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# Determination of 5-Hydroxyindole Acetic Acid by Electrochemical Methods with an Oxidized Glassy Carbon Electrode

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ABSTRACT

The concentration of 5-hydroxyindole acetic acid (5-HIAA), the major metabolite of serotonin, might be the diagnosis basis for some cranial nerve pathology. An oxidized glassy carbon electrode (named OGCE) was employed to detect 5-HIAA with electrochemical methods. Multi-peaks appeared from the second cycle in range of 0.10–0.55 V (vs. AgCl/Ag) were assigned to the oxidative intermediates and products of 5-HIAA after the electrochemically driven oxidation at over 0.6 V in the first cycle. Kinetics studies show that all of these electrode reactions are adsorption-controlled processes, which are contributed to the strong hydrogen-bond interaction of these intermediates with the negatively charged hydroxyl and carboxyl groups on the surface of OGCE. Based on the square wave voltammetry (SWV) method, the linear relationship was shown between the peak current at 0.7 V and the concentration of 5-HIAA in range of 1.56–58.6 μmol/L, so 5-HIAA could be determined with limit of 0.917 μmol/L ($S/N = 3$) even certain interference were co-existed.

## 1. Introduction

5-hydroindole acetic acid (5-HIAA) is the major metabolite of serotonin (5-HT) [1], and the latter is thought as a neurotransmitter or neuromodulator. Different amount of 5-HT might be released when a human being is in certain pathological conditions [2–4], leading to different concentration levels of 5-HIAA found in plasma, urine and cerebrospinal fluid. For a healthy adult, it is about 16–94 μmol/L of 5-HIAA in urine for 24 h. It was reported that 126–1644 μmol/L of 5-HIAA are found for several carcinoid patients [5] and lower as 86.5 nmol/L are found in CSF for some suicide attempters [6,7]. So the determination of 5-HIAA in body fluid is desired urgently in clinical medicine.

In the past several years, 5-HIAA was reported to be detected by liquid chromatography coupled with mass spectrometry [8–10], fluorescence spectra [11,12], or electrochemical detection [13–15]. However, these chromatography-based techniques always require expensive instruments and pretreatment process which consumes long time and environmentally harmful solvents. Therefore, a simple, rapid, economic and environment-friendly technique is demanded for real-time clinic monitoring of 5-HIAA. Electroanalytical methods, which meet these requirements, have been successfully used with electrodes functionalized with metals [16,17], polymers [18,19], and molecularly imprinted materials [20].

In addition, although the physiological roles of 5-HIAA in normal brain are still unclear, researchers proposed the hypotheses that intermediates and products formed in the oxidative reactions might play roles in the neurodegenerative brain disorders. In the field of the oxidation chemistry of indoles, the oxidation reaction was initiated by various chemical, electrochemical, enzyme-mediated reactions and even steady-state radiolysis technology, and the information of the oxidative intermediates could only be speculated by UV spectra or the products should be isolated and identified by HPLC [21] or NMR [22] after the reactions finished. They seem not to be optimal choices for those unstable intermediates only exist during the reaction process. In this respect, electroanalytical method also possesses its advantages for detecting the oxidative intermediates and products of indoles without extra isolation process [23–27].

In this study, 5-HIAA has been determined by an oxidized glass carbon electrode (OGCE) with electrochemical methods. The multi-peaks in the second cyclic voltammetry (CV) curves were assigned to the intermediates and products of 5-HIAA after it was electrochemically oxidized in the first cycle, and the concentration of 5-HIAA was determined by the square wave voltammetry (SWV) method.

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## 2. Experimental

### 2.1. Chemicals and solutions

5-HIAA (≥98%) was purchased from TCI Company (Japan). All other chemicals used were of analytical grade from commercial local source. All dilutions and sample preparations were made using the water (18 M$\Omega$ cm$^{-1}$) purified by an ion-exchange system (Molecule China). Nitrogen gas with high purity (99.999%) was bubbled through the electrolyte during all of the electrochemical tests. Acetate buffer solutions (0.2 M) at various pH values were prepared by 0.2 M HAc and 0.3 M NaAc and were adjusted to the pH with 0.2 M HCl and 0.2 M NaOH.

### 2.2. Instruments

All electrochemical measurements were carried out with a CHI660E potentiostat (Chenhua Instruments, China). The three-electrode system consisted of a bare or electro-modified glassy carbon electrode (GCE) ($\phi$ = 3 mm in diameter) as the working electrode, a platinum plate as the counter electrode, and an Ag/AgCl (in saturated KCl solution) electrode as the reference electrode. The morphology and composition of the electrode was investigated by atomic force microscope (AFM, Dimension Icon, Bruker Nano Surfaces, Germany) and X-ray photoelectron spectroscopy (XPS, ESCALAB 250, Thermo Fisher Scientific, USA).

### 2.3. Pretreatment of electrode

A GCE was polished with alumina slurry (particle size of 1.0, 0.3 and 0.05 μm in order) on a polishing cloth, followed by ultrasonic cleaning in double distilled deionized water, and rinsed by nitric acid (1:1) and acetone subsequently. The bare GCE was then taken as working electrode being potential-scanned in 1 M $H_2SO_4$ in range of −0.6 V and 2.2 V at rate of 50 mV/ s for 10 cycles. After it was washed and dried, the GCE was transferred into acetate buffer solution (pH 4.0) and potential-scanned in range of 0.0 V and 1.0 V at scanning rate of 100 mV/s, until a stable cyclic voltammogram was obtained. The obtained electrode is called OGCE in this study.

### 2.4. Electrochemical behaviors of 5-HIAA

The electrochemical behaviors of 5-HIAA were investigated by cyclic voltammetry (CV), linear sweep voltammetry (LSV) and square wave adsorptive stripping voltammetry (SWV) methods using acetate buffer solution as the supporting solution. The typical steps for detection of 5-HIAA is following: using an OGCE as the working electrode and an Ag/AgCl electrode as the reference, firstly keep a constant potential at −0.2 V for 120 s under stirring, followed by rest for 10 s, and then SWV was recorded from 0.0 V to 1.0 V at frequency of 25 Hz, step potential of 5 mV and amplitude of 50 mV. All measurements were carried out at room temperature.

## 3. Results and discussion

### 3.1. Characterization of GCE and OGCE

The AFM images of the surface of a GCE before and after the anodic oxidation pretreatment are shown in Fig. 1. The surface of the bare GCE had smooth and compact nature with a root mean square roughness (RMS) of 2.2 ± 0.3 nm, and the obvious grooves were recognized as polishing scratches (Fig. 1A). After the pretreatment, the surface turned to be significantly rough surface with uniformly distributed grains (Fig. 1C), and the RMS was calculated as 13.7 ± 0.7 nm.

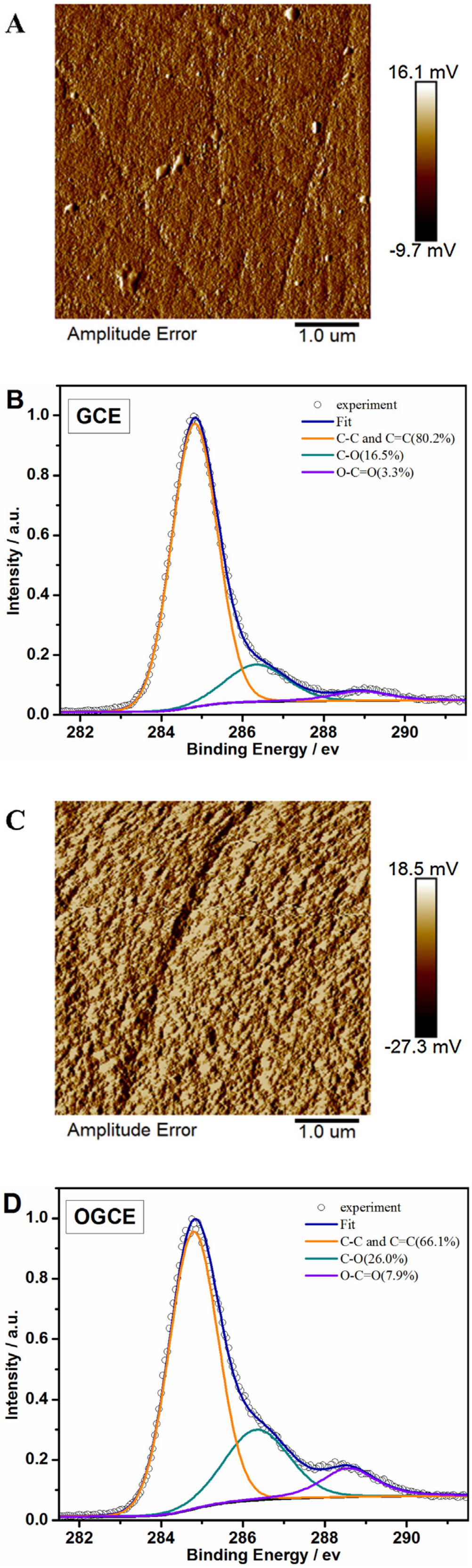

**Fig. 1.** AFM images and XPS $C_{1s}$ on the surface of the glassy carbon electrode before and after the anodic oxidation pretreatment: (A) and (B) bare GCE; (C) and (D) OGCE.

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