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Label-free Lectin Impedimetric Biosensor Based on a Polyaniline/Graphene Nanocomposite for the Detection of Escherichia coli

Haiying Yang[*], *Jianfang Qin, Meng Zhang, Huiyan Shen, Jia Feng, Haoyong Hao*

Department of Chemistry, Yuncheng University, Yuncheng, 044300, PR China
[*]E-mail: haiyingyang79@hotmail.com

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This work reports a label-free impedimetric biosensor based on a polyaniline (PANI) and graphene (G) composite on a glassy carbon electrode (GCE) for the detection of *Escherichia coli* (*E. coli*) with lectin as the recognition molecule. The PANI/G nanocomposite was synthesized by in situ electrochemical oxidative polymerization of aniline onto G and Nafion. The effect of the polymerization on the electron performance of the sensing surface was checked. The results indicated that the heterogeneous electron transfer rate increased from 4.30×10^{-4} cm s^{-1} to 4.70×10^{-4} cm s^{-1} after the incorporation of PANI onto the G/Nafion/GCE with ferrous/ferric as the redox probe. The lectin of *Concanavalin* A (Con A) was used to recognize the carbohydrate moiety on the surface of *E. coli*, which demonstrated the recognition ability of the synthesis interface. The DH5α *E. coli* bacteria strain was chosen as a model target. When the biosensor was incubated with the target under optimized experimental conditions, the electron transfer resistance (R_{et}) increased when the *E. coli* concentration increased from 5.0×10^1 cells/mL to 1.0×10^4 cells/mL. The detection limit for the biosensor was calculated to be 43 cells/mL based on a signal-to-noise ratio of 3. The biosensor was also challenged by incubation with two different bacteria without Con A binding sites, which showed negligible changes in the R_{et} value. The hybrid PANI and G nanocomposite enables us to enhance the biosensor response and reproducibility without sacrificing the electrical conductivity, as found for the use of additives. The developed biosensor highlights a promising approach for the sensitive determination of other desired bacteria via incorporation with a nanocomposite.

Keywords: impedimetric biosensor; electrochemical polymerization, polyaniline and graphene; lectin; *Escherichia coli*

1. INTRODUCTION

Rapid and sensitive detection of pathogens such as bacteria has drawn increasing attention for its great importance in environmental and other related areas [1,2]. Normal *Escherichia coli* (*E. coli*)

plays positive effects in the human and warm-blooded animal intestinal pathway. However, some strains of *E. coli* are a pathogenic bacterium, and the issue of bacterial infection and detection has received considerable critical attention. Colony counting and the polymerase chain reaction have proven promising methods for the highly sensitive and reliable determination of bacteria. However, to culture and count a colony always needs 1-2 days, which is time consuming. Therefore, more rapid, sensitive and cost-effective methods are highly desired [3]. Due to their merits of simple fabrication, rapid detection and low cost, electrochemical biosensors have proven to be a promising way to detect bacteria [1-8]. Considering the crucial effects of a sensing surface on the performance of a biosensor, many researchers have focused on the development of bacterial biosensors. Recently, taking advantage of the exceptional attributes of nanomaterials, the integration of such materials as signal transducers has been considered as an effective way to build biosensors, providing wide applications for the detection of bacteria and biological targets via immobilization of the recognition molecule [2-11].

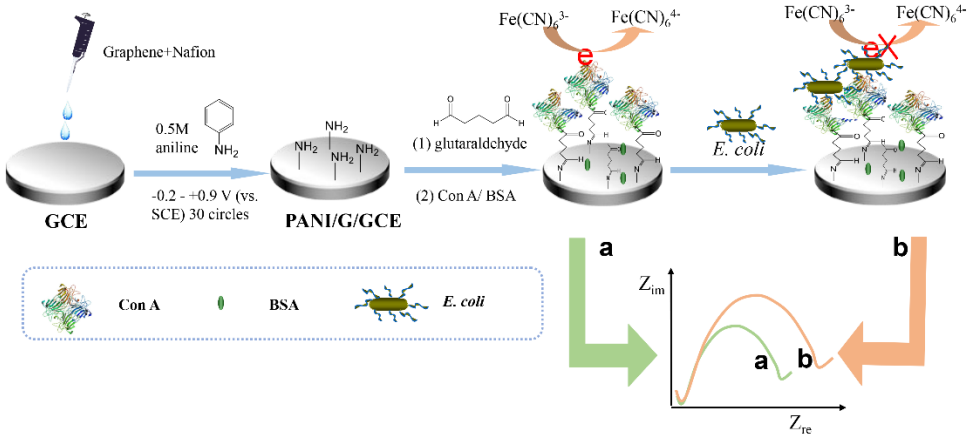
Among nanomaterials, graphene (G) has attracted considerable attention. G is a two-dimensional sheet of carbon atoms bonded with sp^2 hybridization that shows a high specific surface area and high electrical conductivity [5,12-15]. However, it is reported that graphene can easily agglomerate because of van der Waals forces and is rarely able to remain on the electrodes of biosensors during analysis processes performed in a water solution, which can lead to negative impact on the stability and the accuracy of such biosensors [16]. To solve this problem, a Nafion binder is often used to fabricate biosensors by fixing graphene onto the surface of the electrodes, while results in the sacrifice of electrical conductivity.

Conducting polymers, especially polyaniline (PANI), are promising candidates for electrode modification due to their moderate preparation condition and competitive electrochemical performance [17-20]. Graphene sheets have been proven to contain active nucleation sites that can polymerize aniline, and the formed nanocomposite show excellent electron transfer pathways. Recently, the preparation of three-dimensional nanostructures of G/PANI nanocomposites was reported by the method of electrochemical polymerization, with such nanostructures showing high conductivity and mechanical strength [9-12,17,21-26]. The application of hybrid nanomaterials via the integration of G and PANI in bacteria electrochemical biosensors has shown that the doping of G with polyaniline remarkably enhances the conductivity [4,18,19]. The fabrication of PANI nanostructures involves template preparation, wet chemical processing, self-assembly, and electrochemical polymerization. Most other methods rely on the use of binder materials, while electrochemical polymerization is simple and easily controlled. In addition, the electrochemical method also ensures that the active materials are deposited on the support electrodes, which leads to a compact nanocomposite with good adhesion [11,16,17]. Therefore, electrochemical polymerization is used to fabricate PANI and G nanostructures in this work.

For the recognition elements, antibodies [5,6], aptamers [27,28], antimicrobial peptides [29,30] and lectins [8,21,31-33] have been reported to be used in bacterial biosensors due to their ability to selectively bind with the epitopes on the bacterial surface. Among all these recognition elements, the antibodies are widely used but are difficult to produce and are antigens in vivo; the number of aptamers and antimicrobial peptides available for bacteria detection is limited. [3,28-30,34]. Some researchers have developed carbohydrate biosensors, which take advantage of the carbohydrates and

acceptors in bacteria. However, these devices suffer from covalently immobilized carbohydrate residues on the basal supports [35,36]. Interestingly, lectin is a protein that is derived from plants, which can specifically incubate with bacterial carbohydrate moieties. Given their advantages of easy production and stability, lectin-based biosensors are considered as promising devices for detecting bacteria. Some lectin-based biosensors have used lectins incubated with glycoconjugates on bacterial surfaces selectively, with promising results obtained [31-33,37-40]. We have also reported an electrochemiluminescent [32] and impedimetric biosensors [8] incorporating Con A for *E. coli*, with the detection process for such devices needing only 70 min with a detection limit of 127 cells/mL or 75 cells/mL, respectively.

As shown in Scheme 1, we developed a label-free electrochemical biosensor by using a hybrid PANI and G nanocomposite to detect *E. coli*. The method of in situ electrochemical oxidative polymerization of aniline was used to fabricate the PANI onto a G/GCE. The PANI/G nanocomposite was synthesized by in situ electrochemical oxidative polymerization of aniline onto G/GCE. The hybrid nanocomposite modified electrode was then characterized in detail followed by application as a sensing surface based on cross-linking Con A via glutaraldehyde with PANI. The effect of PANI on the electron transfer was assessed quantitatively. The amount of G and Con A concentration for the fabrication process were optimized, and the analytical performance was assessed using the DH5α strain of *E. coli* as a model.



Scheme 1. Schematic showing the biosensor fabrication and detection process for bacteria.

2. EXPERIMENTAL

2.1. Materials and Reagents

Graphene (G) was obtained from Nanjing Xianfeng Nano-Materials Technology Co. Ltd. (Nanjing, China). Potassium ferricyanide ($K_3[Fe(CN)_6]$), bovine serum albumin (BSA), potassium ferrocyanide ($K_4[Fe(CN)_6]$), aniline, H_2SO_4, glutaradehyde, and N,N-dimethylformamide (DMF) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *E. coli* DH 5α was obtained from a microbiological laboratory in the Life science department of Yuncheng University. *Concanavalin* A (Con A), rhamnose, D-galactose, mannose, mannan, lipopolysaccharides (LPS), and

Nafion®117 solution were all obtained from Sigma-Aldrich (St. Louis, USA). Reagents were of analytical grade and used without further purification. Phosphate buffer (PBS, 10 mM, pH 7.4), $CaCl_2$ and $MnCl_2$ were prepared with deionized water. Con A, bacteria and carbohydrate solutions were prepared with a binding buffer: PBS containing 1 mM $CaCl_2$ and 1 mM $MnCl_2$.

The electrochemical measurements were carried out using an experimental system with a three-electrode configuration. A CHI 660 electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China) was used. The fabricated lectin-based biosensor or PANI/G/Nafion/GCE was used as the working electrode, a platinum wire as the auxiliary electrode, and Ag/AgCl (sat. KCl) as the reference electrode, respectively. A Hitachi S-4800 (Japan) was used to acquire scanning electron microscope (SEM) images for the processes of biosensor fabrication and bacteria detection.

2.2. Fabrication of the Biosensor

A GC electrode (3 mm in diameter) was polished using alumina slurry on a polishing pad followed by rinsing with water. Graphene (G, 2 mg) was dispersed into 2 mL DMF and ultrasonicated for 30 min. Nafion (0.2 wt%) was prepared with ethanol. Then, a 1 g/L G suspension was added to the 0.2 wt% Nafion before placing the mixture under mild ultrasonication for 30 min to form a homogeneous suspension. A dispersion containing G (0.5 g/L) and Nafion (0.1 wt%) was obtained at a ratio of 1:1 (v/v). Then, 5 μL of the mixture was drop cast onto a GC electrode following which a G/Nafion/GC electrode was obtained.

The electrochemical polymerization of aniline onto G was completed by the cyclic voltammetry (CV) technique in 0.2 M aniline and 0.25 M H_2SO_4. The potential range was scanned from -0.2 V to +0.9 V at 50 mV/s for 30 cycles. The electrode showed a dark green color due to the formation of the conductive polyaniline emeraldine salt.

The PANI/G/Nafion/GC electrode was kept in 2.5 % glutaraldehyde for 2 h; then, the glutaraldehyde treated films were immersed in a 1 g/L Con A solution for 1 h, in 1 % BSA for 30 min to inhibit nonspecific interactions. Next, the electrode was washed to remove the adsorbent. The obtained ConA/PANI/G/Nafion/GCE biosensor was then stored at 4 °C in PBS (pH 7.4).

According to the culturing guidelines from the manufacturer, different strains of bacteria were cultivated. The bacteria solution was centrifuged after culturing, resuspended and then washed with water twice. The optical density at 600 nm (OD_{600}) was used to estimate the cultivated bacterial concentration. The desired concentration of bacteria samples was sequentially diluted with PBS buffer.

2.3. Electrochemical Measurement

For the electrochemical measurement, the developed biosensor was immersed into 100 μL of bacteria samples or carbohydrate solutions for incubation for 60 min and then rinsed with washing buffer. The EIS technique was used to detect the electron transfer resistance in 3.0 mL of PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. EIS was carried out using a CHI 660D Analyzer at an applied potential of 5 mV across the electrodes over the frequency range of 100 KHz ~ 0.1 Hz. The sample

testing process including data acquisition required a few min. The change in the electron transfer resistance ΔR_{et} ($\Delta R_{et} = R_{et.i} - R_{et.0}$) was used to quantify the concentration of *E. coli*., where $R_{et.0}$ and $R_{et.i}$ is the R_{et} before and after biosensor incubation with the targets. CV was performed from -0.2 V to 0.6 V at 0.1 V/s. The electrochemical experiments were carried out at room temperature.

3. RESULTS AND DISCUSSION

3.1. Characterization of the nanocomposite

Electrochemical polymerization of aniline on G and Nafion-modified GCE results in the firm immobilization of the polyaniline film. The number of cycles in the CV method was kept constant, which ensured a fixed thickness for each case of polymerization. Initially, G and Nafion was drop cast onto a cleaned GCE followed by polymerization with polyaniline (PANI). As shown in Fig. 1, the cyclic voltammetry for the PANI synthesis on the G/Nafion/GCE was carried out in 0.25 M H_2SO_4 containing 0.2 M aniline, with a fixed number of 30 cycles. Three pairs of redox peaks were observed from -0.2 V to +0.9 V on the G/Nafion/GCE, which are ascribed to aniline configuration conversions. The first pair of redox peaks appears at + 0.15 V, which is attributed to the conversion of the reduced form of PANI (leucoemeraldine state) to the half-oxidized emeraldine base (EB) state. At +0.49 V, the second pair of peaks result from the EB transforming into the completely oxidized pernigraniline state and vice-versa. The oxidation of the PANI chain segments to a benzoquinone species gives rise to the third pair of peaks at + 0.67 V [41,42]. The increased peak heights along with the scan number suggests that the PANI polymerization was successful and that the PANI/G/Nafion/GCE was obtained as expected.

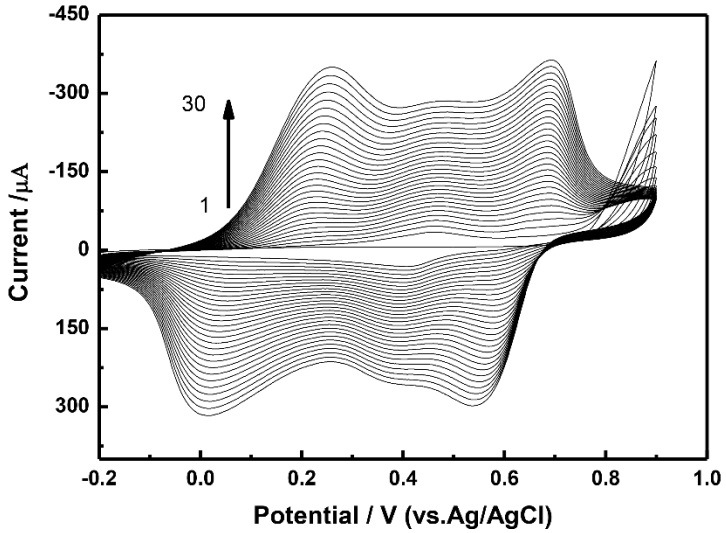


Figure 1. Cyclic voltammograms (30 cycles) recorded during electrodeposition of PANI onto a G-modified GCE in a solution of 0.20 M aniline and 0.25 M H_2SO_4 at a scan rate of 50 mV/s.

The effect of PANI on the performance of PANI/G/Nafion/GCE was evaluated by CV. As shown in Fig. 2, the cyclic voltammograms for the G/Nafion/GCE, PANI/G/Nafion/GCE were recorded in 5.0 mM $Fe(CN)_6^{3-/4-}$ solution at 0.1 V s^{-1}. A slight decrease in the peak current was

observed when the Nafion and G was drop cast onto the GCE, which can be ascribed to the hindering effect of the Nafion for the electron transfer (curve not shown). In contrast, the peak current increased significantly upon PANI polymerization on the G/Nafion/GC electrode. The PANI nanomaterials might account for the current increase via an increase in the electrode active surface and promotion of the electron transfer kinetics at the modified sensing surface.

To evaluate the effect of PANI on the electrochemical performance of the modified electrode, the CV technique was applied. First, the electron-active surface area was calculated based on the Randles-Sevcik equation [43],

$$I_{pc} = 2.69\times10^{5} n^{3/2} A D^{1/2} C v^{1/2} \qquad (1)$$

where n is the electron transfer number, A is the effective electrode area (cm^2), I_{pc} is the reduction peak current (A), v is the scan rate (Vs^{-1})), and C and D is the concentration (mol cm^3) and diffusion coefficient (cm^2 s^{-1}) for $K_3[Fe(CN)_6]$. By analyzing the reduction peak current, the average effective area of the GCE, G/Nafion/GCE and PANI/G/Nafion/GCE was estimated to be 0.037 cm^2, 0.031 cm^2 and 0.063 cm^2, respectively (curves not shown). In comparison, we found that the PANI/G/Nafion/GCE possessed the largest surface area among all the materials studied. PANI/G/Nafion/GCE showed an ~2 times larger effective area than that of the GCE. The reason for this might be ascribed to the PANI/G nanocomposite providing a synergistic effect, which accelerates the electron transfer on the modified electrode. In addition, the high surface-to-volume ratio of PANI also led to an enlarged surface area. The larger surface area contains more active sites that can anchor more recognition molecules on the sensing surface, and, thus, favor the biosensor with high sensitivity.

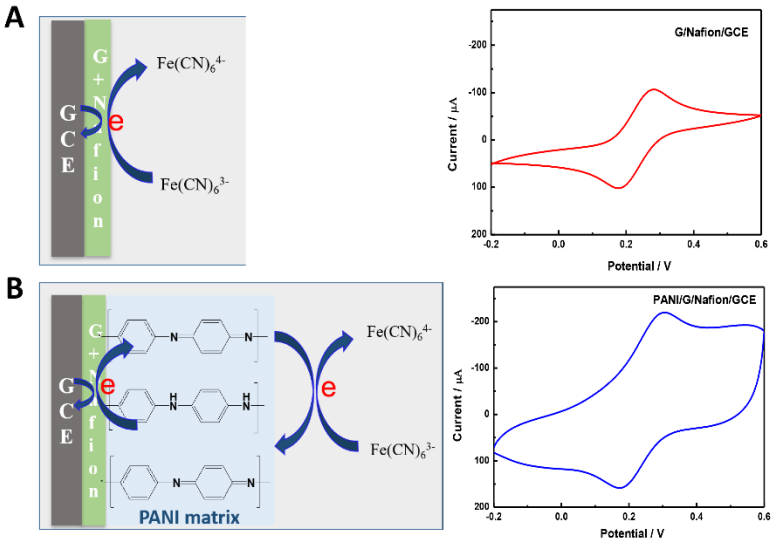


Figure 2. The schemes for the G/Nafion/GCE (A) and PANI/G/Nafion/GCE (B), as well as the corresponding characteristic cyclic voltammograms for the two electrodes in 10 mM PBS containing 5 mM $Fe(CN)_6^{3-/4-}$ and 0.10 M KCl (pH 7.4).

The kinetic parameters for $Fe(CN)_6^{3-/4-}$ were used as a model to evaluate the effect of PANI on the modified electrode using the potential difference (ΔE_p) as a function of the scan rate [44,45]. From Fig. 3, we can conclude that at a higher scan rate, the kinetics are quasi-reversible due to the increase

in ΔE_p. According to Nicholson's curve, one can convert ΔE_p values into a dimensionless kinetic parameter ψ that is proportional to $v^{-1/2}$, as shown in the following equation:

$$\psi = k_0[\pi DnvF/(RT)]^{-1/2} \quad (2)$$

A linear fit to the $\psi - v^{-1/2}$ relationship can be used to calculate the standard heterogeneous electron transfer rate constant (k^0) using equations (3) to (5):

$$k^0 = 2.18[D\beta nvF/(RT)]^{1/2}\exp[-(\beta^2 nF/RT)\cdot(E_{p,a}-E_{p,c})] \quad (3)$$

$$\Psi = (-0.6288-0.0021X)/(1-0.017X) \quad (4)$$

$$X = n\Delta E_p(mV) \quad (5)$$

According to the above equations and the slope in Fig. 3, k^0 was determined to be 4.70×10^{-4} cm s^{-1}, which is larger than the value of 4.30×10^{-4} cm s^{-1} obtained for the G/Nafion/GCE. Therefore, it can be deduced that the incorporation of PANI onto the G/Nafion composite may provide an abundance of effective electron transfer pathways and accelerate the electron transfer [16,42,43,46]. This can be beneficial for realizing high sensitivity for the biosensor in this work.

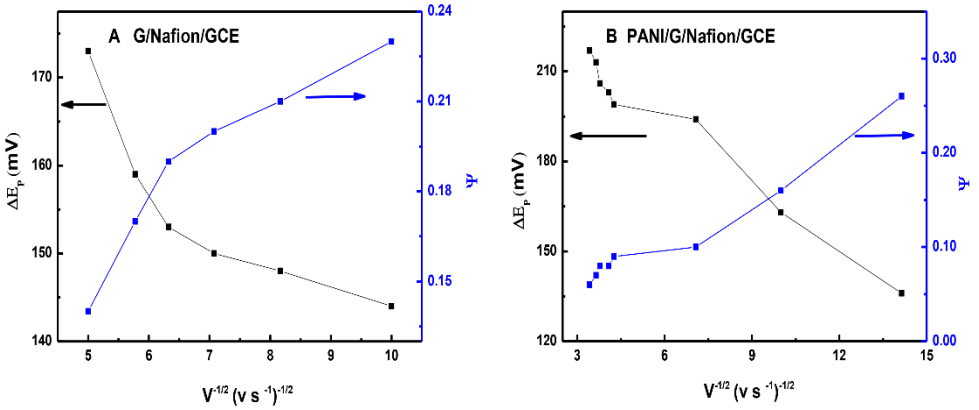


Figure 3. The peak separation (ΔE_p) and Nicholson's kinetic parameter (ψ) versus the reciprocal of the square root of the potential scan rate ($v^{-1/2}$). The linear fit is used to estimate the standard heterogeneous charge transfer rate constant (k^0). The G/Nafion/GCE (A) and PANI/G/Nafion/GCE (B) were immersed into 5 mM $Fe(CN)_6^{3-/4-}$ containing 10 mM PBS (pH 7.4) and 0.1 M KCl.

As shown in Fig. 4, the morphology for the different modified electrodes can be obtained from the SEM technique. A characteristic morphology for G was observed, as shown in image A; however, G showed a much wrinkled, sheet-like structure within Nafion, as shown in image B, which might result in an increase in the effective electrode surface. Fig. 4C shows that the porous nanostructure of PANI was well compounded on the surface. The mutual attraction between the G electron cloud and PANI electrons might provide more active sites for the immobilization of the recognition molecules and promote electron transfer at the electrode surface.

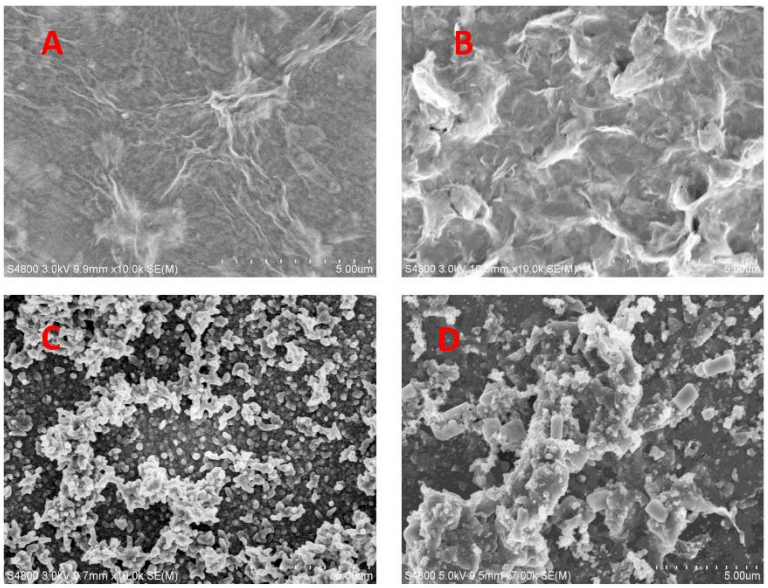


Figure 4. SEM images of (A) G/GCE, (B) G/Nafion/GCE, (C) PANI/G/Nafion/GCE and (D) the biosensor incubated with *E. coli* cells at a concentration of 1.0 $\times 10^3$ cells/mL for 60 min.

3.2. Characterization of the fabrication and detection processes for the biosensor

The hybrid nanomaterials in this work have a crucial effect on the analytical ability of the biosensor. CV and EIS techniques were used to assess the property of the modified surface using the ferri/ferrocyanide redox probe. As shown in Fig. 5A, when G and Nafion were modified on the GCE surface, a R_{et} value of 0.81 KΩ was obtained. It is worth noting that the Nafion molecules suppress the penetration of $Fe(CN)_6^{3-/4-}$ contributing to an increase in the semicircle diameter. The R_{et} decreased to 0.34 KΩ when the PANI was electropolymerized, which demonstrated that PANI can accelerate the electron transfer. Afterwards, Con A was covalently coupled with the amine groups by glutaraldehyde with PANI. Subsequently, when the unoccupied active sites on the electrode were blocked with BSA, R_{et} increased from 10.29 KΩ to 16.25 KΩ.

When the biosensor was incubated with *E. coli* DH5α at 50 cells mL^{-1}, the value of R_{et} greatly increased to 21.50 KΩ (note that considering the toxicity of some bacteria strains such as *E. coli* O157:H7, we employed a nonpathogenic strain, *E. coli* DH5α, to assess the fabricated biosensor). Because the lipopolysaccharides (LPS) present in all gram-negative bacterial cell walls alone with phospholipids and proteins, resulting in Con A could bind with the target of *E. coli* DH5α [31,32,37,40]. The incubated *E. coli* cells on the modified surface hamper the electron transfer between the probes and electrode resulting in an increase in R_{et}. The interaction of Con A and *E. coli* DH5α was proven and the developed biosensor showed successful operation. The CVs were also recorded and the results are displayed in Fig. 5B. As expected, the peak current is decreased when one uses the electrode modified with G/Nafion and Con A, and further electrochemical polymerization of PANI produces an increased peak current. These changes are in agreement with the results observed by EIS.

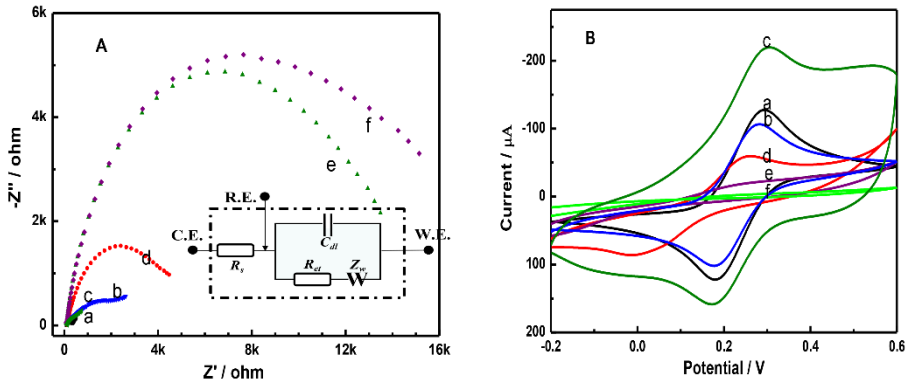


Figure 5. (A) Nyquist plots for the impedance spectra and (B) cyclic voltammograms for the different electrodes in 10 mM PBS containing 5 mM $K_3[Fe(CN)_6]$-$K_4[Fe(CN)_6]$ and 0.1 M KCl (pH 7.4). (a) GCE, (b) G/Nafion/GCE, (c) PANI/G/Nafion/GCE, (d) Con A/PANI/G/Nafion/GCE, (e) BSA/Con A/PANI/G/Nafion/GCE, (f) the biosensor incubated with *E. coli* at 100 cells/mL for 60 min. The biasing potential was 0.24 V, with an applied voltage amplitude of 5 mV in the frequency range of 0.1-100 kHz.

SEM was also used to show the incubation of bacteria by the Con A/PANI/G/Nafion/GCE sensing interface. Briefly, the fabricated electrodes were incubated with *E. coli* cells (1.0×10^3 cells/mL) for 60 min and then characterized by SEM. From Fig. 4D, the rod-shaped cells were found to be distributed independently. The cells show a length and diameter of approximately 1~2 μm and 0.5 μm, respectively, which is in agreement with the literature [31, 32, 36]. These results indicate that *E. coli* cells were captured on the Con A/PANI/G/Nafion nanocomposite. The capability of Con A towards LPS on the *E. coli* surface accounts for the successful incubation. The SEM result proves the interaction between the bacteria and the fabricated biosensor.

3.3. Optimization of the G loading amount and Con A concentration

The amount of G loading and Con A concentration was investigated and optimized. The electrochemical performance of biosensors fabricated with a varying G loading in the range of 0.1-0.6 g/L was tested by EIS. As shown in Fig. 6A, tΔR_{et} values increased with G loading from 0.1 g/L to 0.5 g/L following incubation with *E. coli* at 1.0×10^2 cells/mL and 1.0×10^3 cells/mL, respectively. Nonetheless, ΔR_{et} values decreased slightly at a G loading of 0.6 g/L. The self-agglomeration of G in the hybrid nanocomposites might account for this decrease and result in a decrease in the electrochemical conductivity. A G loading of 0.5 g/L was chosen for further experiments.

By changing the recognition element coverage on the PANI/G/Nafion/GCE, the Con A concentration for the fabrication was optimized. The dependence of ΔR_{et} on Con A concentrations is shown in Fig. 6B. After incubation with *E. coli*, the ΔR_{et} values were found to increase with Con A from 0.25 g/L to 1.0 g/L, and a plateau was observed at 1.0 g/L to 2.0 g/L. The trends for the two curves show that the ΔR_{et} value increases with the Con A concentration in the range from 0.25 g/L to 1.0 g/L, probably due to the increase in the number of binding sites. With a further increase in Con A

concentration, ΔR_{et} is observed to reach a plateau, which might be ascribed to the saturation of Con A on the sensing surface. Therefore, a Con A concentration of 1.0 g/L was used for the fabrication of the biosensor.

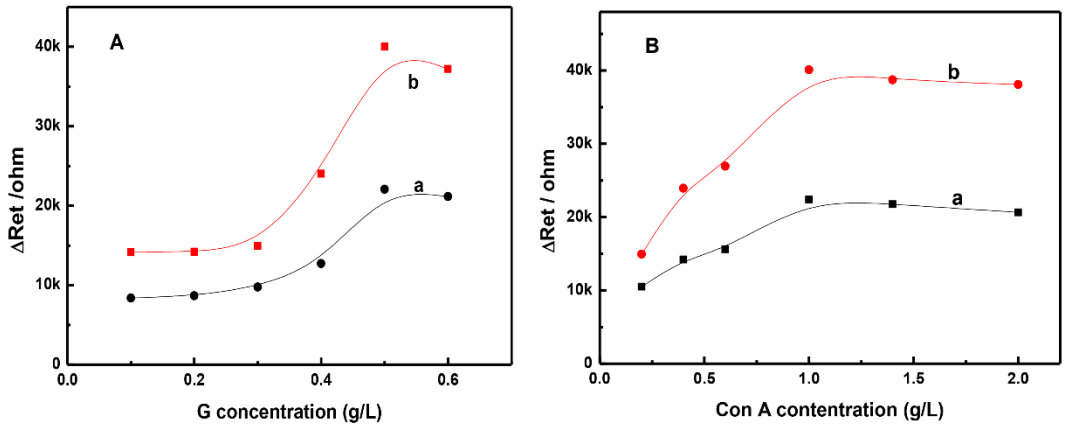


Figure 6. The effect of G concentration (A) and Con A concentration (B) on the EIS response of the biosensor incubated with 100 cells/mL (curve a) and 1000 cells/mL (curve b) in 10 mM PBS containing 5 mM $K_3[Fe(CN)_6]$-$K_4[Fe(CN)_6]$ and 0.1 M KCl (pH 7.4).

3.4. Performance of the biosensor for E. coli

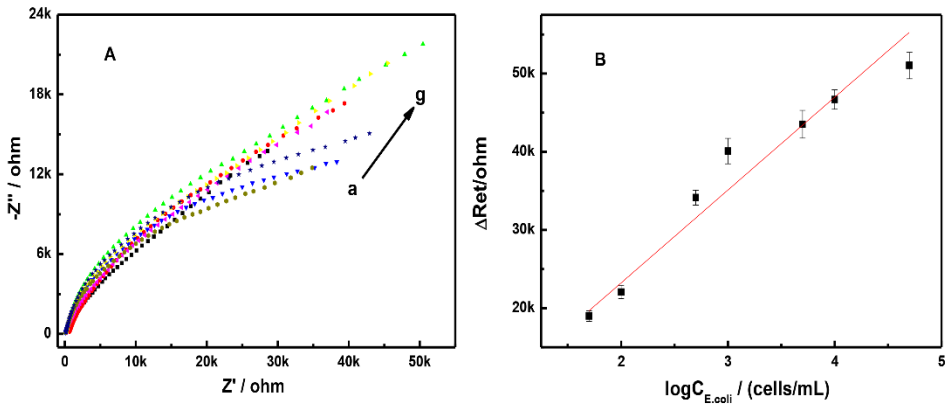


Figure 7. (A) Nyquist plots for Con A/PANI/G/Nafion/GCE interacting with different concentrations of *E. coli* measured in 10 mM PBS containing 5 mM $K_3[Fe(CN)_6]$-$K_4[Fe(CN)_6]$ and 0.10 M KCl (pH 7.4). (B) The linear relationship between ΔR_{et} and *E. coli*. Concentration; (a) 50 cells/mL, (b) 100 cells/mL, (c) 500 cells/mL, (d) 1000 cells/mL, (e) 5000 cells/mL, (f) 10000 cells/mL, and (g) 50000 cells/mL. $\Delta R_{et} = R_{et,i} - R_{et,0}$, where $R_{et,0}$ and $R_{et,i}$ is the electron transfer resistance before and after incubation with the analyte.

The dependence of ΔR_{et} on *E. coli* concentration was used to assess the quantitative performance of the fabricated biosensor. Figure 7A shows the impedimetric spectra obtained following incubation of the biosensor with *E. coli* (5.0×10^1 - 5.0×10^4 cells/mL). A logarithmic relationship between ΔR_{et} and *E. coli* concentration was obtained, as shown in Fig. 7B, which can be described by

the regression equation of ΔR_{et} = 11874 lg C -523 (C is in units of cells/mL). The regression coefficient was determined to be 0.9613 and the detection limit was calculated to be 43 cells/mL *E. coli* based on an S/N ratio of 3 [47].

As shown in Table 1, the detection performance of the fabricated biosensor was compared with other similar bacteria biosensors. The detection limit of the fabricated sensor is lower than the value of 107 CFU/mL obtained for a PANI-based impedimetric biosensor for *E. coli* O157:H7 [24]; the synergistic effect of PANI and G might account for the higher sensitivity. The detection limit of the fabricated biosensor is also lower than the value of 70 CFU/mL obtained for an electrochemical immunosensor with a polyaniline label target and magnetic separation [48]. However, the detection limit is slightly higher than that shown by a differential pulse voltammetric (DPV) technique based on avidin-modified PANI electrochemically deposited onto a Pt disk electrode with methylene blue as a DNA hybridization indicator (11 cells/mL) [49] or that shown by a graphene-polyethylenimine DPV method (10 cells/mL) [50].

Table 1. Comparison of the analytical performance of the proposed method with some reported biosensors for the determination of *E. coli*

Detection technique /[a]	Assay principle	Recognition element	E. coli strain	Linear range (cells/mL)	Detection limit (cells (CFU) /mL)	Refs
EIS	Polypyrrole and MWCNT, and AuNPS	antibody	O157:H7	3.0×10^1 - 3.0×10^7	~30	[**6**]
DPV	Reduced graphene oxide/ polyethylenimine	Anti-fimbria antibody	UT189	10^1 -10^4	10	[**50**]
DPV	avidin-modified PANI electrochemically deposited on a Pt disk	gene	no	no	11	[**49**]
EIS	PANI on gold electrode	antibody	O157:H7	no	107	[**24**]
Electro-chemical	Polyaniline label target and magnetic separation	antibody	O157:H7	7.0- 7.0× 10^4	70	[**48**]
ECL	Ruthenium labeled peptide SAM on gold	Antimicrobial peptide	O157:H7	5.0×10^2 - 5.0×10^5	1.0×10^2	[**29**]
EIS	Mixed MUA and DTT for SAM	Con A	DH5α	1.0×10^2 - 1.0×10^5	75	[**8**]
SWV	Polythiophene with glycosylated quinone moieties on gold	Con A	W 1485	2.5×10^2 - 2.5×10^8	800	[**37**]
ECL	SWCNT coated on SPCE	Con A	O157:H7	5.0×10^2 - 5.0×10^5	1.27×10^2	[**32**]
EIS	Polyaniline and graphene on GCE	Con A	DH5α	5.0×10^1 - 1.0×10^4	43	This work

Note: EIS means electrochemical impedance spectroscopy; ECL means electrogenerated chemiluminescence, SPR means surface plasmon resonance, QCM means quartz crystal microbalance. SAM means self-assemble membrane. SPCE means screen-printed carbon electrode. MUA: 11-mercaptoalkanoic, MH:6-mercapto hexanol; DTT: dithiothreitol.

An E. coli concentration of 1.0×10^3 cells/mL was used to check the reproducibility of the PANI/G-based biosensor. The value of R_{et} was 3.76 % using five biosensors to determine the target and 3.03 % from seven independent measurements with one biosensor. The storage stability for the biosensor was checked by storing in PBS for 7 days at 4 °C, after which the average EIS value was found to be 95.30 % of the initial value. We are currently working on improving the storage ability of the biosensor in our lab. Tap water was used to demonstrate the plausibility of the biosensor. By spiking samples with *E. coli*, the recovery was found to be 91.3 % for 1.0×10^3 cells/mL and 92.4 % for 1.0×10^4 cells/mL, indicating that the developed sensor can be potentially used to detect *E. coli* in real environmental samples.

The fabricated PNAI/G-based biosensor was also assessed with some bacteria (1.0×10^3 cells/mL) including two strains of *Micrococcus luteus* and *Staphyolcoccus aureus*, gram-positive. It was 13.1 % (*Micrococcus luteus*) and 15.3 % (*Staphyolcoccus aureus*) of the ΔR_{et} for *E. coli* DH5α. The results indicated that the biosensor responded to *E. coli* rather than the tested bacteria, which was ascribed to the LPS, Con A incubating with a major component of the gram-negative bacteria rather than other cells [37,40]. Intriguingly, this result agrees well with previous reports [8,32,37,51].

The specificity of the fabricated biosensor was also assessed by challenging the sensing surface with a panel of carbohydrates that included nonspecific carbohydrates (D-galactose, rhamnose) and specific-binding carbohydrates (mannose, LPS and mannan) at a concentration of 5.0 nM. As shown in Fig. 8, the $\Delta R_{et}/R_{et}$ values for mannan, mannose, and glucose were determined to be 0.80, 0.51, and 0.47, respectively. This is ascribed to the fact that Con A binds towards carbohydrates with the vicinal, equatorial hydroxyl groups in 3-OH, 4-OH [52]. Considering that LPS is the main component of the *E. coli* surface, capped with glucose and N-acetylglucosamine, LPS was chosen to be tested by the biosensor [31,37,51,52]. LPS showed a $\Delta R_{et}/R_{et}$ value of 0.78. However, the $\Delta R_{et}/R_{et}$ values for galactose and rhamnose were found to be 0.04 and 0.08, respectively, which might be ascribed to the fact that Con A rarely binds to carbohydrates that contain the axial 4-OH with a totally different stereochemistry. This indicates that the fabricated Con A on the PANI/G sensing surface shows a satisfactory selectivity.

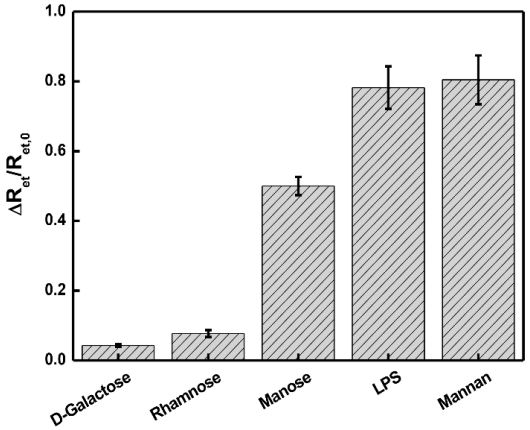


Figure 8. Response of the biosensors incubated with five different carbohydrates at a concentration of 5.0 nM.

Mannan was used to investigate the binding ability of Con A towards *E. coli*. The binding constant (K) for the immobilized Con A with mannan was determined [53]. From a plot of $C_m/\Delta R_{et}$ versus C_m (mannan concentration), which showed a Langmuir adsorption isotherm, K was determined to be 2.18×10^6 M^{-1}. This value is slightly lower than the value of 5.3×10^9 M^{-1} obtained by SPR [54] and 5.2×10^{10} M^{-1} by ECL [32]. The difference in the kinetics of the mannan-Con A interaction might be related to the type of Con A used including that which is spontaneously adsorbed and various covalently immobilized methods [52,54]. The K value shows that the surface-confined Con A has a satisfactory binding strength with mannan.

4. CONCLUSION

In summary, we have developed a label-free impedimetric biosensor by in situ synthesis of a PANI and G composite on a GCE for *E. coli* with the recognition molecule of Con A. The PANI/G nanocomposite was synthesized using electrochemical oxidative polymerization of aniline. The effect of PANI on the electrochemical performance was investigated and the results showed that the heterogeneous electron transfer rate was increased due to the synergistic effect of G and PANI. Lectin of Con A was used as the recognition element. The fabricated biosensor showed a low detection limit of 43 cells/mL for *E. coli*. The hybrid PANI and G nanocomposite enables us to enhance the biosensor response and reproducibility at the same time without sacrificing the electrical conductivity, as found for the use of additives. The developed biosensor highlights a promising approach for highly sensitive determination of other bacteria via incorporation with suitable recognition elements.

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