Positively Charged Gold Nanoparticle - Graphene Oxide Nanohybrid Modified Electrode for Electrochemical Detection of 5-hydroxytryptamine

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Abstract. In this study, positively charged gold nanoparticle - graphene oxide (AuNPs-GO) was synthesized and constructed a sensor for the detection of 5-hydroxytryptamine (5-HT). Various technologies were used to characterize the AuNPs-GO nanohybrid and the proposed sensor. The sensor showed a sufficient linear range from 0.5 to 60 μM 5-HT and the limit was 0.05 μM (S/N = 3). Furthermore, the sensor has been successfully utilized to determine 5-HT in real samples.

1. Introduction
5-Hydroxytryptamine (5-HT), plays the critical roles in controlling of endocrine secretion, mood, sleep, etc [¹]. Hence, creating a rapid detection method for 5-HT would be meaningful in diagnosis of some diseases and elucidating the functions of 5-HT in some neurological disorders [²]. Until now, a lot of methods were developed to detect the concentration of 5-HT, including electrochemical sensors, liquid chromatography, chemiluminescence. Among these methods, electrochemical sensors which are fast response, low cost, high sensitivity, has attracted considerable attention for 5-HT determination.

With the advantage of specific surface area, strong mechanical strength and fast electrode reaction kinetics, graphene oxide (GO) has been widely developed to fabricate electrochemical sensors. The integration of zero-dimensional gold nanoparticle (AuNPs) with two-dimensional GO hybrid materials that combine the characteristics of each material as well as have their unique properties [³]. In addition, GO exhibits negative charge when dissolved in water. Although citrate-capped AuNPs which carry negative charges was extensively used currently, it is inappropriate for negative charged AuNPs to be integrated with GO, due to the electrostatic repulsion.

In this study, we constructed a novel AuNPs-GO/GCE sensor based on the GO- AuNPs (positively charged) for sensitively measuring 5-HT. CTAB-capped AuNPs, which always take positive charges in-situ growth on the surface of GO. Consequently, the AuNPs-GO/GCE new sensor was constructed. At last, the fabricated sensor was successfully utilized to quantify 5-HT in real samples.

2. Materials and Methods

2.1. Experimental Materials
All chemical reagents in this work were analytical or guaranteed reagents. All the solutions used throughout were completely dissolved in HPLC water.
2.2. Synthesis of AuNPs (CTAB-capped) -GO Nanocomposite
The overall process of AuNPs -GO nanocomposite preparation was shown as follows \[^4\]. Firstly, a 3.5 mL mixture containing HAuCl\(_4\)·4H\(_2\)O (3.5×10\(^{-4}\) mol/L) and CTAB (0.1 mol/L) was prepared. Immediately, 1.5 mL ice-cold NaBH\(_4\) (0.01 mol/L) was added to the obtained solution. After being shaken violently for 20 seconds, the solution became light brown and was stored as a seed solution. Secondly, to in-situ synthesis of positively-charged AuNPs - GO nanocomposite, 80 µL CTAB (0.2 mol/L) and 80 µL HAuCl\(_4\)·4H\(_2\)O (2.4 × 10\(^{-2}\) mol/L) were mixed, followed by addition of 9.4 mL 0.8 mg/mL graphene oxide dispersion. Then, 0.6 mL of 0.10 mol/L L-AA solution was added to this mixture. Subsequently, 52 µL Au seed solution mixed into the growth solution and was shaken for 10 s, and the solution turned brownish red. The solution was centrifuged and washed. The residue was heated at 60 °C for a period of 8 h.

2.3. Electrochemical Experiment Procedure
Prior to modification, bare GCE was treated according to the reference \[^5\]. Then, 6 µL AuNPs -GO dispersion (0.8 mg/mL) was carefully deposited onto the surface of the treated GCE and dried naturally. The electrochemical tests were conducted using a 2273 electrochemical workstation. All experiments were carried at 25°C.

3. Results and Discussion

3.1. SEM Characterization of AuNPs-GO Nanocomposite
The surface morphology of the nanocomposite was investigated using SEM. As shown in Figure 1, after in-situ growth of AuNPs on GO, the AuNPs were homogeneously and densely attached to the surface of the layered GO sheets and the average size of AuNPs was about 12 nm. Also, EDX spectrum of the nanocomposite confirmed presence of AuNPs (not shown).

![Figure 1](image1.png)

**Figure 1.** SEM images of AuNPs /GO.

3.2. EIS and Zeta Potential Characterization of AuNPs -GO/GCE
The zeta potentials were measured to study the surface charge of GO and AuNPs-GO (Figure 2). Meanwhile, EIS is a useful technique for characterization of interface property according to the information of impedance change. Therefore, the impedance changes of the different sensors (GO/GCE, Au NPs - GO /GCE) were recorded by EIS. The diameter of semicircle is directly proportional to the electro transfer resistance (R\(_{ct}\)) in the Nyquist plots of EIS. As shown in Figure 3, GO /GCE had a large electron transfer resistance with a big semicircle domain, implying that GO had the larger obstruction effect. This should be ascribed to GO which was negatively charged. Also, the zeta potential of AuNPs /GO was found to be +24.6 mV due to the positive charged AuNPs. Thus, Au NPs -GO /GCE showed a relatively low R\(_{ct}\), due to the excellent electron transfer capability of AuNPs.
3.3. Optimization of Conditions for Sensor

Different influencing factors, including solution pH, and enrichment time of 5-HT would affect the properties of the sensor. DPV was employed to evaluate the electrochemical response of 5-HT under different conditions. In order to investigate the pH effect, 0.1 mol/L PBS was chosen as the buffer solution. As the pH of the PBS solution ranging from 5.2 to 8.1, the peak current response increased gradually and then decreased rapidly when increasing pH, and the current response reached maximum when the pH reached 6.2. Therefore, the PBS buffer solution with pH 6.2 was chosen as the supporting electrolyte.

The influence of accumulation time of 5-HT is also important for sensing performance which was evaluated from 30 to 240 s. The current of 5-HT increased while the accumulation time changing, maximum current was obtained at 210 s, which were adopted for optimal incubation time, implying the saturated sorption of 5-HT at the electrode surface.

3.4. Interferences and Applications

Structural analogues of 5-HT, including dopamine, epinephrine, norepinephrine, ascorbic acid, and various inorganic ions, were selected as competitors to examine the selectivity of the sensor. Based on the anti-interfered experimental results, 20-fold amounts of dopamine, epinephrine, norepinephrine, ascorbic acid exhibited interference in 5-HT sensing due to the structural analogues, but the peak current changes were all less than 5.2%. In addition, 50-fold amounts of Ca$^{2+}$, Mg$^{2+}$, Fe$^{3+}$, NO$_3^-$, SO$_4^{2-}$ had little

![Figure 4. DPV responses for different concentrations of 5-HT on AuNPs-GO/GCE. (a–e) 0.06, 8, 13, 18, 30 µM, respectively.](image-url)

Figure 4 showed amperometric responses gradually increased as the 5-HT concentration increasing from 0.06–30 µM. The calibration curve of the proposed sensor was $I_P (\mu A) = -0.95 - 0.0169 C(\mu M)$, and a detection limit of 0.02 µM (S/N = 3).
effect on the peak current of 5-HT. The reproducibility of AuNPs-GO/GCE was investigated. The measurements were performed at five different electrodes prepared under the same condition. The RSD of the current response for the individual measurement was 4.9%, demonstrating good repeatability of the fabricated sensor. According to the experimental results, the sensor had an acceptable selectivity and reproducibility for detecting 5-HT.

To evaluate the feasibility of AuNPs-GO/GCE sensor, the fabricated sensor was applied to detection of 5-HT in real samples. As 5-HT was not detected in the above samples, a recovery experiment was performed and the results are displayed in Table 1. The recoveries of this sensor were in the range of 94.0–102.0%, showing reliable detection of 5-HT in human serum samples.

| Table 1. 5-HT sensing in serum samples (n=3) at AuNPs-GO/GCE. |
| Samples | Original (µM) | Added (µM) | Found (µM) | Recovery (%) |
| 1 | 0 | 0.5 | 0.47 | 94.0% |
| 2 | 0 | 5 | 4.74 | 94.8% |
| 3 | 0 | 10 | 10.2 | 102% |
| 4 | 0 | 20 | 19.7 | 98.5% |

4. Conclusions
A new AuNPs-GO/GCE sensor was developed via in-situ synthetic method for quantitative analyses of 5-HT. In addition, AuNPs-GO nanocomposite is an excellent electrochemical sensing material which exhibited high sensitivity in detecting 5-HT. What is more, this sensor has been utilized to determine 5-HT in real samples, which is predicted to have wide applications in detecting 5-HT.

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