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ScienceDirect

Biosensors and Bioelectronics 23 (2008) 1680–1685

BIOSENSORS & BIOELECTRONICS

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# Electrochemical determination of uric acid at ordered mesoporous carbon functionalized with ferrocenecarboxylic acid-modified electrode

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Received 21 October 2007; received in revised form 20 December 2007; accepted 29 January 2008
Available online 7 February 2008

## Abstract

Ordered mesoporous carbon (OMC) functionalized with ferrocenecarboxylic acid (Fc) was used to modify the glassy carbon (GC) electrode. The characterization of OMC–Fc shows that, after anchoring ferrocene on the mesoporous, ordered mesostructure of the material (OMC–Fc) remains intact and Fc is electrochemically accessible. The obtained OMC–Fc-modified electrode was used to investigate the electrochemical behavior of uric acid (UA). UA oxidation is catalyzed by this electrode in aqueous buffer solution (pH 7.3) with a decrease of 200 mV in overpotential compared to GC electrode. The detection and determination of UA in the presence of ascorbic acid (AA), the main interferent, were achieved. The voltammetric signals due to UA and AA were well separated with a potential difference of 308 mV, a separation that can allow the simultaneous determination of UA and AA. With amperometric method, at a constant potential of 375 mV, the catalytic current of UA versus its concentration shows a good linearity in the range 60–390 μM ($R = 0.998$) with a detection limit of 1.8 μM (S/N = 3). These results are not influenced by the presence of AA in the sample solution. With good stability and reproducibility, the present OMC–Fc-modified electrode was applied in the determination of UA content in urine sample and satisfactory results were obtained.

*Keywords:* Mesoporous carbon; Ferrocene; Uric acid; Ascorbic acid; Urine sample

## 1. Introduction

Abnormal levels of uric acid (UA), the principal end product of purine metabolism, are usually associated with several disorders such as gout and Lesch–Nyhan syndrome (Harper, 1977). Gout occurs when sodium urate crystals are deposited in the joints, soft tissue, bursae and tendons. Lesch–Nyhan syndrome is an X-linked chromosome disorder that results in the absence of the enzyme hypoxanthine–guanosine phosphoribosyl transferase (HGPRT). Hyperuricemia (elevated concentrations of UA) may indicate other medical conditions such as kidney injury (Heptinstall, 1966), leukemia (Krakoff, 1965) and pneumonia (Puig and Mateos, 1994).

Therefore the determination of the UA with a simple method is essential because it serves as a marker for the detection of the above diseases. For this aim, various methods have been used, such as chemiluminescence (Wu et al., 2005), chromatography (Perello et al., 2005), spectrofluorometry (Martinez et al., 2003), enzymatic system (Akyilmaz et al., 2003) and so forth. The electrochemical techniques for the detection of uric acid and ascorbic acid (the main interferent) have received considerable interest in recent years because of their higher selectivity and rapid detection. The main problem of the electrochemical techniques is the interferences of other electroactive compounds and the oxidation requires high overpotentials (Senthilkumar et al., 2005). To solve this problem, most of the attention has been focused on electrode surface modification by some materials including carbon materials (Ardakani et al., 2006), biomolecule materials (Lin and Jin, 2005), polymers (Roy et al., 2004), organic matters (Raj and Ohsaka, 2003), single-walled carbon nanotubes (SWCNT) (Santhanam et al., 2005) and others.

Besides the above mentioned carbon-based materials, there has been significant interest in the development of a novel carbon material, ordered mesoporous carbons (OMCs), owing to their considerable properties, such as uniform and tailored pore structure, high-specific surface area, large pore volume and chemical inertness (Jun et al., 2000; Joo et al., 2001). To the best of our knowledge, there are few reports on the electrochemical deter-

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doi:10.1016/j.bios.2008.01.026

mination of uric acid at these materials. The electrochemical properties of the OMC and its eletroanalytical application for the determination of dopamine have been reported (Jia et al., 2007), but UA was not studied. In this laboratory, it has also been shown that these electrochemical and electrocatalytic properties of OMC are improved when polyoxometalates are immobilized on the channel surface of these materials (Zhou et al., 2007a).

In the present work, the OMC properties are improved with means of immobilization of ferrocene (Fc) on the OMC. The obtained OMC–Fc-modified glassy carbon (GC) electrode is applied in the detection and determination of uric acid.

## 2. Experimental

### 2.1. Materials

Ferrocenecarboxylic acid (Fc) (from Yixing Weite Petrochemical Additives Plant, China) was 98% purity and used as such. Uric acid (UA) was purchased from Urchem. L-Ascorbic acid (AA) was from Beijing Huagong Chang (China). Nafion was from Sigma–Aldrich. All other chemicals were of analytical reagent grade and were used as received. Double distilled water was used to prepare aqueous solutions.

### 2.2. Preparation of the modified electrodes

SBA-15, as the template, was prepared as discussed in the literature (Zhao et al., 1998) using Pluronic P123 (non-ionic triblock copolymer, $EO_{20}$ $PO_{70}$ $EO_{20}$) as a surfactant and TEOS (tetraethoxysilane) as a silica source. OMC was synthesized according to the previous reported work (Jun et al., 2000) with sucrose as the carbon source.

The obtained OMC was functionalized with ferrocenecarboxylic acid (Fc). 50 mg of OMC were dispersed in 80 ml of 2 M $HNO_3$ and the mixture was stirred for 5 min at room temperature. After dispersing 23 mg of Fc into 20 ml of 2 M $HNO_3$ and stirring, the solution was added to the above dispersion of OMC in $HNO_3$. The mixture was stirred for 6 h and centrifuged at 3500 rpm. The OMC–Fc was separated from the mixture and was washed with ethanol to remove the unreacted Fc.

The GC electrode was polished before each experiment with 1, 0.3 and 0.05 μm alumina powder, respectively, rinsed thoroughly with doubly distilled water between each polishing step. Then it was washed successively with a solution of nitric acid + acetone (v/v = 1:1) and doubly distilled water in ultrasonic bath and dried in air. 3 mg of OMF-Fc were added to 6 ml of PBS solution (pH 6.1). After addition of 0.5 ml of Nafion, the mixture was stirred for 6 h. The colloid (5 μl) was dropped on the surface of the GC electrode and this was allowed to dry at room temperature.

### 2.3. Measurements

Electrochemical experiments were carried out using a CHI 830b Electrochemical Analyzer (CH Instruments, Shanghai Chenhua Instrument Corporation, China) in a conventional three-electrode cell. The working electrode used was GC electrode (Model CHI104, 3 mm diameter) or the modified electrode. A platinum electrode was taken as the counter electrode and an Ag/AgCl (in saturated KCl solution) electrode served as the reference electrode. The sample solutions were purged with purified nitrogen for at least 15 min to remove oxygen prior to the beginning of a series of experiments. Small angle X-ray diffraction (XRD) patterns were obtained on an X-ray Pmax 2200 PC (Rikagu, Japan) operating at 40 kV and 40 mA and using Cu Kα radiation. Amount of ferrocene carboxylic was determined by measuring inductively coupled plasma emission spectrographs with Leeman Prodigy, ICP-AES (USA). Nitrogen adsorption and desorption isotherms were measured on ASAP2020 (Micromeritics, USA). The pore size distributions (PSDs) were calculated by the BJH method.

## 3. Results and discussions

Some physico-chemical characteristics have been determined before and after grafting ferrocene on OMC. Three peaks that can be indexed as (1 0 0), (1 1 0), and (2 0 0) diffractions are associated with *p*6*mm* hexagonal symmetry in OMC and OMC–Fc (Fig. 1 Supporting information). This indicates that, even if a part of the structure is destroyed by the Fc immobilization and the acid treatment before formation of the OH groups, the hexagonal order remains intact after functionalization. Also, all samples exhibited an adsorption–desorption hysteresis loop indicative of the occurrence of pores. Moreover, the samples exhibited narrow size distributions (Fig. 2 Supporting information). These findings indicate that the mesostructure of OMC is not destroyed after immobilization of Fc and it can be concluded that the incorporation of ferrocene as electroactive guest preserves the material properties.

### 3.1. Electrochemical characterization

The electrochemical behavior of OMC has been discussed elsewhere (Jia et al., 2007) and it has been shown that this property can be improved by utilizing some electroactive substances (Zhou et al., 2007a). Here another electroactive substance, ferrocene, is immobilized on OMC. The electrochemical characterization was carried out with GC electrode modified with OMC–Fc in 0.1 M $LiClO_4$ + 0.1 M phosphate buffer solution (pH 7.3). GC electrode modified with OMC is also used for comparison (Fig. 3 Supporting information). The different modified electrodes will be symbolized as OMC electrode and OMC–Fc electrode. Measurements were performed in a blank electrolyte solution free of any electroactive probe.

First, the current of OMC electrode is nearly uniform over the whole potential range, suggesting the electrochemical behavior of carbon materials but with relatively high background current (Brajter-Toth et al., 2000). Second, the appearance of the peaks at approximatively 0.39 and 0.31 V for oxidation and reduction processes, respectively, can confirm the presence of ferrocene in the OMC channel and its electrochemical accessibility. The separation between the anodic and cathodic peak potentials for the OMC–Fc electrode at 20 mV/s is 80 mV. However, the cathodic peak current is a little smaller than the anodic peak current. As