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Electrochemical Determination of Rutin in Herbal Samples Using CuO/CNT composite Modified Glassy Carbon Electrode

Zhiyang Liu¹, Yan Liu^{2,*}, Zhongyu Tan², Ying Liu², Linglong Meng², Sheng Yang²

¹ School of Life Sciences, Changchun Sci-Tech University, Changchun 130600, China
² School of Medicine, Changchun Sci-Tech University, Changchun 130600, China
*CorrespondenceE-mail: soliu365@sina.com

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The goal of this research was to create a nanocomposite based on CuO nanoparticles and functionalized CNTs for modification of the glassy carbon electrode (CuO@f-CNTs/GCE) and utilize it as an electrochemical sensor to detect rutin in Cinnamomum camphora (L.) J. Presl leaves. CuO@f-CNTs nanocomposite consisting of f-CNTs with CuO nanoparticles in a spherical shape that have developed in tiny sizes and irregular forms, as determined by the surface morphology and crystal structure of modified electrodes using SEM and XRD. The electrochemical properties of CuO@f-CNTs/GCE were investigated using DPV analysis, which revealed a selective and sensitive electrochemical response to rutin determination, with a linear range of 10 to 200 μ M and a sensitivity of 0.06087 μ A/ μ M and a detection limit of 11nM. The validity and capability of the developed method for determining rutin content in a prepared real sample of Cinnamomum camphora (L.) J. Presl were investigated, and the results showed that the recovery and RSD values by the standard addition method were 99.13% to 99.56% and 2.21% to 4.03%, respectively, indicating that the proposed system had a high detection accuracy and was valid for rutin determination in real herbal samples.

Keywords: Electrochemical Sensor; CuO@f-CNTs nanocomposite; Functionalized CNTs; Rutin; Cinnamomum camphora (L.) J. Presl

1. INTRODUCTION

Rutin ($C_{27}H_{30}O_{16}$), also known as rutoside, quercetin-3-O-rutinoside, and sophorin, is a plant pigment found in the rind of green citrus fruits, rose hips, and black currants [1-3]. It is a glycoside combining the flavonol quercetin and the disaccharide rutinose [4-6]. Rutin has also been shown to have antioxidant and anti-inflammatory properties, and it has been used in traditional medicine to treat osteoarthritis and other inflammatory illnesses, as well as to improve blood circulation and a healthy heart, and to enhance the activity of vitamin C [7-10]. As a result, it provides some protection from cancer and other disorders [11, 12]. This antioxidant flavonoid is often used for autism, aged skin, exercise-induced airway infections, and a variety of other ailments [13-15].

Thus, many studies have been performed to identify, extract, and determine the rutin concentration in herbal plants through high-performance liquid chromatography [16, 17], UV-Vis spectrophotometry [18, 19], fluorescence spectroscopy [20], Raman spectroscopy [21], FT-IR [22], capillary electrophoresis [23, 24] and electrochemical techniques [25-29]. Between these techniques, electrochemical techniques have demonstrated simplicity, low cost, and speed. Moreover, electrochemical techniques show fast electron transfer and high sensitivity because of use of the mediators and catalysts and improve the sensing properties with nanostructured electrodes [30]. Therefore, this study focused on the synthesis of CuO@f-CNTs nanocomposite for modification by the GCE and its application as an electrochemical sensor for the determination of rutin in the leaves of Cinnamomum camphora (L.) J. Presl.

2. EXPERIMENTAL

2.1. Preparation of modified electrode

In order to functionalize CNTs [31], 200 mg of CNTs (99%, Xiamen Tob New Energy Technology Co., Ltd., China) were treated with 50 mL of a 5 M aqueous acid solution of H_2SO_4/HNO_3 mixture in a volume ratio of 3:1. The mixture was ultrasonically vibrated in a water bath at a temperature of 45 °C for 5 hours. Next, the functionalized CNTs (f-CNTs) were washed thoroughly with deionized water for several times. To make CuO nanoparticles [32], a solution of poly(diallyldimethyl) ammonium chloride (20 wt.%, Sigma-Aldrich) was mixed with an ethanol/water mixture in a volume ratio of 2:3. Subsequently, the obtained solution was mixed with 10 mM CuCl₂ (97%, Sigma-Aldrich) in a molar ratio of 5:1, and the resultant mixture was heated to 55°C for 80 minutes using an N₂ atmosphere at a flow rate of 50 sccm to achieve the dark brown suspension of CuO nanoparticles. After cooling, the CuO nanoparticles were dried in an oven at 80 °C for 5 hours.

2 mg of f-CNTs and 2 mg of were ultrasonically dispersed in 2 mL of a mixture of ethanol (96%, Shanghai Zhongrong Technology Co., Ltd., China) and nafion (20 wt.%, Sigma-Aldrich) solution for 40 minutes to obtain a homogeneous black suspension for the synthesis of the CuO@f-CNTs nanocomposite modified electrode [32]. 50 μ L of dispersed CuO@f-CNTs nanocomposite was dropped onto the clean surface of GCE and dried at room temperature.

2.2. Characterization

For analysis of surface morphology and crystal structure of nanocomposites, scanning electron microscopy (SEM; JSM-5600LV, operating at 15 keV, JEOL, Tokyo, Japan) and X-ray diffraction (XRD; Rigaku, D/max 2550-A Xray diffractometer, Tokyo, Japan) were used. Differential pulse voltammetry (DPV) measurements were performed in 0.1 M phosphate buffer solution (PBS, Sigma-Aldrich) with pH 7.4 using a potentiostat-galvanostat (Wuhan Corrtest Instruments Corp., Ltd., China)

in a three-electrode configuration consisting of modified GCE as the working electrode, Pt wire as the counter, and Ag/AgCl as the reference electrode in a three-electrod.

2.3. Real sample preparation

Fresh Cinnamomum camphora (L.) J. Presl leaves were collected from the Yangtze River delta, particularly in Shanghai, China. The leaves were dried at room temperature, pulverized, and 2.0 g of powder was refluxed in a water bath at 75 °C for 8 hours with 100 ml of methanol. The material was filtered using a filter paper (100 μ m, Whatman) and centrifuged at 1000 rpm for 10 minutes after cooling to room temperature naturally. After filtering, the sample was concentrated under vacuum to an 80 ml volume and then diluted to 100 ml in 0.1 M PBS at a pH of 7.4.

3. RESULTS AND DISCUSSION

3.1. Surface morphology and crystal structure of modified electrodes



Figure 1. SEM micrographs of (a) f-CNTs and (b) CuO@f-CNTs nanocomposite modified GCE.

SEM micrographs of f-CNTs and CuO@f-CNTs nanocomposite modified GCE are shown in Figure 1. The f-CNTs are fully randomly orientated, as shown in Figure 1a, and agglomerated like cotton clumps with an average diameter of 70 nm. CuO@f-CNTs nanocomposite was made up of f-CNTs with CuO nanoparticles in a spherical shape that developed into tiny sizes (average diameter of 30 nm) and irregular forms, as shown in Figure 1b. This is due to the presence of carboxyl functional groups on f-CNTs, which make it easier for them to become ionically bonded to metal ions or covalently coupled through various metal oxides [33-36]. The results demonstrate that the high aspect ratio and high effective surface area of f-CNTs decorated with a high density of CuO nanoparticles were synthesized.

Figure 2 shows the results of XRD examinations of f-CNT powders and CuO@f-CNTs nanocomposite powders. Single strong diffraction peaks at 26.33° were seen in the XRD pattern of f-CNTs, which coincided with the diffraction plane of CNTs (002) [37, 38]. XRD pattern of CuO@f-CNTs shows diffraction peaks at 32.21°, 35.50°, 38.52°, 48.79° and 57.84°, corresponding to (110),

(111), (111), (202) and (202) planes of the monoclinic phase of CuO (CPDS card no. 80-1916) [39, 40], and additional diffraction peak of (002) CNTs. Thus, the results of SEM and XRD analyses are evidence of the successful synthesis of CuO@f-CNTs nanocomposite.



Figure 2. XRD analyses of powders of f-CNTs and CuO@f-CNTs nanocomposite.

3.2. Study of electrochemical property

Figure 3 shows DPV measurements of bare GCE, f-CNTs, and CuO@f-CNTs nanocomposite modified GCE in 0.1 M PBS with pH 7.4 before and after the addition of 100 M rutin at a potential range of 0 V to 0.6 V with a scan rate of 25 mV/s in 0.1 M PBS with pH 7.4 before and after the addition of 100 M rutin. All three electrodes, GCE, f-CNTs/GCE, and CuO@f-CNTs/GCE, do not display any noticeable redox peak before the addition of rutin solution [41, 42]. After adding 100 M rutin to an electrochemical cell, bare GCE shows no electrocatalytic response to rutin oxidation, whereas f-CNTs/GCE and CuO@f-CNTs/GCE show electrocatalytic responses to rutin oxidation at 0.25 V and 0.23 V, respectively [43-45]. The mechanism of the electrooxidation reaction of rutin is proposed as that the catechol structure of rutin is oxidized to 3',4' -diquinone [46-48]. The peak current value is observed to be higher for CuO@f-CNTs/GCE compared to f-CNTs/GCE. It indicates that the CuO@f-CNTs have the most attractive electrocatalysts towards oxidation of rutin which is related to the synergetic effect of excellent electrical conductivity and high porosity and high effective surface area of f-CNTs decorated with a high density of CuO nanoparticles [49-51]. CuO nanoparticles have high surface energy which can be used to enhance electron transfer [52, 53]. The catalytic ability of f-CNTs with well-defined hollow interiors loaded onto CuO nanoparticles is improved. High porosity and nanometer-scale CuO@f-CNTs nanocomposite with a narrow size distribution can improve electrocatalytic activity and stability by increasing the number of electron channels [54-56]. Thus, above DPV measurements clearly illustrated that CuO@f-CNTs nanocomposite possesses higher catalytic activity compared to the other two electrodes, and was selected as a favorable catalyst for following studies on electrochemical sensing of rutin [57, 58].



Figure 3. DPV measurements of bare GCE and f-CNTs and CuO@f-CNTs nanocomposite modified GCE in 0.1 M PBS with pH 7.4 before (dashed line) and after (solid line) addition a 100 μ M rutin at potential range from 0 V to 0.6 V with a scan rate of 25 mV/s.



Figure 4. (a) DPV measurements of CuO@f-CNTs/GCE in 0.1 M PBS with pH 7.4 under different concentration of rutin at scan rate of 25 mV/s; (b) related calibration plot.

Figure 4 shows the electrocatalytic response of CuO@f-CNTs/GCE for the determination of various rutin concentrations, displaying DPV measurements of CuO@f-CNTs/GCE in 0.1 M PBS with pH 7.4 at a scan rate of 25 mV/s in 0.1 M PBS. The proposed electrode has a significant

electrocatalytic reaction when different concentrations of rutin are added, and the DPV response curve indicates a linear increase in rutin concentration from 10 to 200 μ M in 0.1 M PBS [59-61]. Figure 4b shows a calibration plot with a sensitivity of 0.06087 μ A/ μ M and a detection limit of 11 nM (S/N=3). The detecting properties of this study are compared to several of the recently described rutin sensors in Table 1. It indicates that the performance of the CuO@f-CNTs based sensor is comparable with previous rutin sensors, and the wider linear range is observed because of the synergistic catalytic effect of f-CNTs and CuO nanoparticles in the composite [62-64].

Electrodes	Techniqu	Detection	Linear range	Ref.
	e	limit (nM)	(µM)	
CuO@f-CNTs/GCE	DPV	11	10-200	This study
Pt@rGO@MWCNTs	DPV	5	0.05-50	[65]
Methyl-2-pyrrolidone/GO	DPV	3.2	$10^{-3} - 1.25$	[66]
NiCo ₂ O ₄ /rGO	DPV	10	0.1-150	[67]
Polyfurfural film/GCE	DPV	0.025	0.001-10	[68]
Carboxylated-MWCNT@ polythionine @Pt	DPV	1.7	0.01-15	[69]
β-cyclodextrin –Au@ 3, 4, 9, 10-perylene	DPV	4.4	0.01-10	[70]
tetracarboxylic acid – single-walled carbon				
nanohorns/GCE				
Molecularly imprinted polypyrrole/GO-	DPV	5	0.01-1	[71]
MWCNTs/GCE				
Ionic liquid/CPE	SWV	10	0.04 - 10	[72]
Pyridinium-typed ionic liquid/CPE	CV	358	0.5 - 100	[73]
MWCNTs/Au electrode	CV	10	0.02-5	[74]

 Table 1. Comparison the sensing properties of this study with some of the recently reported rutin sensors

CPE: Carbon paste electrode; SWV: Square wave voltammetry; CV: Cyclic voltammetry

The influence of inorganic ions and organic compounds on the detection of rutin was also investigated. Table 2 shows the results of DPV measurements in 0.1 M PBS with pH 7.4 under the addition of rutin and 5-fold excesses of interfering chemicals at a scan rate of 25 mV/s in the presence of rutin and 5-fold excesses of interfering substances [75, 76]. Table 2 demonstrates that there are no significant signal changes for rutin oxidation peak currents at 0.23 V, meaning that the addition of 10-fold excesses of interfering chemicals has no effect on rutin determination. These findings indicate that the proposed sensor has a high selectivity for rutin detection [77-79].

To evaluate the validity and capability of the developed method, the modified electrode was employed for the determination of rutin content in the prepared real sample of Cinnamomum camphora (L.) J. Presl at a potential of 0.23 V in 0.1 M PBS with pH 7.4 at scan rate of 25 mV/s. Figure 5 exhibits the DPV measurements and obtained calibration graph of prepared real sample, demonstrating the rutin concentration in the prepared real sample is 3.02μ M, which is in good agreement with the report of Chen et al. [23].

Table 2. Results of study the interfering effect inorganic ions and organic molecules on determination rutin using CuO@f-CNTs/GCE at potential of 0.23 V in 0.1 M PBS with pH 7.4 at scan rate of 25 mV/s.

Substance	Added (µM)	Peak current (µA) at	RSD (%)
		0.23 V	
Rutin	20	1.2101	±0.0977
Cystine	100	0.1233	±0.0118
Glucose	100	0.2210	±0.0111
Dopamine	100	0.1211	±0.0018
Ascorbic acid	100	0.0222	±0.0012
Oxalic acid	100	0.0704	±0.0033
Uric acid	100	0.0205	±0.010
Citric acid	100	0.1980	±0.0025
Tartaric acid	100	0.1100	±0.0015
p-nitrophenol	100	0.1251	±0.0018
Hydroquinone	100	0.1440	±0.0029
2,4-dinitrophenol	100	0.3004	±0.0102
Histidine	100	0.1451	±0.0038
Glycine	100	0.1305	±0.0045
Asparagine	100	0.1164	±0.0037
Aspartic acid	100	0.0720	±0.0070
Valine	100	0.1100	±0.0100
KCl	100	0.1022	±0.0012
MgSO ₄	100	0.1062	±0.0039
CaCl ₂	100	0.1008	±0.0045
$Zn(AC)_2$	100	0.2018	±0.0019
NaNO ₃	100	0.2209	±0.0100
NH ₄ F	100	0.0904	±0.0021



Figure 5. (a) DPV measurements of CuO@f-CNTs/GCE in in prepared real sample of Cinnamomum camphora (L.) J. Presl at potential of 0.23 V in 0.1 M PBS with pH 7.4 at scan rate of 25 mV/s; (b) related calibration plot.

Moreover, Table 3 presents that the recovery and RSD values by the standard addition method are 99.13% to 99.56% and 2.21% to 4.03%, respectively, indicating the proposed system has a high detection accuracy and is valid for the determination of rutin application in real herbal samples.

added (µM)	Found (µM)	Recovery (%)	RSD (%)
5.00	4.96	99.20	4.03
10.00	9.95	99.50	3.33
15.00	14.87	99.13	3.21
20.00	19.91	99.55	3.44
25.00	24.89	99.56	3.97

Table 3. The analytical results for determination of rutin in the Cinnamomum camphora (L.) J. Presl.

4. CONCLUSION

In this paper, the production of CuO@f-CNTs nanocomposite for GCE modification and application as an electrochemical sensor for rutin measurement in leaves of Cinnamomum camphora (L.) J. Presl was presented. CuO@f-CNTs nanocomposite was found to be made up of f-CNTs with CuO nanoparticles in a spherical shape that was formed in small sizes and irregular forms, according to the surface morphology and crystal structure of modified electrochemical response to rutin determination, with a linear range of 10 to 200 μ M, yielding a sensitivity of 0.06087 μ A/ μ M and a detection limit of 11 nM.

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