

Electrochemical Determination of Nitrite Using a Gold Nanoparticles-modified Glassy Carbon Electrode Prepared by the Seed-mediated Growth Technique

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Seed-mediated growth of gold nanoparticles on glassy carbon (GC) surfaces was developed. The field emission scanning electron microscopy (FE-SEM) and electrochemical characterization confirmed the effective attachment of gold nanoparticles on GC surface with such a wet-chemical method. The as-prepared gold nanoparticles attached glassy carbon electrode (Au/GCE) presented excellent catalytic ability toward the oxidation of nitrite. Compared with bare GCE and planar gold electrode, the Au/GCE obviously decreased the overpotential of nitrite oxidation and improved the peak current. The catalytic current was found to be linearly proportional to the nitrite concentration in the range of 1×10^{-5} – 5×10^{-3} M, with a detection limit of 2.4×10^{-6} M. The Au/GCE was successfully applied to the electrochemical determination of nitrite in a real wastewater sample, showing excellent stability and anti-interference ability.

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Introduction

Because nitrite ions can be made to interact with amines to form nitrosamines, which are well-known carcinogenic substances,¹⁻³ there has been an increasing interest in the development of methods for the quantitative determination of nitrite concentrations, especially in the supervision of the quality of drinking water, in wastewater treatment, and in the food industry. The simple but highly selective and sensitive methods suitable for fast and reliable field measurements are desirable. Besides the traditional methods such as spectrophotometry⁴ and ion chromatography,⁵ more and more emphases have been focused on the electrochemical methods in the determination of nitrite thanks to their convenience, cheapness and simplicity.

The electrochemical determinations of nitrite at traditional electrodes, such as platinum electrode⁶⁻⁸ and glassy carbon electrode (GCE),⁹ have been developed. However, the direct electroreduction/oxidation of nitrite ions requires high overpotential at bare electrode surfaces. In addition, the determination of nitrite at bare electrodes always suffers from the interference from other compounds. Therefore, chemical modification of electrodes using materials such as metallophthalocyanines and metalloporphyrins,¹⁰⁻¹⁷ series of inorganic porous materials¹⁸ and enzyme electrodes¹⁹ have been proposed for nitrite sensing.

Recently, noble metal nanoparticles have become favorite materials in electroanalysis, due to their small dimensions and catalytic activities.²⁰ A novel Pt-Fe(III) nanoparticle

modification method for construction of a nitrite sensor has been proposed.²¹ On the other hand, as one of the most popular materials, gold nanoparticles showing interesting properties have been extensively studied. However, directly determining nitrite, especially in real samples, based on gold nanoparticles has not been described. In this research, we studied the electrochemical oxidation of nitrite on gold nanoparticle-attached glassy carbon electrodes for nitrite sensing in wastewater. The electrode modification is based on a seed-mediated growth approach,²² which has been demonstrated as a new electrode modification method without using peculiar binder molecules.²³ Such a gold nanoparticle modified ITO electrode has provided useful applications in electroanalysis, such as fabricating protein-based biosensors^{24,25} and sensitively determining guanosine²⁶ and epinephrine.²⁷ In the present work, we demonstrate that the seed-mediated growth of gold nanoparticles on glassy carbon (GC) surface is actually feasible. The FE-SEM and electrochemical results confirmed that gold nanoparticles could be successfully attached on GC surfaces. The gold nanoparticles attached on GCE (Au/GCE) showed dramatically electrocatalytic activity toward the oxidation of nitrite. Based on the linear relationship between nitrite concentration and the peak current response of nitrite on Au/GCE, we could illustrate voltammetric determinations of nitrite in wastewater with excellent stability and anti-interference ability.

Experimental

Apparatus and reagents

Field emission scanning electron microscope (FE-SEM)

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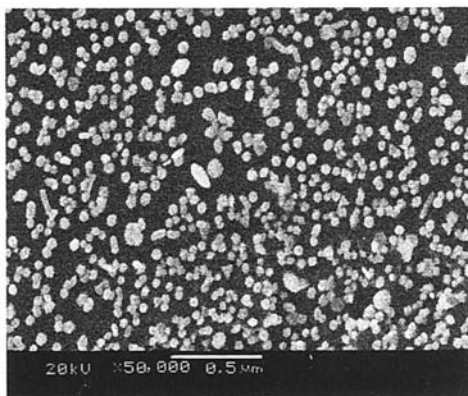


Fig. 1 FE-SEM image of Au/GCE.

images were obtained with a JSM-5510LV FE-SEM instrument. A Unico UV-2000 spectrophotometer (Shanghai Unico Instruments Co., China) was used for spectrophotometric analysis of nitrite content in wastewater, based on the diazotization of sulfanilic amide with nitrite in acidic medium and on a subsequent coupling of the diazonium ions with *N*-(1-naphthyl)ethylenediamine.²⁸ In this method, a color reagent was prepared by adding 250 mL water, 50 mL H₃PO₄ and 20.0 g sulfanilic amide in a 500-mL beaker; next, 1.0 g *N*-(1-naphthyl)ethylenediamine dihydrochloride was dissolved in this solution and the solution volume was adjusted with water to the mark in a 500-mL volumetric flask. Then, a 1.0-mL wastewater sample was pipetted into a 50-mL volumetric flask and diluted with water to the mark. After adding 1.0 mL color reagent into the diluted wastewater solution and allowing the solution to stand for 20 min, we measured the absorbance of the solution at 540 nm for determining the concentration of nitrite according to the standard calibration curve. Electrochemical experiments were carried out with a VMP2/Z electrochemical workstation (Princeton Applied Research, USA) and a CHI 660A electrochemical workstation (Chenhua Instruments Co., Shanghai, China). A conventional three-electrode system was used, consisting of a bare or modified glassy carbon working electrode with an exposed geometric area of *ca.* 0.0314 cm², a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE). All experiments were carried out at room temperature. High purity nitrogen was used for solution deaeration at least 15 min prior to electrochemical measurements and all materials were kept under nitrogen atmosphere during the measurements.

Cetyltrimethylammonium bromide (CTAB) was purchased from Amresco. Other solvents and chemicals were of analytical grade. Phosphate buffer solution (PBS) was prepared by mixing the stock solutions of 0.1 M Na₂HPO₄ and 0.1 M NaH₂PO₄. The nitrite determinations were carried out in the pH range 4.0–6.0 using 0.2 M acetate buffer solution. Ultra-pure fresh water obtained from a water purification system (EASY Pure LF, Dubuque Co., USA) with a specific resistivity of >18.3 MΩ cm⁻¹ was used in all runs.

Preparation of the modified electrode

Prior to the modification, the bare GCE was polished successively with No. 1 to No. 6 emery papers and 0.5 μm diamond slurry to mirror-like smoothness. Then it was sonicated in ethanol and distilled water for 15 min, followed by drying with a stream of high purity nitrogen. The fabrication of an Au/GCE was carried out by a seed-mediated growth method

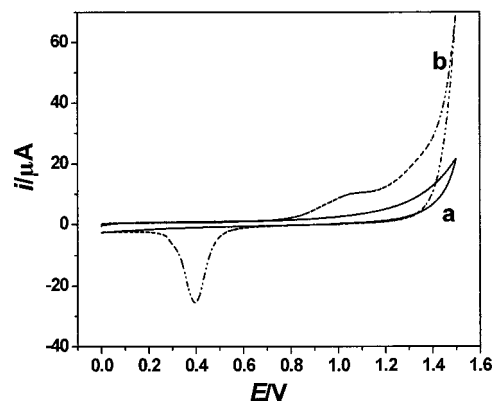


Fig. 2 Cyclic voltammograms of bare GCE (a) and Au/GCE (b) in 0.1 M PBS (pH 7.0) at 100 mV/s.

that was developed by Murphy and coworkers.²² In the first modification, the GCE was immersed in the gold seed solution, which was prepared by mixing 0.5 mL HAuCl₄ (0.01 M) with 0.5 mL trisodiumcitrate (0.01 M), 18 mL water and 0.5 mL NaBH₄ (0.1 M) ice-cold fresh aqueous solution with stirring, and the solution was left undisturbed for 2 h.

After the seeding procedure, the substrate was thoroughly rinsed with distilled water, dried with nitrogen and then immersed in the growth solution, which was developed by mixing 18 mL CTAB (0.1 M), 0.5 mL HAuCl₄ (0.01 M), and 0.1 mL ascorbic acid (0.1 M) solutions. The substrate was taken out of the solution after 24 h and washed thoroughly with distilled water and then dried with nitrogen.

Results and Discussion

Morphological and electrochemical characterization of Au/GCE

The FE-SEM image of Au/GCE is shown in Fig. 1, confirming the attachment of gold nanoparticles directly on the glassy carbon electrode surface with the seed-mediated growth method. The nanoparticles are all spherical with diameters of 60–100 nm.

Figure 2 shows the cyclic voltammograms of Au/GCE and bare GCE in PBS (pH 7.0). For bare GCE, no faradaic current is shown, while for Au/GCE, a small anodic wave at 1.10 V and a remarkable cathodic wave at 0.40 V corresponding to the characteristic peaks of gold are observed, further confirming the attachment of gold nanoparticles on glassy carbon surfaces.

Similar to the case of ITO, on GCE, the formation of gold nanoparticles by this wet-chemical method can also be attributed to the unusual properties of ultra-small-sized particles. It has been demonstrated that gold seeds with the diameter of about 4 nm are formed during the seeding procedure;²³ and it is speculated that 4 nm gold seeds exhibit a strong adsorptivity, which allows them to deposit directly on the GCE surface even without a thiol linker.²⁷ Then, in the growth procedure, the growth of gold nanoparticles on GCE surface may also reinforce the compact attachment of these nanoparticles onto the substrate. Thus, gold nanoparticles with such a seed-mediated growth method can be directly attached on GCE.

Electrocatalytic oxidation of nitrite at Au/GCE

In order to evaluate the electrocatalytic activity of gold

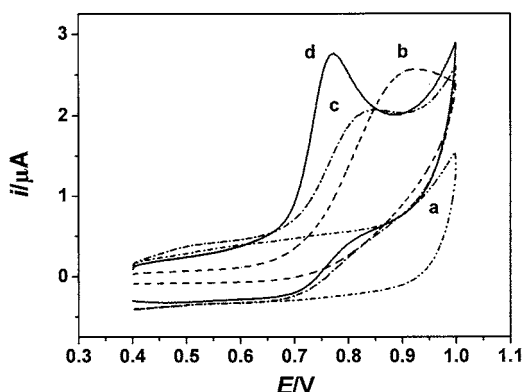


Fig. 3 Cyclic voltammograms of Au/GCE (a, d), bare GCE (b) and planar gold electrode (c) in the absence of nitrite ions (a) and in the presence of 1×10^{-4} M NO_2^- (b, c, d) in 0.2 M acetate buffer solution (pH 4.6) at 100 mV/s.

nanoparticles toward the oxidation of nitrite, we recorded the cyclic voltammograms of a bare GCE, planar gold electrode and Au/GCE in 0.2 M acetate buffer solution (pH 4.6) in the absence and presence of 1×10^{-4} M NO_2^- (Fig. 3). In the absence of nitrite, no redox peak was observed on the Au/GCE in pH 4.6 buffer solution, while with the addition of 1×10^{-4} M NO_2^- , a significant oxidation peak for NO_2^- appeared at 0.77 V vs. SCE. Compared with the results obtained at bare GCE, the potential was shifted negatively by about 150 mV at Au/GCE, accompanied with an enhancement of 0.8 μA in peak current. On the other hand, the Au/GCE exhibits a sharper oxidation peak with almost 1 μA enhancement in peak current for nitrite as compared with the planar gold electrode. These results illustrate that the gold nanoparticles could present a favorable activity toward the oxidation of nitrite by reducing the oxidation overpotential and increasing the peak current, suggesting that the Au/GCE will be an excellent sensor for NO_2^- determination.

Figure 4 shows the cyclic voltammograms obtained at different potential scan rates at Au/GCE in 1×10^{-4} M NO_2^- . The inset in the figure shows the linear increase of the oxidation peak current (i_p) with the square root of the scan rate ($v^{1/2}$) in the range of 10–200 mV/s. This result demonstrates that the oxidation process of nitrite at Au/GCE is controlled by the mass transport of nitrite ion from the bulk solution to the electrode surface.

In addition, it was observed that the catalytic oxidation peak potential (E_p) shifted slightly to more positive potentials with increasing the scan rate. The analysis of these data shows that the plot of E_p vs. the logarithm of scan rate presents a linear relation and the slope of this plot was equal to 20.2 mV decade⁻¹, indicating that the electrocatalytic oxidation of nitrite on the Au/GCE surface is irreversible.²⁹ According to the following equation, which is valid for a totally irreversible diffusion-controlled process,³⁰

$$E_p = A + \frac{2.3RT}{2(1-\alpha)n_aF} \log v \quad (1)$$

where α is the transfer coefficient, n_a is the number of electrons involved in the rate determining step, v is scan rate, α is calculated to be 0.3.

Effect of pH on the Au/GCE response to nitrite

In strongly acidic media, the rate of disproportionation of

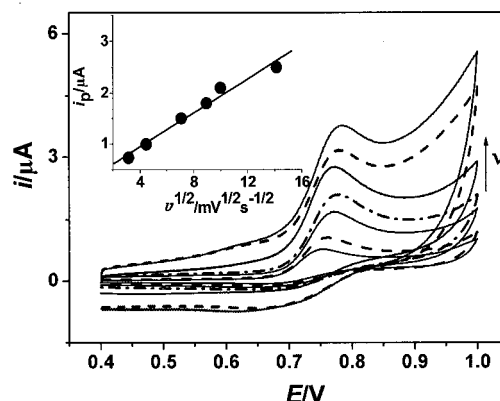


Fig. 4 Cyclic voltammograms for the oxidation of nitrite (1×10^{-4} M) on Au/GCE at various potential scan rates. Electrolyte: 0.2 M acetate buffer solution (pH 4.6). Inset: Linear relationship between the oxidation peak current of NO_2^- and the square root of scan rate.

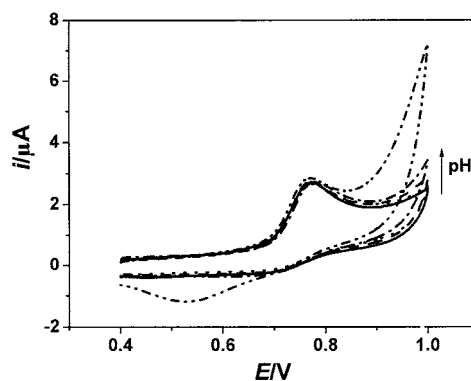
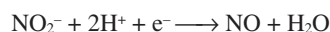


Fig. 5 Cyclic voltammograms of the Au/GCE in 0.2 M acetate buffer solution with 1×10^{-4} M nitrite at pH 4.0, 4.6, 5.0, 5.6, 6.0.

nitrite is significant according to the following reaction.



Therefore, to make sure that nitrite is the dominant species, we selected the pH range from 4.0 to 6.0 to observe the pH effect on the electrocatalytic behavior of nitrite oxidation on Au/GCE. However, the results show that the peak current and the peak potential for the Au/GCE in 1×10^{-4} M nitrite solution are almost not influenced by pH over this pH range (Fig. 5). Similar results have also been found on palladium pentacyanonitrosylferrate modified aluminum electrode¹⁴ and on Co(II) porphyrin modified carbon paste electrode¹⁷ for nitrite electrocatalytic oxidation. The pH-independent result seems to be inconsistent with the pH-dependent characteristic of the nitrite oxidation reaction to nitrate. We speculate that the reaction is an electrocatalytic procedure and is controlled by kinetics rather than thermodynamics.

From Fig. 5, one can also notice that, when the pH increased to 6.0, the reduction peak of gold was presented at 0.53 V, which induced the enhancement of background current. To make sure of the sensitivity of the Au/GCE response to nitrite, we selected the pH of electrolyte to be 4.6 in the following experiments.

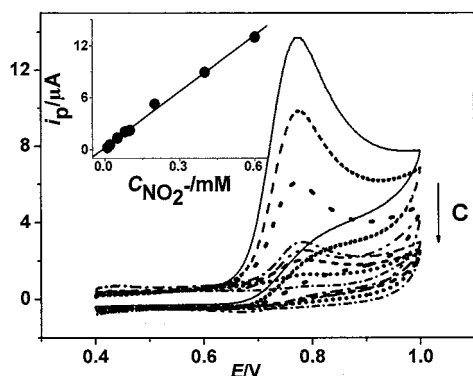


Fig. 6 Cyclic voltammograms of the Au/GCE in 0.2 M acetate buffer solution (pH 4.6) with 6×10^{-4} , 4×10^{-4} , 2×10^{-4} , 1×10^{-4} , 8×10^{-5} , 5×10^{-5} , 2×10^{-5} and 1×10^{-5} M nitrite at 100 mV/s. Inset: Linear relationship between peak current and concentration of nitrite.

Calibration curve for nitrite determination

The oxidation current of nitrite at the Au/GCE in acetate buffer solution is found to present an excellent linear relationship with the nitrite concentration in the range of 1×10^{-5} – 5×10^{-3} M. The linear range was broader than the reported ranges obtained from CV analysis^{15,31,32} or from amperometry using flow injection analysis.³³ The regression equation is expressed as $I_p (\mu\text{A}) = 0.098 + 22.2C_{\text{NO}_2^-} (\text{mM})$, with a correlation coefficient of $r = 0.9995$ ($n = 12$). Figure 6 illustrates the cyclic voltammograms at lower concentration range and the corresponding calibration curve. The detection limit ($S/N = 3$) is estimated to be 2.4×10^{-6} M, lower than that on the palladium pentacyanonitrosylferrate-modified aluminum electrode¹⁴ and the 3-mercaptopropionic-modified gold electrode.³⁴

Determination of nitrite in wastewater

To test the analytical application of this electrochemical method, we collected four real wastewater samples in flaxen color from the outflow of a bioreactor for removing the total nitrogen from cesspool wastewater. The concentrations of nitrite in these wastewater samples were determined by cyclic voltammetry using Au/GCE as proposed above, after the pH of the wastewater samples were adjusted to 4.6 by adding acetate. At the same time, the concentrations of nitrite in these samples were also determined by spectrophotometry. The concentration values obtained from the two methods are listed in Table 1. It can be seen, although the range of determined nitrite concentration is wide, there is no remarkable difference between the conventional spectrophotometric results and our electrochemical determination results. That is to say, the gold nanoparticles-modified glassy carbon electrode can act as an effective method for the detection of nitrite in wastewater.

Stability and selectivity

To evaluate the operational stability of Au/GCE, we used one modified electrode to determine the nitrite in the real wastewater sample by 22 successive measurements in one day. The relative standard deviation (RSD) was calculated as 2.4% and only a small decrease of current (about 8%) was observed after 22 measurements, illustrating an excellent stability of Au/GCE for electrochemical determination of nitrite in wastewater. The operational stability of Au/GCE in nitrite determination is much better than that of a metallophthalocyanines-modified electrode,¹¹ a carbon nanotube-modified

Table 1 Comparison between the nitrite concentrations in real wastewater samples determined by proposed electrochemical method and by standard spectrophotometric method

Water sample	Nitrite concentration/mM	
	Voltammetry	Spectrophotometry
1	0.0639	0.0638
2	0.481	0.471
3	0.823	0.872
4	1.367	1.421

electrode³⁵ or an enzyme-modified electrode.¹⁹

Moreover, the long-term stability of Au/GCE was also studied by storing the electrode for 7 days at room temperature. Compared with the response for nitrite in the real wastewater sample measured at the first day, the current response was only decreased by 2.3% after 7 days, further revealing that this modified electrode has good long-term stability and repeatability for nitrite determination.

On the other hand, the selectivity and anti-interference ability of Au/GCE were tested by studying the effects of common ions in the determination of the real sample. No obvious interference was seen in nitrite determination when adding 20-fold uric acid, 100-fold Ca^{2+} , Cu^{2+} , SO_4^{2-} , K^+ , PO_4^{3-} , CO_3^{2-} , NO_3^- and plenty of Ac^- and Na^+ in real samples. In addition, a 200-fold amount of Cl^- did not interfere with the nitrite determination using such an Au/GCE, similar to the voltammetric determination of nitrite on other modified electrodes.^{10,34,35} However, 50-fold ascorbic acid was found to have noticeable interference in nitrite determination on the Au/GCE.

Conclusion

In this work, a gold nanoparticles-attached glassy carbon electrode was prepared with the seed-mediated growth method. By comparison of the responses of nitrite on the Au/GCE, on the bare GCE and on the planar gold electrode, the gold nanoparticles were found to have favorable activity toward the oxidation of nitrite by reducing the oxidation peak potential and increasing the peak current. The oxidation current response at the modified electrode was found to be linearly proportional to the nitrite concentration in the range of 1×10^{-5} – 5×10^{-3} M with a low detection limit of 2.4×10^{-6} M. Accordingly, the Au/GCE was applied to the determination of nitrite in a wastewater sample, which exhibited a desirable stability, repeatability and selectivity.

Acknowledgements

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