

基于Pt/Au双金属修饰针灸针的非酶葡萄糖传感器

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摘要: 通过在不锈钢针灸针(AN)表面依次电沉积金(Au)纳米颗粒和铂(Pt)纳米颗粒, 基于它们在AN表面的协同作用, 实现了一种用于非酶葡萄糖检测的电化学传感器。首先, 通过扫描电子显微镜对其功能界面(Pt/Au/AN)进行表征, 结果显示类似卷心菜的纳米材料均匀致密地分布在AN表面。然后, 通过循环伏安法和电化学阻抗法对Pt/Au/AN电极的电化学特性进行了研究。结果表明, 与Au/AN或Pt/AN电极相比, Pt/Au/AN电极对葡萄糖氧化表现出优越的电催化活性。这表明双金属Pt/Au的接触界面是葡萄糖氧化的重要电催化位点。在pH 7.4的模拟生理介质中, 制得传感器的线性范围为0.1~35 mmol·L⁻¹, 检测限为0.076 3 mmol·L⁻¹, 对葡萄糖的检测表现出较高的灵敏度和良好的抗干扰性能、稳定性。此外, 该传感器已成功用于人体血清葡萄糖的检测。

关键词: 针灸针; Pt/Au双金属接触界面; 非酶传感器; 葡萄糖

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Non-enzyme Glucose Biosensor Based on Bimetallic Pt-Au Nanoparticles Decorated Acupuncture Needle

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Abstract: An electrochemical biosensor for non-enzyme glucose detection was constructed based on the synergistic action of gold (Au) nanoparticles and platinum (Pt) nanoparticles on the surface of a stainless steel acupuncture needle (AN), which was achieved by respectively electrodeposition. The functional interface (Pt/Au/AN) was characterized by a scanning electron microscope, showing that cabbage-like nanomaterials were uniformly and densely distributed on the surface of AN. Pt/Au/AN electrode also possessed outstanding electrochemical characteristics, which were studied by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Significantly, Pt/Au/AN electrode exhibited remarkably electrocatalytic activity toward glucose oxidation compared with Au/AN or Pt/AN electrode. The results indicated that the contact interface of bimetallic Pt/Au was the vital electrocatalytic site for glucose oxidation. A further study proved that the contact interface exhibited intrinsic features and distinct selec-

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tivity for sensing glucose. The prepared sensor showed a wide linear range from 0.1 to 35 mmol·L⁻¹, and the detection limit of glucose was 0.076 3 mmol·L⁻¹. The sensor showed great stability, excellent selectivity, and miniaturization. Furthermore, the sensor was successfully used for the detection of glucose in human serum.

Keywords: acupuncture needle; contact interface of bimetallic Pt/Au; non-enzyme sensor; glucose

0 Introduction

Diabetes mellitus, a chronic disease, is caused by insufficient insulin secretion in the body or inefficient use of insulin by cells^[1-2]. This disease is characterized by high blood sugar, which can induce a variety of chronic complications, such as blindness, coronary heart disease, cerebrovascular disease, and diabetic nephropathy^[3-4]. Nowadays, there were approximately 463 million people suffer from diabetes in the world. Unfortunately, the number of diabetic patients has continued to increase for over 50 years according to the latest report from International Diabetes Federation^[5]. In the future, it is predicted that there will be 700 million diabetic patients worldwide by 2045, which will be seriously threatened human health^[6]. Given the definitive functional relationship between human health and glucose, the development of a glucose sensor with high sensitivity, wide linearity, low cost, and strong anti-interference would be of great significance for assisting the clinical assessment of diabetes.

At present, there are various technologies that were used for glucose detection, such as colorimetry^[7], Raman spectroscopy^[8], fluorescence spectroscopy^[9], photoelectrochemical measurement^[10], gas chromatography-mass spectrometry^[11], and electrochemical method^[12]. Among these methods, electrochemical sensors have received widespread attention in the multiplex biological matrix because of their high discernibility, fast response, low detection limit, simplicity, as well as low cost^[13-14]. Generally, the most widely-used electrochemical glucose sensors are enzyme-based sensors^[15]. However, enzymatic electrochemical sensors have some poor stability, complicated immobilization procedures, critical operating situation, and high cost that limit their practical application in glucose detection^[16-17]. Because of the above-mentioned drawbacks, non-enzyme electrochemical glucose sensors have

become a new research hotspot. For non-enzyme electrochemical glucose sensors, the key factor is electrode materials. From this perspective, the foremost thing is to find novel electroactivity materials, which could catalyze glucose oxidation with fast kinetics and lower overpotential.

In this direction, various nanomaterials have been studied for fabricating non-enzyme glucose sensors, such as carbon nanomaterials^[18], metal-organic frameworks nanomaterials^[19], and quantum dots^[20], metal and metal oxide nanomaterials^[21]. Thereinto, Pt-based materials expressed prominent catalytic activity for the oxidation of glucose and were widely used to construct an electrochemical interface for non-enzyme-sensors^[22]. For instance, Guo et al. used ultrasonic electrodeposition to deposit Pt nanoparticles onto gold electrodes, which showed good catalytic performance in a neutral solution with a linear range of 1-16 mmol·L⁻¹ and a detection limit of 48 μmol·L⁻¹^[23]. However, the intermediate products produced in the process of glucose oxidation easily adhere to the Pt nanomaterials, which leads to the passivation or poisoning of the interface and reduces the electrocatalytic activity subsequently^[24]. To settle down this limitation, developing bimetallic nanomaterials is considered to be an effective way. Gold (Au) nanomaterial is another interesting and attractive material due to the merits of high surface reactivity, good solubility, and excellent bioactivity^[25]. Meanwhile, Au also has strong anti-interference, which is attributed to its full-filled 3D orbit. In previously published reports, bimetallic Pt/Au showed excellent electrocatalytic activity toward glucose and high tolerance to toxicity. However, the high price and large size of electrode substrate are inconvenient for the *in-vivo* detection and limited their application.

Acupuncture is a kind of traditional Chinese medicine treatment technique, which can relieve pain and

promote blood flow by piercing a needle into the human body^[26]. Acupuncture needles (ANs) are made of stainless steel, gold, silver, *etc.* Among these materials, the stainless steel AN is cheap and commonly used. Because ANs are made of metal with high conductivity, which provides their potential applications as electrochemical electrodes. Currently, relative reports have confirmed that ANs can be used as electrodes for the detection of nitrate^[27], lead^[28], rutin^[29], ascorbic acid^[30], dopamine^[31], hydrogen peroxide released from living cells^[32], *etc.* Compared with the common glassy carbon electrode, the AN electrode only needs to be cleaned rather than polished before electrochemical experiments. What's more, it has been reported that the unique needle-shaped structure provides a larger surface area for the modifier and improves electrode performance^[33-34]. In a word, stainless steel AN has the advantages of low price, simple to operate, acicular structure, and high specific surface area. However, the detection of glucose based on ANs has not been reported so far, although it is very significant and strong practicable, which should be anastomosed with traditional medicine acupuncture.

In this work, a bimetallic Pt/Au nanocatalyst was modified onto stainless steel AN tip by electrodeposition, and the interface possessed the intrinsic capability for electrochemical non-enzyme sensing glucose effectively. Electrodeposition is a feasible method to synthesize metal, in which the size and morphology of alloy can be adjusted by applied potential, deposition time, and concentration. The catalytic performance of the non-enzyme electrochemical sensor (Pt/Au/AN) for glucose oxidation was systematically studied. Compared with Pt/AN and Au/AN electrodes, Pt/Au/AN electrodes significantly improved the electrocatalytic performance of glucose oxidation. Meanwhile, Pt/Au/AN electrode possesses not only simple preparation and low price, but also high selectivity, sensitivity, and a wide linear range for detecting glucose.

1 Experimental

1.1 Reagents

Gold chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), chloro-

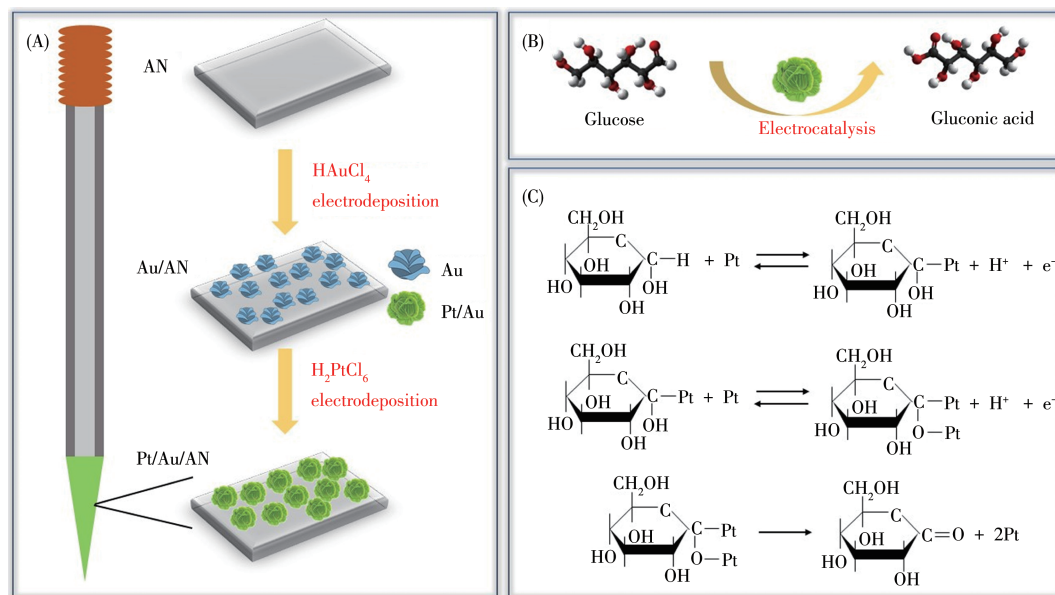
platinic acid hexahydrate ($\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$), glucose, sodium hydroxide (NaOH), uric acid (UA, >98%), ascorbic acid (AA, $\geq 99\%$) were purchased from Aladdin Reagent Co., Ltd. Sulfuric acid (H_2SO_4) and absolute ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. Stainless steel ANs (size: 0.25 mm $\times$ 40 mm) were purchased from Suzhou Medical Appliance Factory Co., Ltd. All reagents were analytical grade and the aqueous solution was prepared using $18.25 \text{ M}\Omega \cdot \text{cm}^{-1}$ ultrapure water throughout the experiment. Human serum samples were taken from Hangzhou Cancer Hospital, affiliated with Zhejiang University, China.

1.2 Apparatus

The morphology of the prepared electrode was characterized by Gemini scanning electron microscope 300 (SEM, ZEISS, Germany) operating at 1, 2, 3, or 5 kV. On the CHI660 electrochemical workstation (Chenhua, Shanghai, China), the conventional three-electrode mode was used for electrochemical measurement. The modified electrode, saturated calomel electrode (SCE), and Pt wire electrode were used as the working electrode, the reference electrode, and the counter electrode, respectively.

1.3 Preparation of the modified electrode

Scheme 1 illustrates the preparation procedure of Pt/Au/AN electrode for the determination of glucose. As shown in Scheme 1A, Pt/Au/AN electrode was fabricated by a two-step sequential electrodeposition strategy. Firstly, the AN was ultrasonically cleaned in absolute ethanol and ultrapure water for 5 min and then dried with nitrogen. Subsequently, Au/AN electrode was achieved by depositing gold nanomaterials onto AN in the range of 1.5 mm from the tip via applying an accumulation potential of -8.0 V for 180 s in $0.75 \text{ mmol} \cdot \text{L}^{-1}$ HAuCl_4 solutions ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was dissolved in $0.2 \text{ mol} \cdot \text{L}^{-1}$ H_2SO_4). Finally, Pt/Au/AN electrode was obtained by modifying Pt nanomaterials onto Au/AN surface via performing an accumulation potential of -0.8 V for 300 s in $1.25 \text{ mmol} \cdot \text{L}^{-1}$ H_2PtCl_6 solutions ($\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ was dissolved in $0.4 \text{ mol} \cdot \text{L}^{-1}$ H_2SO_4). Pt/AN and Au/AN electrodes were used as the control, which were prepared by a one-step electrode-



Scheme 1 (A) Fabrication procedure of Pt/Au/AN electrode; (B, C) Mechanism of glucose oxidation on Pt/Au/AN electrode

position procedure via immersing the bare AN electrode into $1.25 \text{ mmol} \cdot \text{L}^{-1}$ H_2PtCl_6 solutions or $0.75 \text{ mmol} \cdot \text{L}^{-1}$ HAuCl_4 solutions, and then performing an electrodeposition procedure, respectively.

1.4 Electrochemical measurements

Firstly, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to characterize the electrochemical properties of the electrodes. CV was performed in the range of -0.2 – 0.8 V at a scan rate of $0.1 \text{ V} \cdot \text{s}^{-1}$ in $0.1 \text{ mol} \cdot \text{L}^{-1}$ KCl containing $1 \text{ mmol} \cdot \text{L}^{-1}$ $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Fe}(\text{CN})_6]^{4-}$. EIS was carried out with an Autolab electrochemical analyzer (Eco Chemie, Netherlands) in $20 \text{ mmol} \cdot \text{L}^{-1}$ $\text{K}_3[\text{Fe}(\text{CN})_6]$ and $\text{K}_4[\text{Fe}(\text{CN})_6]$ ($0.1 \text{ mol} \cdot \text{L}^{-1}$ KCl) as the supporting electrolyte at a bias potential of 0.18 V , within the frequency range of 0.001 to 100 kHz . Secondly, chronoamperometry was performed at a potential of -0.1 V for 50 s in $0.1 \text{ mol} \cdot \text{L}^{-1}$ phosphate buffer solution (PBS, $\text{pH}=7.4$) containing $0.1 \text{ mol} \cdot \text{L}^{-1}$ NaCl to establish the calibration plots of glucose. The amplitude was 50 mV with a pulse width of 0.167 s , and a pulse period of 0.5 s .

1.5 Detection of glucose in real samples

To investigate the practicability of the proposed sensor under clinical conditions, human serum samples were used as real samples to conduct the electrochemical measurements. The human serum samples were diluted by 10 times with $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS ($\text{pH}=7.4$)

solution. For glucose recovery in serum samples, different amounts of glucose were spiked into the above-diluted serum samples. Chronoamperometry measurements were carried out to detect the concentration of glucose using Pt/Au/AN electrodes. The recovery rates were obtained by the ratio of the calculated concentration to the spiked concentration.

2 Results and discussion

2.1 Characterizations of the modified electrode

The surface morphology of Pt/Au/AN electrode was characterized by SEM, as shown in Fig.1. For the bare AN electrode, a smooth tip surface with a tip diameter of around $5 \mu\text{m}$ can be observed (Fig.1A, 1B). It had a stainless steel metallic luster. In addition, we calculated that the diameter of the modified AN was about $160 \mu\text{m}$ at a 1.5 mm position from the tip (Fig. S1, Supporting information). After the modification of gold nanomaterials, Au/AN electrode exhibited a golden appearance (Fig.1A). A stone-like morphology with an average diameter of 300 nm was further observed (Fig.1C, 1D). Subsequently, the surface of Au/AN electrode was coated with the bimetallic Pt/Au nanoparticles through the electrodeposition strategy. It can be observed that Pt/Au/AN electrode displayed a grey slush appearance (Fig.1A). A cabbage-like morphology was uniformly and densely distributed on the surface of

AN electrode (Fig.1E, 1F), which effectively increases the surface area of the electrode and then provides abundant electro-active sites for glucose oxidation^[35]. These characterizations demonstrated that the functional needle electrode was successfully fabricated. AN is integrated as the base layer and provides a large surface for follow-up growth of Au and Pt, developing needle electrodes with a large surface-to-volume ratio and much active sites for the following determinations.

EIS is an effective tool to study the conductivity of modified electrodes as well as to characterize the modification process^[36]. The EIS spectrum contains a semicircle part and a linear portion, where the semicircle portion at high frequency represents the electron transfer limited process, and the linear part at low frequency represents a diffusion-limited process. According to the EIS data of the electrode, Randle's equivalent circuit of the electrode can be obtained, which is consisted of

ohmic resistance (R_s), double layer capacitance (CPE), and transfer resistance (R_{ct}), and Warburg impedance (Z_W). Among these components, the R_{ct} was calculated by the semicircle diameter of EIS spectrum, which controls the electron-transfer kinetics of the active species on the electrode surface. Fig.2A and 2B show the typical Nyquist plots of AN, Pt/AN, Au/AN, and Pt/Au/AN electrodes, which were recorded in 0.1 mol·L⁻¹ KCl solution containing 20 mmol·L⁻¹ [Fe(CN)₆]³⁻ and [Fe(CN)₆]⁴⁻ at a frequency from 0.001 to 100 kHz at a potential of 0.005 V. As shown in Fig.2A, the Nyquist plot of bare AN possessed a large semicircle portion that corresponded to a large electron transfer resistance (30 656.0 Ω), which means the electron transfer is difficult. After modification of Au or Pt, the electron transfer resistance was found to be 7.4 and 296.9 Ω, as shown in Fig.2B. The downward trend of electron transfer resistance was ascribed to the excellent conductivi-

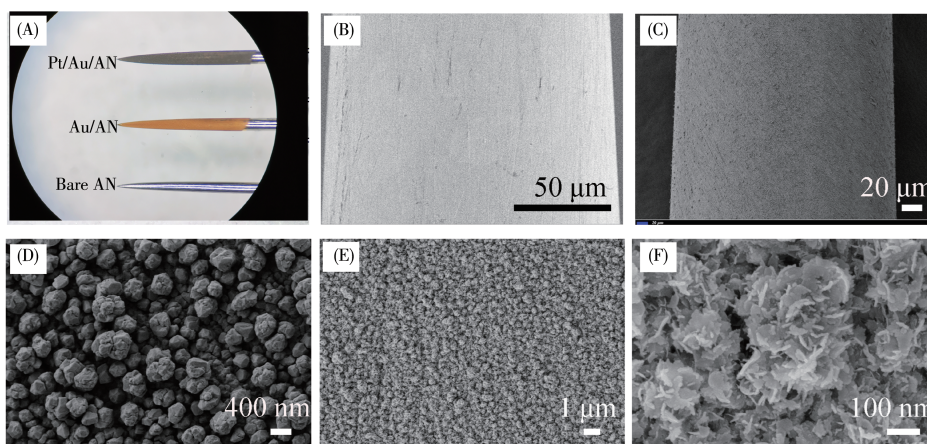


Fig.1 (A) Bright-field image of bare AN, Au/AN, and Pt/Au/AN electrode; SEM images of AN electrode (B), Au/AN electrode (C, D), Pt/Au/AN electrode (E, F)

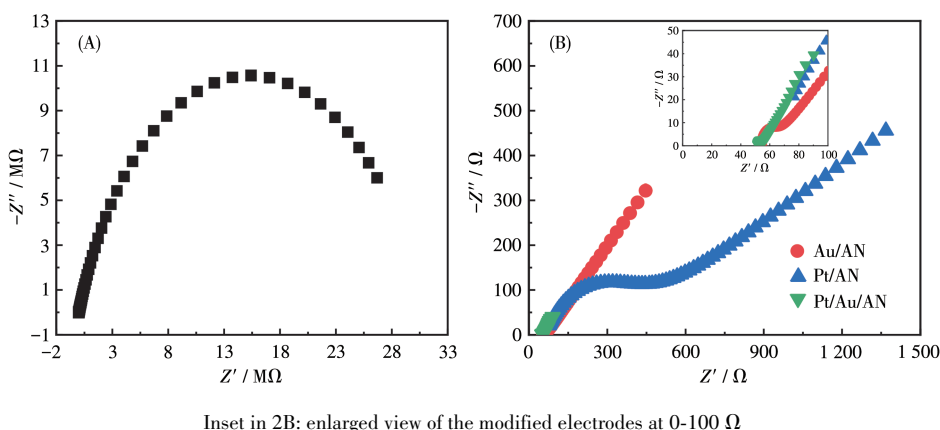


Fig.2 EIS spectra of (A) bare AN and (B) different modified electrodes of Au/AN, Pt/AN, and Pt/Au/AN

ty of Au or Pt, which could promote the electron transfer between solution and electrode interface. After further modification of Pt on Au/AN, the electron transfer resistance was reduced continuously and the value of electron transfer resistance was $3.2\ \Omega$ (Fig. 2B). The lowest electron transfer resistance value indicates the highest conductivity. The results indicate that the bimetallic Pt/Au nanoparticles are successfully modified onto the ANs and can effectively enhance the electron-transfer ability of electrodes compared with single metal. In addition, the Randle's equivalent circuit of Pt/Au/AN electrode is shown in Fig.S2.

CV is a useful technique for studying the electrochemical performance of electrode surfaces^[37]. Herein, the performance of four different electrodes, including AN, Pt/AN, Au/AN, and Pt/Au/AN were investigated by performing CV experiments (Fig.3). For bare AN, it can be seen that there was no obvious redox peak, indicating the poor conductivity and electron transfer rate of $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Fe}(\text{CN})_6]^{4-}$ on AN. For Au/AN and Pt/AN, it was found that there were a pair of symmetrical redox peaks with a peak potential separation (ΔE_p) of 90 and 138 mV respectively, which could be attributed to Au and Pt nanoparticles for improving the electron transfer and enhancing the conductivity of the electrode. Moreover, Pt/Au/AN electrode generated a pair of symmetrical redox peaks with a peak potential separation (ΔE_p) of 90 mV. The peak current of Pt/Au/AN electrode increased by 4 times and 1.8 times com-

pared to Pt/AN and Au/AN. These observations indicated that the bimetallic Pt/Au can increase the electron transfer rate more effectively than single metal, which may be due to the synergistic effect of bimetal. To confirm the best electrochemical performance of Pt/Au/AN electrode, the electrochemically active surface areas of different electrodes were calculated according to the Randles-Sevcik equation (Eq.1).

$$i_p = 2.69 \times 10^5 n^{3/2} A c D^{1/2} v^{1/2} \quad (1)$$

Herein, i_p , n , A , c , D , and v represent the peak current, the number of electrons involved in the redox couple, electrode area, analyte concentration, analyte diffusivity, and scan rate, respectively. By calculation, the active areas of Pt/AN, Au/AN, and Pt/Au/AN were 0.014 5, 0.032 8, and 0.058 4 cm^2 , respectively. It is generally known that the larger the electrode surface area, the better the performance. The largest surface area achieved at Pt/Au/AN verified the best electrochemical performance of Pt/Au/AN electrode compared with other electrodes.

2.2 Electrooxidation of glucose on the modified electrodes

Before implementing Pt/Au/AN electrode for glucose sensing, its electrochemical behavior was investigated by performing CV experiments in $0.1\ \text{mol}\cdot\text{L}^{-1}$ PBS (pH=7.4) solution containing $0.1\ \text{mol}\cdot\text{L}^{-1}$ NaCl in the presence and absence of glucose ($5\ \text{mmol}\cdot\text{L}^{-1}$ glucose of Fig.4A; 5 and $10\ \text{mmol}\cdot\text{L}^{-1}$ glucose of Fig.4B) at a scan rate of $50\ \text{mV}\cdot\text{s}^{-1}$ within the scan range from -0.65 to $0.5\ \text{V}$. No obvious redox peak was observed at bare AN and Pt/AN in the absence and presence of glucose, which suggests that these electrodes do not catalyze the oxidation of glucose in this potential window. In contrast, Au/AN generated two tiny oxidation peaks of glucose at -0.04 and $0.24\ \text{V}$, indicating low electrocatalytic activity of Au/AN electrode towards glucose oxidation. On the other hand, the CV of Pt/Au/AN without glucose has three simple and clear parts: two pairs of redox peaks at negative potentials are ascribed to hydrogen adsorption/desorption peaks, a flat double layer region at intermediate potentials, and metal oxide formation/reduction peaks at positive potentials (Fig.4B). After the addition of glucose, the CV curves

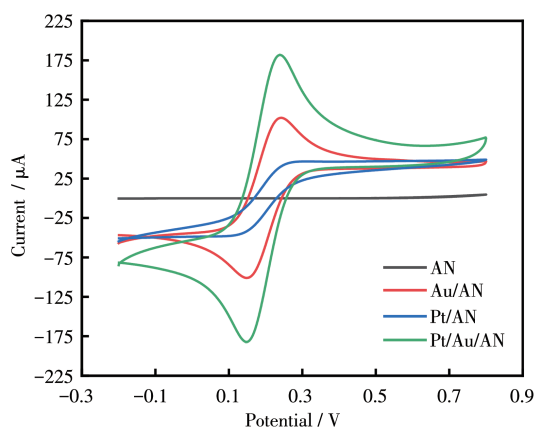


Fig.3 CV responses of bare AN, Au/AN, Pt/AN, and Pt/Au/AN electrodes at the scan rate of $50\ \text{mV}\cdot\text{s}^{-1}$

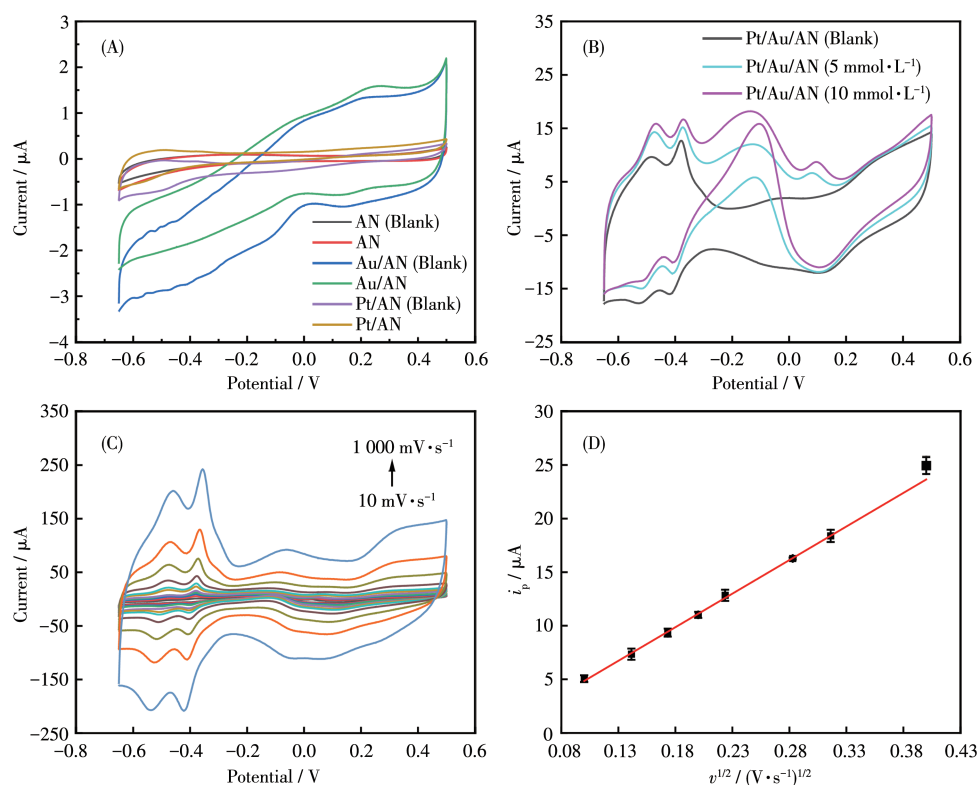


Fig.4 CV responses of (A) bare AN, Au/AN, Pt/AN, and (B) Pt/Au/AN in 0.1 mol·L⁻¹ PBS (pH=7.4) solution containing 0.1 mol·L⁻¹ NaCl in the presence and absence of glucose at the scan speed of 50 mV·s⁻¹; (C) CV response of Pt/Au/AN electrode at different scan rates from 10 to 1 000 mV·s⁻¹ in 0.1 mol·L⁻¹ PBS (pH=7.4) containing 0.1 mol·L⁻¹ NaCl and 5 mmol·L⁻¹ glucose; (D) Plot of i_p at -0.09 V vs square root of scan rate

of Pt/Au/AN were similar at various concentrations of glucose, indicating that Pt/Au/AN electrode is stable for glucose electro-oxidation under the CV method. Meanwhile, Pt/Au/AN electrode had multiple obvious oxidation peaks in the forward scan, among which three obvious oxidation peaks were located at -0.46, -0.37, and -0.09 V. According to the number of oxidation peaks, it can be inferred that the oxidation of glucose may be completed in multiple steps. The first oxidation peak may be caused by the adsorption and dehydrogenation of glucose on the electrode, and the following oxidation peaks may be attributed to the further oxidation of intermediate products^[38]. The reduction peak at 0.09 V in the backward scan may be caused by the reduction of surface oxides at high potential. As the surface oxide decreased, more active sites were exposed to the oxidation of glucose, resulting in a larger oxidation current. Therefore, according to the electrocatalytic results detected by CV, the prepared Pt/Au/AN electrode provided a higher catalytic activity toward glu-

cose oxidation than AN, Pt/AN, and Au/AN.

Furthermore, the glucose oxidation process on Pt/Au/AN electrode was also discussed, as shown in Scheme 1C. Firstly, glucose was oxidized to gluconolactone. Then, gluconolactone was directly oxidized to gluconic acid^[39]. To further investigate the mechanism, the effect of scan rate on oxidation peak current at Pt/Au/AN electrode was measured in a range of 10 to 1 000 mV·s⁻¹, in which 0.1 mol·L⁻¹ PBS (pH=7.4) solution containing 0.1 mmol·L⁻¹ NaCl and 5 mmol·L⁻¹ glucose (Fig.4C). It can be seen that the anodic peak currents increased with the increase of the scanning rate. As shown in Fig.4D, the anodic i_p at -0.09 V showed a good linear relationship with the square roots of scan rates (v). The linear regression equation is $i_p = 62.65v^{1/2} - 1.41$ ($R^2 = 0.998$), implies that the electrocatalytic for glucose oxidation on Pt/Au/AN electrode is a diffusion-controlled electrochemical process.

2.3 Optimization of the experimental conditions

According to the above discussion, Pt/Au/AN

electrode exhibited great electrocatalytic performance toward glucose oxidation. To further improve the electrochemical performance of Pt/Au/AN electrode for detecting glucose, several experimental parameters, including electrodeposition potential, time, the concentration of H_2SO_4 , the molar ratio of HAuCl_4 to H_2PtCl_6 were optimized. CV was carried out and the current difference (Δi_p) was recorded at Pt/Au/AN electrode in the absence and presence of $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose. Firstly, the potential and time for the electrodeposition procedure were optimized. As shown in Fig.5, the value of Δi_p increased significantly with increasing the electrodeposition potential and time. The reason may be attributed to the increase in the amount of metal nanoparticles deposited onto the electrode surface to improve the surface area and conductivity of the electrode. When the deposition potential and time increased to a certain extent, the value of Δi_p began to decrease, which may be caused by the accumulation of metal covering the active sites. Therefore, the best potential and time of the electrodeposition procedure for Au were -8.0 V and 180 s , and the best electrodeposition

parameters of Pt were -0.8 V and 300 s .

Subsequently, the effect of H_2SO_4 concentration on the electrochemical catalysis of Pt/Au/AN electrode toward glucose was studied (Fig. 6A and 6B). It was found that the current elevated with increasing H_2SO_4 concentration, and started to level off after a certain value. Based on the experimental results, the optimized H_2SO_4 concentrations for the electrodeposition of Au and Pt were 0.2 and $0.4 \text{ mol} \cdot \text{L}^{-1}$. Most importantly, the influence of the molar ratio of HAuCl_4 to H_2PtCl_6 on the electrochemical catalysis of Pt/Au/AN electrode toward glucose was explored. The total concentration of HAuCl_4 and H_2PtCl_6 was controlled to be $2 \text{ mmol} \cdot \text{L}^{-1}$. As shown in Fig. 6C, the value of Δi_p increased with decreasing the molar ratio of H_2PtCl_6 to HAuCl_4 , which may be due to the large increase in the poisoning ability of Pt catalyst resulting from the increased loading of Au. After reaching the maximum value of Δi_p at the molar ratio of $3:5$ (H_2PtCl_6 to HAuCl_4), the electrocatalytic activity of Pt/Au/AN electrode started falling, which implies that the electrocatalytic activity may be mainly determined by the amount of Pt. The results

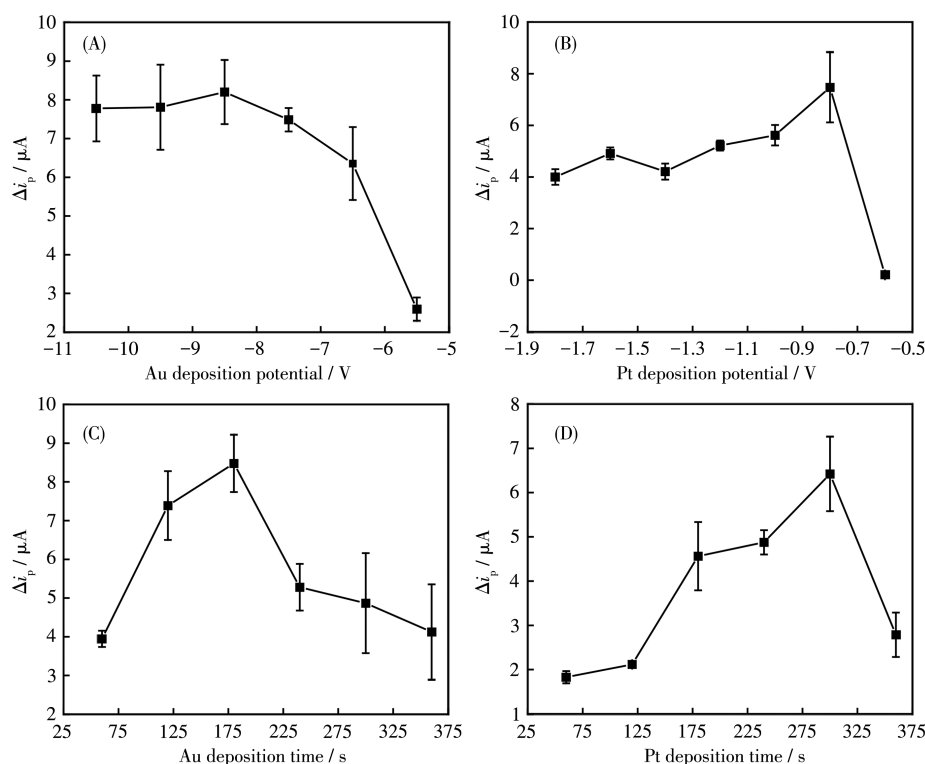


Fig.5 Effect of (A) electrodeposition potential for Au, (B) electrodeposition potential for Pt, (C) electrodeposition time for Au, and (D) electrodeposition time for Pt on the value of Δi_p

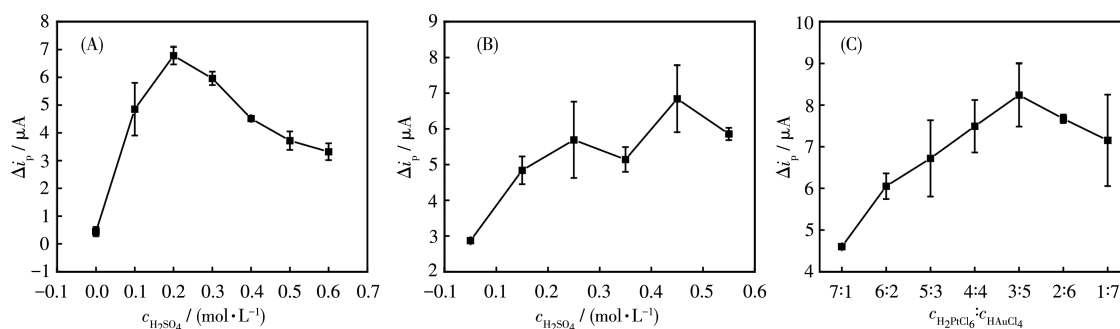


Fig.6 Effects of the concentration of H_2SO_4 in preparing $HAuCl_4$ solution (A), and H_2PtCl_6 solution (B), the concentration ratio of H_2PtCl_6 to $HAuCl_4$ (C) on the value of Δi_p

indicated that the contact interface of the bimetallic Pt/Au is the vital electrocatalytic site for glucose oxidation.

2.4 Amperometric detections of glucose on Pt/Au/AN electrode

To valid the potential application of Pt/Au/AN electrode, the capacity of Pt/Au/AN electrode for the determination of different glucose concentrations was measured by chronoamperometry at a potential of -0.1 V for 50 s in $0.1 \text{ mol} \cdot L^{-1}$ PBS (pH=7.4) solution containing $0.1 \text{ mol} \cdot L^{-1}$ NaCl. As shown in Fig.7A, the amperometric current of Pt/Au/AN increased linearly with the increase of glucose concentration (c) from 0.1 to 35 $\text{mmol} \cdot L^{-1}$. The linear calibration curve of Pt/Au/AN can be expressed as $i = 0.968c^{1/2} + 0.133$ ($R^2 = 0.996$) and the limit of detection (LOD, $S/N=3$) was calculated to be $76.3 \text{ } \mu\text{mol} \cdot L^{-1}$. The reason why Pt/Au/AN electrode possesses high sensitivity and wide linear range

may be attributed to the synergy of bimetals and their contact interface. Meanwhile, the comparison between the previous electrochemical sensors for non - enzyme determination of glucose is shown in Table 1. Our proposed biosensor exhibited an acceptable detection limit and a wider detection range, which helps diagnose hypo and hyperglycemic cases with highly significant in the real-time monitoring of glucose. Moreover, the preparation of Pt/Au/AN electrode is great cheap, and simple, which is expected to be used for actual sample analysis.

2.5 Selectivity, reproducibility, and stability of Pt/Au/AN electrode

Selectivity, which is a crucial indicator for sensors, can evaluate the ability to distinguish the interference and other targets. For the detection of glucose in human serum, the main interfering substances are some electrically active substances, such as AA, UA,

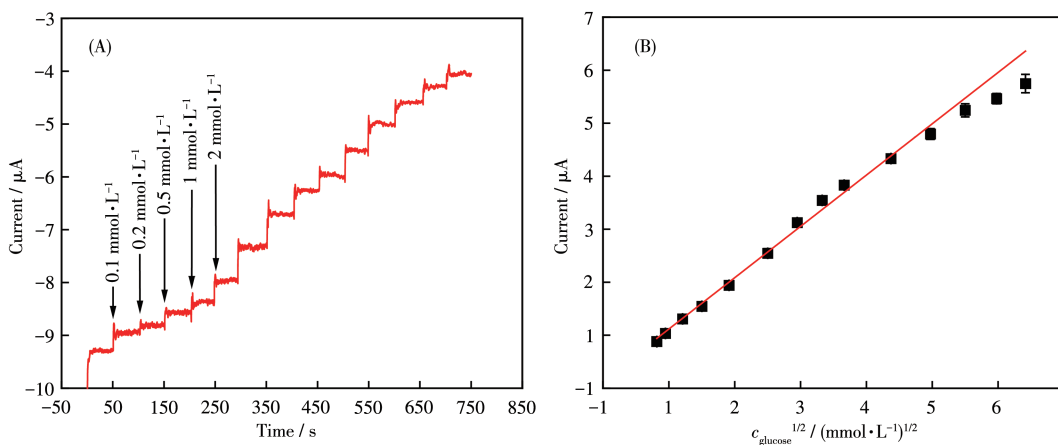


Fig.7 (A) Amperometric response of Pt/Au/AN electrode to different glucose concentrations (0, 0.1, 0.2, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 $\text{mmol} \cdot L^{-1}$) at -0.1 V; (B) Calibration curves of current vs square root of glucose concentration from Pt/Au/AN electrode by chronoamperometry

Table 1 Comparison of non-enzyme glucose sensors with different materials

Material	Linear range / ($\text{mmol} \cdot \text{L}^{-1}$)	LOD / ($\mu\text{mol} \cdot \text{L}^{-1}$)	Sensitivity / ($\mu\text{A} \cdot \text{mmol}^{-1} \cdot \text{L} \cdot \text{cm}^{-2}$)	Ref.
Pt/Au nano-alloy/Pt	1.39-13.9	482	2.82	[39]
Pt/Au/BDD	0.01-7.5	6.5	—	[24]
PtAu/GCE	0.01-10	3	—	[16]
Cu-Al LDH/SPE	3-27	17.6	1 111.15	[13]
Cu_2O -Au/Cu foil	1-20	10	2 430	[35]
Pt/Au/AN	0.1-35	76.3	16.58	This work

4-acetaminophen (AP), and DA. To assess the selectivity of Pt/Au/AN electrode, interferences have been added into $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH=7.4) solution containing $0.1 \text{ mol} \cdot \text{L}^{-1}$ NaCl and $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose, and then the current intensity changes were recorded. As illustrated in Fig.8, it was found that an obvious current response was observed after injection of glucose, while the current response did not change obviously after adding $0.1 \text{ mmol} \cdot \text{L}^{-1}$ AA, $0.5 \text{ mmol} \cdot \text{L}^{-1}$ UA, $0.2 \text{ mmol} \cdot \text{L}^{-1}$ AP, and $0.2 \text{ mmol} \cdot \text{L}^{-1}$ DA respectively. Therefore, it shows that the sensor has high selectivity and anti-in-

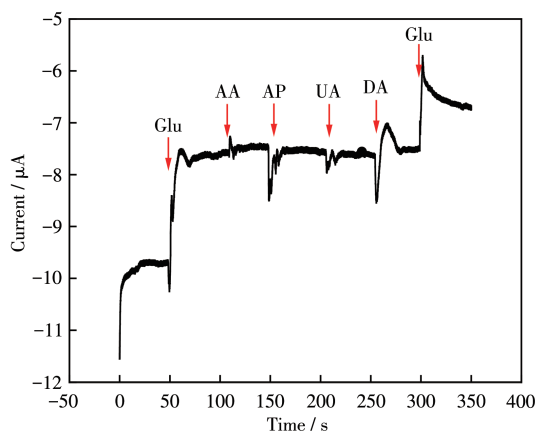


Fig.8 Amperometric response of Pt/Au/AN electrode upon the injection of glucose, AA, UA, AP, DA, and glucose

terference from the anti-interference experiments.

Reproducibility and stability are also important evaluation indicators for sensors. After comparing the current response of six Pt/Au/AN electrodes in $0.1 \text{ mol} \cdot \text{L}^{-1}$ PBS (pH=7.4) solution containing $0.1 \text{ mol} \cdot \text{L}^{-1}$ NaCl and $5 \text{ mmol} \cdot \text{L}^{-1}$ glucose by CV, the result of relative standard deviation (RSD) from six parallel experiments was 4.02%, which shows good repeatability. For the evaluation of stability, the prepared Pt/Au/AN electrode was stored at room temperature for 15 d to compare the response current before and after 15 d. The result showed that Pt/Au/AN electrode remained at 90.5% of the initial current, indicating that the electrode has great stability.

2.6 Determination of glucose in real sample

Pt/Au/AN sensor was used to test human serum samples to further verify its potential application. Firstly, each real sample was diluted by 10 times with PBS (pH=7.4) and then the concentrations of glucose were measured by chronoamperometry. The obtained results were compared with the concentration value measured by Hangzhou Cancer Hospital. Finally, glucose was added to the diluted sample and the concentration of glucose was measured by chronoamperometry again. The measurement results are shown in Table 2. It can

Table 2 Using Pt/Au/AN sensor to measure glucose in real human serum samples

Sample	Hospital measured concentration / ($\text{mmol} \cdot \text{L}^{-1}$)	Detected concentration / ($\text{mmol} \cdot \text{L}^{-1}$)	RSD / % ($n=6$)	Added concentration / ($\text{mmol} \cdot \text{L}^{-1}$)	Detected concentration / ($\text{mmol} \cdot \text{L}^{-1}$)	Recovery / %	RSD / % ($n=6$)
1	5.07	4.96	5.42	5	9.84	97.6	4.76
2	4.66	4.43	8.63	5	9.70	105.4	5.76
3	4.70	4.75	1.59	5	9.87	102.4	2.17
4	4.74	4.59	4.65	10	13.64	90.5	4.84
5	4.99	5.16	3.92	10	15.77	106.10	5.27
6	4.36	4.50	2.66	10	15.29	107.90	8.45

be seen that RSD and recovery were acceptable, indicating that the sensor has good accuracy for glucose detection and a wide range of potential applications in clinical monitoring.

3 Conclusions

In this work, we successfully modified bimetallic Pt/Au cabbage-like nanoparticles onto acupuncture needles by electrodeposition, which can be used to construct a non-enzyme glucose sensor (Pt/Au/AN). Compared with Pt/AN or Au/AN sensors, Pt/Au/AN sensor has better electrocatalytic performance for glucose oxidation, high sensitivity, and selectivity, which is mainly due to the synergistic effect and their contact interface of Pt and Au. Under the optimized conditions, Pt/Au/AN electrode has a good linear relationship over the glucose concentration ranging from 0.1 to 35 mmol·L⁻¹ with the detection limit of 0.076 3 mmol·L⁻¹. Finally, the obtained sensor exhibited an excellent ability to detect glucose in human serum samples. In addition, Pt/Au/AN sensor was constructed based on the acupuncture needle, which possesses a low cost and simple preparation method. In general, these features make Pt/Au/AN sensor have a wide range of potential applications in clinical monitoring.

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Supporting information is available at <http://www.wjhxxb.cn>

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