CV), and the potential change rate was 100mV/s. The electrode was taken out and rinsed with water and dried under an infrared lamp. Then, the GCE was used as a base electrode, and stewing in 10ml of o-phenylenediamine (1.00 mol/l) and 10 ml of β - cyclodextrin (5 mmol/l). In the above conditions, the CV scans 20 times to the CV curve to stabilize, then takes out the electrode and dries under the infrared lamp. The MWNTs were carboxylated by V (HNO_3): V (HCl) =1:3, circulation reflux for 12 h, washed with NaOH (0.01 mol/L) until the pH was near neutral, and dried after centrifugation. 5.0mg of pretreated MWNTs was added to 5.0ml DMF (N, N-dimethylformamide) solution in the ultrasonic dispersion 30min, and finally obtained stable black dispersion solution. [8]. Weighing 7mg of MWNTs into 10ml of $AgNO_3$ solution (0.1mg / ml) mixed, the modified GCE into this mixed solution, ultrasonic dispersion 5 ~ 10min, in the above conditions scanning 10 laps, and then take out the electrode, drying under the infrared lamp, that is, o-PD /β-CD/Ag-MWNTs/GCE, stored in the buffer solution [9].

Electrochemical Test Method.

Electrochemical testing uses a three-electrode system. The working electrode is an activated glassy carbon electrode, the reference electrode is an Ag/AgCl electrode, and the auxiliary electrode is a platinum wire electrode. Setting of relevant parameters for cyclic voltammetry (CV): the potential change rate was 100mv/s.

Results and Analysis

Characterization of Electropolymerization Membranes by Cyclic Voltammetry.

In turn, the bare glassy carbon electrodes, o-PD/β-CD/GCE, o-PD/β-CD/Ag-MWNTs/GCE were scanned in the 5mmol/l concentration K_3 [Fe (CN) $_6$] solution using CV method (potential range -0.2 - +0.6v, potential change rate 100mv/s). The oxidation peak of the K_3 [Fe (CN) $_6$] probe molecule on the bare glassy carbon electrode is good symmetry with the reduction peak, and has good reversibility.

The oxidation peak for o-PD/β-CD/GCE is positively shifted, the peak shape is broadened, and the peak current is significantly reduced. It is possible that o-phenylenediamine, which has a crosslinking effect on the surface of the modified electrode, is firmly bonded to β-CD [10], which has a special cavity structure, and forms a film with a certain coverage on the surface of the electrode and has weak conductivity [11], thus impeding the electron transfer of K_3 [Fe (CN) $_6$] on the surface of the electrode. For the o-PD/β-CD/Ag-MWNTs/GCE, the redox peak current is much larger than the above two electrodes, which may be due to the excellent conductivity of silver ions, which facilitates the electron's transfer speed at the electrode surface. Carbon nanotubes have large specific surface area and many active sites, which can improve the sensitivity of electrode detection [12]. The results show that the electrode has a good modification effect. When o-phenylenediamine, β-cyclodextrin and Ag-MWNTs are modified together on the surface of glassy carbon electrode, it can not only prevent interference and prolong the service life, but also transfer electrons efficiently.

Electrochemical Response of Adenine for O-PD/ β-CD/Ag-MWNTs/GCE.

Adenine standard solution with 0.01 mg/ml concentration was scanned by cyclic voltammetry in the range of -0.2 - +0.6V potential at a potential change rate of 100mV/s with different modified electrodes. As can be seen, the bare glassy carbon electrode has almost no response to adenine; Adenine is slightly more responsive to electrical signals for o-PD/β-CD/GCE than bare glassy carbon electrodes, suggesting that these modifiers have some catalytic enrichment effect on adenine, possibly due to cross-linking agent o-PD and β-CD bonding with each other, making the modified electrode relatively stronger for adenine adsorption. On o-PD/β-CD/Ag-MWNTs/GCE, the peak current increased significantly. Comparing with the two modified electrodes, the modified electrode has the strongest electrochemical response to adenine, probably because the silver ion has good conductivity; MWNTs have multiple active sites, large specific surface area, so the electrode has more obvious catalytic activity. The results show that the oxidation of adenine is easier on o-PD/β-CD/Ag-MWNTs modified electrodes due to the interaction of the modified layers. The electrical oxidation of adenine on the o-PD/β-CD/Ag-MWNTs modified electrode is an irreversible process. In the structure of adenine , there are carbon-carbon double bonds between carbon atoms and nitrogen atoms at the C-8 and C-2 positions, and the π-electron cloud will transfer to the nitrogen atom, so that the electron cloud density on the carbon atoms decreases; And in the adjacent position of the carbon atom, there is also a relatively strong electronic attraction of nitrogen atoms, the interaction of them lead to C-H bond polarity enhancement, C-H bond easily disconnected, hydrogen atoms off, so it can be speculated that this step reaction is involved in two electron oxidation process.

Optimization of Experimental Conditions

Selection of Carbon Nanotube Dosage.

The 4, 7, 10, 13 and 16mg of carbon nanotubes were prepared for o-PD/β-CD/Ag-MWNTs/GCE, and the contrast experiments were carried out in adenine standard liquid in the same conditions. Results as shown in Fig. 1, when the carbon nanotubes is 7mg, the electrochemical response is the strongest, and the peak current is the highest, and the peak type is the best. Therefore the 7mg carbon nanotube modified electrode is selected.

Selection of Concentration of Silver Nitrate Modification Solution.

The o-PD/β-CD/ Ag-MWNTs/GCE was prepared by the solution of silver nitrate concentration of 0.05, 0.1, 0.15, 0.2 and 0.25mg/ml, and the contrast experiments were carried out in adenine standard liquid in the same conditions. Results as shown in Fig. 2, when the concentration of silver nitrate is 0.1 mg / ml, the peak current is the highest and the peak type is the best, so 0.1mg / ml $AgNO_3$ modified electrode is selected.

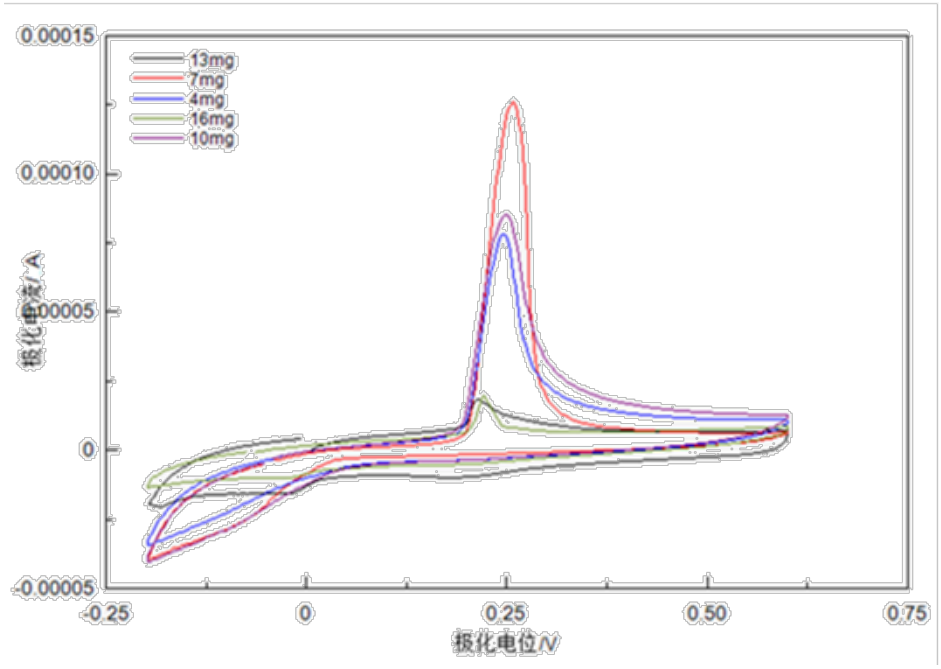

Fig. 1 Cyclic Voltammograms of Adenine in Modified Electrodes with Different Dosage of Carbon Nanotubes

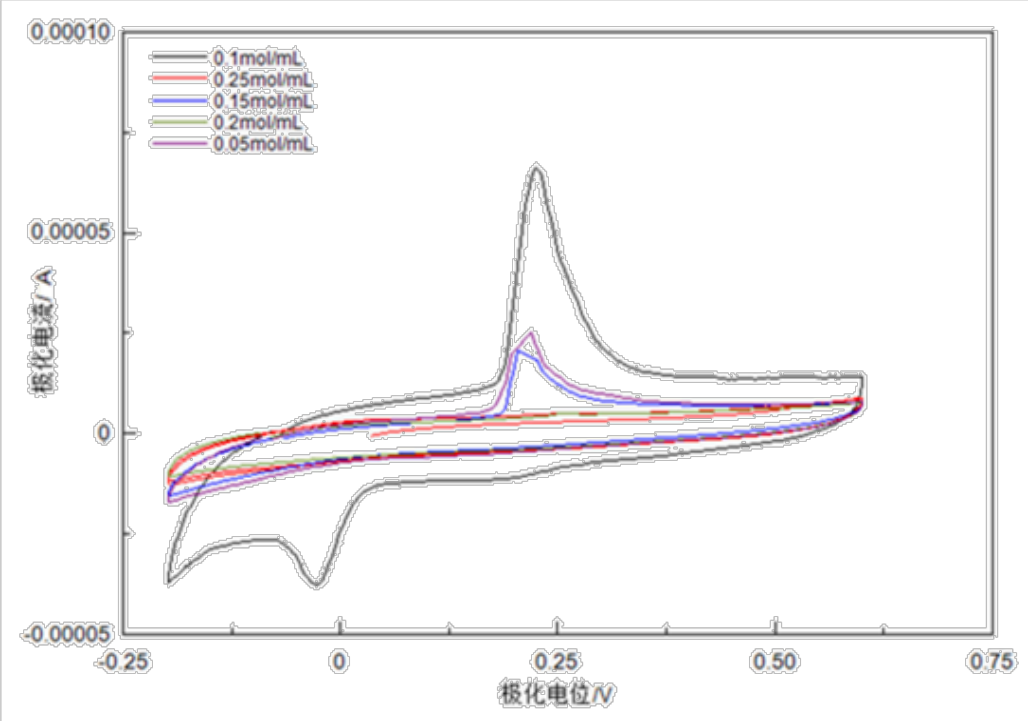

Fig. 2 Cyclic Voltammograms of Adenine on Modified Electrodes with Different Concentrations of Silver Nitrat

Impact of Scan Rate.

In the 10~150mV/s scanning rate range, the scanning speed is changed and the peak current is 100mV/s, so the 100mV/s scanning speed is selected in this experiment.

Selection of the Base Fluid.

According to the electrochemical method as described above, the adenine was compared experimentally in three kinds of TISAB, sodium acetate and PBS respectively. The redox peak appeared in the sodium acetate solution and the peak current was extremely high. It can be concluded

that the sodium acetate solution interfered with the detection of adenine; the peak current in TISAB is larger than that in PBS buffer solution, so it is considered that PBS buffer solution is more suitable for the determination of adenine in this experiment.

Influence of pH Value of Base Solution on Polarization Current.

The pH value of the bottom liquid was changed in the range of 2.0 - 11.0, and the peak current of 1.0mg/ml concentration by o-PD/β-CD/Ag-MWNTs/GCE was determined by the electrochemical method, and the peak current of adenine increased with the increase of pH in the range of 2.0 - 5.0. The peak current decreases with the increase of pH value in the range of 5.0 - 7.0. And it increases with the increase of pH value in the range of 7.0 - 11.0. Therefore, PBS with pH=7.0 was chosen as the best base solution.

Reproducibility and Stability.

Using o-PD/β-CD/Ag-MWNTs/GCE for 8 time's consecutive measurements of adenine standard solution with 0.01 mg / ml concentration, the relative standard deviation (RSD) of the results was 0.2%, indicating good reproducibility of the modified electrode. The o-PD/β-CD/Ag-MWNTs/GCE is kept for 18 days under the condition of room temperature and air circulation, and the response current of adenine standard solution is basically unchanged, which shows that the electrode has good stability.

Linear Range and Detection Limit.

The relationship between the oxidation peak current of adenine and its concentration was studied by cyclic voltammetry under optimal test conditions. It is found that there is a good linear relationship between the oxidation peak current of adenine and its concentration in 0.2 - 1.0 mg / ml. For the linear relationship, the linear equation is: $y = -0.0558x + 0.2935$ (I: mA, c: mg/ml , $R_2 = 0.9419$) , and the minimum detection limit is 0.06 mg/ml.

Recovery Rate Experiment.

Take samples of the original plants of the maca, crush, sieve (40 meshes), and get the maca powder. The 5g of maca powder was extracted with 150ml acetone under 90 ° C conditions, and the 30ml extract was obtained. The solution is condensed by the rotary evaporation instrument to about 10ml, sieving 0.45um microporous filter membrane, and pH=7 the PBS buffer into 50ml solution, shake evenly, then make the sample solution to be tested. Take appropriate amount of sample to test, and scan it by cyclic voltammetry under optimal experimental conditions to obtain peak height and current value, and then obtain the concentration index of adenine in Maca according to the standard curve, and the linear equation $y = -0.0558x + 0.2935$.The calculated adenine content in 1g maca is 0.037mg. Three samples of the maca sample solution were taken from the whole samples to test, and the modified electrode was used for the detection, and then it was added to 0.01 mg / ml concentration of standard solution of adenine recovery experiment, the recovery rates are 104.1%, 100.0%, 102.9%. The results show that the modified electrode has good accuracy and precision for the determination of adenine.

Conclusion

The paper studied the electrochemical behavior of adenine on o-PD/β-CD/Ag-MWNTs/GCE. Since β-cyclodextrin has a strong cavity structure due to the cross-linking action of o-phenylenediamine , β-cyclodextrin has a special cavity structure, and silver ions fill it to form an envelope with it, forming an inert state under electrochemical oxidation. The layer enhances the anti-interference ability and durability of the electrode; Moreover, carbon nanotubes with large specific surface area and more active sites can improve the sensitivity of adenine detection, so that adenine on o-PD/β-CD/Ag-MWNTs/GCE showed good redox activity. At the same time, the results show that in pH=7 PBS buffer, the glassy carbon electrode modified by 7mg and silver ion concentration in 0.1mg/ml solution is used, and the -0.2 - +0.6v potential range, 100mv/s scanning speed, The cyclic

voltammetry can be used to establish a working curve for the determination of adenine content in MA, and the content of Maca adenine is successfully determined by this method. The method has the advantages of simple operation and high sensitivity, and the modified electrode has good reproducibility and stability, and has certain application value.

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