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An Amperometric Hydrogen Peroxide Sensor Based on Hollow Carbon Spheres/Pt NPs Modified Glassy Carbon Electrode

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A nanoenzyme hydrogen peroxide (H₂O₂) electrochemical sensor was prepared by fixing a hollow carbon sphere (HCS) and platinum nanoparticles (Pt NPs) nanocomposite onto a glassy carbon electrode (GCE) through physical adsorption and electrodeposition. The morphology and element distribution of HCS-Pt/GCE were characterized by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). Cyclic voltammetry (CV) and chronoamperometry (i-t) were used to study the electrocatalytic reduction of H₂O₂ on HCS-Pt/GCE. The synergistic effect of HCS and Pt NPs enhances the conductivity and catalytic ability of the sensor, which makes HCS-Pt/GCE reach high sensitivity (42.8 μ A mM⁻¹ cm⁻²), wide linear range (25 μ M~1.7 mM) and low detection limit (6.25 μ M). In addition, the sensor has good anti-interference performance. It has been successfully used to evaluate the concentration of H₂O₂ in seafood soaking solution samples, which provides a new and convenient method for monitoring whether H₂O₂ in seafood exceeds the standard.

Keywords: Amperometric sensor; Nanozymes, Hydrogen peroxide; Electrochemical catalysis; Real sample analysis

1. INTRODUCTION

Hydrogen peroxide (H_2O_2) is a strong oxidant, which is widely used as bleach and antiseptic in food processing. Some food processing industries may use H_2O_2 as bleaching preservative in violation of regulations during processing, resulting in excessive H_2O_2 residues in food, such as wine, raw milk, tofu and tremella [1]. Excessive H_2O_2 entering the human body will cause extensive damage to skin mucosa and cells, and produce a large number of hydroxyl free radicals during metabolism, thus causing apoptosis and cancer, accelerating human aging and even inducing cardiovascular diseases [2]. Therefore, it is necessary to strengthen the analysis and control of hydrogen peroxide residues in food.

At present, methods for analyzing hydrogen peroxide in food include high performance liquid chromatography [3], spectrophotometry [4], potassium permanganate method [5], iodometry [6] and electrochemical method [7]. China has formulated the Method for the Determination of Hydrogen Peroxide Residue in Food (GB/T 23499-2009). In this standard, iodometry and titanium salt colorimetry are used for detection [8, 9]. Although these methods are accurate, they are cumbersome and time-consuming. The electrochemical method stands out because of its simple operation, high sensitivity and real-time detection [10].

Generally, H_2O_2 can directly participate in an oxidation-reduction reaction on ordinary electrode. However, due to the high electromotive force and slow electrode dynamics of H_2O_2 , its sensitivity is reduced when detected by ordinary electrodes [11]. In addition, H_2O_2 is easy to form intermediate products that can poison the electrode surface or inactivate the electroactive substances on the electrode surface in the process of catalysis. The reduction of electrode material activity and poor stability caused by this are serious problems in electrode application [12-14]. Therefore, the current research mainly focuses on finding new materials with high electrocatalytic activity and good stability to build new enzyme free sensors [15]. The unique chemical and physical properties of nanomaterials make them very suitable for designing new and improved sensing devices, especially electrochemical sensors and biosensors [16]. Different nanomaterials, especially nanomaterials with different properties, have been widely used in many analytical methods through compounding to give full play to their synergistic effect [17].

The research on nanozymes has developed rapidly in the past decade. Among many redox like nanozymes, catalase like nanozymes, as an important part of regulating active oxygen, have been particularly favored in recent years [18]. Because of its low cost, high catalytic activity, high stability, controllable and adjustable enzyme activity, catalase like nanozyme has been widely used in the field of electrochemical sensing detection. Commonly used nanozymes mainly include metal nanomaterials led by Pt, Au and Ag [19] and carbon nanomaterials [20] such as graphene [21], carbon nanotubes [22] and mesoporous carbon [23]. Metallic nanomaterials have good electrochemical stability and excellent catalytic properties, while carbon nanomaterials have good conductivity, large specific surface area, high stability and excellent mechanical strength [24]. In recent years, most of the researches have used the existing material preparation technology to optimize and recombine substances with different properties and compositions to obtain efficient multi-component composites, and then used them to construct nanozyme sensors [25]. Nanocomposites combine the properties of a variety of nanoparticles, which have a strong attraction in the preparation of electrochemical biosensors. For example, Sabury et al. [26] synthesized graphene-Au nanoparticle composite and constructed a sensor to detect glucose. Zhang et al. [27] modified the glassy carbon electrode with graphene-ferric oxide/Pt nanocomposite to prepare a highly sensitive H₂O₂ sensor, which was used for the detection of hydrogen peroxide in the actual sample milk. Saengsrichan et al. [28] successfully constructed a highly sensitive H₂O₂ sensor through hydrothermal synthesis of carbon spot Pt nanoparticle composite, and the detection limit can reach nanomolar.

Therefore, we used a simple and efficient method to construct an electrochemical sensor for the detection of H_2O_2 using hollow carbon spheres and platinum nanoparticles (HCS-Pt) functionalized modified electrodes (Scheme 1). The electrochemical detection technology has many advantages, such as high sensitivity, high selectivity and real-time in situ detection, and is used to detect H_2O_2 in food.



Scheme 1. Schematic diagram of the construction process of HCS-Pt modified GCE for monitoring H_2O_2 .

2. EXPERIMENT SECTION

2.1. Materials and Instrumentation

Chloroplatinic acid (K₂PtCl₆·6H₂O), and hydrogen peroxide (H₂O₂) are from Sinopharm Chemical Reagent Co., Ltd. β -D (+) glucose (Glu), fructose (Fru), glutathione (GSH), dopamine (DA), cysteine (Cys), uric acid (UA), and ascorbic acid (AA) were purchased from Macklin Biological Reagent Co., Ltd. NaH₂PO₄ and Na₂HPO₄ are used to prepare 0.02 M, pH 7.4 phosphate buffer (PBS). The ultrapure water (18.2 M Ω ·cm) used in the experiment comes from the Thermo scientific nanopure water purifier.

Electrochemical workstation (CHI660E), glassy carbon electrode (GCE, CHI104), platinum wire electrode (CHI115), Ag/AgCl electrode (CHI104) and shielding box (CHI200B) were bought from Shanghai Chenhua Instrument Co., Ltd. and used to complete all electrochemical experiments. JSM-7800F FESEM (JEOL, Japan) was used for the morphology characterization and element analysis of the modified electrode.

2.2. Preparation of HCS-Pt/GCE

The hollow carbon spheres (HCS) were obtained according to the reference [29]. First, an HCS aqueous solution with a concentration of 2 mg/mL was prepared, and mixed with ultrasound for 30 minutes, then prepare a chitosan solution with a mass fraction of 1% and a mixed solution of 1% acetic

acid, and mix the HCS aqueous solution and the mixed solution with ultrasound at a volume ratio of 4:1 for 15 minutes to obtain a HCS solution with certain viscosity.

Glassy carbon electrodes (GCE, diameter 3 mm) were polished with 0.3 μ m and 0.05 μ m aluminum oxide powder on the chamois until the surface appeared mirror smooth before use. Then, it was rinsed with ultrapure water continuously for 5 minutes. After it was dried with a N₂ air flow, a certain volume of HCS solution was added to the electrode surface immediately. The modified electrode HCS/GCE can be obtained after drying at room temperature for three hours. Before use, it needs to be soaked with distilled water and dried twice to remove the unfixed nano materials on the surface of the modified electrode.

Platinum nanoparticles (Pt NPs) were electrodeposited onto HCS/GCE by cyclic voltammetry (CV) technology. That is, HCS/GCE was put into a PBS solution containing 2 mM K₂PtCl₆ and 20 mM KCl to scan for a certain number of cycles. The scanning potential window is -0.4V to +0.6V, and the scanning speed is 50 mV/s. After electrodeposition, the electrode was washed with a large amount of ultrapure water, dried at room temperature, and stored at room temperature.

2.3. Morphology characterization of modified electrode

The S-3700 scanning electron microscope produced by Hitachi Co., Ltd. was used to characterize the morphology of modified electrodes, and the images were taken at 1000 times and 30000 times, and then EDS was used to characterize the elements of the modified electrodes.

2.4. Electrocatalytic reduction of H₂O₂ on HCS-Pt/GCE

In order to study the electrochemical behavior of HCS-Pt/GCE electrode, the electrocatalytic behavior of HCS-Pt/GCE on hydrogen peroxide in PBS (0.02 M, pH 7.4) was studied by cyclic voltammetry (CV). PBS (0.02 M, pH 7.4) was bubbled with nitrogen to remove oxygen interference. The potential window is - 0.2 V to + 0.6 V, and the scanning speed is 100 mV/s.

2.5. Electrochemical detection of H_2O_2 in real samples

The hairy crab soaking solution is randomly selected from the local supermarket and filtered with 0.25 μ m filter membrane to obtain the real sample soaking solution. The detection of H₂O₂ in hairy crab soaking solution was performed by chronoamperometry curve. First, a certain volume of sample soaking solution was added to PBS (0.02 M, pH 7.4) to evaluate whether the soaking solution contains H₂O₂. Next, the soaking solution sample containing H₂O₂ was prepared using the standard dropping methods. The recovery was calculated by fitting equation determined by i-t curve according to the response current.

3. RESULTS AND DISCUSSION

3.1. Preparation optimization of HCS-Pt/GCE

The preparation of the modified electrode involves many factors, the most important of which is that the different modification amount of the nanocomposite directly determines the performance of the modified electrode[27, 30].



Figure 1. (A) The CV curve of HCS/GCE obtained by scanning in $K_3[Fe(CN)_6]$ (1 mM) after modified with 0, 4, 5, 6, and 7 µL of HCS respectively; (B) Its corresponding histogram analysis of the current increase times for A. The i-t curve of the current response of HCS-Pt/GCE to H₂O₂ (C) and its corresponding sensitivity histogram (D) after electrodeposition of 5, 8, 10, 11, 12, 13 and 14 CV cycles respectively in 2 mM K₂PtCl₆. Error bar is the standard error of the average value after 3 repetitions.

In the experiment, we found that both HCS and Pt NPs have catalytic effects on the oxidation and reduction of H₂O₂, and the catalytic effects of different modified amounts are different. Therefore, whether it is physically modified HCS or electrodeposited Pt NPs, it is necessary to determine the appropriate amount to achieve the maximum catalytic effect. As shown in Fig. 1A and 1B, the current of HCS modified electrode was significantly higher than that of bare electrode. When the modification volume of HCS was 6 μ L, the current increased the most, and the potential difference was about 96 mV. This is because HCS has a good adsorption effect on small molecule organics in water due to the limitation of pore size. However, too much or too little modified volume of HCS is not conducive to electron transfer [27, 30, 31]. The modification amount of Pt NPs is controlled by changing the number of CV scanning cycles of electrodeposition in a given concentration of K₂PtCl₆ solution (2 mM). When the number of CV scanning cycles of electrodeposition of Pt NPs is 13, the electrocatalytic reduction sensitivity of the modified electrode to H₂O₂ is the highest (Fig. 1C, D). Therefore, we choose HCS to modify the volume of 6 μ L and Pt NPs electrodeposited for 13 cycles as the best modification conditions.

3.2. Morphology characterization of HCS-Pt/GCE

Fig. 2A and 2B show the SEM image of HCS/GCE and HCS-Pt/GCE respectively. It can be clearly seen that the HCS presents a clear two-dimensional structure, similar to a pleated pattern, and HCS is evenly distributed on the electrode surface.



Figure 2. (A, B) SEM morphology characterization of HCS (A) and HCS-Pt (B) modified on detachable GCE respectively; (C, D) EDS of HCS/GCE (C) and HCS-Pt/GCE (D).



Figure 3. (A) EDS layered image of HCS/GCE; (B) Element mapping images of C on HCS/GCE; (C) EDS layered image of HCS-Pt/GCE; Element mapping images of C (D), Pt (E) on HCS-Pt/GCE

After depositing Pt NPs, HCS-Pt is denser than HCS alone. EDS results indicate that the HCS/GCE is mainly composed of carbon element, and HCS-Pt/GCE is composed of carbon element and platinum element. At the same time, EDS imaging shows that there is only carbon element in HCS/GCE and it is evenly distributed, while carbon element and platinum element are evenly distributed in HCS-Pt/GCE.

3.3. Electrocatalytic reduction of H₂O₂ on HCS-Pt/GCE

Fig. 4 shows the CV curves of bare GCE, HCS/GCE, Pt/GCE and HCS-Pt/GCE in K_3 [Fe(CN)₆] solution. Compared with the peak potential, the peak currents of the modified electrodes HCS/GCE, Pt/GCE and HCS-Pt/GCE are all higher than those of the bare GCE. The peak current of HCS-Pt/GCE is the largest, which is owed to the large specific surface area and good electrical conductivity of the composite of HCS and Pt NPs [27, 30, 31].



Figure 4. CV curves of GCE, Pt/GCE, HCS/GCE, HCS-Pt/GCE scanning in 1 mM K₃[Fe(CN)₆]. Scan rate: 100 mV/s.

According to Randles Sevcik equation [31], the effective area of the modified electrode can be calculated:

 $Ip = 2.69{\times}10^5 AD^{1/2}n^{3/2}\gamma^{1/2}C$

Where Ip represents the redox peak current value, in amperes; A is the effective area of electrode, in cm²; D is the diffusion coefficient of molecules in solution, which is $(6.70 \pm 0.02) \times 10^{-6}$ cm² s⁻¹ in potassium ferricyanide solution; N represents the number of electron transfers when redox occurs, for $[Fe(CN)_6]^{3-/4-}$, n=1; γ represents the scanning speed of cyclic voltammetry curve, unit: V s⁻¹; C is the concentration of redox probe molecules in solution, in mol cm⁻³. According to the formula, the effective areas of the four different modified electrodes are GCE (0.06886 cm²), HCS/GCE (0.7913 cm²), Pt/GCE (0.7508 cm²), HCS-Pt/GCE (1.0004 cm²), which shows that the effective areas of the modified electrodes are significantly increased. This makes it possible for HCS-Pt/GCE to be used as an enzyme free sensor for highly sensitive detection.

Fig. 5 displays the CV curve of HCS-Pt/GCE in deoxygenated PBS (0.02M, pH 7.2) before and after adding H_2O_2 successively. When the concentration was 0.50 mM, the reduction peak of hydrogen peroxide appeared and moved to the negative potential along with the concentration rise. However, with the adding of H_2O_2 , the reduction peak current near - 0.2 V still increased significantly, indicating that H_2O_2 can be catalytic reduced by HCS-Pt/GCE in a wide concentration range (0.25 mM~5.20 mM). The excellent electrocatalytic reduction performance of HCS-Pt/GCE for H_2O_2 is mainly owed to the unique nanostructure of HCS-Pt and the synergistic effect between HCS and Pt NPs.



Figure 5. CV curves of HCS-Pt/GCE in PBS (0.02M, pH 7.4) with different concentrations of hydrogen peroxide. The concentration of hydrogen peroxide is 0, 0.25, 0.5, 1, 1.49, 2.11, 2.73, 3.97 and 5.20 mM respectively; Scan rate: 100 mV/s.

In order to illustrate the catalytic performance of the constructed HCS-Pt/GCE sensor, the amperometric current time response (i-t) technique was used to compare the sensitivity of several modified electrodes for H₂O₂ detection. We compared the current response of GCE, Pt/GCE, HCS/GCE and HCS Pt/GCE after continuously adding H₂O₂ to PBS (0.01 M, pH 7.2) at - 0.2 V. As shown in Fig. 6A, compared with Pt/GCE and HCS/GCE, HCS Pt/GCE has greater current response when the same H₂O₂ concentration is added. The excellent electrocatalytic activity of HCS-Pt/GCE results from the good synergistic effect between Pt NPs and HCS [31]. First, the HCS with excellent electronic conductivity has a good surface area [29]. Then, the Pt NPs dispersed on HCS provide better catalytic activity than single component Pt NPs.

As shown in Fig. 6B, we can get that the linear equation of HCS-Pt/GCE is $I(\mu A) = 18.18 \text{ C}+3.12$ (linear correlation coefficient $R^2 = 0.990$; C is the concentration of H₂O₂), and the sensitivity is 257.2 ± 5 mA M⁻¹ cm⁻²; The linear equation of Pt/GCE is $I(\mu A) = 3.94 \text{ C}+2.69$ ($R^2 = 0.925$), the sensitivity is 55.7 ± 3 mA M⁻¹ cm⁻²; The linear equation of HCS/GCE is $I(\mu A) = 3.03 \text{ C}+0.14$ ($R^2 = 0.952$), the sensitivity is 42.8 ± 2 mA M⁻¹ cm⁻². The sensitivity of HCS-Pt/GCE is significantly higher than that of Pt/GCE and HCS/GCE, which further indicates that HCS-Pt/GCE is an excellent nanocomposite for H₂O₂ detection.



Figure 6. (A) i-t curves obtained by adding H₂O₂ into deoxygenated PBS (0.02 M, pH 7.2) by GCE, Pt/GCE, HCS/GCE and HCS-Pt/GCE. Potential: - 0.2 V. (B) Linear calibration curves of response current and H₂O₂ concentration corresponding to GCE, Pt/GCE, HCS/GCE and HCS-Pt/GCE. Error bar is the standard error of the average value after 3 repetitions.



Figure 7. (A) i-t curve of HCS-Pt/GCE with successive adding of 0.025, 0.0875, 0.25, 0.38, 0.50, 0.80, 1.18 and 1.70 mM H₂O₂ to deoxygenated PBS (0.02 M, pH 7.4). Potential: - 0.2 V; (B) The fitting line between the response current and H₂O₂ concentration in the range of 0.025 to 1.70 mM.

Fig. 7A shows the current response curves of HCS-Pt/GCE for adding H₂O₂ to PBS (0.01 M, pH 7.2) continuously at the potential of - 0.2 V. When H₂O₂ was added to PBS, HCS-Pt/GCE show a good and sharply increased current response, and the response time is about 2~3 s, which obviously shows the rapid electron transfer on HCS-Pt/GCE. With the increase of concentration, the current step is continuously strengthened. At the concentration range of $2.5 \times 10^{-5} \sim 1.7 \times 10^{-3}$ mol L⁻¹, there is a good linear relationship between the response current and H₂O₂ concentration. The linear regression equation is I = 18.558C + 3.119, and the correlation coefficient is 0.990. As shown in Fig. 8, through experiments,

the detection limit of H_2O_2 can be reduced to 6.25 μ M. Compared with the H_2O_2 sensor reported in the literature, the sensor is comparable in performance index, such as detection sensitivity, detection limit and detection range (Table 1). These results attribute the synergistic effect of HCS-Pt composite materials, which promotes the electron transfer rate of H_2O_2 on the electrode surface and enhances the electrocatalytic effect of Pt NPs on H_2O_2 .



Figure 8. i-t curve of HCS-Pt/GCE with successive adding of 6.25, 18.75, and 31.25 mM H₂O₂ to deoxygenated PBS (0.02 M, pH 7.4). Potential: - 0.2 V.

Sensors	Sensitivity (µA mM ⁻¹ cm ⁻²)	LOD (µM)	Linear range (µM)	References
PRGO-AuNPs-GOx	15.04	0.06	0.4-4	[26]
Fer/rGO-Pt	340	0.38	0.4-10800	[27]
CDs/PtNPs		0.01	1-100	[28]
RGO-Pt	459	0.2	0.5-3475	[31]
ERGO	300	0.7	1-16	[32]
Ag NPs-L-pro	12.17	0.05	0.1-5410	[33]
CoFe ₂ O ₄ -CNTs	3310	0.005	5-50	[34]
Mc-AgNPs	7067	0.05	0.1-41000	[35]
PtNPs-MWCNTs- SDS		0.0019	0.0058-1100	[36]
Pt-Au/rGSs		0.31	0.001- 1.78 ; 1.78-16.8	[37]
CNTs-TiO ₂ /Pt	36.4	5	1-100	[38]
Cu ₂ O/PANI/rGO	39.4	0.5	0.8-12780	[39]
HCS-Pt	42.8	6.25	25-1700	This work

Table 1. Comparison of performance indexes of various electrodes for H₂O₂ detection

3.4. Selectivity of HCS-Pt/GCE

In the experiment, the anti-interference ability of the sensor is tested by selecting several potential interfering substances in practical application, which is one of the main factors to be investigated. 0.04 M H_2O_2 and 0.4 M interfering substances uric acid (UA), ascorbic acid (AA), dopamine (DA), glutathione (GSH) and glucose (Glu) were continuously added to 0.02 M PBS (pH 7.4) solution, and then the chronoamperometric response curve was recorded.

Fig. 9 shows a typical chronoamperometric reaction when 0.04 M H_2O_2 , 0.4 M UA, AA, DA, GSH and Glu are continuously added to PBS (0.01 M, pH7.2) solution. It was found that the HCS-Pt/GCE showed a clear current response to H_2O_2 , while other interferences did not show a clear current response. The results show that the prepared HCS-Pt/GCE has good selectivity for H_2O_2 .



Figure 9. i-t curve of HCS-Pt/GCE with adding 0.04 M H₂O₂, 0.4 M UA, AA, DA, GSH, Glu and a second addition of 0.04 M H₂O₂ in deoxygenated PBS (0.02 M, pH 7.4).

3.5. Real sample detection

In order to prove the feasibility of the sensor, the target analyte H_2O_2 in the actual sample was studied. The sample of hairy crab soaking solution was taken from the local supermarket. Before analysis, the hairy crab immersion solution sample was tested to estimate whether there is endogenous H_2O_2 . Fig. 10A shows the amperometric current response changes obviously after adding hairy crab immersion solution, which indicates that the hairy crab immersion solution contains H_2O_2 . Therefore, we prepared H_2O_2 of different concentrations with hairy crab immersion solution, and measured the fitting equation of HCS-Pt/GCE in hairy crab immersion solution by i-t technology. As displayed in Fig. 10B, within the concentration range of $0.0 \sim 1.7 \times 10^{-3}$ mol L⁻¹, it shows a good linear relationship between the response current and H_2O_2 concentration. Then, the endogenous H_2O_2 concentration in the sample was calculated by using this equation, which is 24.3 μ M_o



Figure 10. (A) i-t response curves obtained by continuously dropping hairy crab immersion solution into deoxygenated 0.02 M PBS, pH 7.4. Potential: - 0.2 V; The arrow position represents the time point for adding H₂O₂ of different concentrations. (B) Linear calibration curve of ampere current and H₂O₂ concentration of HCS-Pt/GCE in the range of 0-2.5 mM.

Table 2. The recovery of H₂O₂ from hairy crab immersion solution samples using the H₂O₂ sensor

Hairy crab soaking solution ^a	Concentration of H ₂ O ₂ (µM)			
	Original	Amount added	Amount detected ^b	Recovery (%)
1	24.30	0.61	0.64±0.03	104.92±4.92
2	24.30	1.06	1.06 ± 0.06	100.00 ± 5.66
3	24.30	1.51	1.49 ± 0.04	98.68±2.65
4	24.30	1.71	1.69 ± 0.04	98.83±2.34
5	24.30	2.11	2.04±0.04	96.68±1.90

^a Samples 1-5 are added with four different concentrations of H₂O₂: 0.61, 1.06, 1.51, 1.71 and 2.11 μ M. ^b The result is the average of the parallel analysis (*n* = 5).

This study demonstrates the availability of sensors. Table 1 shows that the recovery rate of the sample added with hairy crab soaking solution is 96.77-105.26%, and the RSD is 4.17%, which indicates that the sensor can be used to determine the residual H_2O_2 in hairy crab soaking solution, providing a new method for the evaluation of H_2O_2 in food.

4. CONCLUSIONS

A novel enzyme free biosensor with high performance was prepared by physically fixing HCS and electrodepositing Pt NPs onto a GCE. The HCS-Pt/GCE enzyme free sensor has a wide detection

range (25 μ M~1.7 mM), lower detection limit (6.25 μ M), and anti-interference capability. Furthermore, the sensor has good anti-interference performance and has been successfully used to evaluate H₂O₂ residues in seafood soaking samples. Therefore, as an effective, reliable and convenient detection tool, the sensor has an important application prospect in the field of food safety monitoring.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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