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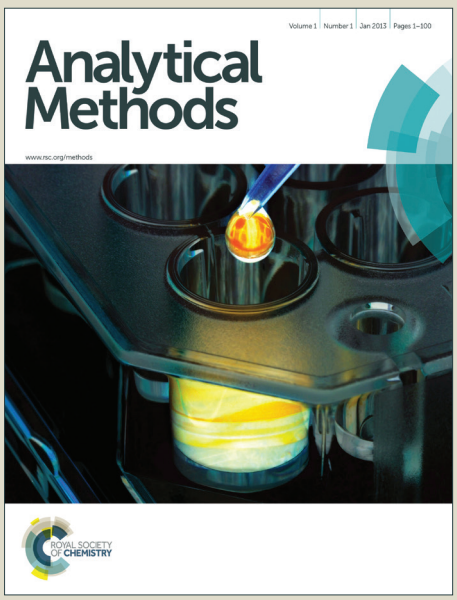

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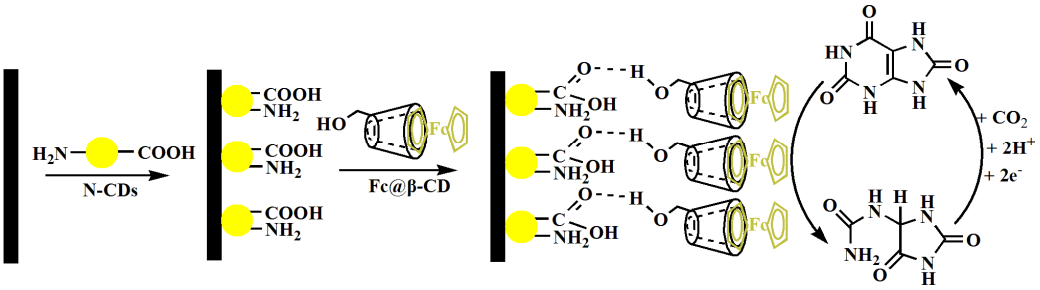
Graphical abstract



Scheme The reaction process of UA on the surface of Fc@β-CD/CNDs/GCE.

Nitrogen-doped carbon dots/ferrocene@β-cyclodextrin composite as an enhanced material for sensitive and selective determination of uric acid

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Abstract

A simple and convenient method for the synthesis of nitrogen-doped carbon dots (N-CDs) was reported. Layers of N-CDs and ferrocene@β-cyclodextrin (Fc@β-CD) host-guest complex were modified on a glassy carbon electrode (GCE) which was used for highly selective and sensitive detection of uric acid (UA). Under the optimal conditions, Compared to bare, N-CDs and Fc@β-CD modified electrodes, the Fc@β-CD/N-CDs/GCE had higher catalytic activity toward the oxidation of UA. The differential pulse voltammetry (DPV) was as the analytical technique for detection of UA, the observed linear range for the determination of UA concentration was from 5 μM to 120 μM with the detection limit was estimated to be 0.08 μM (3S/N). Meanwhile, it was applied to determine uric acid in spiked samples with satisfactory results.

Keywords: *Nitrogen-doped carbon dots, β-Cyclodextrin, Ferrocene, Uric acid, Electrosensor*

1. Introduction

Uric acid (2, 6, 8-trihydroxypurine, UA) is the end product of purine metabolism and the important biological molecule in the body fluid. UA abnormalities are the symptoms of several diseases, such as gout, hyperuricaemia, Lesch-Nyhan syndrome and renal failure. Therefore, it is very important to monitor the concentration of UA in biological fluids.[1] Till now many instrumentals methods for UA determination have been developed, such as electrochemical techniques,[2-4] spectrofluorometry,[5] chemiluminescence,[6-8] chromatography,[9,10] enzymatic[11,12] and so on. The direct electrochemical method for UA analysis has attracted wide attention in recent years because of a simple procedure, high selectivity and rapid detection. Unfortunately, other electroactive compounds which coexist in the body fluid, such as ascorbic acid (AA) and dopamine (DA), will interfere with monitoring UA. Therefore, it is necessary to develop a new electrochemical sensor to monitor the UA in the presence of AA and DA.

Carbon dots (CDs) are a class of 'zero-dimensional' carbon nanomaterials[13-16] that are accidentally discovered during the electrophoretic purification of single-walled carbon nanotubes derived from arc-discharge soot.[17,18] Because of unique and novel properties, such as excellent water solubility, biocompatibility, resistance to photo-bleaching, low toxicity, high stability and remarkable conductivity, [19,21] CDs had been used in biological imaging,[22,23] electrochemical sensors,[24] light-emitting diodes,[25] nano-carriers for gene delivery[26] and so on. Doping heteroatoms can effectively tune CDs intrinsic properties, including electronic characteristics, surface and local chemical features.[27] The N dopant can inject electrons into carbon-based materials, thus changing the electronic and transport properties and will further enhance their versatile properties.[28] Ferrocene (Fc) is an excellent electron-transfer mediator in the redox reaction of various

electroactive compounds, and can increase current response. β-cyclodextrin (β-CD) is a class of macrocyclic molecule with a cavity. The Fc and β-CD host-guest complex can improve their solubility, electro-stability and bioavailability.[29, 30] These compounds also can be accumulated on the electrode surface. Hence, we describe N-CDs and Fc@β-CD host-guest complex layer modified GCE to detect UA in phosphate buffer solution (PBS) by cyclic voltammetry (CV) and DPV. Meanwhile, this method is efficient, sensitive and simple for routine analysis.

2. Experimental

2.1. Reagents and apparatus

Glucose, ammonia solution, sodium dihydrogen phosphate and disodium hydrogen phosphate dodecahydrate were purchased from Xilong Chemical Co., Ltd. (Guangdong, China), uric acid, dopamine, β-cyclodextrin and ferrocene were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

All other reagents are analytical reagents. All the solutions were prepared with deionized water (18.2 MΩ). All electrochemical experiments were performed with CHI 650D electrochemical workstation (Shanghai Chenhua Instruments Co., China). A conventional three-electrode cell was used for all electrochemical experiments. A bare or modified GCE (3 mm in diameter) as the working electrode, a platinum wire served as the counter electrode, an Ag/AgCl as the reference electrode. The pH measurements were carried out on a PHS-3C type precise pH meter (Shanghai Kangyi Instrument Co., Ltd, China). Ultrasonic instrument was performed on a KQ-250B (Kunshan Ultrasonic Instruments Co., Ltd, China). Ultraviolet visible (UV–vis) absorption spectra were recorded by Mapada UV-1800PC (Shanghai, China). The fluorescence spectra were obtained

by a Varian (America) Cary Eclipse fluorescence spectrophotometer with a 1.0 cm quartz cell (Ex slit 10 nm, Em slit 10 nm). Transmission electron microscope (TEM, JEM-1230, Japan) was used to observe the surface morphology of the film. All experiments were carried out at room temperature.

2.2. Synthesis of N-CDs

N-CDs have been prepared following a previous report.[28] Glucose (2.0000 g) was added to the ammonia solution (30%, 40 mL) and deionized water (100 mL) solution to form pellucid solution. Then the pellucid solution was ultrasonicated (100 W) for 24 h at room temperature, and a yellow solution was obtained. After that, the yellow solution was concentrated by rotary evaporator and dialyzed 24 h with a dialysis tube (molecular weight 1000).

2.3. Electrode preparation and modification

Prior to modification, a GCE was polished with 1.0 μm, 0.3 μm and 0.05 μm alumina powder and then ultrasonic cleaning with distilled water, ethanol and distilled water each for 5 min and dried at room temperature.

Synthesis of the Fc@β-CD/N-CDs composite film: with a micro injector, 5.0 μL of N-CDs solution (1.17 $mg \cdot L^{-1}$) was modified on a clean GCE and left it dry in air at room temperature to obtain N-CDs/GCE. The Fc (0.0010 g) was added to β-CD (10^{-3} M, 1 mL) solution then vigorous ultrasonic treatment 30 min and obtained Fc@β-CD host-guest complex solution. Then 5 uL of Fc@β-CD solution was modified on the surface of the N-CDs/GCE to obtain Fc@β-CD/N-CDs/GCE. For comparison, N-CDs/GCE and Fc@β-CD/GCE were also prepared by the similar method.

3. Results and Discussion

3.1. Characterization of N-CDs and Fc@β-CD

Figure 1 (a) shows the TEM image of N-CDs whose size is approximately 2 nm and the Figure 1 (b) shows the TEM image of Fc@β-CD. The UV-vis absorption spectra and fluorescence spectra of N-CDs are shown in Figure 2a. An intense ultraviolet absorption peak appears at 283nm; the fluorescence excitation and emission wavelengths appear at 326 nm and 426 nm. The groups of N-CDs were identified by FT-IR spectra (Figure 2b). The FT-IR peaks at 3373 cm^{-1} and 1642 cm^{-1} are attributed to the stretching vibrations of O-H and C=O, demonstrate the existence of hydroxyl and carbonyl groups; the peak at 1401 cm^{-1} belong to the stretching vibrations of C-N; and the peak at 1560 cm^{-1} pertain to the deformation vibration of N-H. These properties are consistent with a previous report.[28]

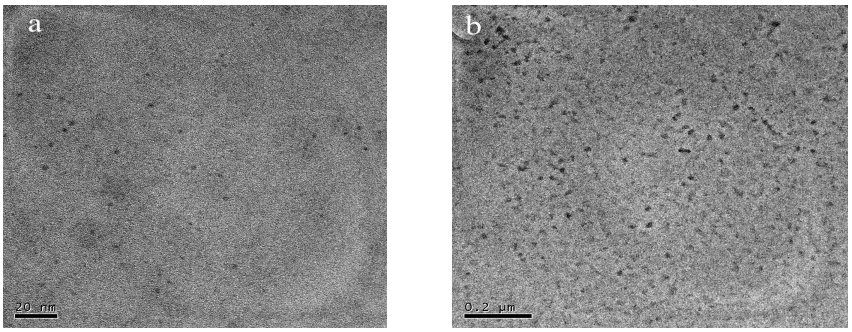


Figure 1. TEM image of the N-CDs (a) and Fc@β-CD (b).

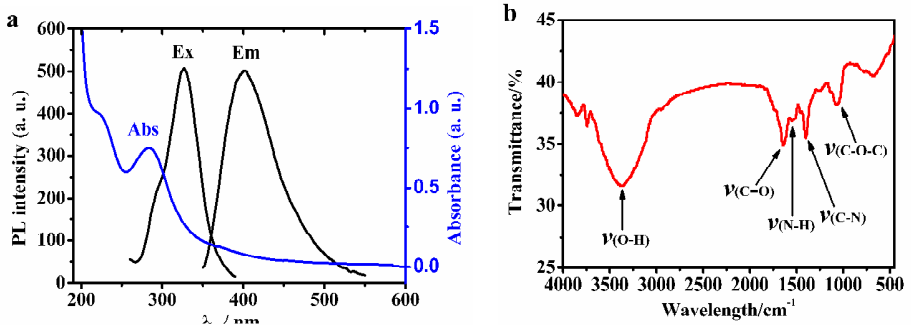


Figure 2. (a) UV-vis absorption spectra (Abs) and fluorescence spectra (Ex, Em) of the N-CDs. (b)

FT-IR spectra of the N-CDs.

3.2. Electrochemical properties of UA on the Fc@β-CD/N-CDs/GCE

The oxidation peak currents of UA on different electrodes in 0.1 M PBS with pH 4.0 were shown in Figure 3. When the Fc@β-CD/N-CDs was modified on the bare electrode, the oxidation peak current of UA increased significantly than the bare GCE, N-CDs/GCE and Fc@β-CD/GCE. A possible reaction mechanism of the Fc@β-CD/N-CDs/GCE with UA was discussed, which was shown in Scheme 1. N-CDs could increase surface area of the GCE because of the small size.[31] Meanwhile, N-doped carbon structures display excellent electrocatalytic performance due to the additional electrons contributed by the N atom.[32] The Fc@β-CD host-guest complex can enhance their solubility, electro-stability and bioavailability. Carboxyl groups on N-CDs with hydroxyl groups on β-CD form hydrogen bonds which promote the electrons transfer of the redox of UA. So the oxidation peak current of UA increased significantly with the Fc@β-CD/N-CDs/GCE than other modified electrode.

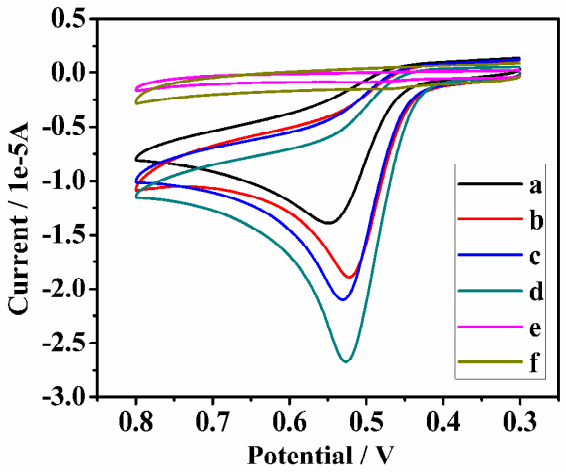
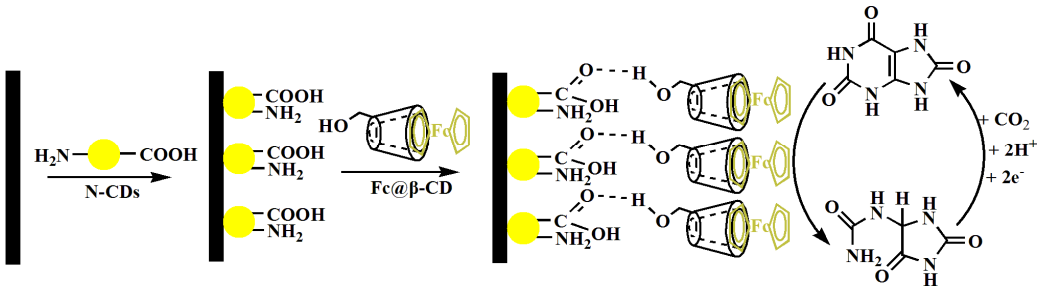


Figure 3. CV of 1 mM UA in 0.1 M PBS with pH 4.0 on GCE (a), N-CDs/GCE (b), Fc@β-CD/GCE (c) and Fc@β-CD/N-CDs/GCE (d), CV of 0.1 M PBS with pH 4.0 on

Fc@β-CD/GCE (e) and Fc@β-CD/CNDs/GCE (f) (scan rate: 0.1 $V \cdot s^{-1}$).



Scheme 1. The reaction process of UA on the surface of Fc@β-CD/N-CDs/GCE.

3.3. Effects of scan rate

In order to better understand the electrochemical mechanism of UA on Fc@β-CD/N-CDs/GCE, the scan rate of UA was also investigated. Figure 4 shows the CV of 0.1 mM UA on the Fc@β-CD/N-CDs/GCE with different scan rate. As the scan rate increases, the oxidation peak current of UA increases linearly well. The relationship of the oxidation peak currents with the square root of scan rate is shown in the inset of Figure 4. The oxidation peak currents (I_{pa}) show a linear relationship with the square root of the scan rate whose equation is I_{pa} / uA = -5.98552 $v^{1/2}$ ($V^{1/2} \cdot s^{-1/2}$) - 0.43391 (R = 0.9953), which suggests that redox process of UA on the Fc@β-CD/N-CDs/GCE is a diffusion process.[11, 33, 34]

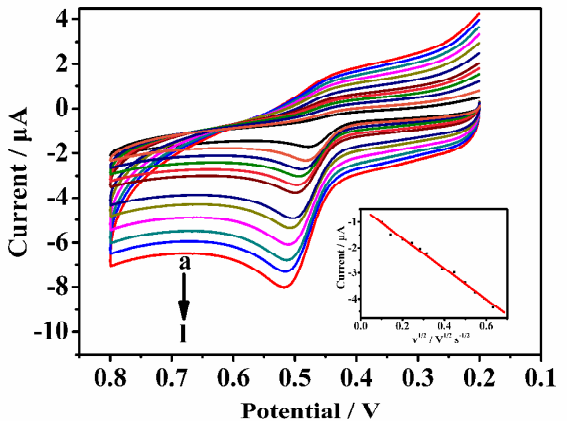


Figure 4. CV of 0.1 mM UA on the Fc@β-CD/N-CDs/GCE in 0.1 M PBS with pH 4.0 at different

scan rate (a → l: 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 $V \cdot s^{-1}$). Inset: the plot of the oxidation peak current relation with the square root of the scan rate.

3.4. Effect of pH

The pH values of the supporting electrolyte have an important effect on the peak current of UA. As shown in Figure 5, oxidation peak potentials (E_{pa}) of UA on the Fc@β-CD/N-CDs/GCE are shifted to more negative values as pH increased ranging from 3.0 to 5.5. Peak potentials is a linearly related to the pH value, and the linear equation is E_{pa} / V = -0.05526 pH + 0.77034 (R = 0.9887) (inset of Figure 5). It indicates that two protons take part in the electrode reactions.[35, 36]

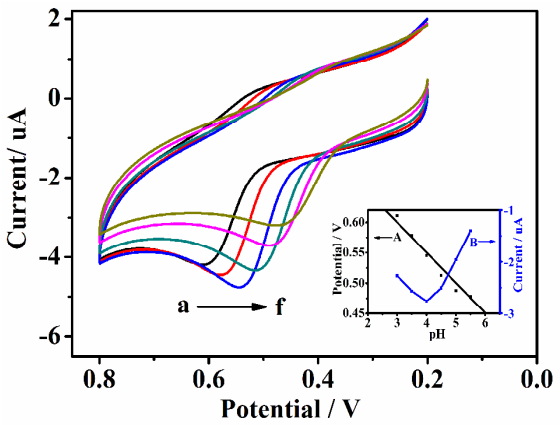


Figure 5. CV of 0.1 mM UA on the Fc@β-CD/N-CDs/GCE in 0.1 M PBS at different pH (a → f: 3.0, 3.5, 4.0, 4.5, 5.0, 5.5) (scan rate: 0.1 $V \cdot s^{-1}$). Inset: the plots of I_{pa} (B) and E_{pa} (A) with pH values.

3.5. Interference study

As we all known, AA and DA often coexists with UA in body fluids. Therefore, it is important to accurately monitor UA in the presence of AA and DA. Figure 6 (a) and (b) shows that when the concentration of AA in the range of 0 to 0.1 mM and DA in the range of 0.01 to 0.1 mM, the peak current of UA is almost constant. Additionally, the linear equation of peak current with different

concentrations of UA is very close in the presence and absence of AA and DA (inset of Figure 7). It indicates that presence of AA and DA are not interfere with monitoring UA.

In addition, other influences from common co-existing substances were also investigated, such as Na^{+}, K^{+}, Ca^{2+}, SO_4^{2+}, Zn^{2+}, glucose and urea. The oxidation peak potential and current of UA was observed almost constantly in the presence of all interferents.

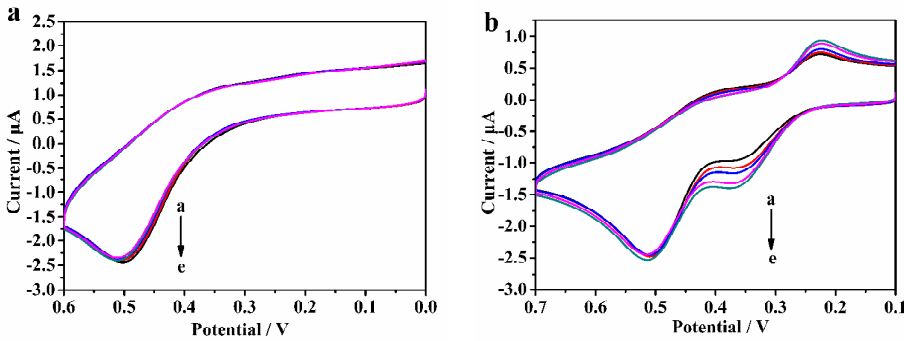


Figure 6. CV of 50 μM UA with the different concentrations of AA (a) (a → e: 0-0.1 mM) and DA (b) (a → e: 0.01-0.1 mM) on the Fc@β-CD/N-CDs/GCE in 0.1 M PBS with pH 4.0. Scan rate: 0.1 $V \cdot s^{-1}$.

3.6. Selective determination of UA in the presence of DA

Owing to high sensitivity, the DPV technology was applied to the quantitative determination UA in the presence of DA. As shown in Figure 7, with increases in the concentration of UA, the peak current intensity gradually increases. Peak intensity is a good linearly related to the concentration of UA in the presence of 20 μM DA on the Fc@β-CD/N-CDs/GCE. The linear equation is I_{Pa} = -0.00897C_{UA} / μM - 0.02189 (R = 0.9980) (inset of Figure 7. A), and the limit of detection is estimated to be 0.08 μM based on S/N of 3. The detection limit of the present biosensor less than or similar to other electrochemical sensors described in the literature. Comparison to other electrochemical sensors is presented in Table 1.

The Fc@β-CD/N-CDs/GCE electrochemical biosensor is stable at least in five days. It demonstrates that Fc@β-CD/N-CDs/GCE electrochemical biosensor can be applied to detect UA.

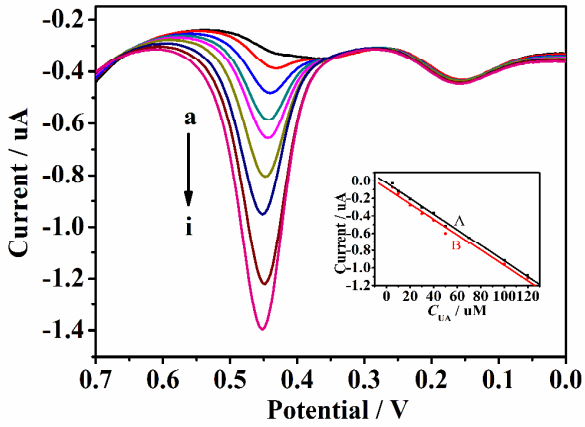


Figure 7. DPV for different concentrations of UA (a → i: 5, 10, 20, 30, 40, 50, 70, 100, 120 μM) in the presence of 20 μM AA and DA on the Fc@β-CD/N-CDs/GCE in 0.1 M PBS with pH 4.0 (scan rate: 0.1 $V \cdot s^{-1}$). Inset: the plots of I_{pa} with C_{UA} in the presence (A) and absence (B) of 20 μM DA.

Table 1. Comparison of some modified electrodes for the determination of UA.

Electrode	Methods	Linearity range (μM)	Detection limit (μM)	Refs.
MWCNT-Plys/GCE	DPV	10-100	2.2	37
OMC-Fc/GCE	DPV	60-390	1.8	38
ERGO-ITO/GCE	DPV	0.3-100	0.3	39
RuOHCF/MWCNT/GCE	DPV	0.90-250	0.599	40
Au/CPE	DPV	6-180	0.71	41
Fc@β-CD/N-CDs/GCE	DPV	5-120	0.08	this work

3.7. Analysis of spike sample

In order to examine the practical performance of the Fc@β-CD/N-CDs/GCE, determinations of

UA in spike samples were investigated. A certain amount of UA was added to the solution, then the recovery can be determined (Table 2). It indicates that the Fc@β-CD/N-CDs/GCE can be used for determining UA.

Table 2. Results of determination of UA in spike samples.

Samples (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD%
40	0	40.70	101.75	0.53
40	10	49.70	99.4	0.44
40	20	62.45	104.08	2.73
40	30	70.12	100.17	0.94

4. Conclusion

In summary, ultrasonic technology，as a fast and simple method，was used to synthesize N-CDs. Then N-CDs and Fc@β-CD complex were layer deposited on GCE to obtain Fc@β-CD/N-CDs/GCE. The Fc@β-CD/N-CDs/GCE can quickly, efficiently and sensitively to detect UA in the presence of DA and the limit of detection is reached to 0.08 μM. It is credible through monitoring of some samples. So the Fc@β-CD/N-CDs/GCE can be used as electrochemistry sensors of UA.

Acknowledgement

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