Electrochemical Sensor for Determination of Baicalin Based on Carbon Nanospheres

L. Shi, W. Liu[*] and Z.F. Wang[*]

Department of Chemistry, Honghe University, Mengzi 661199, P.R. China

*Corresponding authors: E-mail: liuwei4728@126.com; wangzefeng841006@163.com

A novel electrochemical sensor based on the carbon nanospheres for the sensing of baicalin is reported. The electrochemical sensing platform was developed using the prepared carbon nanospheres. The colloidal carbon nanospheres were successfully synthesized by hydrothermal method. The resident porosity of porous carbon nanospheres will promote diffusion of baicalin molecules through interconnected micropores and will be benefited for increasing the detection sensitivity. The results show that the linear response range for baicalin is 0.1-2 μM, with the detection limit of 0.033 μM. The method maybe open up a new possibility for the wide spread use of electrochemical sensors for monitoring of flavonoid drugs owing to its advantages of simple preparation, low cost, high sensitivity.

Keywords: Carbon nanospheres, Baicalin, Electrochemical sensor, Shuanghuanglian oral liquid.

INTRODUCTION

Flavonoid is a class of polyphenolic compounds distributed throughout the plant kingdom, which has been widely studied in drug exploitation due to their specific effects on human health. Baicalin belonging to the class flavonoid, are mainly isolated from the crude drug[1]. Recently, baicalin has been widely used in medical practice as therapeutical medicine due to the multiple bioactivities including antioxidant[2], antivirus[3], anti-inflammation[4,5] and anticancer activities[6,7] and antiallergy[8]. Based on the above pharmacological effects, different kinds of concentrated composite herbal preparations that contain baicalin as the active component have been used clinically as therapeutical medicine. However, baicalin overdose will result in severe side effects. Therefore, the accurate determination of baicalin is an important and significant work in clinical. Therefore, establishment of simple, fast, practical and low cost analytical techniques for the determination of baicalin is of great significance in clinics and pharmaceutics.

Several methods have been developed for the analysis of baicalin such as attenuated-total-reflectance infrared and near infrared[9], high performance liquid chromatography[10], liquid chromatography-tandem mass spectrometric[11], one-step immuno chromatographic assay and indirect competitive enzyme-linked immunosorbent assay[12], capillary liquid chromatography with electrochemical detection[13]. Although these methods have been documented, these methods either required complicated sample preparation, or suffered from low sensitivities and specificities. The electrochemical methods, with the merits of accuracy, simplicity and ease of on-site determination, have attracted considerable attention for the analysis of flavonoid drugs. At present, some nanomaterials such as nano-metal oxide[14], carbon nanotubes[15] and gold nanoparticles[16] have been used to improve the electrochemical performance for these flavonoid drugs. All of these demonstrated that nanomaterials play an important role in constructing baicalin sensors. Therefore, development a highly sensitive and simple detection method for baicalin is one of most important analytical challenges.

Carbon nanomaterials have attracted considerable attention in electrochemical biosensors because of their extraordinary physical properties and remarkable conductivities[17]. Recently, carbon nanospheres have received special attention in the areas of nanoscience and nanotechnology because of the tunability of particle size and shape as well as the resident porosity that promotes diffusion of guest molecules through interconnected micropores. The excellent property of carbon nanospheres is benefit for improving the electrochemical performance of sensors. Moreover, the carbon nanospheres can be prepared by an absolute "green" approach that involves none of the organic solvents, initiators, or surfactants[18]. More importantly, few reports have been documented about the determination of flavonoids drugs by electrochemical technique. Meantime carbon nanospheres were used to detect the flavonoids drugs hasn't been reported.

In this paper, we report an electrochemical sensor for sensitive detection of baicalin based on carbon nanospheres as the sensor platform. Carbon nanospheres can act as an enhanced electrochemical material for the determination of

baicalin. **Scheme-I** shows the procedure for sensing baicalin by an electrochemical strategy. The electrochemical behavior of baicalin at the carbon nanospheres modified glassy carbon electrode exhibited much better electrochemical performance than that at the bare glassy carbon electrode. The linear range of baicalin is 0.1-2 µM, with the detection limits of 0.033 µM. The applicability of the sensor was also successfully demonstrated for the analysis of baicalin in Shuanghuanglian oral liquid.

EXPERIMENTAL

Baicalin were purchased from Certification Institute of Chinese Pharmaceutical and Biological Products. Other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with doubly distilled water. Shuanghuanglian oral liquid (a kind of oral Chinese herbal medicine) was diluted about 12,000 times before determination.

Synthesis of colloidal carbon spheres: The colloidal carbon spheres were prepared followed by previously reported method[18,19]. Glucose (3.6 g, analytical purity, Sinopharm Chemical Reagent Co. Ltd.) was dispersed in double-distilled water (40 mL) by sonicating for 0.5 h and formed a clear solution. And then the resulting solution was placed in a 40 mL Teflon-sealed autoclave and maintained at 180 ℃ for 4 h. The black or puce products were isolated by centrifugation (12000 rpm, 8 min) cleaned four times with double-distilled water and finally redissolved in double-distilled water.

Electrochemical measurement: Electrochemical measurements were carried out on CHI660D electrochemical workstation (ChenHua Instruments Co., Shanghai, China). The glassy carbon electrode (GCE, 3 mm in diameter) was polished carefully with alumina slurry (1.0, 0.3 and 0.05 µm) and rinsed with distilled water followed by sonication in nitric acid (1:1), ethanol and distilled water, then dried in a stream of nitrogen gas. A conventional three-electrode system was used, including an Ag/AgCl reference electrode, a Pt wire counter-electrode and the modified electrode as the working electrode. 6 µL of 0.5 mg/mL carbon nanospheres were carefully cast on the surface of the well-polished glassy carbon electrode and dried in air. And then, the modified electrode was used as the working electrode for all electrochemical studies. The electrochemistry behavior was characterized by cyclic voltammograms and differential pulse voltammograms.

RESULTS AND DISCUSSION

Preparation and characterization of the carbon nanospheres: The monodisperse colloidal carbon nanospheres were prepared from aqueous glucose solutions by hydrothermal synthesis. The approach is an absolute 'green' method and the synthetic procedure involves none of the organic solvents, initiators, or surfactants that are commonly used for the preparation of polymer nanospheres. Moreover, the obtained carbon nanospheres possess some functional groups at the surface of carbon nanospheres that will be benefit for the detection of baicalin.

Electrochemical behaviour of baicalin on carbon nanospheres modified glassy carbon electrode: It is well known that carbon nanospheres are able to promote diffusion of guest molecules through interconnected micropores of carbon nanospheres. This characteristic makes it increase the sensitivity of detection for some common organic compounds (including baicalin) through the formation of supermolecular complexes. The baicalin structure presents functional OH groups attached to ring structures that can be electrochemically oxidized. For illustrating the catalytic effect of carbon nanospheres for baicalin, a comparison experiment was performed using a carbon nanospheres modified glassy carbon electrode and bare glassy carbon electrode. Fig. 1 shows the cyclic voltammograms of 0.56 µM baicalin for bare glassy carbon electrode (a) and carbon nanospheres modified glassy carbon electrode (b) in 0.2 M CH$_3$COOH-CH$_3$COONa (pH=4.7) buffer solutions. The baicalin exhibits very weak redox peaks at the bare glassy carbon electrode. However, for carbon nanospheres modified glassy carbon electrode, a pair of significant redox peaks are observed in the potential window between -0.2 and 0.5 V with Epa = 0.29 V and Epc = 0.27 V (ΔEp = 20 mV). The peak currents show a remarkable increase compared to those observed

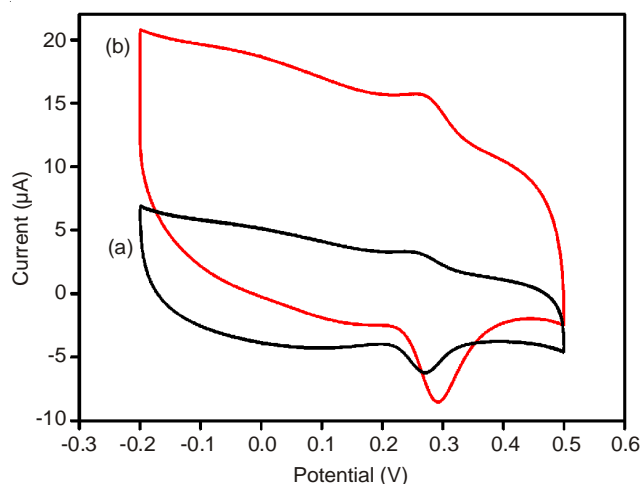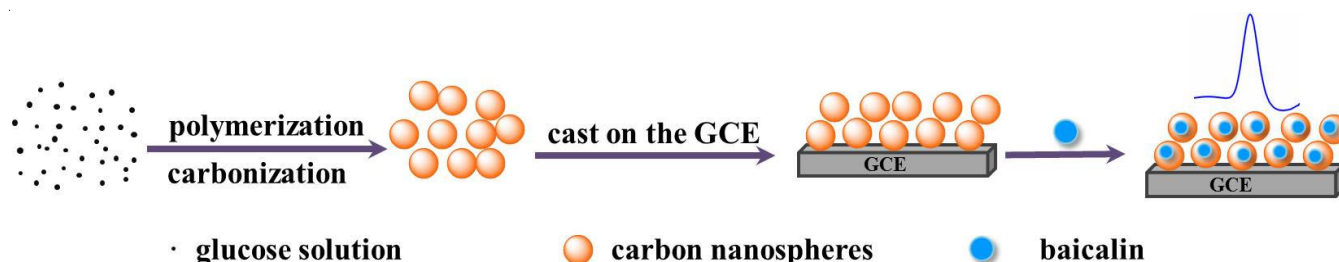Fig. 1. Cyclic voltammograms of 0.56 µM baicalin at bare glassy carbon electrode (a) and carbon nanospheres modified glassy carbon electrode (b) in pH=4.7 CH$_3$COOH-CH$_3$COONa buffer solutions (scan rate: 20 mV s^{-1}). And the accumulation time is 5 min

· **glucose solution**   ◯ **carbon nanospheres**   ● **baicalin**

Scheme-I: Schematic of the procedure for the synthesis of carbon nanospheres and sensing baicalin

on the bare glassy carbon electrode. These results demonstrate that the carbon nanospheres have sensitive catalytic effect for baicalin. That maybe due to the interaction between carbon nanospheres and baicalin can further enhance the accumulation effect of arbon nanospheres modified glassy carbon electrode, accordingly increase the concentration of the analytes on the interface of the modified electrode, which results in remarkable peak currents enhancement.

Optimization of the determination conditions

Influence of supporting electrolyte: The types of supporting electrolytes played a key role in the voltammetric responses of baicalin. So the influence of some electrolytes, such as acetate buffer solution, citric acid buffer solution, NaCl buffer and KCl buffer, were studied in a 0.56 µM baicalin solution. The effect of types of supporting electrolytes on the electrochemical response of the carbon nanospheres modified glassy carbon electrode towards the detection of baicalin was investigated by cyclic voltammograms. As shown in Fig. 2, the type of supporting electrolytes has a significant effect on the peak potentials of baicalin. A very weak oxidation peak is observed at +0.35 V and no any reduction peak is observed between -0.2 V and 0.5 V in pH=3.0 HCl-sodium citrate (Fig. 2a). A pair of redox peaks are observed in the potential window between -0.2 and 0.5 V with the oxidation peak at 0.33 V and the reduction peak at 0.28 V in pH=3.6 citric acid-sodium citrate buffer solution (Fig. 2b). When pH=4.6 CH$_3$COOH-CH$_3$COONa buffer solutions were used as supporting electrolytes, a pair of very significant redox peaks are observed with the oxidation peak at 0.29 V and the reduction peak at 0.27 V (Fig. 2c). However, further increase the pH value of supporting electrolytes caused an extremely significant decrease in the peak current and the peak potentials shifted to negative values. As can be seen in Fig. 2d and 2e, a very weak oxidation peak is observed in KCl (pH=6.9) solution and in NaCl (pH=7.0) solution, respectively. Meantime, the oxidation peak potential at 0.26 V for KCl (pH=6.9) solution and at 0.25 V for NaCl

(pH=7.0) solution. And the reduction peaks are almost disappeared. The results show that the peak potentials shifted to negative values as the pH increased. The higher peak current and the best peak shape could be obtained in CH$_3$COOH-CH$_3$COONa solution. Therefore, 0.2 M CH$_3$COOH-CH$_3$COONa (pH=4.6) buffer solutions was chosen for the determination of baicalin.

Electrochemical sensing of baicalin on carbon nanospheres modified glassy carbon electrode: Differential pulse voltammetry was employed to investigate the electrochemical sensing performance of carbon nanospheres modified glassy carbon electrode towards baicalin under the selected conditions mentioned above. As can be seen in Fig. 3, the peak currents are increased with the gradual addition of baicalin, showing the catalytic property of the modified electrode in oxidation of baicalin. As presented in the inset of Fig. 3, the peak current are linear with the concentration of baicalin in the range of 0.1-2 µM. The linear equation of ip (µA) = 21.20 + 35.79C (µM) with a correlation coefficient of 0.9987 is obtained. The detection limit of baicalin is 0.033 µM at the signal-to-noise ratio of 3, which is lower than that of other electrochemical sensors for flavonoid drug detection, such as multiwall carbon nanotubes modified glassy carbon electrode (0.1 µM)[20], capillary electrophoresis with electrochemical detection (0.548 µM)[6], gold nanoparticles modified glassy carbon electrode (0.1 µM)[16]. This excellent electrochemical sensing performance is ascribed to the good properties of carbon nanospheres.
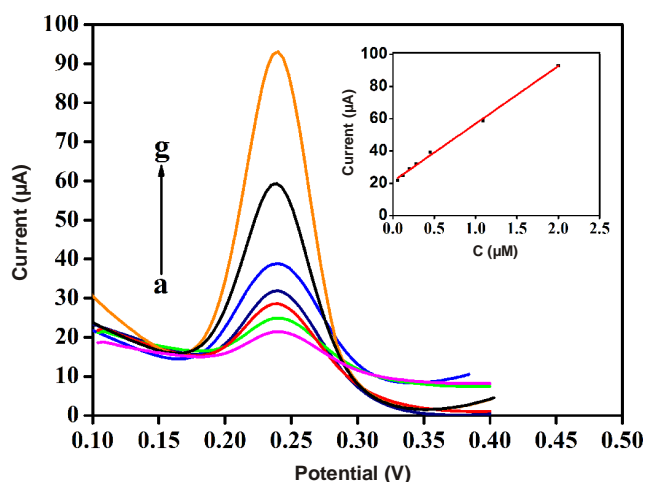
Fig. 3.   Differential pulse voltammograms of carbon nanospheres modified glassy carbon electrode in 0.2 M HAc-NaAc buffer solutions (pH 4.6) in the presence of baicalin with different concentrations (from bottom to up: 0.06, 0.12, 0.2, 0.28, 0.45, 1.09, 2.00 µM). Acumulation time: 5 min; pulse amplitude: 50 mV. The inset is the calibration curves of baicalin

Study on interfering agents: In consideration of the possible analytical application of the method, the effects of several possible interferences on the detection ability were evaluated. We fixed the concentration of baicalin at 5.6 µM and then added various amounts of the species in excess under the same experimental conditions. The results indicate that 10-fold excess of uric acid and 50-fold excess of ascorbic acid and glucose are acceptable. Furthermore, the interference of metal ions was investigated use the same method. It was also
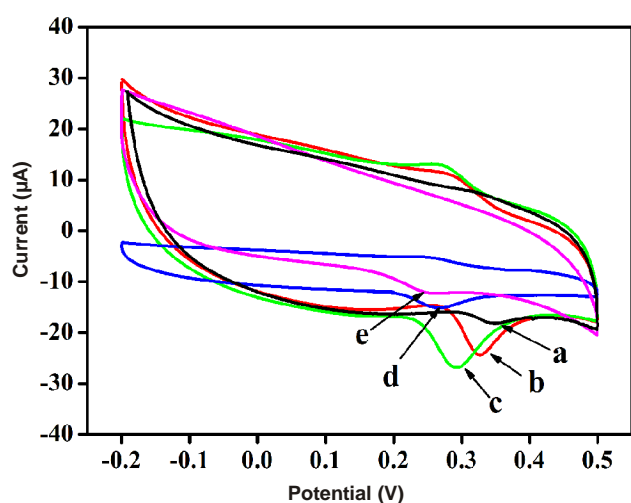

Fig. 2.   Cyclic voltammograms of 0.56 µM baicalin at carbon nanospheres modified glassy carbon electrode in different solution: (a) 0.1 M HCl-sodium citrate (pH=3.0), (b) 0.1 M citric acid-sodium citrate (pH=3.6), (c) 0.2 M HAc-NaAc buffer solutions (pH=4.6), (d) 0.5 M KCl solution (pH=6.9) and (e) 0.5 M NaCl solution (pH=7.0), the scan rate is 50 mV s^{-1}

observed that 50-fold excess of Ca(II), Fe(III), Zn(II) and Mg(II) metal ions have no effects on the detection of baicalin. Therefore, it suggested that our method has a good selectivity for the determination of baicalin.

Determination of baicalin in real samples: To demonstrate the performance of the sensor in real sample analysis, the content of baicalin in Shuanghuanglian oral liquid was employed to evaluate the applicability of method. Shuanghuanglian oral liquid was diluted about 12,000 times before determination and the standard curve method was employed to calculate the baicalin content. For six parallel determinations, the obtained relative standard deviation (RSD) was 3.50 % and the average content of baicalin in Shuanghuanglian oral liquid was 10.28 g L^{-1}. After determination, standard baicalin was added in the six samples respectively and the average recoveries were calculated again (Table-1). For testing the accuracy of the method, the same samples (Shuanghuanglian oral liquid) were also determined by high performance liquid chromatography (HPLC) method and the results were listed in Table-1. A RSD value is 2.65 % for detection of baicalin in Shuanghuanglian oral liquid by HPLC. The electrochemistry method is simpler and more time-saving than HPLC. Therefore, a more convenient, accurate and reliable method was developed for baicalin detection in commercial samples.

TABLE-1
RECOVERIES OF BAICALIN IN
SHUANGHUANGLIAN ORAL LIQUID

Electrochemical sensor (n=6)			HPLC (n=3)			
Amount found (mg L^{-1})	RSD (%)	Stand added (mg L^{-1})	Total found (mg L^{-1})	Average recovery (%)	Amount found (mg L^{-1})	RSD (%)
0.857	3.50	0.840	1.670	96.79	0.88	2.65

Conclusion

The present work shows that colloidal carbon nanospheres have been successfully synthesized by hydrothermal synthesis. The obtained carbon nanospheres are able to promote diffusion of guest molecules through interconnected micropores of carbon nanospheres and make it increases the sensitivity of detection for some common organic compounds (including baicalin) through the formation of supermolecular complexes. A simple and enhanced electrochemical sensing platform for the determination of baicalin has been successfully constructed. It is found that the resultant sensor has good sensitivity and broad linear range for the analysis of the studied targets. Moreover, the method was also applied for the determination of baicalin in Shuanghuanglian oral liquid samples with satisfactory results. The research provides a promising basis for analytical baicalin.

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