Disposable enzyme-free glucose biosensor based on H-rGO-Pt@Pd NPs/Au NPs

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Abstract: In this study, we constructed a H-rGO-Pt@Pd NPs with the characteristics of glucose oxidase. Then, the H-rGO-Pt@Pd NPs and Au NPs was modified on the surface of screen printed electrode to form an enzyme-free glucose biosensor. Among them, H-rGO-Pt@Pd NPs/Au NPs has a strong ability to capture electrons and catalyze the hydrolysis of glucose, resulting in a current-time change. The i-t curve is used to record the current-time change under different glucose concentration. When the glucose concentration was in the range of 0.6-1.4 mg/mL, the sensor current response value (Y) showed a linear relationship with the glucose concentration (X). The linear regression equation was Y=4.8515X-9.9361, the correlation coefficient was 0.9844, and the minimum detection limit was 0.2 mg/mL. In addition, the sensor has good specificity and stability.

1. Introduction
The detection of glucose has been widely used in clinical blood glucose detection, catering industry, environmental monitoring and other fields[1]. Among the many glucose detection methods, glucose oxidase, glucose kinase and other biological enzymes as specific recognition molecules are the most widely studied in glucose detection[2, 3]. However, the use of biological enzymes as specific identification substances is easily affected by humidity, temperature, pH and other environmental factors[3]. Therefore, in order to overcome these shortcomings, biosensors based on enzyme-free glucose detection have received extensive attention.

The key to design an enzyme-free sensor is to select sensitive materials with good catalytic activity. In the research of non-enzymatic glucose sensor, platinum based nanomaterials have become one of the most commonly used practical electrode materials to detect glucose due to their good oxidation catalytic activity for glucose[4, 5]. Among them, Pt@Pd NPs heterozygote, through the synergistic effect of two metal nanoparticles, has better catalytic oxidation performance than Pt NPs alone[4]. Au NPs has a strong ability to capture electrons and catalyze the hydrolysis of glucose[6, 7]. Hence, in this study, H-rGO-Pt@Pd NPs with the properties of glucose oxidase has been synthesized. Then, H-rGO-Pt@Pd NPs and Au NPs was modified on the surface of screen printed electrode (SPE) to form an enzyme-free glucose biosensor. The content of glucose can be obtained by analyzing the changes of current caused by different samples to be tested.
2. Experimental

2.1. Chemicals and reagents
Graphene oxide (GO) was purchased from xianfeng nano Co., Ltd. (Nanjing, China); \( \text{H}_2\text{O}_2 \), \( \text{NaCl} \), ammonium hydroxide, hydrazine hydrate, ethylene glycol, ascorbic acid, \( \text{Na}_2\text{PtCl}_6 \), \( \text{Na}_2\text{PtCl}_4 \), \( \text{Na}_2\text{HPO}_4 \), \( \text{Na}_2\text{H}_2\text{PO}_4 \), was purchased by xilong scientific Co., Ltd. (Shantou, China); \( \text{NaOH} \), concentrated sulfuric acid, glutaraldehyde, heme, \( \text{HAuCl}_4 \), glucosum anhydricum, purchased from Shanghai aladdin biochemical technology Co.Ltd. (Shanghai, China).

2.2. Apparatus
All electrochemical measurements were implemented on CHI660D electrochemical workstation (Shanghai Chenhua Instrument, China) at room temperature. Amperometric \( i-t \) curve (\( i-t \)), cyclic voltammetry (CV) was performed in phosphate buffer saline (PBS, pH 7.4). All aqueous solutions used in this experiment were deionized water from 18.2 \( \Omega \cdot \text{cm}^{-1} \) of Milli-Q purified purification system (Milli-Pore, Bedford, MA, USA).

2.3. Preparation of the H-rGO-Pt@Pd NPs nanohybrid
The GO suspension was obtained after dissolving 10 mg GO in 10 mL ultra-pure water and ultrasonic crushing for 2 h. The solution of rGO was obtained by adding 10 mg AA into 10 mL GO suspension and stirring continuously at room temperature for 12 h. Dissolve 30 mg heme in 10 \( \mu \text{L} \) ammonia solution and add 30 mL pure water to obtain heme solution. The H-rGO suspension was obtained by mixing 10 mL heme solution, 8 \( \mu \text{L} \) hydrazine hydrate, and 10 mL rGO solution and stirring continuously for 4 h at 60\(^\circ\)C.

2.0 mL PDDA (0.2\%) and 0.0585g \( \text{NaCl} \) were added to 10 mL H-rGO solution, which was continuously stirred for 12 h. After centrifugation at 10000 \( r/\text{min} \) for 15 min, PDDA-modified H-rGO was obtained by precipitation. 0.0225g \( \text{Na}_2\text{PtCl}_6 \), 0.0118g \( \text{Na}_2\text{PdCl}_4 \), and 10 mL ethylene glycol were added into the PDDA-modified H-rGO solution, and continuous stirring for 12 h. The pH value of the mixed solution was adjusted to 12 with 1.0 mol/L \( \text{NaOH} \) to obtain H-rGO-Pt@Pd NPs solution. The H-rGO-Pt@Pd NPs solution was centrifuged at a rotating speed of 10000 \( r/\text{min} \) for 10 min, and the precipitate was taken to obtain the H-rGO-Pt@Pd NPs nanohybrid material.

2.4. Preparation of the non-enzymatic biosensor
The SPE was immersed in 0.5 mol/L \( \text{H}_2\text{SO}_4 \) solution and scanned for 20 cycles in the voltage range of -0.4V to 1.2V. Then, the activated SPE electrode was placed in 4 mL 0.01% \( \text{HAuCl}_4 \) solution and deposited for 120 s at -0.5V. After that, the activated SPE electrode was washed with pure water for 3 times and blown dry to obtain Au NPs/SPE electrode. Next, the Au NPs /SPE electrode was immersed in 2.5% glutaraldehyde for 15 min and rinsed with PBS solution. Then, dripping with 5 \( \mu \text{L} \) H-rGO-Pt@Pd NPs suspension was added and incubated for 30 min, washed with PBS solution and dried to obtain the H-rGO-Pt@Pd NPs/Au NPs/SPE.

3. Results and discussion

3.1. Analytical Principle of the H-rGO-Pt@Pd NPs/Au NPs/SPE non-enzymatic biosensor
Fig. 1 shows a schematic diagram of an enzyme-free electrochemical sensor for detecting glucose. When glucose was added to the biosensor interface, the redox reaction occurred at the biosensor interface due to the catalytic oxidation of the nanohybrid material. The electrochemical signal of glucose in PBS solution was detected by \( i-t \) method.
3.2. Characterization of H-rGO-Pt@Pd NPs nanohybrid

It can be seen from Fig. 2A that the granular metal Pt and Pd uniformly adhere to the H-rGO material, indicating that the preparation of H-rGO-Pt@Pd NPs is successful.

As shown in Fig. 2B, rGO has an obvious absorption peak at the wavelength of 260 nm; Heme has an obvious absorption peak at the wavelength of 390 nm; The UV absorption peaks of H-rGO are at 270 nm and 410 nm because Pt, Pd has no UV absorption peak. Therefore, the above results indicate that the preparation of H-rGO-Pt@Pd NPs nanomaterials is successful.

3.3. Characterization of non-enzymatic biosensor

As shown in Fig. 3, compared with bare electrode (Fig. 3A), the surface of the electrode modified by Au NPs has obvious granular feeling (Fig. 3B). As shown in Fig. 3C, when Pt@Pd NPs were modified on the surface of Au NPs/SPE electrode, a layer of crystal sensitive materials could be seen, which indicated that the preparation of biosensor was successful.

As shown in Fig. 3D, there is no oxidation peak in curve a and curve b. After the deposition of Au NPs, the oxidation peak of curve c appeared obviously, which is because Au NPs can catalyze the decomposition of glucose. The oxidation peak of curve d was higher than that of curve c, which indicated...
that the H-rGO-Pt@Pd NPs could further catalyze the decomposition of glucose. The above CV results show that it is feasible to use the sensor to detect glucose.

As shown in Fig. 3E, the impedance value of bare electrode (curve a) is very large, which is 991 Ω; When a layer of Au NPs is deposited on the bare electrode (curve b), the impedance of the electrode decreases rapidly to 362 Ω. After, further deposition H-rGO-Pt@Pd NPs (curve c), the impedance of the electrode decreased to 305 Ω. These results indicate that the preparation of enzyme-free glucose sensor is successful.

Figure 3  SEM for bare SPE (A), Au NPs/SPE (B), and H-rGO-Pt@Pd NPs/Au NPs/SPE (C); CV characterization of (bare SPE (a), SPE+glucose (b), Au NPs/SPE+glucose (c), and H-rGO-Pt@Pd NPs/Au NPs/SPE+glucose (d)) (D); EIS characterization of (bare SPE (a), Au NPs/SPE (b), and H-rGO-Pt@Pd NPs/Au NPs/SPE (c)) (E)

3.4. The performance of the H-rGO-Pt@Pd NPs/Au NPs/SPE non-enzymatic biosensor

Take H-rGO-Pt@Pd NPs/Au NPs/SPE electrode was put into PBS buffer and the glucose concentration in the buffer was continuously changed. The i-t curves of different glucose concentrations are shown in Fig. 4A. As shown in Fig. 4B, when glucose concentration is in the range of 0.6-1.4 mg/mL, the linear regression equation is \( Y = 4.8515X - 9.9361 \) (Sensor current response value \( Y \) and glucose concentration \( X \)), and the correlation coefficient is 0.9844. According to the minimum detection limit formula \( LOD = K \times S_b/S \) (\( S_b \) is the standard deviation calculated by repeated detection of blank samples, \( S \) is the slope of the standard curve, and \( K \) is the coefficient determined by certain confidence degree \( K=3 \)), the calculated minimum detection limit of the enzyme-free glucose sensor is 0.2 mg/mL.

Ascorbic acid (AA), uric acid (UA), dopamine (DA) and acetic acid (DOPAC) were used as interfering substances to verify the specificity of the enzyme-free glucose sensor. As shown in Fig. 4C, the current measured by the i-t curve did not change significantly after the interference substance was added, indicating that the sensor had good specificity.

The prepared electrodes were stored at room temperature and taken out at 1 day, 2 day, 3 day, 5 day, 7 day and 13 day, respectively, for glucose detection to verify the stability of the enzyme-free glucose sensor. As shown in Fig. 4D, after 13 days of storage, the response current remains at 85.6%, which indicates that the sensor has good stability.
4. Conclusion
In summary, the enzyme free glucose biosensor was constructed based on the excellent conductivity, large specific surface area, and good catalytic activity for glucose of H-rGO-Pt@Pd NPS hybrid materials and Au NPs. In the range of 0.6 -1.4 mg/mL, the current response was linearly correlated with glucose concentration, the regression equation was $Y=4.8515X-9.9361$, the correlation coefficient was 0.9844, and the minimum detection limit was 0.2 mg/mL. The sensor also showed good specificity and stability.

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