A label-free electrochemical biosensor based on screen-printed electrodes modified with gold nanoparticles for quick detection of bacterial pathogens

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Abstract

In this study, carbon screen-printed electrodes (SPEs) modified with gold nanoparticles (AuNPs), were prepared for label-free detection of *Escherichia coli (E. coli)* O157. AuNPs were synthesized by an electrochemical method and then modified on the carbon SPEs to improve the stability and effectiveness of the biosensor. Anti-*E. coli* O157 antibody was immobilized on the modified SPEs via -NHS cross-linking. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were selected to investigate electrochemical properties of modified carbon SPEs in a 5.0 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ added with 0.1M KCl as well as to detect *E. coli* O157 bacteria. Results showed that the carbon SPEs were successfully modified with AuNPs of 18.0 ± 1.6 nm. The electrochemical signal of modified SPEs was stable after CV cycles, and the charge transfer resistance (R_{ct}) decreased approximately to half of its initial value. Importantly, electrochemical biosensors based on AuNPs-modified carbon SPEs could detect *E. coli* O157 in the range of 10–10^6 CFU/ml without labels. The limit of detection was found at 15 CFU/ml with a signal-to-noise ratio of 3:1, and the time of detection was about 30 min. The success of as-prepared biosensor could open a strategy of portable diagnostics for label-free and quick detection of bacterial pathogens causing food-borne diseases, hospital-acquired infections as well as the emerging and re-emerging infectious diseases.

Keywords: gold nanoparticle; carbon screen-printed electrode; *Escherichia coli* O157 bacteria; electrochemical biosensor; label-free detection

1. Introduction

In recent decades, electrochemical biosensors have attracted attention from researchers worldwide for quick detection of pathogens [1][2][3]. Among them, screen-printed electrodes (SPEs) have gained popularity as electrochemical transducers owing to their easy operation, low cost, compact and high sensitivity compared to traditional diagnostics [4]. SPEs can be fabricated using several types of materials, including gold, platinum, and carbon ink [5]. Carbon-based SPEs are inexpensive but have a high surface impedance. Depending on different types of targets for detection, they can be modified with suitable materials to improve their stability and effectiveness. SPEs are preferable for enzyme-based biosensors, where the reaction of enzyme-substance complex can generate a number of electrons on the surface of electrodes [6][7]. They are also developed for small molecules sensing [8]; food control [9]; and early detection of carcinoembryonic antigens [10]; and environmental monitoring [11].

The most useful function of electrochemical biosensors is its capability to integrate into portable devices for on-site detection of pathogenic agents in a short time [5,11,12]. Recently, Simoska and Stevenson [12] have reported an overview of electrochemical sensors for real-time diagnosis of pathogens. In which, there are many types of SPEs modified with different materials for bacteria detection, such as gold, silver, carbon nanotubes or graphene oxide. The modification is expected to reduce the surface impedance of the working electrode to the value that can sense a small change in the electron transfer on the interface [13]. Gold nanoparticles (AuNPs) are common materials selected to use in electrochemical biosensors for detection of pathogens [14][15][16]. Because that AuNPs posse high biocompatibility and retain the activity of biological molecules over time [17–19]. AuNPs also have high conductivity, enabling them to transfer electrons quickly between the electrolytic solution and transducer [20][21][22].

Escherichia coli (*E. coli*) O157:H7 is known as one of the most dangerous bacteria of food-borne diseases [23][24]. The Centers for Disease Control and Prevention [25] in the United

States reported that about 48 million people get sick, 128,000 are hospitalized, and 3,000 die because of food-borne illness each year. There are several traditional diagnostics used to verify bacterial infections, but results can be only received after several hours to days [26]. Hence, the success of electrochemical biosensors can be potential for quick detection of bacterial pathogens, providing timely treatment, and minimizing the loss of human health [27][28][29]. For example, Liu *et al.* [30] developed disposable amperometric strips based on AuNPs-modified carbon SPEs to detect *E. coli* O157:H7 via the second antibody conjugated with horseradish peroxidase. Results showed that these strips could detect *E. coli* O157: H7 in the range of 10^2–10^7 CFU/ml. In addition, AuNPs-modified carbon SPEs have also been developed to detect other pathogens with high sensitivity and selectivity [8,31,32]. To our best knowledge, there are challenges of electrochemical biosensors, which should be optimized, such as the analysis time, pre-treatment of samples, mobility and stability [12].

In our strategy for the development of portable devices for quick and direct diagnosis of bacterial pathogens, electrochemical biosensors based on AuNPs-modified carbon SPEs have been developed for detection of *E. coli* O157 without labels.

2. Materials and methods

2.1. Materials

Carbon SPEs were commercially purchased from Dropsens, Spain. (3-mercaptopropyl) trimethoxysilane (MTS; 95%), *N*-(γ-Maleimidobutyryloxy)succinimide (GMBS), trisodium citrate ($Na_3C_6H_5O_7$; 99.0%), $K_3[Fe(CN_6)]$, $K_4[Fe(CN_6)]$, phosphate-buffered saline (PBS, pH 7.4), bovine serum albumin (BSA), ethanol absolute, and potassium chloride (KCl; 99.0%) were purchased from Sigma-Aldrich (USA).

Goat anti-*E. coli* O157 IgG polyclonal antibody (Ab) was purchased from Abcam. *E. coli* O157 (ATCC 43888,10^6 CFU/ml), *Salmonella enteritidis* (ATCC 13076, 10^6 CFU/ml), and AuNPs were provided by the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam.

2.2. Preparation of AuNPs-modified carbon SPEs

AuNPs have been synthesized by a modified electrochemical method [33]. Briefly, two gold electrodes (purity: 99.99%) were connected to a direct current power (9V) as an anode and cathode. The gold electrodes were immersed in a jar filled with 50 ml bi-distilled water and 0.1% $Na_3C_6H_5O_7$. The electrochemical synthesis of AuNPs was performed for 2 hrs. in heating condition. The solution of AuNPs has been centrifuged at 7,000 rpm for 10 min to collect the sediment.

The prepared AuNPs were checked under transmission electron microscopy (TEM, JEM1010-JEOL). A drop of AuNPs solution was directly transferred onto a formvar carbon-coated copper grid (200 meshes), dried in the air, and then investigated under TEM.

Initially, 100 ppm of AuNPs was deposited onto the working area of carbon SPEs for 60 min at room temperature. Afterwards, the AuNPs-modified SPEs were rinsed three times by bi-distilled water, and cyclic voltammetry of five cycles, from –0.3 V to 0.6 V at a scan rate of 50 $mV.s^{-1}$ in a 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ to remove all unbound AuNPs on the surface. Finally, AuNPs-modified carbon SPEs have rinsed three times again with bi-distilled water and kept them in the dark condition at 4 °C for further testing.

Scanning electron microscopy (SEM, S-4800, Hitachi), and energy-dispersive X-ray (EDX) spectroscopy (EMax, Horiba) were used to study the morphology and structure of the carbon SPEs before and after modification with AuNPs.

2.3. Antibody immobilization

The working area of AuNPs-modified carbon SPEs was incubated with a drop of 2% MTS/ethanol for 1 h at room temperature. After that, it was washed three times with drops of bi-distilled water (50 µl/drop) before incubation with 0.04 M GMBS (6 µl) for 30 min at room temperature to create -NHS groups. Finally, the functionalized surface of modified SPEs was immobilized with Ab after washing three times with drops of PBS (pH 7.4) (50 µl/drop) [34][35].

For the development of electrochemical biosensors, the working electrode of modified SPEs was incubated with a drop of goat anti-*E. coli* O157 IgG polyclonal antibody (1.0 µg/ml, diluted in PBS pH 7.4) for one hour at room temperature. Unbound antibodies on the modified SPEs were thoroughly rinsed by drops of PBS (pH 7.4) (50 µl/drop), and all non-specific binding sites were blocked by a drop of 2% BSA/PBS for 30 min at room temperature.

Fourier-transform infrared (FTIR) spectroscopy (IRAffinity-1S, Shimadzu) was then performed in the wavelength range (λ) of 400–4000 cm^{-1} to verify the bonds of biological molecules linked to modified SPEs.

2.4 Electrochemical measurements

After antibody immobilization, the biosensors were incubated with *E. coli* O157 bacteria for 30 min at different concentrations (diluted in PBS pH 7.4) and then washed three times with drops of PBS (pH 7.4) before measurements. Electrochemical properties were investigated with a portable system (PalmSens 3.0, Netherlands) in a 5.0 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ added with 0.1 M KCl (Fig. 1) [36]. In this study, *Salmonella enteritidis* was served as a control sample using the same procedure described above.

For electrochemical measurements, we have selected cyclic voltammetry (CV) from –0.3 V to 0.6 V, and a scan rate of 50 $mV.s^{-1}$, electrochemical impedance spectroscopy (EIS) in the range of frequency from 0.01 Hz to 50 kHz.

<Figure 1>

3. Results and Discussion

3.1. Formation of AuNPs and the surface of modified SPEs

The morphology, and structure of AuNPs as well as the surface of the SPEs before and after modification were verified by TEM and SEM. Fig. 2A shows that AuNPs have been successfully synthesized by the modified electrochemical method. AuNPs are spherical and well dispersed, with an average diameter of 18.0 ± 1.6 nm. Fig. 2B shows the SEM image of the raw carbon working electrode with a lot of pores and small cavities. Owing to the porous structure of the carbon membrane, AuNPs could easily go inside the network and locate stably on the surface of SPEs [36]. All unbound AuNPs could be removed during the rinse by five CV cycles. In fact, there are some AuNPs still found on the surface of SPEs after washing steps (Fig. 2C). The EDX spectra also confirmed the presence of AuNPs on the carbon membrane (Fig. 2D). The EDX spectra show that there are only gold and carbon elements with characteristic peaks, and confirm that the working surface of the modified carbon SPEs has no impurity.

<Figure 2>

Anti-*E. coli* O157 IgG polyclonal antibody was immobilized on AuNPs-modified carbon SPEs to become electrochemical biosensors. FTIR was used to verify the surface functionalization and the binding between the antibody and modified SPEs. Fig. 3 shows the FTIR spectra of the surface of modified SPEs in the wavelength range of 400–4000 cm^{-1}. Two peaks are clearly found at 3240 and 1630 cm^{-1} (Fig. 3a, red line), which are characteristic of the double bonds, such as C=C, C=O, and C=N from GMBS molecules, and hydroxyl (-OH) groups generated from the surface of AuNPs-modified SPEs [37]. After immobilization with antibody, the FTIR spectra reveal more peaks at 2921 and 2822 cm^{-1} and other sites at wavenumbers lower than 1630 cm^{-1}. These peaks are attributed to the bonds of C–H, C–O, and N–H stretching,

respectively. The peak at 1320 cm^{-1} is assigned to the vibration of amide III in the protein structure [37]. Determining the vibration of amides individually, such as N–H stretching, C–N stretching, and N–H bending, is difficult because these peaks may overlap with those of the electrodes before antibody immobilization [37,38]. However, under the interaction of antibody molecules, the FTIR spectra of modified SPEs could show differential changes.

<Figure 3>

3.2. Electrochemical properties of AuNPs- modified carbon SPEs

The electrochemical properties of modified carbon SPEs were measured in a 5.0 mM $Fe_3(CN)_6/Fe_4(CN)_6$ added with 0.1 M KCl. Fig. 4A shows CV curves of the carbon SPE before and after modification with AuNPs. It can be seen that the anodic and cathodic currents of AuNPs- modified SPE are significantly higher than those of bare SPEs (e.g., the anodic current of modified SPEs is 149.08 μA compared to 76.81 μA of bare SPEs). These CV curves were obtained after five cycles to remove all unbound molecules. This step is beneficial for the development of electrochemical biosensors, because if SPEs are not well modified and washed, nanomaterials and biological molecules may be easily detached during the electrochemical process, and result in the instability of biosensors [36]. Furthermore, EIS curves showed that the charge transfer resistance (R_{ct}) of bare SPEs markedly reduced from 1.8 kΩ to 0.7 kΩ (AuNPs-modified SPEs, and ΔR_{ct} was approximately 1.1 kΩ, Fig. 4B). The decrease in R_{ct} could help the electron transfer more convenient between the electrolytic solution and electrode. It means that modified SPEs can be more sensitive to a small change of electrons on the surface than that of bare SPEs.

<Figure 4>

3.3. Electrochemical detection of E. coli O157

Fig. 5A shows CV cycles of SPEs before and after modification and functionalization, including the bare SPE, SPE/AuNPs, SPE/AuNPs/Ab, and SPE/AuNPs/Ab/BSA, corresponding to curves “a” to "d", respectively. The figure illustrates that the peak current of the SPE is increasing after modification with AuNPs (Fig. 5A, curves “a” and “b”). However, the functionalization with chemical molecules and immobilization with antibody lead to the decrease in peak currents of modified SPEs (Fig. 5A, curves “b”– “d”). The findings could be explained that the SPEs have become more conductive to improve the electron transfer between the electrolytic solution and electrode with the presence of AuNPs. By contrast, after functionalization with MTS and GBMS, immobilization with antibody, and blocking with BSA, these molecules could hinder the electron transfer on the surface of the electrode. The details of peak currents can be seen in Table S1 (Supplementary material). Fig. 5B shows the stability of the SPE/AuNPs/Ab/BSA during the electrochemical process. After washing steps and seven CV cycles, there is no change found in peak currents. It means that the biosensors are stable and ready for measurements.

<Figure 5>

In this study, both CV and EIS techniques were applied for AuNPs-modified SPEs to detect *E. coli* O157 bacteria at different concentrations after 30 min of incubation. Fig. 6A shows CV curves corresponding to the detection of *E. coli* O157 bacteria in the range of 0 CFU/ml to 10^6 CFU/ml, (Fig. 6A, curves "a" to "g", respectively). The change in concentrations of bacteria resulted in changing peak currents of biosensors. Fig. 6B also shows EIS curves with the increase in R_{ct} corresponding to the increase of bacterial concentrations from 0 CFU/ml to 10^6 CFU/mL (Fig. 6B, curves “a" to "g", respectively). The values of peak currents and R_{ct} can be seen in Table S2 (Supplementary material). By using the Z-view software, their equivalent circuit was fitted and expressed in the inset of Fig. 6B [30].

<Figure 6>

In comparison between the values of R_{ct} calculated from EIS curves of electrochemical biosensors tested with and without the presence of *E. coli* O157, the change of R_{ct} could be fitted by the linear equation $y(\Delta R_{ct}) = 1.36667x - 0.12222$ ($R^2 = 0.96999$) (Fig. 7).

<Figure 7>

In this study, *Salmonella enteritidis* bacteria were served as a control. The biosensors were incubated with *Salmonella enteritidis* at a concentration of 10^2 CFU/ml. By EIS measurements, the average value of R_{ct} was found about 1.96 kΩ, a little bit lower than that of the biosensor tested with *E. coli* O157 at 10 CFU/ml (2.02 kΩ). It was also recognized as the R_{ct} of noise. With serial dilutions of *E. coli* O157 sample from 10^2 CFU/ml down to 10 CFU/ml. The lowest concentration of *E. coli* O157, which could give the value of R_{ct} with a signal-to-noise (S/N) ratio of 3:1, was determined as the limit of detection (LoD) of the biosensor (Fig. 8A). In our experiments, the LoD was found at 15 CFU/ml, which was lower than those published previously [30,39,40]. When the biosensors tested with both bacterial strains at the same concentration of 10^2 CFU/ml, ΔR_{ct} was found approximately 1.8 kΩ (inset of Fig. 8A).

<Figure 8>

Fig. 8B shows the life time of electrochemical biosensors stored at 4 °C on day 0, 7, 14, and 21. It reveals that there is no change found in peak currents during 21 days of the storage. On day 21, the biosensors were tested with *E. coli* O157 at a concentration of 10^2 CFU/ml using CV measurement, and results showed a decrease in the peak current of about 44.14 µA (inset of Fig. 8B). It means that the biosensors remain their bioactivity after 21 days stored at 4ºC. When the biosensor was tested with *E. coli* O157, the bacteria could be captured by specific antibody immobilized on the working electrode, leading to an increase in the surface impedance, and a decrease in the electron transfer rate on the surface of the biosensors.

5. Conclusions

In this study, electrochemical biosensors were successfully developed from carbon SPEs, which modified with AuNPs of 18.0 ±1.6 nm, for label-free detection of *E. coli* O157. Both CV and EIS measurements were performed in a solution of 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6$ added with 0.1 M KCl. Results showed that the biosensor could detect *E. coli* O157 in the range of 10–10^6 CFU/ml, with a low LoD of 15CFU/ml without labels. The time of detection was just about 30 min. The study showed that AuNPs-modified carbon SPEs could be developed for quick and direct detection of bacterial pathogens. This biosensing platform is potential for the development of point-of-care devices for monitoring highly pathogenic agents, specifically hospital-acquired infections and emerging and re-emerging infectious diseases.

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Captions for Figures

Figure 1. Preparation of the electrochemical biosensor based on AuNPs-modified carbon SPEs for label-free detection of *E. coli O157* bacteria

Figure 2. (A)TEM image of AuNPs; (B) SEM images of carbon SPEs before and (C) after modification with AuNPs; (D) EDX spectra of AuNPs-modified SPEs

Figure 3. (A) FTIR spectra of AuNPs-modified SPEs before and (B) after immobilization with anti-*E. coli* O157 IgG polyclonal antibody

Figure 4. (A) CV curves and (B) EIS profiles of bare and AuNPs-modified SPEs

Figure 5.(A) CV curves of (a) the bare SPE, (b) SPE/AuNPs, (c) SPE/AuNPs/Ab, and (d) SPE/AuNPs/Ab/BSA at a scan rate of 50 $mV.s^{-1}$; (B) the stability of SPE/AuNPs/Ab/BSA after seven CV cycles

Figure 6. Electrochemical detection of *E. coli* O157 using (A) CV and (B) EIS at concentrations of (a) 0 CFU/ml, (b) 10 CFU/ml, (c) 10^2 CFU/ml, (d) 10^3 CFU/ml, (e) 10^4 CFU/ml, (f) 10^5 CFU/ml, and (g) 10^6 CFU/ml. The inset shows the equivalent circuit of the electrochemical biosensor.

Figure 7. The change in ΔR_{ct} of electrochemical biosensors tested with different concentrations of *E. coli* O157

Figure 8. (A) EIS profiles of electrochemical biosensors tested with *E. coli* O157, and a control of *Salmonella* bacteria at a concentration of 10^2 CFU/ml, respectively (inset shows the change in ΔR_{ct}); (B) CV curves show the life time of electrochemical biosensors stored at 4°C during 21 days (n = 5).

Figure 1

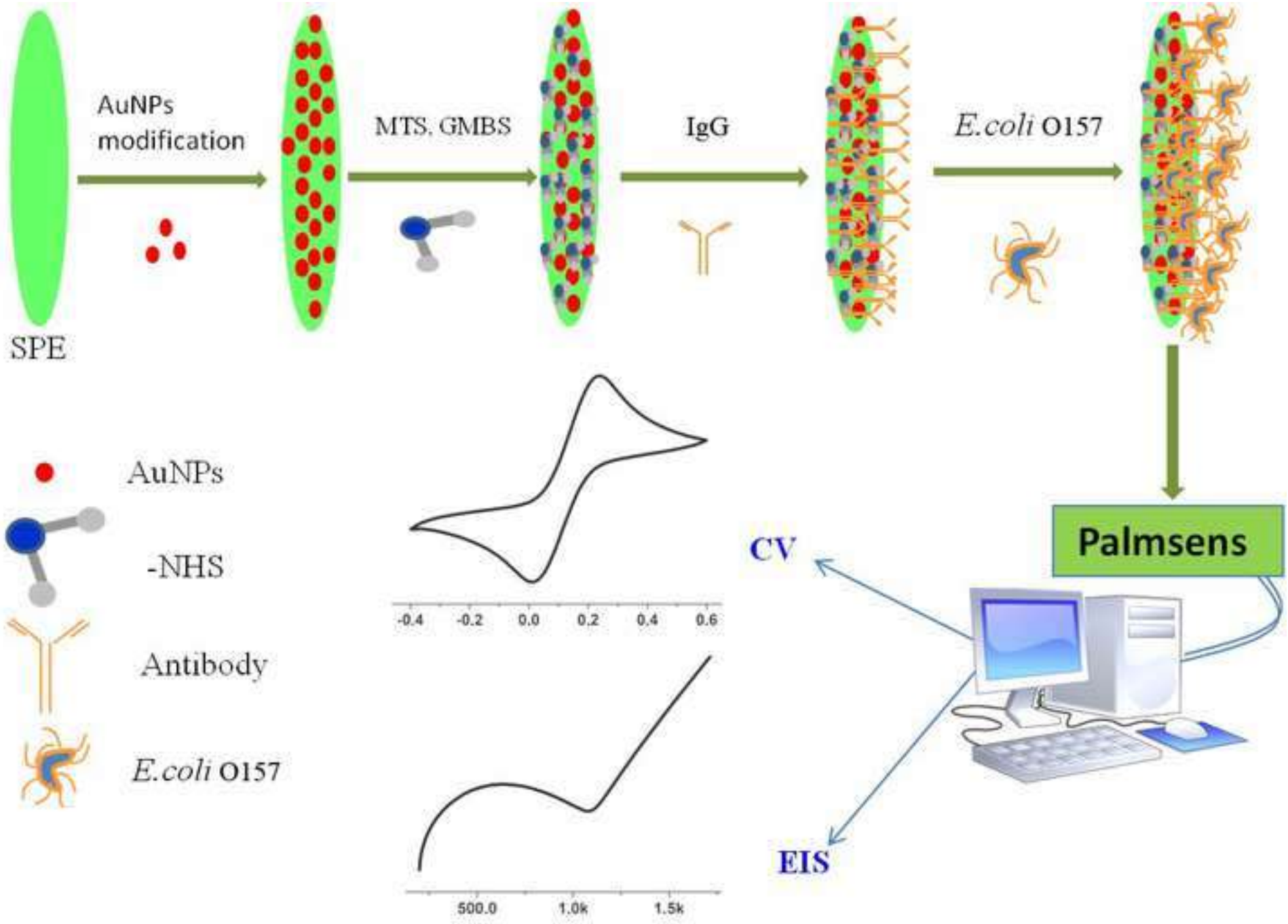
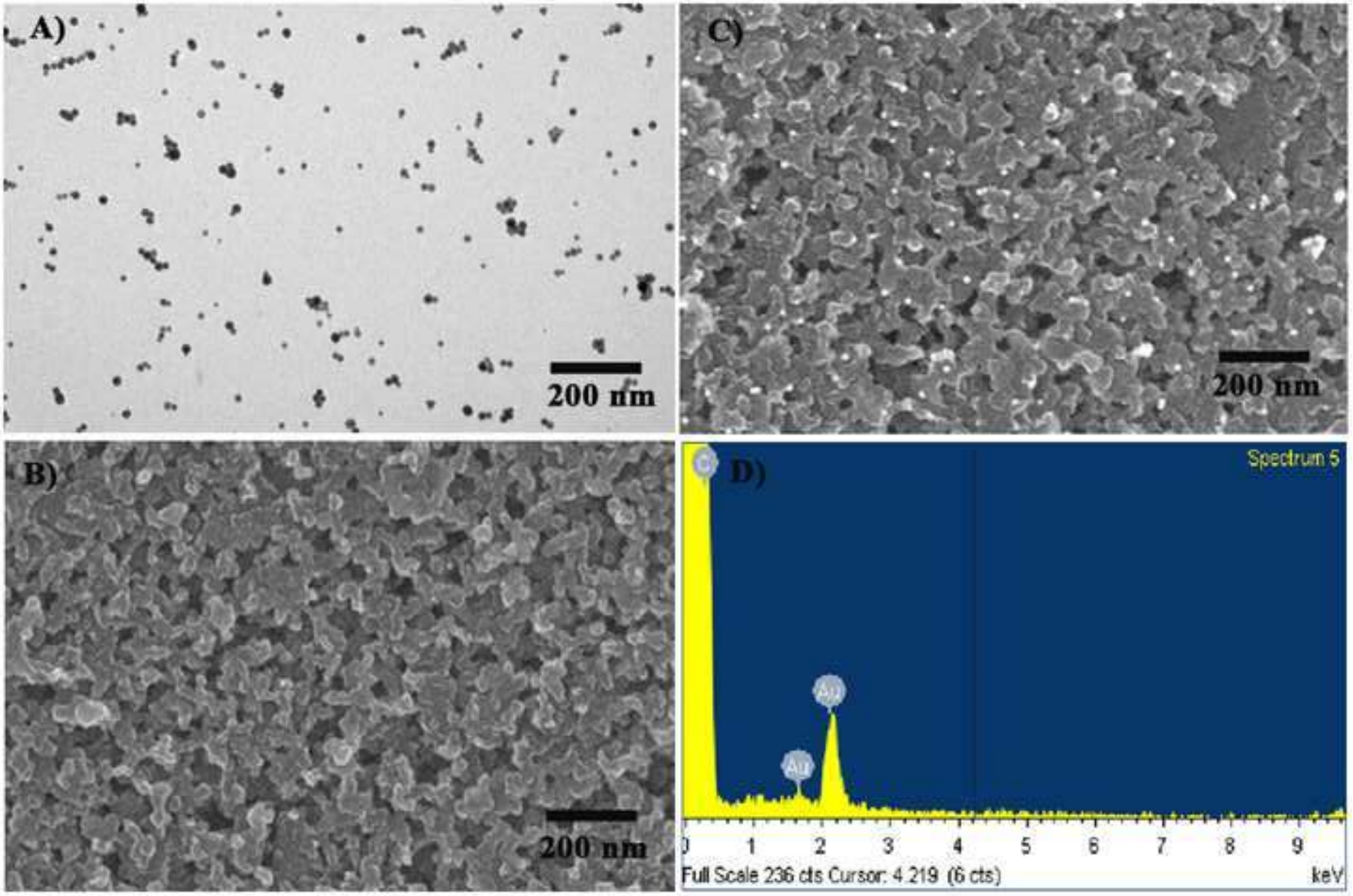


Figure 2



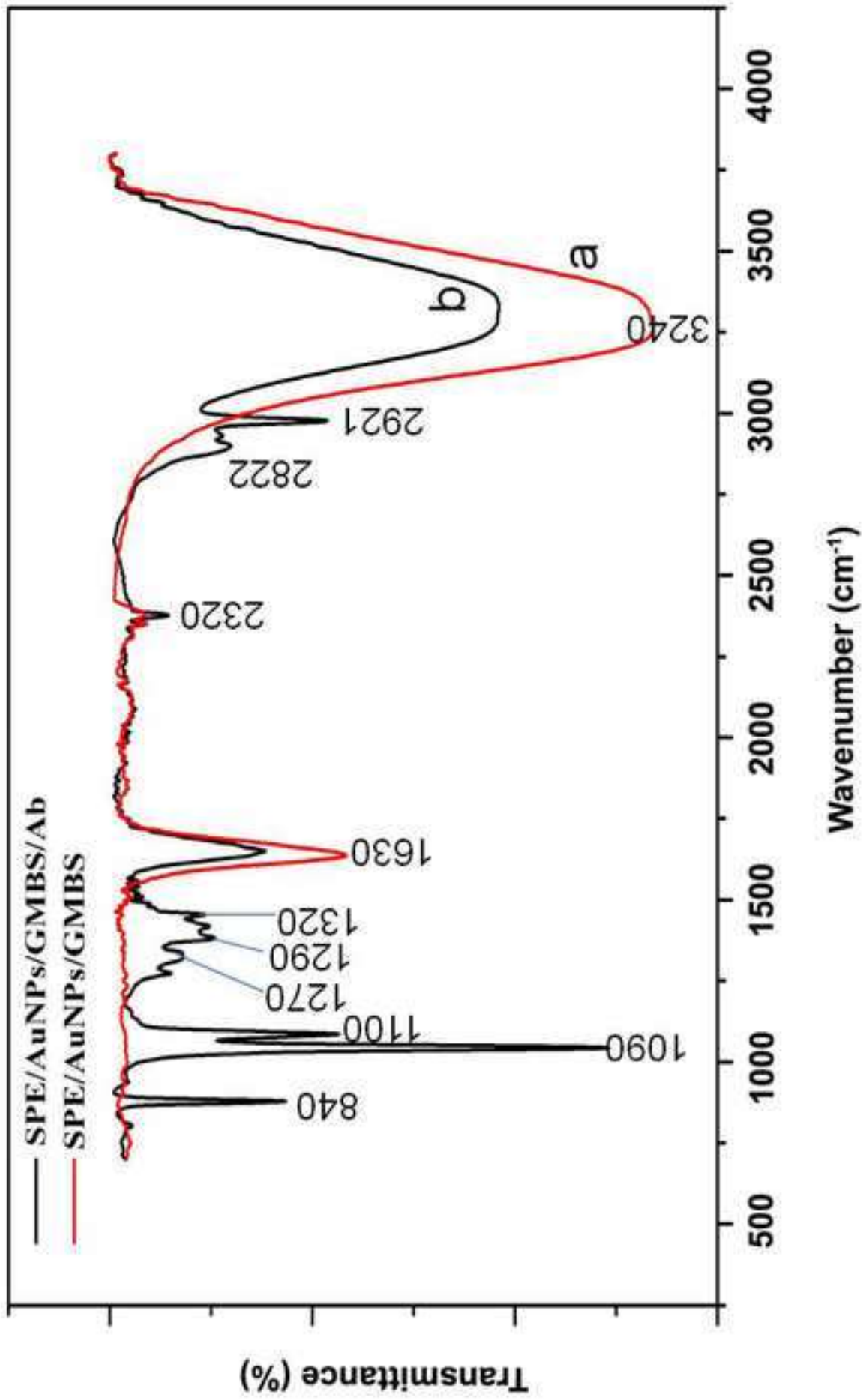


Figure 3

Figure 4

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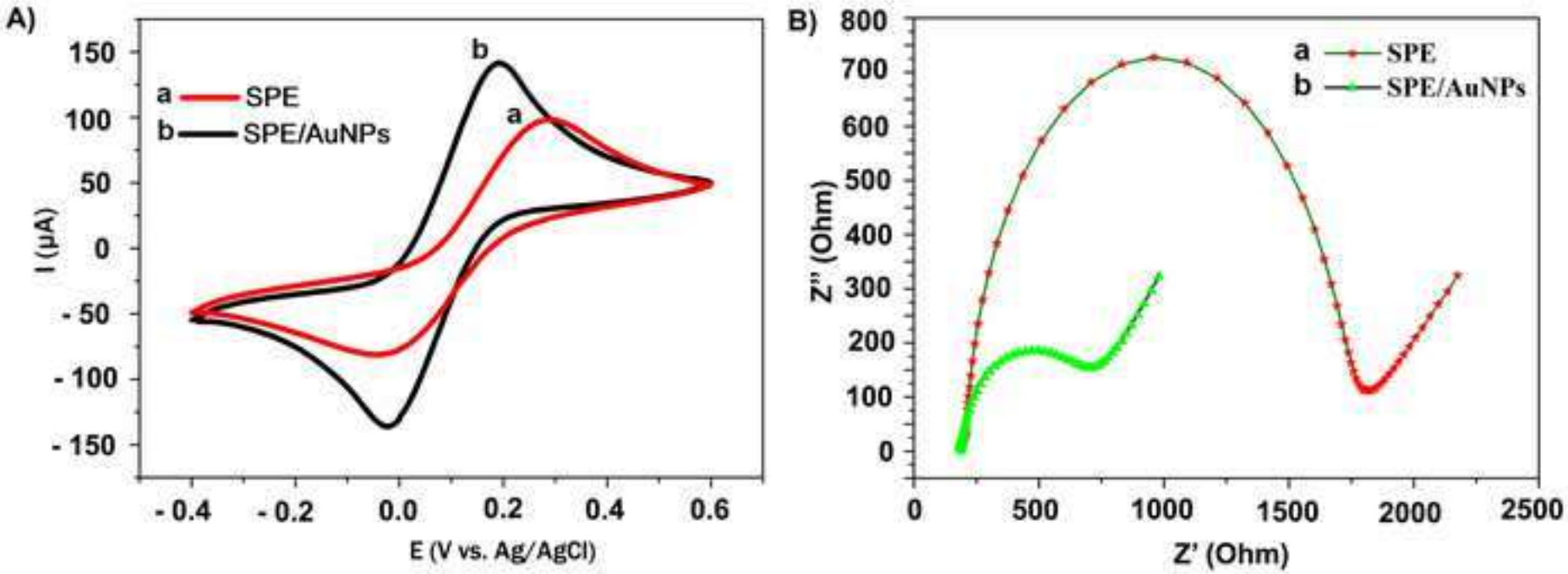

Figure 5

Click here to access/download;Figure;Fig 5AB.jpg

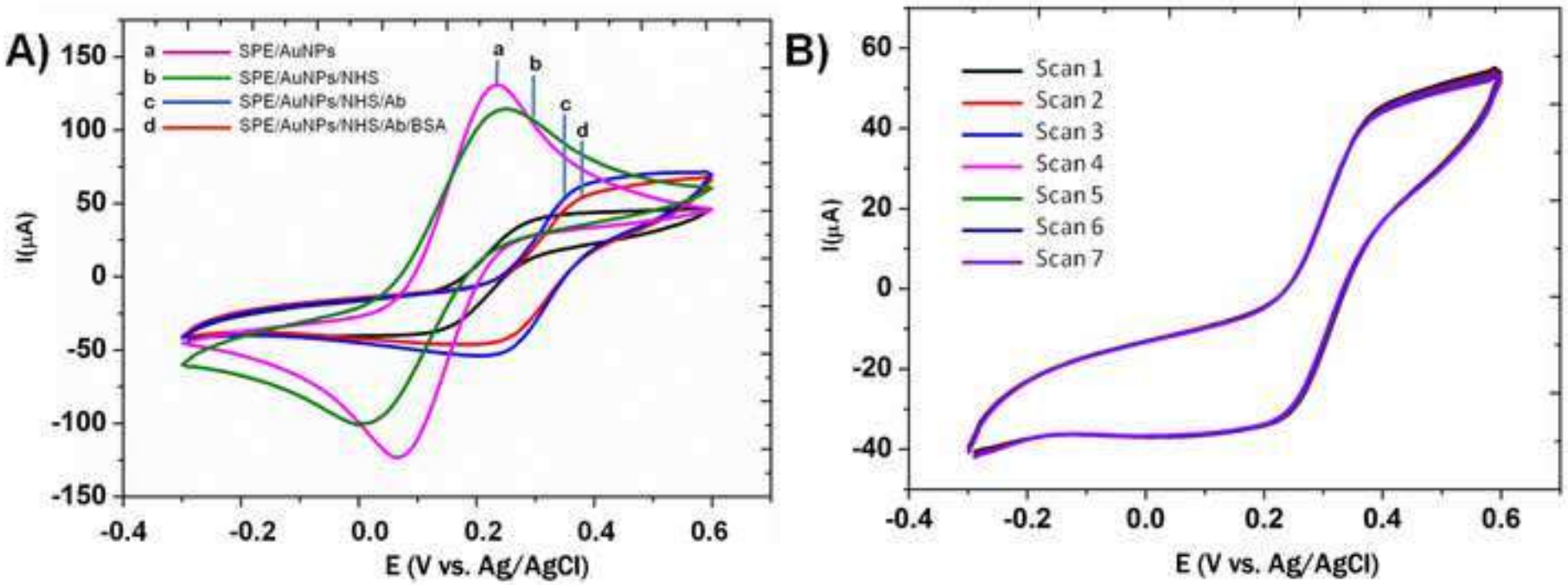

Figure 6

Click here to access/download;Figure;Fig 6AB.jpg

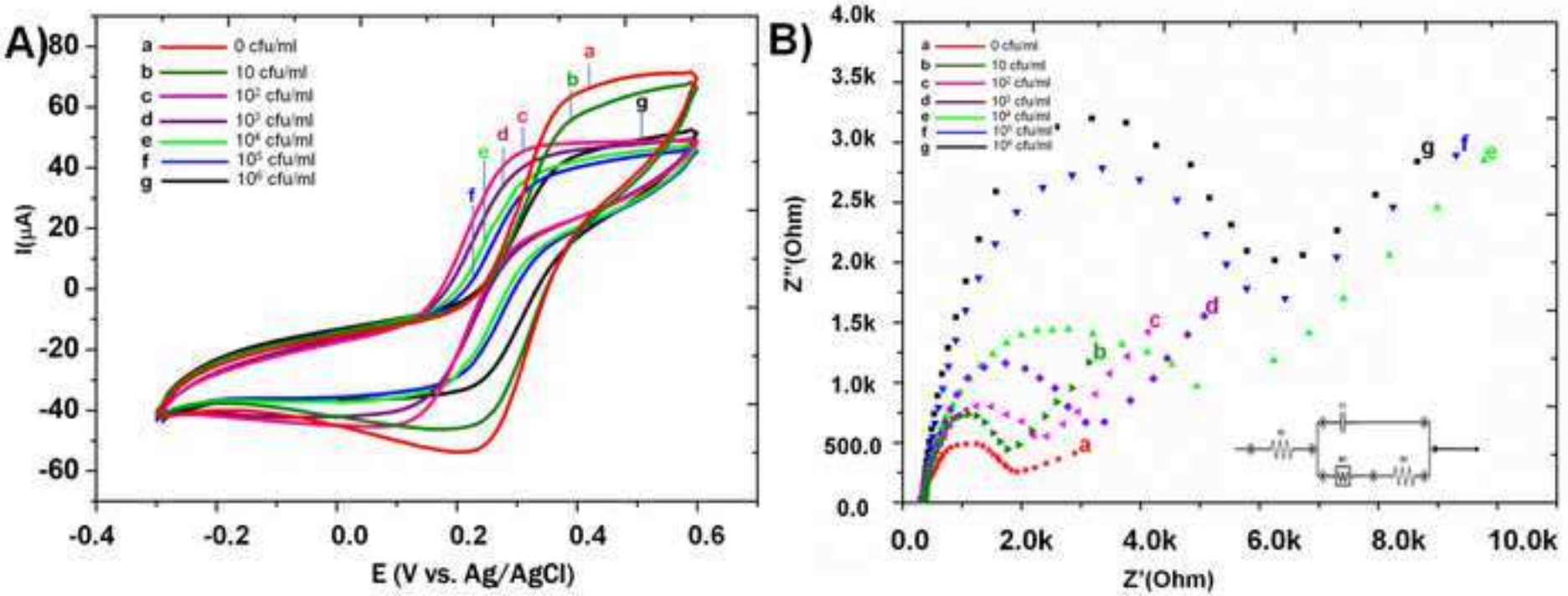

Figure 7

Click here to access/download;Figure;Fig 7.tif

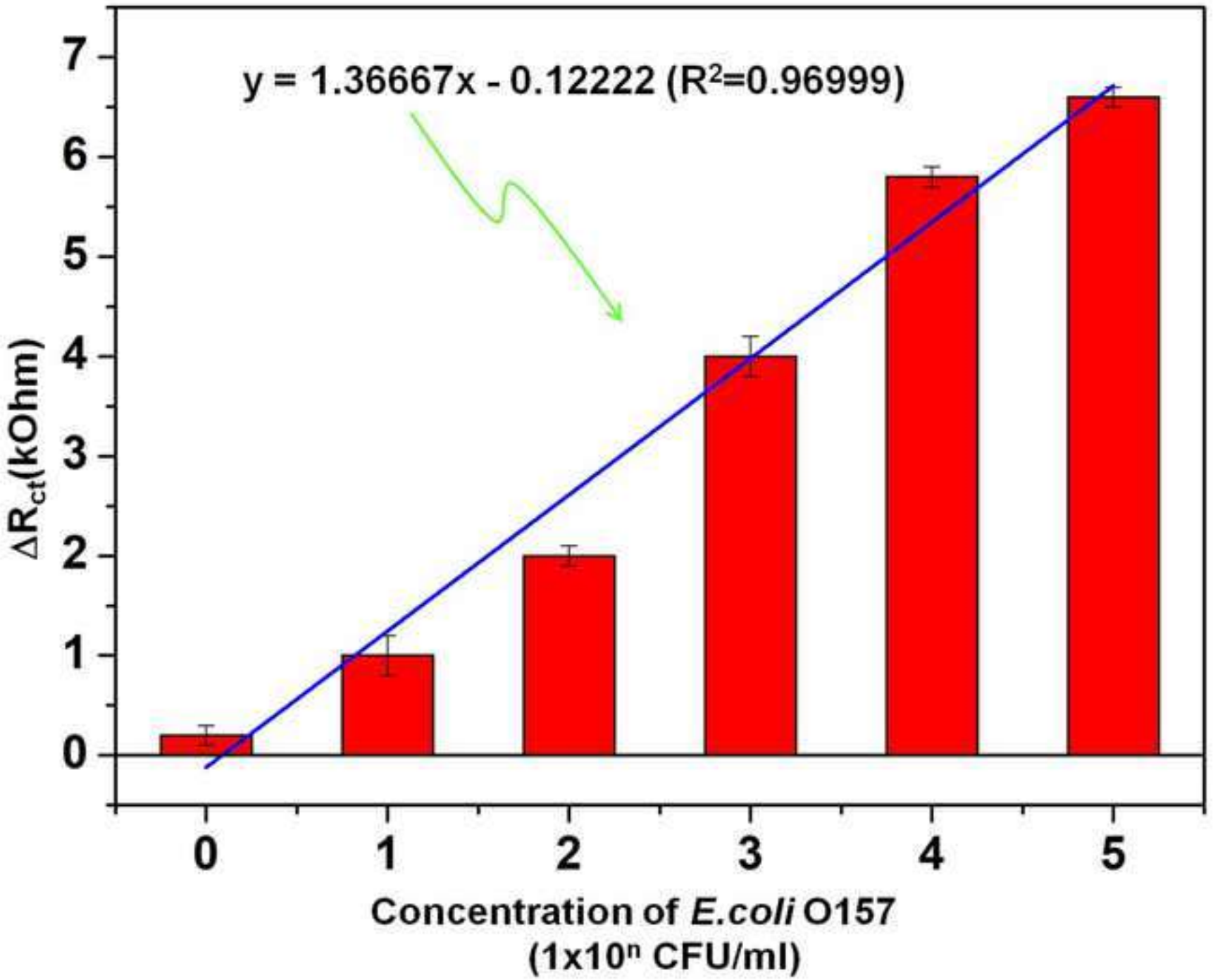

Figure 8

Click here to access/download;Figure;Fig 8A-B.tif