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Application of Single-Walled Carbon Nanotubes/Au Nanosol Modified Electrode for the Electrochemical Determination of Esculetin in *Cortex Fraxini*

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A novel simple, sensitive and selective electrochemical sensor was successfully prepared for the determination of esculetin in *Cortex Fraxini* based on the carboxylic acid-functionalized single-walled carbon nanotubes-Nafion–Au nanosol nanocomposite modified glassy carbon electrode (c-SWCNTs-NF–AuNs/GCE). Scanning electron microscopy, energy dispersive X-ray spectroscopy, electrochemical impedance spectroscopy and cyclic voltammetry were carried out to characterize the properties of c-SWCNTs-NF–AuNs nanocomposite. Owing to the synergistic effects of large surface area, superior electrical conductivity, and large amount of chemically active sites of c-SWCNTs, together with the good biocompatibility and high conductivity of AuNs, the c-SWCNTs-NF–AuNs/GCE exhibited a good electrocatalytic activity to esculetin with wide linear range of 0.004–55 μM and low detection limit of 0.12 nM. Additionally, the modified electrode was employed for analysis of esculetin in *Cortex Fraxini* with satisfactory results.

Keywords: Electrochemical determination; Differential pulse voltammetry; Single-walled carbon nanotube; Au nanosol; Esculetin;

1. INTRODUCTION

Cortex Fraxini, as one of the important traditional Chinese herbal medicine, has been clinically used for treatment of cough, diarrhea, asthma, dysentery, and some gynecopathy [1, 2]. Esculetin (6,7-dihydroxycoumarin, shown in Scheme 1), as the most important bioactive components in *Cortex*

fraxini, has multiple biological and pharmacological effects, including inhibition of the xanthine oxidizing activity and platelet aggregation, anti-oxidative, anti-inflammatory, anti-arrhythmic, anti-cancer, anti-tumor, and inhibitory effect on the growth of human breast cancer and leukemia cells [2-6]. Consequently, it is vital and significant to construct some simple, rapid, and effective approaches to quantify esculetin in plant medicines and pharmaceutical formulations.

In recent years, various analytical technologies have been employed for the determination of esculetin, for instance, capillary electrophoresis [2, 7], fluorimetry [8], high performance liquid chromatography [9], and liquid chromatography–tandem mass spectrometry [10, 11]. Generally, these methods performed at centralized laboratories, requiring analytical resources and extensive labor, and often costly and time consuming. On the contrary, electrochemical method is a good alternative for the determination of esculetin due to the advantages of simple operation, time-saving, low-cost, sensitivity, selectivity, and real-time online detection. Up to now, diverse electrodes composed of glassy carbon electrode (GCE) [12], carbon paste electrode (CPE) [13], or GCE modified with CdSe nanoparticles-decorated poly-(diallyldimethylammonium chloride)-functionalized graphene (CdSe–PDDA-G) [3], carbon nanotube/poly(ethylene-co-vinyl acetate) (CNT/EVA) [6], carbon nanotube/poly(ethylene-co-vinyl acetate) (CNT/EVA) [7], electrochemically reduced grapheme oxide (ERGO) [14], carboxylic acid-functionalized multi-walled carbon nanotubes (c-MWCNTs) [15] have been reported for the determination of esculetin. However, application of carbon nanotube combines with Au nanocomposite for the development of esculetin sensor has not been reported until now.

Owing to the good chemical and physical stability, high electrical conductivity and electrocatalytic activity, large specific surface area, and good biocompatibility, carbon nanotubes (CNTs) have been considered as one of the most popular novel carbon nanomaterials using as sensing materials for electrochemical and biochemical applications [16-18]. Single walled carbon nanotubes (SWCNTs) have attracted tremendous interests for the establishment of chemo/bio-sensors. Our previous works indicated that carboxylic acid-functionalized SWCNTs (c-SWCNTs) was a promising chemo/bio-sensing material, applying to develop different electrochemical sensors for the determination of molluscicide niclosamide [19], organophosphorus pesticide methyl parathion [20], and antioxidant catechin [21]. Moreover, c-SWCNTs, containing terminal carboxylic acid groups, can provide sites for the stabilization and anchoring of metal nanoparticles to produce nanotube-reinforced composite materials [22, 23]. Recently, Au nanomaterials have focused on much attention on the designing and constructing of chemo/bio-sensors due to the good electronic and catalytic properties, high surface areas, strong adsorption ability, and excellent biocompatibility. In addition, the hybrid material combined CNTs with metal nanomaterial may lead to a successful integration of properties of the two components [24-26]. Therefore, combining the merits of c-SWCNTs with Au nanosol (AuNs) may be a good choice to develop a novel electrochemical sensor for the determination of esculetin sensor.

In this paper, a novel esculetin electrochemical sensor was prepared by taking advantages of c-SWCNTs and AuNs. Using as a binding agent, Nafion (NF) was employed to improve the binding force and adhesion between electrode and films. The characterizations of c-SWCNTs-NF–AuNs nanocomposite were investigated by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS). Owing to the synergistic effect of c-SWCNTs with electrocatalytic

activity and AuNs with good electronic and catalytic properties, high surface areas and excellent biocompatibility, the c-SWCNTs-NF–AuNs modified glassy carbon electrode (c-SWCNTs-NF–AuNs/GCE) showed good electrocatalytic activity toward esculetin. In addition, c-SWCNTs-NF–AuNs/GCE was applied to detection of esculetin in the traditional Chinese herbal medicine *Cortex Fraxini*.

2. EXPERIMENTAL

2.1. Regents

Esculetin (≥ 98%) was purchased from Nationals Institutes for Food and Drug Control. AuNs (0.2 g/L) was obtained from Nanjing XFNANO Materials Tech Co., Ltd. c-SWCNTs suspensions (1.0 wt%) were purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences. 5% Nafion (NF) solution was obtained from Sinopharm chemical reagent Co., Ltd. All the other reagents were purchased from Aladdin reagent Co., Ltd. Phosphate buffer solutions (PBS, 0.1 M) with different pH values were applied as supporting electrolyte solution. The esculetin stock solution (1×10^{-3} M) was prepared with methanol and stored at 4 ºC in refrigerator.

2.2 Instruments

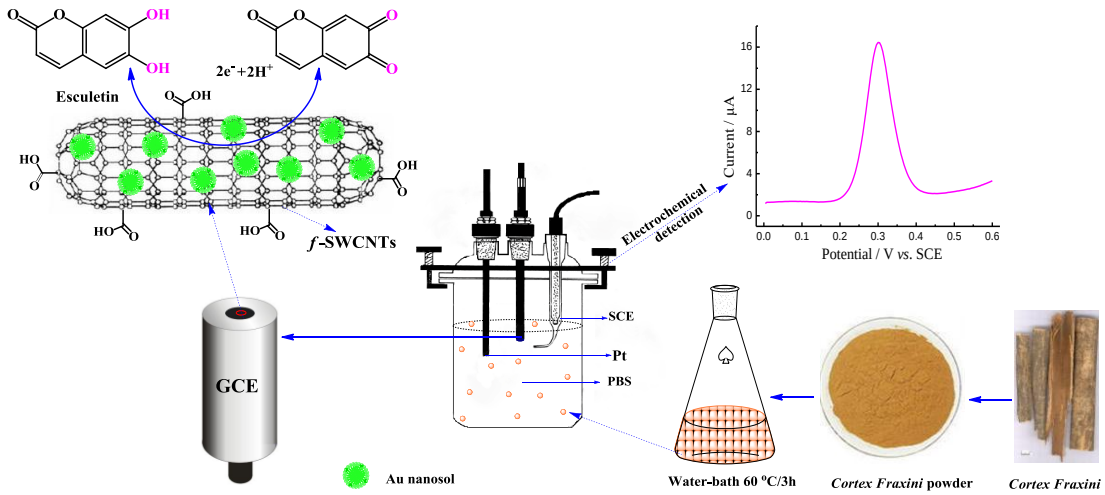
Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were performed on a model CHI660B electrochemical workstation with a conventional three-electrode system. Bare GCE (Φ = 3 mm), c-SWCNTs-NF/GCE and c-SWCNTs-NF–AuNs/GCE were used as the working electrode, the saturated calomel electrode (SCE) was used as a reference electrode, while the platinum wire (Φ = 1 mm) was used as a counter electrode. Infrared spectra were performed with a Bruker Vertex 70 Fourier transform infrared (FT-IR) spectrometer. Scanning electron microscopy (SEM) images were recorded by a JEOL JSM-6700F scanning electron microscope.

2.3. Preparation of modified electrodes

Bare GCE was polished with chamois leather containing 0.05 μm γ-alumina before modification. Until a mirror-shine surface was obtained, GCE was successively sonicated in doubly-distilled deionized water, ethanol, and doubly-distilled deionized water for 5 min, respectively, and then dried and standby. c-SWCNTs-NF suspensions were prepared by doping different concentration of c-SWCNTs (from 0 wt% to 0.5 wt%) with 0.5 wt% NF. The c-SWCNTs-NF–AuNs suspensions were prepared by adding different concentration of AuNs (0-0.2 mg mL^{-1}) to the c-SWCNTs-NF suspensions, following by ultrasound continuously. 5 μL c-SWCNTs-NF and c-SWCNTs-NF–AuNs suspensions were drop-coating on the surface of bare GCE, respectively and dried in infrared lamp, and then the c-SWCNTs-NF/GCE and c-SWCNTs-NF–AuNs/GCE were constructed.

2.4 Sample preparation

500 mg commercial *Cortex Fraxini* (medicinal market of Chongqing) was accurately weighed, powdered and shifted through a 60 mesh sieve. Then, the *Cortex Fraxini* powders were mixed with 30 mL absolute ethanol in an Erlenmeyer flask with water-bath for 3h at 60 ºC. Finally, filtered and diluted to 250 mL volume with absolute ethanol and stored at 4 ºC, as shown in Scheme 1.



Scheme 1. The mechanism for the determination of esculetin and the process of practical sample preparation.

2.5. Experimental measurements

Five milliliters of 0.1 M PBS (pH 6.0) with different concentration of esculetin or samples were added to the sealed electrochemical cell. The potential of EIS was 0.25 V and the supporting electrolyte was 5 mM equal concentrations of $[Fe(CN)_6]^{3-/4-}$ redox coupled with 0.1 M KCl at the frequency range from 100 mHz to 10 kHz. CVs were performed in the potential ranging from 0 V to 0.6 V at a scan rate of 50 mV s^{-1}. DPVs were recorded from 0 V to 0.6 V, potential increase, 0.004 V; amplitude, 0.05 V; pulse width, 0.05 s; pulse interval, 0.2 s. Prior to each experiment, all detection solutions were deoxygenated by purging with nitrogen for 10 min. Every working electrode was transferred into a stirring 0.1 M PBS (pH 6.0) solution with a specific concentration of esculetin for 120 s at 0 V.

3. RESULTS AND DISCUSSION

3.1. Characterization of modified electrodes

The surface morphologies of different modified electrode films were characterized by SEM. As shown in Fig. 1A, c-SWCNTs film shows a three-dimensional (3D) network structure with the

diameter of 120 nm. When the c-SWCNTs-NF combined with the AuNs (Fig. 1B), the c-SWCNTs-NF–AuNs composite shows 3D network structure and nanoparticles, which improved the roughness of the surface of modified electrode and offered much more electroactive sites, beneficial to accelerated the electron transfer between the solution and electrode interface [20, 27]. In addition, Fig. 1C shows energy dispersive spectrum (EDS) spectral data of c-SWCNTs-NF–AuNs nanocomposite, which contains C, O, F and Au with 13.94 wt%, 40.54 wt%, 19.78 wt% and 25.74 wt% respectively. These results further confirmed the existence of c-SWCNTs-NF dispersed AuNs on the surface of the GCE.

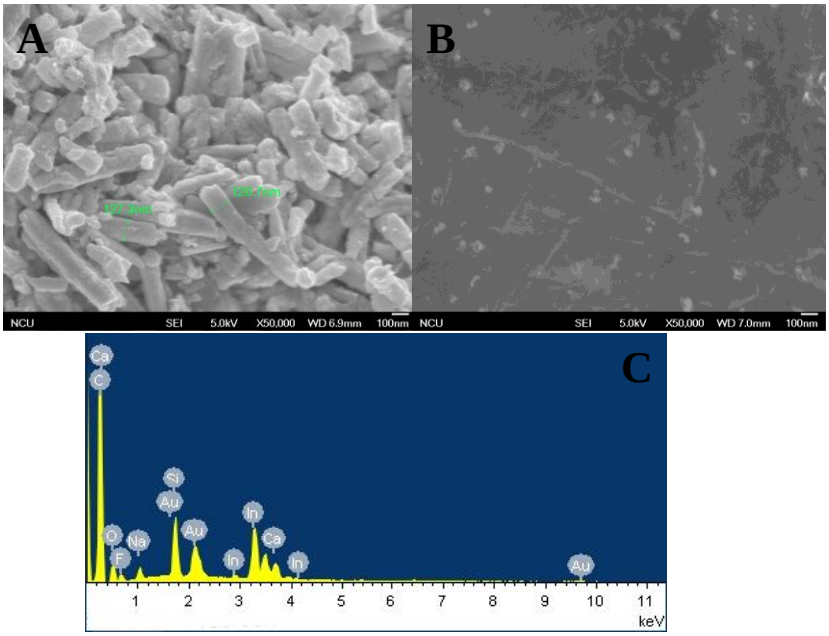


Figure 1. SEM of c-SWCNTs (A) and c-SWCNTs-NF–AuNs (B); EDS of c-SWCNTs-NF–AuNs nanocomposite (C).

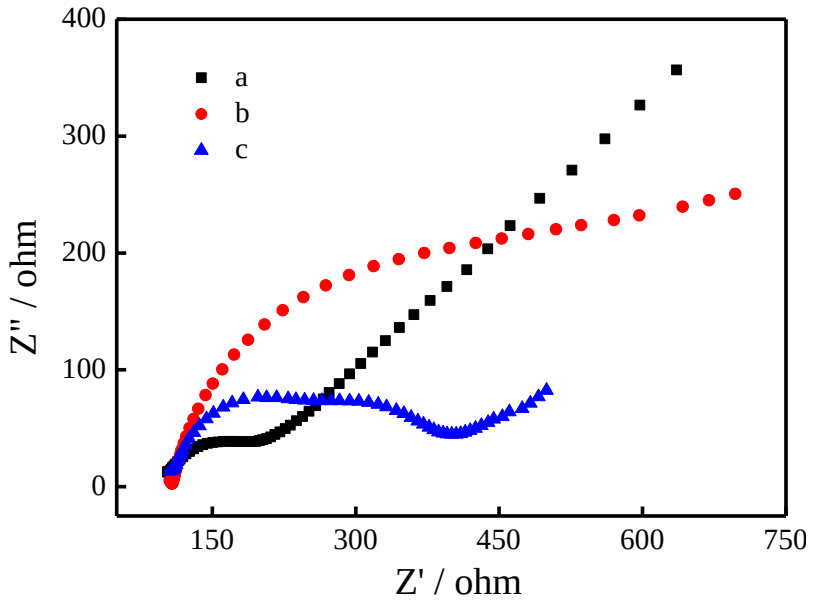


Figure 2. Nyquist plots of bare GCE (a), c-SWCNTs-NF/GCE (b) and c-SWCNTs-NF–AuNs/GCE (c), in 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl.

To illustrate the electron transfer capability of different modified electrodes: bare GCE (a), c-SWCNTs-NF/GCE (b) and c-SWCNTs-NF–AuNs/GCE (c), EIS was investigated. As shown in Fig. 2, GCE shows a small quasi-semicircle. When c-SWCNTs-NF modified on the surface of GCE, the electron transfer resistance (R_{ct}) of c-SWCNTs-NF/GCE increased sharply, attributing to the NF that put off the electron transfer [28]. For c-SWCNTs-NF–AuNs/GCE, the R_{ct} value was much smaller than c-SWCNTs-NF/GCE, which is contributed to the good conductivity and electrocatalytic ability of AuNs that improved the diffusion of ferricyanide toward the electrode interface [29]. It also suggested that c-SWCNTs-NF–AuNs/GCE is an excellent modified electrode material, which is beneficial to the electron-transfer between electrolyte and electrode interface [27].

3.2. Voltammetric behavior of esculetin

CVs of bare GCE (a), c-SWCNTs-NF/GCE (b), and c-SWCNTs-NF–AuNs/GCE (c) in 0.1 M PBS (pH 6.0) containing 20 μM esculetin were investigated. As shown in Fig. 3, all the modified electrodes showed a couple of redox peaks, illustrating that the electrochemical reaction of esculetin is a reversible process [3]. For bare GCE, a relatively poor redox peak current was observed (curve a). When c-SWCNTs-NF and c-SWCNTs-NF–AuNs suspensions were drop-coating on the surface of GCE, the redox peak current of esculetin improved significantly owing to the synergistic effect of c-SWCNTs and AuNs with excellent electrocatalytic activity [20]. In addition, the anode peak potential (E_{pa}) and cathode peak potential (E_{pc}) of c-SWCNTs-NF–AuNs/GCE (curve c, E_{pa} = 0.355 V; E_{pc} = 0.307 V) shifted much more negatively than c-SWCNTs-NF/GCE (curve b, E_{pa} = 0.372 V; E_{pc} = 0.327 V). These results suggested that AuNs could accelerate the electron transfer rate and reduce the overpotential of esculetin on account of the high surface areas and good electronic and catalytic properties of AuNs. Moreover, c-SWCNTs-NF–AuNs/GCE (curve c) had the largest peak current than the other electrodes. Therefore, c-SWCNTs-NF–AuNs nanocomposite can be applied as a promising modified electrode material for the electrochemical determination of esculetin.

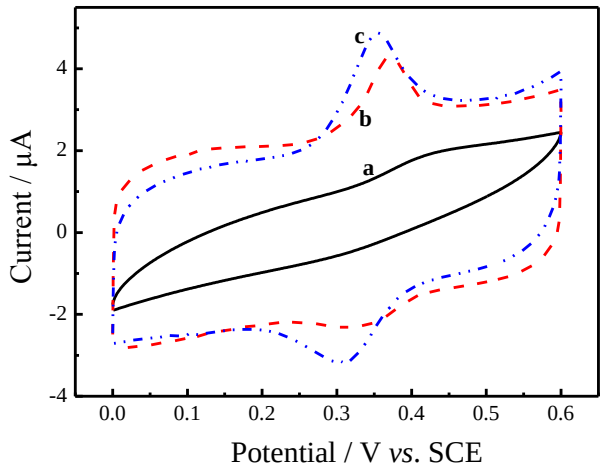


Figure 3. CVs of bare GCE (a), c-SWCNTs-NF/GCE (b), and c-SWCNTs-NF–AuNs/GCE (c) in 0.1 M PBS (pH 6.0) containing 20 μM esculetin; Accumulation potential: 0 V; Accumulation time: 120 s; Scan rate: 50 mV s^{-1}.

3.3. Optimization of conditions

To obtain a highly sensitive detection of esculetin, experimental parameters including accumulation time, accumulation potential, the concentrations of c-SWCNTs and AuNs, and pH value have been optimized.

Sometimes, the accumulation time and accumulation potential can increase the absorption of esculetin on the modified electrode surface, which can improve detection sensitivity and decrease the detection limit. Thus, the influences of accumulation potential and accumulation time were studied. Effect of accumulation time on the oxidation peak current of esculetin at c-SWCNTs-NF–AuNs/GCE was carried out using DPV. The c-SWCNTs-NF–AuNs/GCE was dipped into 0.1 M PBS (pH 6.0) containing 20 μM esculetin with different accumulation times. As shown in Fig. 4A, the oxidation peak current of esculetin grow rapidly with the increase of accumulation time until reach a platform at 120 s, and then decrease slightly. Hence, 120 s was selected as the accumulation time. Moreover, effect of accumulation potential on the oxidation peak current of esculetin at c-SWCNTs-NF–AuNs/GCE was also investigated. The c-SWCNTs-NF–AuNs/GCE was dipped into 0.1 M PBS (pH 6.0) containing 20 μM esculetin with 120 s accumulation. The accumulation potential was in the range from –0.2 to 0.8 V. However, with the variation of accumulation potential, there was almost no change for the peak current of esculetin, implying that the accumulation potential has no obvious influence on the adsorption of esculetin [30]. Therefore, we chose the initial potential (0 V) as the accumulation potential.

The concentrations of c-SWCNTs and AuNs were also investigated. Fig. 4B shows the influence of c-SWCNTs concentration on the oxidation peak current of esculetin. The concentration of AuNs was maintained constantly at 0.1 mg mL^{-1} and the c-SWCNTs-NF–AuNs/GCE was accumulated in 0.1 M PBS (pH 6.0) containing 20 μM esculetin for 120 s. The concentration of c-SWCNTs was ranged from 0 wt% to 5.0 wt%. As shown in Fig. 4B, with the increasing of c-SWCNTs concentration, the oxidation peak current of esculetin increased gradually and reached a maximum value at 0.35 wt%, which was attributed to the addition of electroreactive sites with the increase of c-SWCNTs concentration at composite electrode surface [20, 31]. However, the oxidation peak current rapidly decreased when the concentration of c-SWCNTs further increased. This might be ascribed to the increase of film thick that restricted the electron transfer between analyte and electrode [32]. Therefore, 0.35 wt% *f*-SWCNT was employed for the development of c-SWCNTs-NF–AuNs/GCE. Meanwhile, the influence of AuNs concentration on the oxidation peak current of esculetin was shown in Fig. 4C. The concentration of c-SWCNTs was maintained constantly at 0.35 wt% and the c-SWCNTs-NF–AuNs/GCE was also accumulated in 0.1 M PBS (pH 6.0) containing 20 μM esculetin for 120 s. When the concentration of AuNs increased from 0 mg mL^{-1} to 0.10 mg mL^{-1}, the oxidation peak current increased obviously, but decreased sharply with the increase concentration of AuNs, which might be due to the high concentration AuNs that prevented the electron transfer and influenced the dispersion stability of nanocomposite. Therefore, 0.10 mg mL^{-1} AuNs was applied in subsequent experiments.

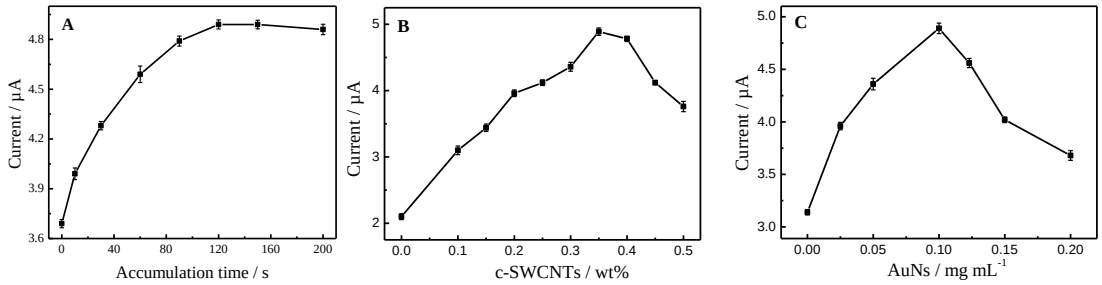


Figure 4. Effect of accumulation time (A), c-SWCNTs concentrations (B), and AuNs concentrations (C) on the oxidation peak current of esculetin at c-SWCNTs-NF–AuNs/GCE.

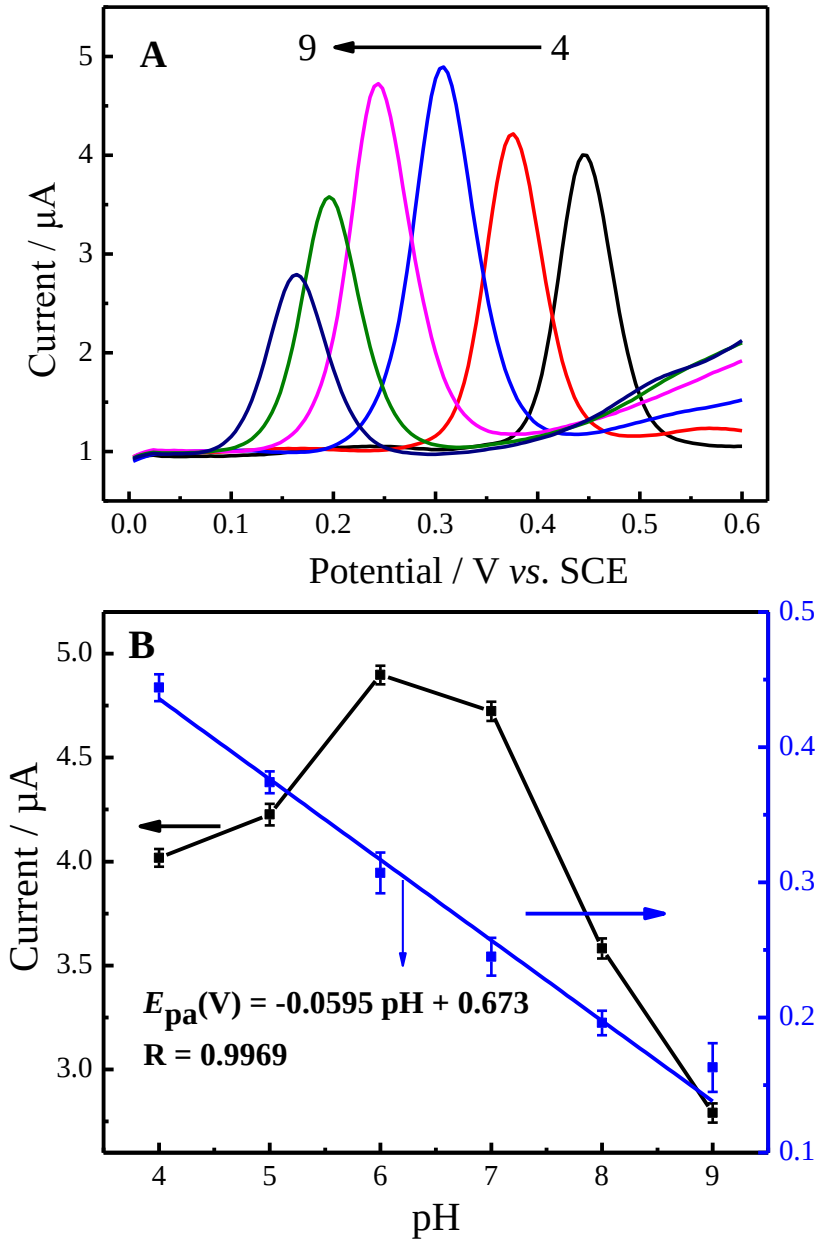


Figure 5. (A) DPVs of 20 μM esculetin at c-SWCNTs-NF–AuNs/GCE with different pH values (pH: 4.0, 5.0, 6.0, 7.0, 8.0, 9.0); (B) Effect of pH values on the oxidation peak currents (black line) and oxidation peak potentials (blue line). Accumulation potential: 0 V; Accumulation time: 120 s.

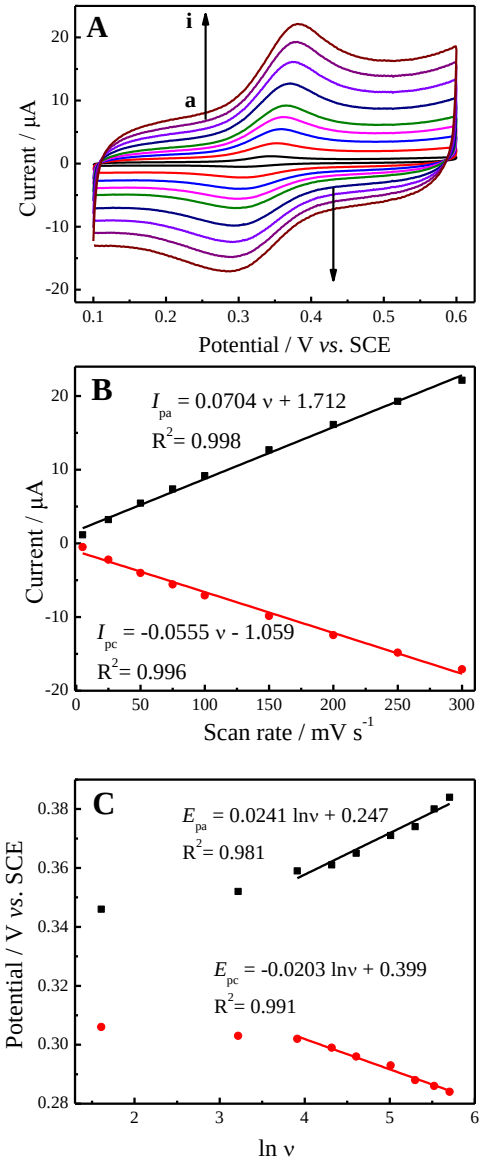


Figure 6. CVs of 20 μM esculetin at c-SWCNTs-NF–AuNs/GCE with different scan rates (from a to i: 5, 25, 50, 75, 100, 150, 200, 250, and 300 mV s^{-1} in 0.1 M PBS (pH 6.0). The plot of redox peak current *vs.* scan rate (B) and redox peak potential *vs.* Napierian logarithm of scan rate (C)

Effect of pH value on the oxidation peak current of esculetin at c-SWCNTs-NF–AuNs/GCE by DPVs was shown in Fig. 5A. The pH value was in range of 4.0 – 9.0. With the increase of pH value, the peak potential shifted negatively, suggestion that protons took part in the electrochemical reaction process of esculetin. The relationship of oxidation peak current (I_{pa}) and peak potential (E_{pa}) of esculetin with various of pH values were exhibited in Fig. 5B. The oxidation peak current of esculetin at c-SWCNTs-NF–AuNs/GCE reached the maximum value at pH 6.0. Hence, pH 6.0 is chose for the electrochemical detection of esculetin. The relationship of E_{pa} and pH value was also investigated. As can be seen from Fig. 5B, E_{pa} changed linearly with pH values and the equation was E_{pa} (V) = –0.0595 pH + 0.673 (R^2 = 0.994). The slope (59.5 mV pH^{-1}) is close to the theoretical value (58.5 mV pH^{-1}), replying that electrochemical redox process of esculetin involves equal numbers of proton-transfer and electron-transfer.

3.4. Effect of scan rate towards esculetin

Fig. 6A shows the effect of scan rate on the electrochemical behavior of esculetin at c-SWCNTs-NF–AuNs/GCE by CVs in 0.1 M PBS (pH 6.0) containing 20 μM esculetin. As shown in Fig. 6B, the I_{pa} and reduction peak currents (I_{pc}) were increased linearly with the increase of scan rate (ν) from 5 to 300 mV s^{-1}. The corresponding regression equations were I_{pa} (μA) = 0.0704 ν + 1.712 (ν in mV s^{-1}) (R^2 = 0.998) and I_{pc} (μA) = –0.0555 ν – 1.059 (R^2 = 0.996), indicating that the electrochemical reaction of esculetin is an adsorption-controlled process [14]. Similarly, the E_{pa} and reduction peak potential (E_{pc}) were increased linearly with the Napierian logarithm of scan rate (ln ν) in the range of 50 – 300 mV s^{-1} (Fig. 6C). The linear regression equations were E_{pa} (V) = 0.247 + 0.0241 lnν (R^2 = 0.981) and E_{pc} (V) = 0.399 – 0.0203 ln ν (R^2= 0.991). According to the Laviron's equations [33]:

$$E_{pc} = E^0 + \frac{RT}{\alpha nF} \ln\left(\frac{RTK_s}{\alpha nF}\right) - \frac{RT}{\alpha nF} \ln \nu \qquad (1)$$

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF} \ln\left[\frac{RTK_s}{(1-\alpha)nF}\right] - \frac{RT}{(1-\alpha)nF} \ln \nu \qquad (2)$$

R, *T* and *F* have their usual meaning (*R* = 8.314 J mol^{-1} K^{-1}, *T* = 298 K and *F* = 96480 C mol^{-1}). According to Eq. (1) and Eq. (2), the number of electrons transfer *n* was calculated approximately to be 2 and the charge transfer coefficient *α* was 0.543. In section 3.3, the electrochemical redox process of esculetin was indicated that there were equal numbers of proton-transfer and electron-transfer involved in the electrochemical redox process. Therefore, the electrochemical behavior of esculetin at c-SWCNTs-NF–AuNs/GCE is a two-electron and two-proton process. The mechanism for the electrochemical redox reaction of esculetin was shown in Scheme 1.

3.5. Detection of esculetin

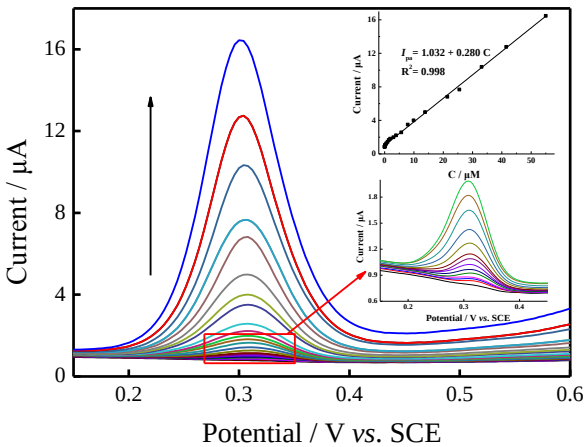


Figure 7. DPVs of different concentration of esculetin at c-SWCNTs-NF–AuNs/GCE esculetin (from bottom to top: 0.004, 0.008, 0.028, 0.048, 0.088, 0.128, 0.188, 0.38, 0.58, 0.98, 1.38, 1.88, 2.98, 2.65, 3.88, 5.68, 7.88, 9.88, 13.8, 21.4, 25.5, 33.1, 41.5 and 55 μM). Inset: the calibration plot of oxidation peak current *vs.* concentration of esculetin in 0.1 M PBS (pH 6.0); Accumulation potential: 0 V; Accumulation time: 120 s.

Under optimum experimental conditions, DPVs of esculetin at c-SWCNTs-NF–AuNs/GCE over the range of concentrations from 0.004 to 55 μM were investigated (Fig. 7). As can be seen from the inset of Fig. 7, the peak currents are proportional to the concentrations of esculetin and the corresponding linearization equations is I_{pa} (μA) = 1.032 + 0.280 C (μM) (R^2 = 0.998). The limit of detection and the limit of quantitation were calculated to be 1.2 nM and 4.0 nM, respectively. Compared with the other reported electrodes for the determination of esculetin (Table 1), the c-SWCNTs-NF–AuNs/GCE exhibited shorter accumulate time, wider linear range, and lower detection limit, which might be attributed to the synergistic effect of c-SWCNTs and AuNs. Furthermore, the constructed c-SWCNTs-NF–AuNs/GCE was turned out to be a good modified electrode for the determination of esculetin.

Table 1. Comparison of the constructed electrode with the other reported electrodes for the determination of esculetin.

Electrodes	Accumulation time	Buffer solution	Linear range (μM)	Limit of detection (μM)	Real sample	Ref.
CdSe–PDDA-G/GCE	300 s	0.1 M sodium citrate–hydrochloric acid (pH 1.5)	0.01–50	0.004	*Cortex Fraxini*	[3]
CuS/GN/GCE	60 s	0.1 M sodium citrate–hydrochloric acid (pH 1.5)	0.1–100	0.058	*Cortex Fraxini*	[6]
CNT/EVA	–	50mM borate buffer (pH 9.2)	1.0–1000	0.22	*Cortex Fraxini*	[7]
GCE	–	0.1 M PBS (pH 4.5)	0.2–3.2	0.026	*Cortex Fraxini*	[12]
CPE	2 min	0.1 M PBS (pH 4.0)	0.5–4.5	0.06	*Cortex fraxini*	[13]
ERGO/GCE	–	0.1 M PBS (pH 3.0)	0.04–5	0.02	*Viola yedoensis Makino*	[14]
c-MWCNTs-GCE	2 min	0.1 M PBS (pH 4.5)	0.06–8	0.005	*Cortex Fraxini*	[15]
c-SWCNTs-NF–AuNs/GCE	120 s	0.1 M PBS (pH 6.0)	0.004–55	0.0012	*Cortex Fraxini*	This work

3.6. Stability, reproducibility and selectivity

The operation stability of c-SWCNTs-NF–AuNs/GCE for the electrochemical determination of esculetin was investigated through 30th successive measurements, and the relative standard deviation (RSD) was 1.6%, indicating that the c-SWCNTs-NF–AuNs/GCE has good operation stability. Meanwhile, the long-term stability of c-SWCNTs-NF–AuNs/GCE was also investigated. The c-SWCNTs-NF–AuNs/GCE immersed in sealed 0.1 M PBS (pH 6.0) and stored in 4 °C refrigerator for one month. Then, the c-SWCNTs-NF–AuNs/GCE was employed for the detection of esculetin every other day. In the first week, there was no obvious variation for the current response of esculetin, following on decrease gradually. After one month later, there still remained 92.5% initial response, suggesting that c-SWCNTs-NF–AuNs/GCE has long-term stability. In addition, six c-SWCNTs-NF–AuNs/GCE were employed for the detection of esculetin and the RSD was 3.1%, implying that c-SWCNTs-NF–AuNs/GCE has good reproducibility. All the results demonstrated that the c-SWCNTs-NF–AuNs/GCE has high stability.

To evaluate the selectivity of c-SWCNTs-NF–AuNs/GCE for the detection of esculetin, 10-fold concentration of citric acid, oxalic acid, ascorbic acid, mannitol, glucose, urea, lysine and 100-fold Na^+, K^+, Zr^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Al^{3+}, NO_3^{2-}, CO_2^{2-}, SO_4^{2-}, and PO_4^{3-} were applied to investigate the interference studies. The variation of peak current was less than 5% (i.e. 97.6-103.9%), proving that the c-SWCNTs-NF–AuNs/GCE has good selectivity.

3.7. Practical determination of esculetin in Cortex Fraxini samples

To evaluate the feasibility and validity of fabricated modified electrode for the detection of esculetin, the standard addition method was used to determination the esculetin in real sample *Cortex Fraxini*, as list in Table 2. The recovery of three independent experiments is in the range of 98.86–101.06 and the RSD is less than 5%, indication that the modified electrode c-SWCNTs-NF–AuNs/GCE is practicability for the determination of esculetin in real sample without sample purification step.

Table 2. Determination of esculetin in *Cortex Fraxini* using the standard addition method (n = 5).

Samples		Measured (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Cortex Fraxini	1	3.26	0.005	3.27 ± 0.24	100.15	4.38
	2	3.25	0.10	3.32 ± 0.32	99.10	3.21
	3	3.24	5.50	8.64 ± 0.18	98.86	3.82
	4	3.26	25.0	28.56 ± 0.14	101.06	2.61

4. CONCLUSION

In this work, a novel simple, sensitive and selective electrochemical sensor was successfully constructed for the determination of esculetin by taking advantages of c-SWCNTs with electrocatalytic activity and AuNs with good electronic and catalytic properties, high surface areas and excellent biocompatibility. NF was employed as a binding agent to improve the binding force and adhesion between electrode and films. Owing to the synergistic effect, c-SWCNTs-NF–AuNs/GCE exhibited a good electrocatalytic activity toward esculetin with rapid response, wide linear range, low detection limit, good stability, reproducibility and sensitivity. Moreover, the modified electrode was employed for the analysis the esculetin in the traditional Chinese herbal medicine *Cortex Fraxini*, offering a sound for the practical detection of esculetin in Chinese herbal medicines and pharmaceutical formulations.

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