

Solid-state voltammetry based electrochemical immunosensor for *Escherichia coli* using graphene oxide-Ag nanoparticle composites as labels

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1. Preparation of AgNPs

AgNPs were prepared by reduction of AgNO₃ by sodium citrate dihydrate according to the literature with some modifications.¹ Briefly, 5 mL of 10 mM AgNO₃ was added to 45 mL of water at 45 °C and heated rapidly to boiling. Then 1 mL of 1% sodium citrate dihydrate and 300 μL of 3 mM NaBH₄ were injected under vigorous stirring, and the resulting solution was held at boiling for 60 min. The resulting solution was cooled in the ambient conditions. The solution was subsequently filtered through a polycarbonate membrane (0.22 μm). Finally, the obtained solution was centrifugated at 8000 rpm and the precipitate was re-dissolved in water. They displayed an absorption band at 393 nm, which corresponded to the characteristic surface plasma resonance band of AgNPs. The final concentration of resultant AgNPs was 0.1 mg mL⁻¹.

2. Preparation of AuNPs

AuNPs were synthesized according to the as-reported method.² Briefly, 500 μL of 2% HAuCl₄ was diluted in 100 mL of ultrapure water and brought to reflux while stirring and then 1.3 mL of 1% trisodium citrate solution was added quickly, which resulted in a color change of the solution from pale yellow to deep red. After the color changed, the solution was refluxed for an additional 15 min. The final concentration of resultant AuNPs was 0.1 mg mL⁻¹.

3. Preparation of P-GO

First, the graphite oxide was obtained from natural graphite powder by the modified Hummers method.³ Then, the resulting solid (50 mg) was dispersed in 100 mL water by ultrasonication for 2 h to make an aqueous dispersion; the obtained dispersion was subjected to 30 min of centrifugation at 3000 rpm to get a black-brown supernatant. Finally, the homogeneous exfoliated graphite oxide aqueous dispersion was used to prepare P-GO according to a previous work.^{1b} In a typical procedure, PVP-capped graphene oxide was firstly prepared by adding 80 mg of PVP to 20

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mL of 0.25 mg mL⁻¹ graphene oxide solution and stirring for 30 min, which was washed and centrifuged three times and dissolved in 5 mL of water. Then, 0.1 mL of 20 wt % PDDA was mixed well with 16.8 mL of 0.625 M KCl, followed by injecting 4.2 mL of PVP-capped graphene oxide, and the resulting solution was sonicated for 1.5 h. The products were washed and centrifuged three times. Finally, the P-GO was redispersed in 4 mL of water, forming a brown suspension with a final concentration of 1.0 mg mL⁻¹.

4. The protocols of solid-state voltammetry and stripping voltammetry in Fig. 4A

The AgNPs modified electrodes used in Fig. 4A were prepared by the following procedure. A pretreated Au electrode was immersed in 0.1 M of cysteamine aqueous solution for 10 h, followed by washing with water, and then dried with high-purity nitrogen steam. Twenty microliters of P-GO-Ag composites were dropped on the center of the pretreated electrode and allowed to dry at room temperature for over 3 h. Then the modified electrode was washed with PBST (PBS with 0.05% Tween, pH 7.4) for 15 min to remove unabsorbed materials. Solid-state voltammogram was obtained by CV in 0.2 M of KCl solution. As shown in Fig. 4A (curve b), two well-defined current peaks were observed. That corresponded to the oxidation of Ag to forming insoluble AgCl and the reduction of AgCl, respectively. Stripping voltammogram was obtained by CV in 1 M of KNO₃ solution. In the anodic potential sweep, a very sluggish process was observed in the potential range of 0.4–0.55 V vs Ag/AgCl, corresponding to the oxidation of AgNPs. The Ag was oxidized to form Ag⁺ and stripped from the electrode. So in the reverse cathodic potential cycle, no distinct cathodic peak was observed. It is well observed that the Ag/AgCl solid-state voltammetric process produced a sharper and higher peak at a lower potential than that of stripping voltammetric process.

Table 1S Comparison of character of the proposed method with previously published electrochemical methods for *E. coli* determination.

Detection technique	Liner range (cfu mL ⁻¹)	Detection Limits (cfu mL ⁻¹)	R	Reference
EIS	6.0×10 ⁴ - 6.0×10 ⁷	6×10 ³	0.978	4
EIS	3.0×10 ³ - 3.0×10 ⁷	1×10 ³	0.994	5
ASV	50 - 5.0 × 10 ⁴	30	0.992	6
Amperometric i-t curve	20 - 1.0 × 10 ⁶	10	0.998	7
DPV	1.0×10 ² - 1.0×10 ⁶	50	0.997	8
Solid-state voltammetry	50 - 1.0×10 ⁶	10	0.999	This work

EIS: Electrochemical impedance spectroscopy; ASV: anodic stripping voltammetry; DPV: Differential pulse voltammetry.

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