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The Electrochemical Redox Mechanism and Antioxidant Activity of Oleanolic Acid Based on Multi-walled Carbon Nanotuber Screen-printing Electrode

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This article emphasis on the application of the multi-walled carbon nanotubes screen printing electrodes(MWCNTs/SPEs) to reseach of plant active constituent (oleanolic acid, OA) by electrochemistry analytic procedure. The work explores the optimization of reaction conditions of OA. The stoichiometric ratio of OA on DPPH about 1:1, electrochemical redox mechanism and antioxidant activity of OA were obtained by the determination of electrochemical kinetics parameters such as electron transfer numbers (n), propons (m), electron charge coefficient (α), and standard electron transfer rate constant (k_s) are 1, 1, 0.66, 0.53 respectively based on electrochemical behavior at MWCNTs/SPEs. This method is fast, convenient, low-cost, practicable and can be used to the trace amount determination of the content of triterpenes in natural product.

Keywords: Screen-printed electrodes; Oleanolic acid; Electrochemical redox mechanism; Antioxidant activity;

1. INTRODUCTION

Oleanolic acid(OA, (3β)-3-Hydroxyoliean-12-en-28-oic acid) is one of hydroxyl pentacyclic triterpenoic acids. It is main isomeric triterpene compound in plants widely, such as white snake in the grass, western sichuan swertia, cloves and jujube, it's the main active ingredients of natural products. it is believed to have an antioxidative constituents[1,2] which is suggested to play an important role in

pharmacology with the function of anti-inflammatory[3], protect liver, hypoglycemic, antitumor activity[4], and can inhibit cancer cell proliferation, resistance to cancer cells and induce cancer cells differentiation, anti-platelet aggregation, and so on. OA has been used in the treatment of chronic hepatitis, liver cirrhosis, improve human immunity, etc.[6] in Clinically.

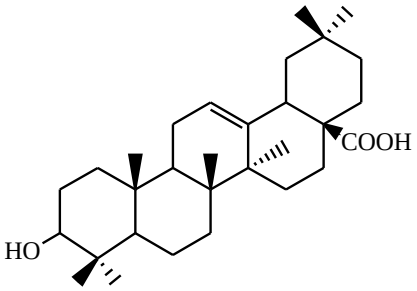


Figure 1. The structure of Oleanolic acid

There are various methods have been reported for antioxidant detection in different Oil samples, such as RP-HPLCPA[5], micellar electrokinetic capillary chromatography (MECC)[6], high-performance thin-layer chromatographic(HP-TLC)[7], thin layer chromatography(TLC)[8], and High pressure liquid chromatography(HPLC) combined with means of spectral analysis(IR, MS, 1H-NMR, 13C-NMR)[9], all with the features of rapid, precise, sensitive, repeatable, suitable, and economical, but high-cost, complexity, and the pretreatment of sample verbosely.

Electroanalytical techniques[10] are highly recognized as a favorable tool in the quantification of various natural antioxidants(NAO) and in natural organic compounds. Moreover, electrochemical instrumentation is compact and portable, allowing on-site analysis, obtaining results in only minutes without preliminary sample treatment. In addition, these methods have a sensitivity comparable to HPLC methods.

Multi-walled carbon nanotubes（MWCNTs）as a one-dimensional nanomaterials with special mechanical properties in the development of electrochemical devices, screen-printed electrodes (SPEs)[11], which are widely used as economical electrochemical substrates, because of their advantageous material properties, such as disposability, simplicity, and rapid responses, over the past 20 years, a great development in screen-printed sensors for several analytical applications have been observed, about the application of screen-printed technology in such as phloroglucinol derivative [12], tumor markers [13]. SPEs can combine ease of use and practicality with simple and inexpensive fabrication. These devices are ideally used for voltammetric measurements using portable electrochemical instrumentation[14,15], it can reduce size, ease of mass production, disposability, practicality and ability to be miniaturized to reduce consumption of sample and electrolyte[16,17]. However, multi-walled carbon nanotubes modified screen-printing electrode(MWCNTs/SPE), which can not only greatly enhance the sensitivity of the modified electrode, but also improve electronic transfer properties, and are described as useful electroanalytical tools[18].

The aim of this work was to use simple and fast electrochemical analysis method to revelate free radical scavenging mechanisms of natural antioxidant based on multi-walled carbon nanotubes screen-printed electrode was employed to improve sensitive, availability and reliability. In this paper, The electrochemical reaction mechanism of OA was investigated to determine stoichiometric ratio of OA and DPPH and the electrochemical free radical savenging mechanism of OA was obtained. On this base, the electrochemical redox mechanism and antioxidant activity of oleanolic acid.

2. EXPERIMENTAL SECTION

2.1 Instruments and reagents

MEC - 12 b type multifunction electrochemical analysis system (jiangsu jiang electric analysis instrument Co., Ltd.). A three-electrode system. pH–213 meter.

Oleanolic acid standard stock solution (purchased from xi 'an grass plant

technology Co., Ltd., 4 ℃ storage Standby); 0.1mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$ buffer solution (pH=4.0)**.** KCl solution for supporting electrolyte.

All the chemicals used were of analytical-reagent grade. Twice-distilled water was used throughout the experiments.

2.2 Preparation and modification of the electrode

Before modifying the working electrode at integrated SPEs, the SPEs were first washed with distilled water and dried by N_2 stream. Then the SPEs was pre-anodized in a 0.1M (pH7.4) PBS containing 0.1M KCl by applying an anodic potential of +1.9 V (vs. Ag/AgCl) for 120s. The MWCNTs/SPEs was prepared by coating 5μL 0.3mg/mL of the MWCNTs homogeneous suspension onto the SPEs and then dried at room temperature overnight. All modified electrodes were cleaned by cyclic voltammetric technique betwe -0.5V and +0.5V at a scan rate of 50mV·s^{-1} in PBS (pH7.4) until a stable cyclic voltammetric response was obtained, and then rinsed with water and dried under a nitrogen stream[19].

2.3 Experimental Methods

2.3.1. Electrochemical analysis

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed in the three-electrode cell in 0.10 mol·L^{-1} citric acid buffer solution (pH=4.0) between the potential range of -0.2 V and +0.5 V at a scan rate of 0.05 V·s^{-1}**.** The DPV conditions were: pulse width of 150 ms, pulse amplitude of 180 mV and pulse interval of 100 ms.

3. RESULTS AND DISCUSSION

3.1 Electrochemical behavior of OA on the screen-printing electrode

Fig.2 displays the CV curve of OA in the 0.1 mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$ buffer solution (pH4.0) at MWCNTs/SPE with scan rate is 0.08V·s^{-1} with the potential range from 0.0V to 1.0V as shows in Fig.2 .

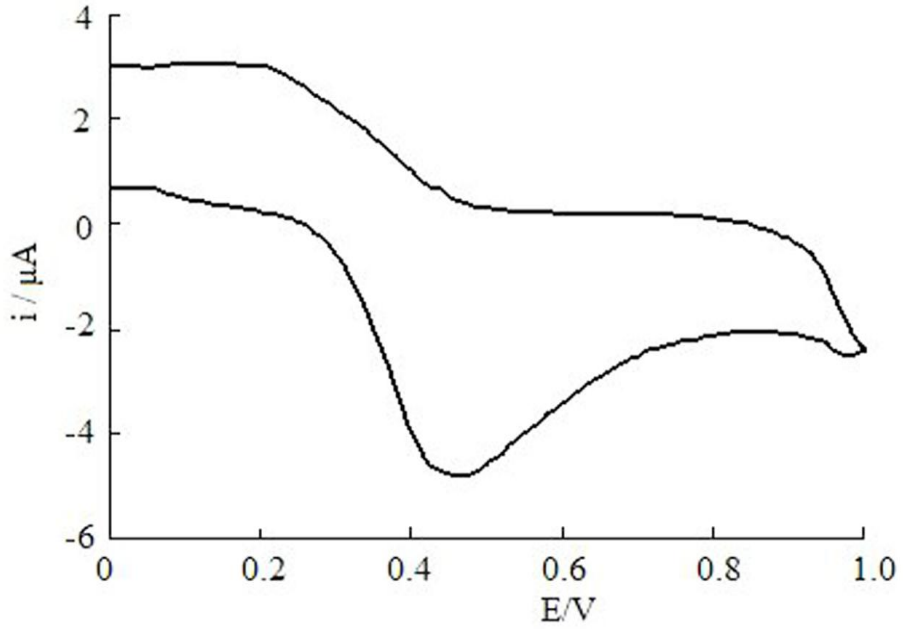


Figure 2 Electrochemical behavior of oleanolic acid at the MWCNTs/SPE
v: 0.08V·s-1, 0.1 mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$ pH4.0, E/V(0.0V to 1.0V)

Fig.2 shows the electrochemical behavior of oleanolic acid at the MWCNTs/SPE, the REDOX reaction of OA was happened on the electrode surface, there is a quasi reversible REDOX peaks at 0.45V(Epa) and 0.19V(Epc) (vs. SCE) respectively, the potential for $\Delta E = E_{pa} - E_{pc}$ =0.26 V(vs. SCE).

3.2 Influence of supporting electrolyte and pH

Several supporting electrolytes such as 0.10 mol·L^{-1} sodium hydrogen phosphate-potassium dihydrogen phosphate buffer, 0.10 mol·L^{-1} phosphate buffer solutions(PBS), 0.10 mol·L^{-1} citric acid buffer, 0.10 mol·L^{-1} acetic acid-sodium acetate buffer, were tested at MWCNTs/SPEs. A pair of CV redox peak is observed in the four supporting electrolyte, while, well-defined CV response with high redox peak of OA was obtained in 0.10 mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$buffer.

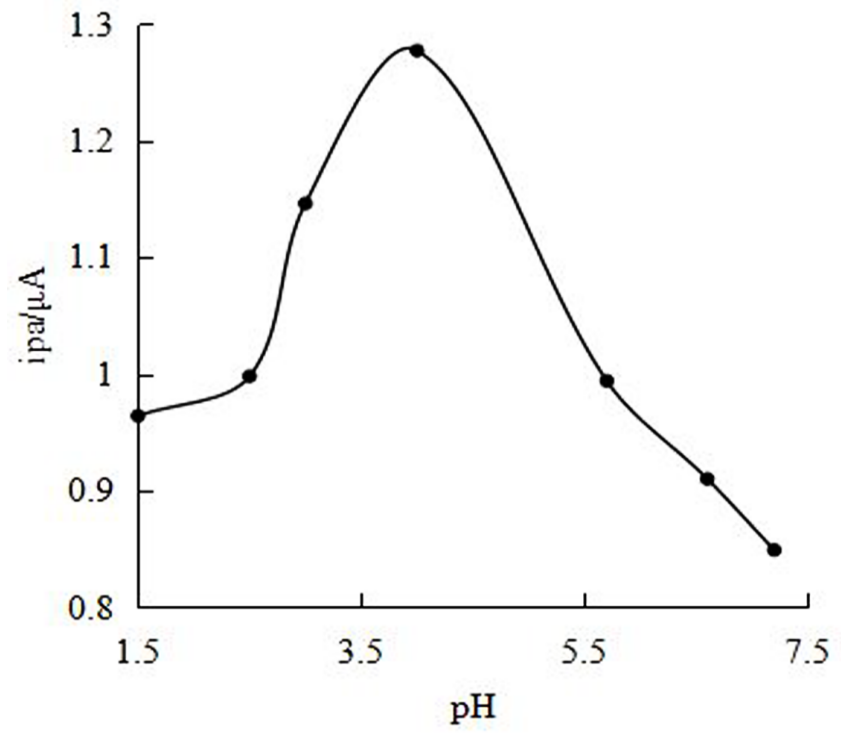


Figure 3a The influence of supporting electrolyte pH to peak current at MWCNTs/SPEs (v: 0.08V·s-1, 0.1mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O7$)

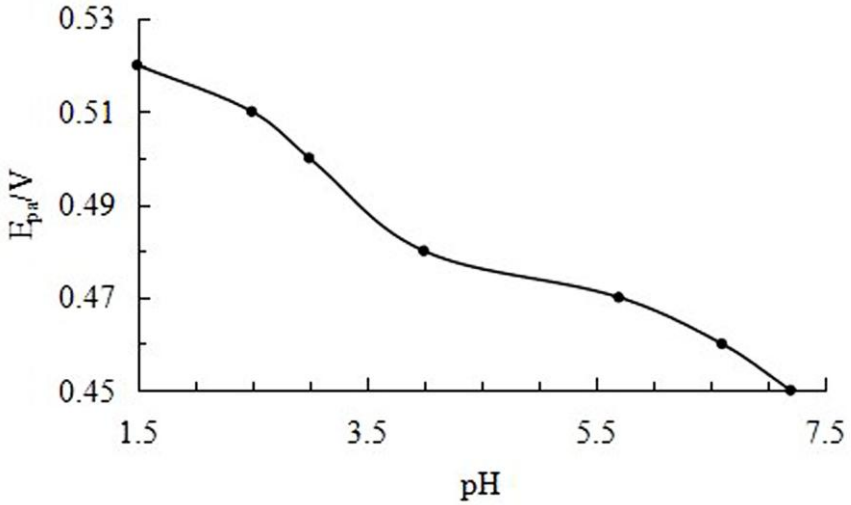


Figure 3b The influence of supporting electrolyte pH to peak current at MWCNTs/SPEs (0.08V·s-1, 0.1mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O7$)

Fig.3a-Fig.3b The influence of pH was investigated in 0.1mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O7$ buffer solution, the oxidation peak current of OA increases with the increasing of pH (from 1.5 to 7.2), and then reaches its maximum 1.277μA at pH4.0. And then, the oxidation peak current decreases as pH increasing above 4.0 shown in Fig.3a. While, the CV peak potential moves to the negative direction with the increase of pH as shown in Fig.3b. Therefore, pH4.0 was identified the optimal supporting electrolyte for subsequent experiments.

3.3 Electrochemical response of different concentrations of oleanolic acid at the MWCNT/SPE

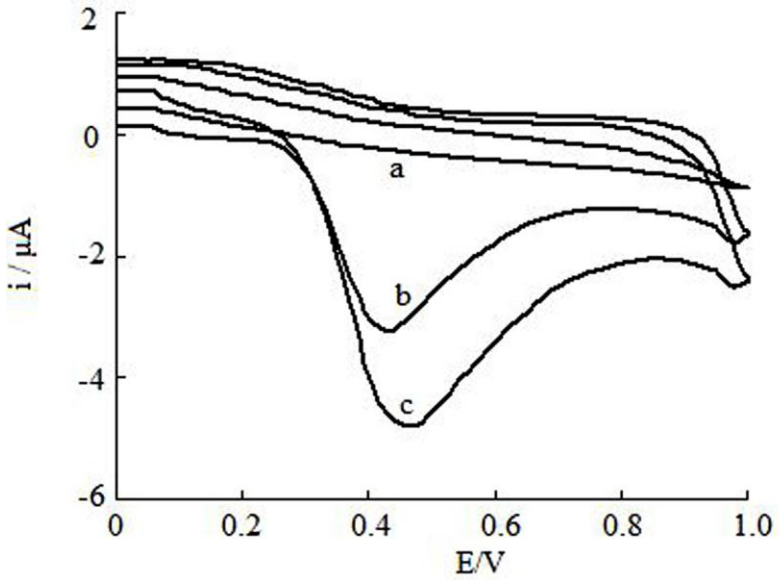


Figure 4. Electrochemical behaviors of different concentrations of OA at the MWCNT/SPE (0.1mol·L-1 C6H8O7-Na3C6H5O7 pH4.0, V: 0.08 V/s) (a) 0.0 mg·mL-1, (b) 2.0 mg/mL, (c) 4.0 mg·mL-1

The CV analysis was performed for the different concentrations of OA at MWCNT/SPE in 0.1mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$ buffer solution with concentrations of 0.0 mg·mL^{-1}(a), 2.0 mg/mL(b), 4.0 mg·mL^{-1}(c). The oxidation peak current of OA increases with the increase of concentration .

3.4 The effect of the scan rate to the electrode process

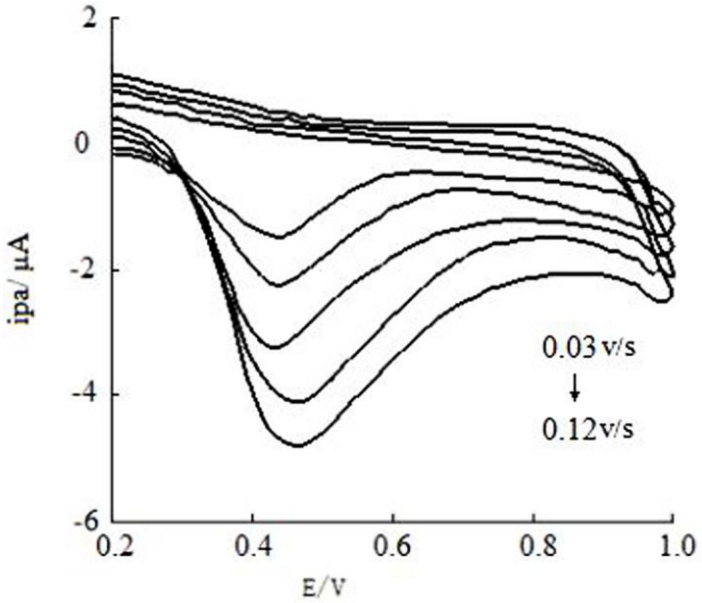


Figure 5. The cyclic voltammetry curves of OA with different scan rate at MWCNT/SPE in 0.1mol·L-1 C6H8O7-Na3C6H5O7 (pH4.0)

Fig.5 shows the effect of different scan rate on the CV response of OA at MWCNT/SPE in the 0.10 mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$ buffer solution(pH4.0). we obtained that the oxidation peak current is proportional with the scan rate. And the linear regression equation is: i_{pa} = 3.5243v+0.7337 (r=0.9969); It shows that the electrode reaction of OA at the MWCNT/SPE is controlled by the adsorption[20]. The maximum peak signal-to-noise ratio for OA was obtain at the scan rate 0.08V/s, the scan rate 0.08V/s was therefor selected for this work.

4. THE DETERMINATION OF KINETIC PARAMETERS

4.1 The determination of the electron transfer number (n)[21]

The electron transfer number is one of the key parameter in electrode reaction, the peak current and scan rate of OA show a good linear relationship in the graph, The results indicate that electrode process in the 0.1mol·L^{-1} $C_6H_8O_7$-$Na_3C_6H_5O_7$ buffer solution and MWCNT/SPE system is governed by the surface adsorption-controlled electrochemical process [22]. And the adsorption system meets the Langmuir isotherm of Laviron [23]:

$$i_p = \frac{n^2 F^2 A \Gamma_T \nu}{4RT} = \frac{nFQ\nu}{4RT} \qquad (2.1)$$

$$Q = n\ F\ A\ \Gamma_T \qquad (2.2)$$

In the formula, Q is the quantity of charge measured from the oxidation or reduction peak area of voltammogram, i_p represents the peak current(μA), F= 96485 C·mol^{-1} as the Faraday constant; R= 8.1345 J/K/mol, T= 298.15K, Γ_T is the surface coverage of the electroactive(mol/cm^{-2}); A(cm^2) represents surface area of electrode, which is about 0.095 cm^2. In this work, the electron transfer number (n) was calculated to be 1.14 at 0.08 V·s^{-1} of scan rate, and Γ_T=1.42×10^{-11} mol/cm^2 according from (2.2) to (2.2).

4.2 The determination of the proton number (m) of the electrode reaction

As known from this vestigation, according to the Nernst equation by the linear relationship of physical peak potential and pH as shown in Fig.6:

$$E = E^{\Theta} + \frac{RT}{nF}\ln\frac{[O][H^+]}{R} = E^{\Theta} + \frac{RT}{nF}\ln\frac{[O]}{R} + \frac{mRT}{nF}\ln[H^+] \qquad (2.3)$$

$$E = k - \frac{mRT}{nF}pH = k - \frac{m}{n}\times 0.059(25^{\circ}\mathrm{C}) \qquad (2.4)$$

The linear relationship of oxidation peak potential (E_{pa}) and pH was: E_{pa} (V)= -0.0479 pH +0.8228 (r=0.9910), the slope(k)= 0.059 m/n, the proton number (m=1) is obtained according to the electron transfer number (n).

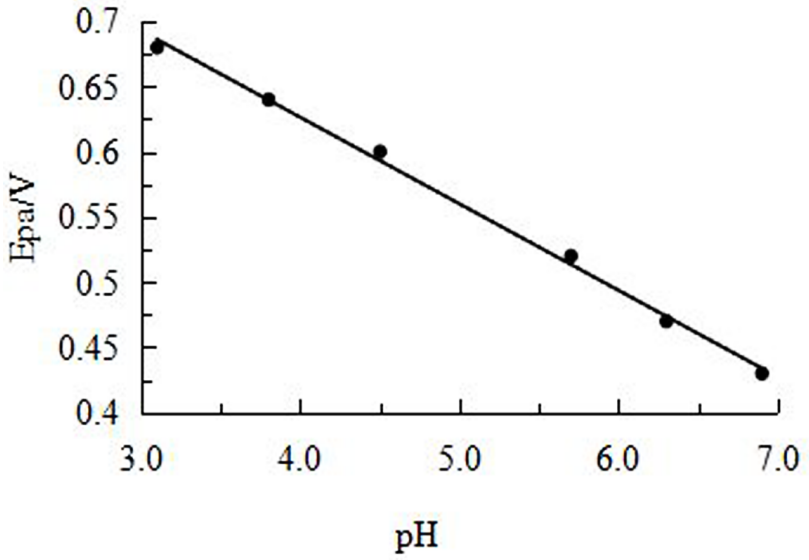


Figure 6. the linear relationship of Epa and pH

4.3 The determination of the electron transfer coefficient alpha (α)

The electron transfer coefficient alpha (*α)* was calculated according to the following equation:

$$E_{\mathrm{p}} = k + \frac{RT}{\alpha nF}\lg \nu \qquad (2.5)$$

According to the linear diagram of the lgv argument mapping with E_{pa} as shown in Fig.7, the slope(k)= *k*= 2.303 R T/(*α n* F), the electron transfer coefficient alpha (*α)* is acquired via the electron transfer number (n) .

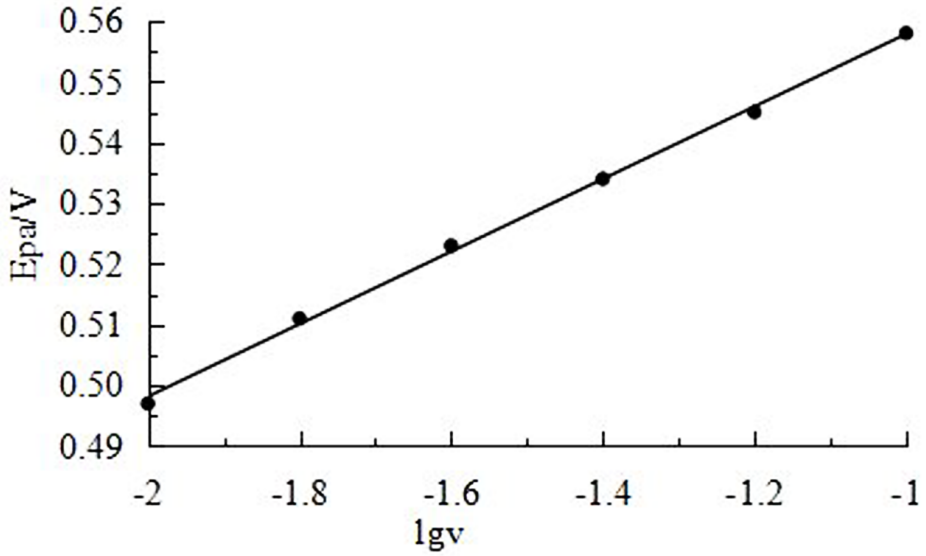


Figure 7. the linear relationship of Epa and lg *v*

4.4 The determination of the apparent constant of electron transfer rate（k_s）

In this work, the apparent constant of electron transfer rate（ k_s）is calculated to be 0.53 by following equation:

$$\lg k_s = \alpha \lg(1-\alpha) + (1-\alpha)\lg\alpha - \lg\frac{RT}{nF\nu} - (1-\alpha)\alpha\frac{nF\Delta E_p}{2.303RT} \qquad (2.6)$$

In conclusion, the determination of kinetic parameters of OA are calculated according to the correlation formulas as shown in table 1.

Table 1. The determination of the electron transfer coefficient alpha (α) and apparent constant of electron transfer rate（ k_s）

sample	the linear relationship	r	*n*	*m*	α	k_s
OA	y = 0.0497x + 0.6049	0.9922	1	1	0.66	0.53

Above all, one electron and proton electrochemical oxidation reaction of OA at the MWCNT/SPE could be obtained according to the above results, which is shown in Fig.8：

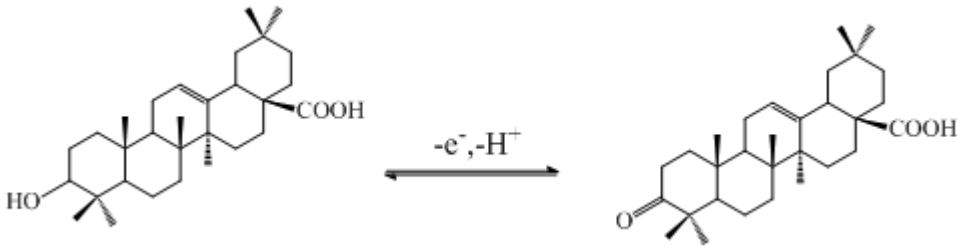


Figure 8. the oxidation mechanism of OA at

4.5 The electrochemical Redox Mechanism and antioxidant activity of OA

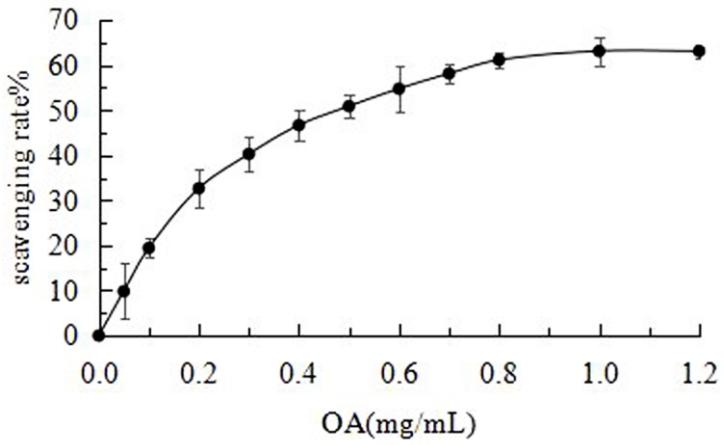
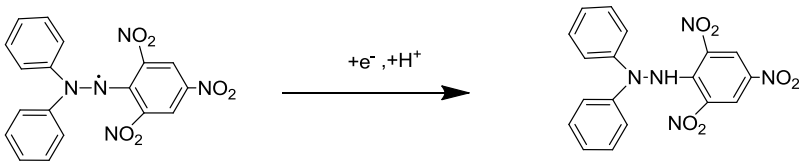


Figure 9. The scavenging rate of OA on DPPH (n=5)

The scavenging capacity of OA on DPPH was determined using spectrophotometric method [24]. And the scavenging rate of OA on DPPH was caculated according to the work[24].

Fig.9 shows the effect of concentrations of OA on the scavenging rate of DPPH. The scavenging rate of OA on DPPH increases with the increase of the concentrations of OA and tend to its max-scavenging rate(64.3%) as the concentration of OA above 1.0 mg/mL. And the half maximal inhibitory concentration(IC50)value of OA was caculated to be 0.55mg/mL(1.21mmol/L) for initial concentration(2.51mmol/L) of DPPH, which means that the stoichiometric ratio of OA on DPPH about 1:1. So, the reaction mechanism of OA and DPPH could be infered together with the facts that the stoichiometric ratio, mechanism of electrochemical redox and one electron and one proton transfered redox reaction mechanism of DPPH [26] as shown in Fig.10.



DPPH free radical $DPPH_2$

Figure 10. the reactive mechanism of DPPH

The 3-hydroxy of Oleanolic acid was oxidated to be ketone compound via DPPH free radical according to two equations as shown in Fig.8 and Fig.10. In conclusion, the electrochemical redox reaction of OA could be infered as shown in Fig.11. Furthermore, it indicated that oleanolic acid had a strong antioxidant activity, and it played a significant role in antioxidant activity field.

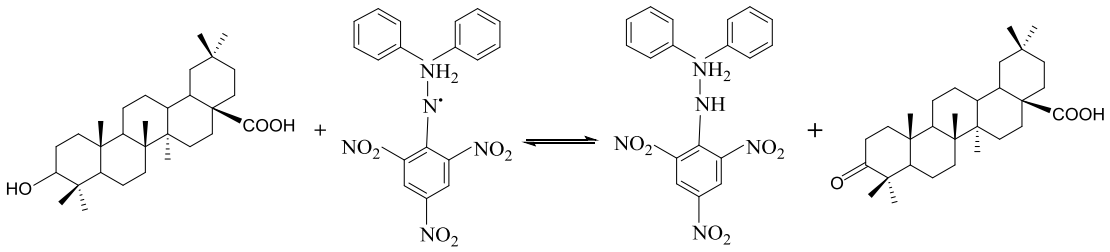


Figure 11. redox reaction equation of oleanolic acid at MWCNT/SPE

5. CONCLUSION

This work, we proposed a electrochemical method for the investigation of scavenging reaction mechanism of oleanolic acid on DPPH based on redox mechanisms of oleanolic acid and the determination of stoichiometric ratio of oleanolic acid and DPPH using multi-walled carbon nanotubes screen-printed electrode. One proton transfered redox reaction mechanism of oleanolic acid and DPPH was obtained using this method. Benifited from the sensitization of multi-walled carbon nanotubes and

the accessibility of screen-printed electrode, the proposed method is sensitive, fast and convenient to investigate antioxidant free radical scavenging mechanisms of natural antioxidant.

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