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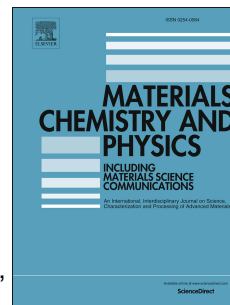
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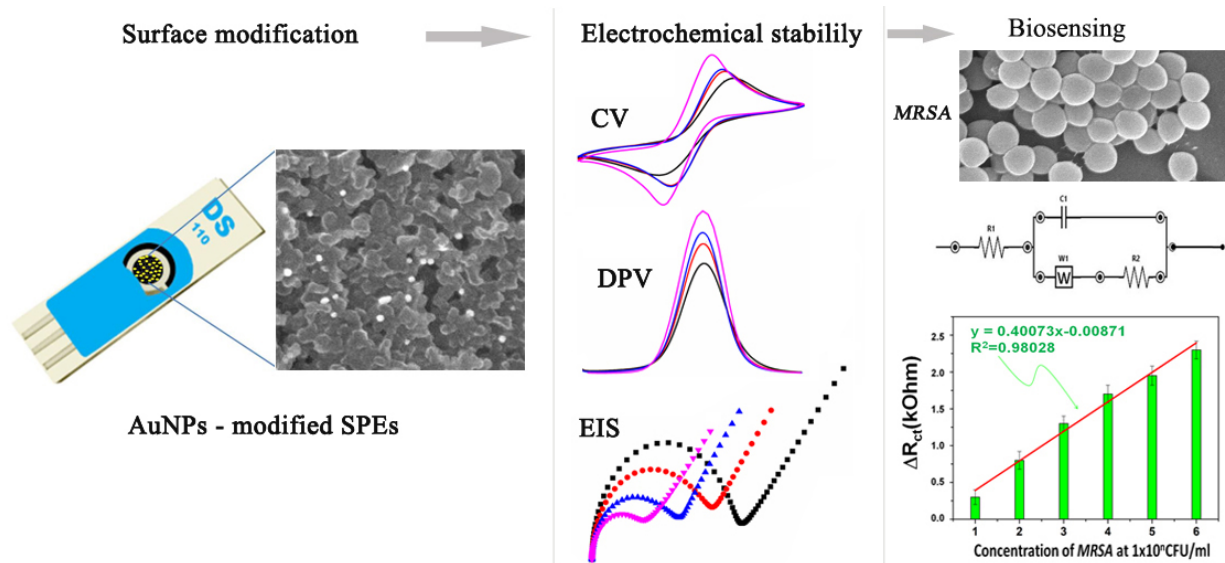
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**Vu Quang Khue:** investigation, formal analysis, data curation, and writing- original draft; **Tran Quang Huy:** conceptualization, methodology, resources, writing – review and editing, and supervision; **Vu Ngoc Phan:** investigation, formal analysis, and writing- original draft; **Anh Tuan-Le:** conceptualization, methodology, and review; **Dang Thi Thanh Le:** investigation, visualization, and review; **Matteo Tonzzer:** conceptualization, visualization, review, and supervision; **Nguyen Thi Hong Hanh:** funding acquisition, validation, project administration, review and supervision.



**Electrochemical stability of screen-printed electrodes modified with Au nanoparticles  
for detection of methicillin-resistant *Staphylococcus aureus***

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**Abstract**

In this study, the surface of carbon screen-printed electrodes (SPEs) was modified with Au nanoparticles (AuNPs) of different sizes to investigate their electrochemical stability for detection of methicillin-resistant *Staphylococcus aureus* (*MRSA*). AuNPs were electrochemically synthesised and used to modify the working electrode of SPEs via drop-casting method. Electron microscopic techniques were conducted to investigate the change in the morphology of AuNP-modified SPEs. The electrochemical behaviour of the AuNP-modified SPEs was studied by cyclic voltammetry (CV), differential pulse voltammetry, and electrochemical impedance spectroscopy in 5.0 mM  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  solution added with 0.1 M KCl. The AuNP-modified SPEs were also functionalised and prepared for the electrochemical detection of *MRSA*. Results showed that spherical AuNPs were successfully synthesised, the mean sizes were 25, and 15 nm. The electrochemical behaviour of modified SPEs strongly depended on the size of AuNPs. The AuNP-modified SPEs were stable after 25 CV cycles and can detect *MRSA* in the range of  $10-10^6$  CFU/ml, with a limit of detection (LoD) of 13 CFU/ml. The study revealed that carbon SPEs modified with AuNPs of suitable sizes can provide high-stability in the electrochemical behaviour for biosensing devices, especially for the rapid detection of highly pathogenic microorganisms.

**Keywords:** carbon screen-printed electrode; AuNPs; electrochemical detection; *MRSA*

## 1 **1. Introduction**

2 Biosensing technologies have been widely considered by scientists worldwide, and various  
3 types of biosensors have been developed for the rapid determination of pathogenic agents [1].  
4 Thanks to their compactness, portability, quick response time, high sensitivity, and high  
5 specificity, biosensors are expected to be alternative diagnostics for the rapid screening of  
6 dangerous pathogens on-site. However, there are few commercial biosensors available in the  
7 market. The major problems concerning the potential development of biosensors are stable  
8 over time and production cost [2,3]. Carbon screen-printed electrodes (SPEs) are a great  
9 solution for sensing technologies due to their ease of use, low-cost, and availability [4], [5],  
10 [6]. SPEs have been modified and widely applied for the electrochemical determination of  
11 glucose [7], thiamphenicol residue in milk [8], vitamin C [9], acetylcholine [10], and  
12 bentazone [11]. SPEs have also been developed to detect highly pathogenic agents, such as  
13 carcinoembryonic antigen [12], *Staphylococcus aureus* [13], *Salmonella* [14], and *Escherichia*  
14 *coli* O157:H7 [15]. Several nanostructured materials, including Au nanoparticles (AuNPs)  
15 [16], [17], AuNPs-MoS<sub>2</sub> [18], Ag nanoparticles [19], [20], Pt nanowires [21], magnetic beads  
16 [22], Prussian blue [23], [24], carbon nanotubes [25], [26], [27], graphene oxide [28], [29],  
17 [30], and zinc oxide nanorods [31], are used to modify the surface of SPEs for biosensing.  
18 Among these nanostructured materials, AuNPs are preferably used to improve the biosensing  
19 performance because of their unique properties, such as high surface free energy, excellent  
20 conductivity, and biocompatibility [32]. Biomolecules can easily bound to the surface of  
21 AuNPs and retain their bioactivity and stability of the latter during storage and measurement  
22 [33], [34]. In electrochemical detection, the surface stability and sensitivity of electrodes are  
23 keys to ensure the successful development of biosensors, and AuNPs can act as electron wires  
24 to enhance the electron transfer rate between the electrolytic solution and electrode [32], [35].

1 Although scientific papers have described the use of nanostructured materials to modify  
2 carbon SPEs for enhancing the sensitivity of such devices, the surface stability of these  
3 modified SPEs has not been clearly reported [5,6]. Active nanomaterials and functionalisation  
4 processes are selected depending on the type of specific interactions between the target  
5 analyte and probe. Specifically, bio-functionalisation and electrochemical measurement may  
6 change the surface properties of the modified SPEs and the quality of the developed  
7 biosensors.

8 Methicillin-resistant *S. aureus* (*MRSA*) is a Gram-positive, one of the leading causes of  
9 hospital-acquired infections, and a major problem in the global public health [36]. At present,  
10 there are a few diagnostics available to detect *MRSA* except the traditional culture-based  
11 method, and molecular techniques in clinical laboratories such as quantitative PCR or loop-  
12 mediated isothermal amplification (LAMP) [37], [38]. Hence, the development of biosensing  
13 devices for rapid detection of *MRSA* has become crucial for screening and identifying  
14 outbreaks caused by this bacterial strain. In 2015, Hiremath *et al.* [39] developed a  
15 magnetoelastic biosensor based on a lytic phage for detection of *MRSA*, and its LoD was  
16 found at 3.0 log CFU/ml. The selectivity and binding kinetics of a lytic phage to *MRSA* were  
17 also demonstrated by testing the biosensor in individual cultures of *MRSA* mixed with other  
18 foodborne pathogens [40]. Recently, Maldonado *et al.* [41] have been developed a  
19 nanophotonic interferometric biosensor for label-free detection of nosocomial bacteria.  
20 Results showed that the biosensor could detect *MRSA* at a concentration of 800 CFU/ml, and  
21 LoD was found at 29 CFU/ml.

22 In our strategy for the development of electrochemical biosensors for direct detection of  
23 bacterial pathogens, carbon SPEs were modified with AuNPs of different sizes to investigate  
24 their electrochemical stability during measurements. The AuNP-modified SPEs were then  
25 functionalised and tested to detect *MRSA* directly without any labels. The findings of the work



1 are expected to provide useful information to develop AuNP-modified carbon SPEs for quick  
2 and label-free detection of highly pathogenic bacteria and other microorganisms causing  
3 infectious diseases.

4

## 5 **2. Materials and methods**

### 6 *2.1. Materials*

7 Carbon SPEs (SPEs-DS110) were purchased from DS Dropsens, Spain. (3-Mercaptopropyl)  
8 trimethoxysilane (MTS; 95%), (N-gamma-maleimidobutyloxy) sulphosuccinimide (GMBS;  
9 98.5%),  $K_3[Fe(CN)_6]$  and  $K_4[Fe(CN)_6]$  (98.5%), KCl ( $\geq 99\%$ ), phosphate-buffered saline (PBS,  
10 pH 7.4), ethanol absolute, and bovine serum albumin (BSA, 96%) were purchased from Sigma-  
11 Aldrich (USA). All chemicals were of analytical grade.

12 Mouse monoclonal anti-*MRSA* antibody was purchased from Abcam. *MRSA* ( $10^6$  CFU/ml)  
13 and *E. coli* O157:H7 ( $10^6$  CFU/ml) were isolated from clinical samples and provided by the  
14 Department of Bacteriology, National Institute of Hygiene and Epidemiology (NIHE), Hanoi,  
15 Vietnam.

### 16 *2.2. AuNP preparation*

17 AuNPs were electrochemically synthesised and supplied by NIHE, Hanoi, Vietnam. Briefly,  
18 AuNPs were synthesised from bulk gold bars using a modified electrochemical method  
19 published previously [42]. Two electrodes were washed by bi-distilled water to remove rough  
20 and dirt residues under ultrasonic vibration for 30 min and then connected to the power as an  
21 anode and a cathode. The electrodes were dipped in an electrochemical jar containing 50 ml bi-  
22 distilled water and added with 0.1% trisodium citrate. Direct current power was supplied to the  
23 system, and the electrochemical process was conducted for 2 h under stirring condition. The  
24 formation, size, and morphology of AuNPs were checked by transmission electron microscopy  
25 (TEM, JEM1010-JEOL).

### 1 2.3 Preparation of carbon SPEs modified with AuNPs

2 The surface of bare SPEs was checked by scanning electron microscopy (SEM, S4800-  
3 Hitachi) before modification. Afterwards, bare SPEs were incubated with 50 ppm AuNPs by  
4 drop-casting method for 60 min at room temperature. Next, AuNP-modified SPEs were  
5 electrochemically cleaned in a 5 mM  $\text{Fe}_3(\text{CN})_6/\text{Fe}_4(\text{CN})_6$  solution with five cyclic  
6 voltammetry (CV) cycles to remove all unspecific molecules, including unbound AuNPs and  
7 other residues on the SPE surface. Finally, the AuNP-modified SPEs were rinsed thrice with  
8 bi-distilled water before use.

9 Differential pulse voltammetry (DPV, a scan rate of  $20 \text{ mV s}^{-1}$ ,  $E_{\text{pulse}}$  step of 10 mV, and  $t_{\text{pulse}}$   
10 of 0.02 s, in the range of  $-0.3 \text{ V}$  to  $0.5 \text{ V}$ ), cyclic voltammetry (CV, a scan rate of  $50 \text{ mV s}^{-1}$   
11 in the range of  $-0.4 \text{ V}$  to  $0.6 \text{ V}$ ), and electrochemical impedance spectroscopy (EIS, an  
12 amplitude of 10 mV, in the frequency range of 0.01 Hz to 50 kHz), were conducted in a 5.0  
13 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$  solution added with 0.1 M KCl.

### 14 2.3. Surface functionalisation and antibody immobilisation

15 The working surface of the AuNP-modified SPEs was immersed in a solution of 2%  
16 MTS/ethanol for 1 h at room temperature to create  $-\text{SH}$  groups. After washing with bi-distilled  
17 water, the surface was continuously incubated with  $6 \mu\text{l}$  GMBS (0.04 M) for 30 min at room  
18 temperature to create  $-\text{NHS}$  groups. Finally, the electrodes were washed thrice with PBS (pH  
19 7.4) before immobilisation with antibody (Ab) [31][43].

20 In the preparation of electrochemical biosensors, a drop of anti-MRSA Ab ( $10 \mu\text{g/ml}$ , diluted in  
21 PBS at pH 7.4) was incubated with the working surface of AuNP-modified SPEs for 1 h at  
22 room temperature. The sensors were then thoroughly washed thrice with PBS at pH 7.4 to  
23 remove unbound antibodies. Afterwards, a drop of blocking buffer (2% BSA/PBS) was added,  
24 and the biosensors were incubated for 30 min at room temperature to block all non-specific  
25 binding sites on the biosensor surface [44,45].

1 To test the biosensing ability, we incubated the biosensors with *MRSA* at different  
2 concentrations from 10 to  $10^6$  CFU/ml for 30 min, and electrochemical measurements were  
3 conducted using a portable electrochemical system (Palmsens 3.0, Netherlands; Fig. 1).  
4 Interference control tests were tested with *E. coli* O157:H7 at a concentration of  $10^6$  CFU/ml  
5 with the same protocol. For positive control, after functionalisation to create –NHS groups, the  
6 electrodes were incubated with *MRSA* at a concentration of  $10^3$  CFU/ml for 30 min without the  
7 presence of anti-*MRSA* antibody, the EIS was then measured to see the change in  $R_{ct}$ .

8 (Figure 1)

### 9 3. Results and discussion

#### 10 3.1 Surface characterisation

11 In this study, AuNPs were electrochemically synthesised in two different sizes, namely, 15  
12 (Fig. 2A), and 25 nm (Fig. 2B). TEM observation showed the spherical shape and  
13 homogeneous status of AuNPs. The homogeneity of AuNPs was expected to help them bind  
14 firmly to the SPE surface, reduce its surface impedance, and enhance the conductivity and  
15 stability of SPEs during the electrochemical process.

16 (Figure 2)

17 SEM observation showed that the SPE surface contained a porous structure with pores that  
18 were less than 25 nm in diameter (Fig. 2C), and there are numerous AuNPs firmly adhered to  
19 the carbon surface (white dots in Fig. 2C). Different AuNP sizes were tested to observe  
20 whether these nanoparticles bind tightly to the SPE surfaces and deeply penetrate the porous  
21 structure. The presence of AuNPs can result in the best possible Ab binding and ensure that  
22 their contact space with the samples would remain unaffected during electrochemical  
23 measurement. Energy-dispersive X-ray spectroscopic (EDX) analysis also revealed that there  
24 was only Au element found in the carbon electrode structure (Fig. 2D), and no impurity  
25 affected the SPE surface.

### 1 3.2 Electrochemical behaviour of AuNP-modified SPEs

2 The electrochemical behaviour of the modified SPEs was analysed by CV at  $50 \text{ mV s}^{-1}$ ; EIS in  
3 the frequency ranging from 0.01 Hz to 50 kHz, DPV at a scan rate of  $20 \text{ mV s}^{-1}$ ,  $E_{\text{pulse}}$  step of  
4 10 mV, and  $t_{\text{pulse}}$  of 0.02 s in 5.0 mM  $\text{Fe}_3(\text{CN})_6/\text{Fe}_4(\text{CN})_6$  solution added with 0.1 M KCl.

5 (Figure 3)

6 Fig. 3A shows the different CVs of the modified SPEs. After modification, the  $I_{\text{peak}}$  of the  
7 AuNP-modified SPEs can be found at 210.66 (Fig. 3A, curve “b”), and 215.06 (Fig. 3A, curve  
8 “c”), corresponding to the 25, and 15 nm AuNPs, respectively. The redox current of the AuNP-  
9 modified SPEs was considerably higher than that of bare SPEs ( $154.01 \mu\text{A}$ , Fig. 3A, curve  
10 “a”). The highest redox current obtained corresponded to SPEs that were modified with the  
11 small AuNPs (15 nm). The small size of AuNPs ensured their good adherence to the electrode  
12 surface, and penetration of the porous structure of the carbon working electrode resulted in the  
13 surface stability under the washing steps or electrochemical process. Hence, the small size of  
14 AuNPs has also the advantage of stabilising the electron transfer rate on the SPE surface [29].  
15 As shown in Fig. 3B, EIS demonstrated that the charge transfer resistance ( $R_{\text{ct}}$ ) of the bare SPE  
16 (Fig. 3B, curve “a”) was 1.81 kOhm, and it reduced to 1.48, and 1.13 kOhm for SPEs modified  
17 with 25 (Fig. 3B, curve “b”), and 15 nm (Fig. 3B, curve “c”), respectively. As shown in Fig.  
18 3C, the DPV responses of the SPEs before and after modification with AuNPs significantly  
19 changed. The experimental data revealed that the anode current of the bare SPE was  
20 approximately  $146.01 \mu\text{A}$  (Fig. 3C, curve “a”). After modification with AuNPs of two different  
21 sizes, the anode current significantly increased to 174.20 (Fig. 3C, curve “b”), and  $185.61 \mu\text{A}$   
22 (Fig. 3C, curve “c”), corresponding to AuNP sizes of 25, and 15 nm, respectively. The highest  
23 anode current was also obtained from the SPEs modified with 15 nm AuNPs. These techniques  
24 confirm that the electrochemical behaviour of SPEs strongly depends on the size of AuNPs  
25 used to modify the porous structure of the carbon working electrode. The size of NPs should be

1 smaller than that of pores in the porous carbon structure. It ensures that small signals that are  
2 generated from biochemical or immunological reactions on the SPE surface could be detected.  
3 AuNPs should also adhere firmly on the carbon SPEs to provide the necessary stability during  
4 the electrochemical process for biosensing applications.  
5 Among the carbon SPEs which were modified with AuNPs of two different sizes, 15 nm  
6 AuNP-modified SPEs were selected to investigate their stability during the electrochemical  
7 process for biosensing ability because it was also to ensure that the AuNPs in 15 nm firmly  
8 adhered on the electrode after different treatments and measurements. As shown in Fig. 4A, the  
9 DPV responses of 15 AuNP-modified SPEs for  $E_{\text{pulse}}$  step values (10 mV) from 0.1 V to 0.35 V  
10 were measured in 5 mM  $\text{Fe}_3(\text{CN})_6/\text{Fe}_4(\text{CN})_6$  solution added with 0.1 M KCl at a scan rate of 20  
11  $\text{mVs}^{-1}$ , and  $t_{\text{pulse}}$  of 0.02 s ranging from -0.3 V to 0.5 V. The inset in Fig. 4A indicates that the  
12 anode current increased linearly, corresponding to the increase in  $E_{\text{pulse}}$  (Fig. 4A, inset).

13 (Figure 4)

14 Fig. 4B shows the stability of the 15 nm AuNP-modified SPEs in CV cycles. No change was  
15 observed in the redox current after 25 CV cycles at a scan rate of  $50 \text{ mV s}^{-1}$ , which proved that  
16 the surface of these modified SPEs remained stable after functionalisation and electrochemical  
17 process. Hence, carbon SPEs modified with AuNPs in 15 nm, the size is less than that of pores  
18 in the carbon structure, is suitable for the electrochemical detection of small signals generated  
19 from biochemical reactions on the electrode surface.

### 20 3.3 Electrochemical detection of MRSA

21 The 15 nm AuNP-modified SPEs were functionalised and prepared for label-free detection of  
22 MRSA by CV and EIS. Before measurements, the bacterial sample of MRSA was checked by  
23 SEM. Fig. 5A shows the typical morphology of MRSA, that is, a spherical shape, and  
24 agglomeration. Electrochemical measurements were conducted in 5.0 mM  $\text{Fe}_3(\text{CN})_6/\text{Fe}_4(\text{CN})_6$   
25 solution added with 0.1 M KCl. Fig 5B. shows the CVs of the SPEs before and after

1 modification with 15 nm AuNPs, after each step of the surface functionalisation, and the  
2 incubation of biological molecules. The changes in the redox current of the modified and bare  
3 SPEs were 215.10 and 154.05  $\mu\text{A}$ , respectively (Fig. 5B, curves “a” and “b”). After surface  
4 functionalisation to form –NHS groups and antibody immobilisation, the redox current  
5 decreased significantly from 215.10  $\mu\text{A}$  to 150.81 and 96.70  $\mu\text{A}$ , respectively (Fig. 5B, curves  
6 “c” and “d”). The incubation with the sample of *MRSA* at a concentration of  $10^2$  CFU/ml also  
7 resulted in decreasing the redox current, because it is assumed that these *MRSA* hindered the  
8 electron transfer between the electrolytic and electrode surfaces (Fig. 5B, curve “f”).

9 (Figure 5)

10 These results can be explained considering that the surface modification of carbon SPEs with  
11 15 nm AuNPs could increase the electron transfer rate between the electrolytic solution and  
12 modified electrode compared with that of bare SPE. However, the functionalisation of the  
13 modified SPE surface with chemical and biological molecules reduced the electron transfer rate  
14 significantly. This result is important evidence showing the necessity of the surface  
15 modification of carbon SPEs with conductive nanomaterials for capturing small signals  
16 generated from biochemical reactions on the electrode for biosensing applications.

17 To demonstrate such a phenomenon, we conducted the EIS of the 15 nm AuNP-modified  
18 SPEs. Fig. 6A shows the Nyquist plots of modified SPEs after functionalisation steps, and it  
19 can be seen the increase of  $R_{ct}$  of AuNPs-modified SPE from 1.13 kOhm (Fig. 6A, curve “a”)  
20 to 1.65 kOhm (Fig. 6A, curve “b”) after functionalisation to create -NHS groups, and 1.79  
21 kOhm (Fig. 6A, curve “c”) after immobilization with anti-*MRSA* Ab. In this study, a positive  
22 control of modified SPEs was tested with *MRSA* at  $10^3$  CFU/ml without the presence of anti-  
23 *MRSA* Ab; the EIS curve showed a slight increase in  $R_{ct}$  to 1.83 kOhm (Fig. 6A, curve “d”). It  
24 can be explained that the increase may not come from *MRSA*, because the size of *MRSA* is  
25 much larger than that of pores of the carbon membrane. Without the specific binding with

1 antibody, bacteria can be easily detached during the electrochemical process. However, the  
2 increase in  $R_{ct}$  may result from small molecules in the media, which go deep inside the carbon  
3 network during the incubation. This was also demonstrated when the SPEs immobilized with  
4 the antibody for *MRSA* detection at different concentrations in the range of  $10$ – $10^6$  CFU/ml.  
5 Fig 6B shows the increase in  $R_{ct}$  values, corresponding to the increase in bacterial  
6 concentrations. The smallest and highest  $R_{ct}$  values are approximately 2.10, and 4.26 kOhm,  
7 corresponding to *MRSA* concentrations of 10 (Fig. 6B, curve “a”) and  $10^6$  CFU/ml (Fig. 6B,  
8 curve “f”), respectively. In Figure 6 A-B,  $R_{ct}$  was calculated by the diameter of the semicircle  
9 fitted by the equivalent circuit and analysed by the Z-view software.  $\Delta R_{ct}$  can be calculated by  
10 comparing the  $R_{ct}$  obtained from the EIS curves of AuNP-modified SPEs tested as a positive  
11 control and after incubation with *MRSA* at different concentrations. The calibration curve was  
12 also obtained using the linear equation,  $y = 0.40073x + 0.00871$ , with a correlation coefficient  
13 of 0.98028 (Fig. C).

14 (Figure 6)

15 To confirm the selectivity and stability of the electrochemical biosensors, they were also tested  
16 with *E. coli* O157:H7 at a concentration of  $10^6$  CFU/ml for 30 min at room temperature, using  
17 the same protocol used when measuring *MRSA*. The EIS results showed that the  $R_{ct}$  value was  
18 approximately 2.12 kOhm for *E. coli* O157:H7 detection (Fig. 6D, curve “a”), a little increase  
19 in  $R_{ct}$  compared to that of modified SPEs detected with *MRSA* at a concentration of 10 CFU/ml  
20 (Fig. 6B, curve “a”). However, this value was considerably lower than that of *MRSA* at the  
21 concentration of  $10^2$  CFU/ml (Fig. 6D, curve “b”). According to serial dilutions of *MRSA*  
22 concentrations from 100 to 10 CFU/ml for testing with the biosensor, the limit of detection  
23 (LoD) was found approximately 13 CFU/ml. As shown in Table 1, this biosensor could detect  
24 *MRSA* at a low concentration compared to that of SPEs – based biosensors published

1 previously for the electrochemical detection of other pathogenic bacteria [13], [22], [23], [46],  
2 [47], [48].

3 (Table 1)

#### 4 **4. Conclusions**

5 In this study, we investigated the surface stability and electrochemical behaviour of carbon  
6 SPEs modified with AuNPs of different sizes for biosensing applications. The results  
7 demonstrated that the electrochemical stability of modified carbon SPEs strongly depended on  
8 the size of AuNPs. The size of these NPs should be smaller than that of pores in the carbon  
9 structure of the working electrode. The AuNP-modified SPEs were stable after 25 CV cycles.  
10 The electrochemical biosensors based on 15 nm AuNP-modified SPEs could detect *MRSA* in  
11 the range of  $10\text{--}10^6$  CFU/ml after 30 min of incubation, with the LoD equal to 13 CFU/ml.  
12 This work provides evidence for the electrochemical stability of AuNP-modified carbon SPEs.  
13 Biosensors based on these modified SPEs may have potential use for the rapid and label-free  
14 detection of highly pathogenic bacteria and other agents causing emerging infectious diseases.

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#### 18 **References**

- 19 [1] S. Vigneshvar, C.C. Sudhakumari, B. Senthilkumaran, H. Prakash, Recent advances in  
20 biosensor technology for potential applications - an overview, *Front. Bioeng.*  
21 *Biotechnol.* 4 (2016) 1–9.
- 22 [2] M.L. Sin, K.E. Mach, P.K. Wong, J.C. Liao, Advances and challenges in biosensor-  
23 based diagnosis of infectious diseases, *Expert Rev. Mol. Diagn.* 14 (2014) 225–244.
- 24 [3] N.F.D. Silva, J.M.C.S. Magalhães, C. Freire, C. Delerue-Matos, Electrochemical  
25 biosensors for *Salmonella*: State of the art and challenges in food safety assessment,  
26 *Biosens. Bioelectron.* 99 (2018) 667–682.
- 27 [4] D. Antuña-Jiménez, M.B. González-García, D. Hernández-Santos, P. Fanjul-Bolado,  
28 Screen-Printed Electrodes Modified with Metal Nanoparticles for Small Molecule  
29 Sensing, *Biosensors.* 10 (2020) pii: E9. <https://doi.org/10.3390/bios10020009>.
- 30 [5] G. Hughes, K. Westmacott, K.C. Honeychurch, A. Crew, R.M. Pemberton, J.P. Hart,



- 1       Recent advances in the fabrication and application of screen-printed electrochemical  
2       (bio)sensors based on carbon materials for biomedical, agri-food and environmental  
3       analyses, *Biosensors*. 6 (2016) 50. <https://doi.org/10.3390/bios6040050>.
- 4       [6]   M. Li, Y.T. Li, D.W. Li, Y.T. Long, Recent developments and applications of screen-  
5       printed electrodes in environmental assays-A review, *Anal. Chim. Acta*. 734 (2012)  
6       31–44.
- 7       [7]   V. Polan, J. Soukup, K. Vyřas, Screen-printed carbon electrodes modified by rhodium  
8       dioxide and glucose dehydrogenase, *Enzyme Res.* 2010 (2010) 324184.  
9       <https://doi.org/10.4061/2010/324184>.
- 10      [8]   A. Muhammad, R. Hajian, N.A. Yusof, N. Shams, J. Abdullah, P.M. Woi, H.  
11      Garmestani, A screen printed carbon electrode modified with carbon nanotubes and  
12      gold nanoparticles as a sensitive electrochemical sensor for determination of  
13      thiamphenicol residue in milk, *RSC Adv.* 8 (2018) 2714–2722.
- 14      [9]   J.K. Jadav, V. V. Umrania, K.J. Rathod, B.A. Golakiya, Development of silver/carbon  
15      screen-printed electrode for rapid determination of vitamin C from fruit juices, *LWT -*  
16      *Food Sci. Technol.* 88 (2018) 152–158.
- 17      [10]  S.Z. Mohammadi, H. Beitollahi, S. Tajik, Nonenzymatic coated screen-printed  
18      electrode for electrochemical determination of acetylcholine, *Micro Nano Syst. Lett.* 6  
19      (2018). <https://doi.org/10.1186/s40486-018-0070-5>.
- 20      [11]  A. Geto, J.S. Noori, J. Mortensen, W.E. Svendsen, M. Dimaki, Electrochemical  
21      determination of bentazone using simple screen-printed carbon electrodes, *Environ. Int.*  
22      129 (2019) 400–407.
- 23      [12]  K.F. Chan, H.N. Lim, N. Shams, S. Jayabal, A. Pandikumar, N.M. Huang, Fabrication  
24      of graphene/gold-modified screen-printed electrode for detection of carcinoembryonic  
25      antigen, *Mater. Sci. Eng. C*. 58 (2016) 666–674.
- 26      [13]  A.C. Ward, A.J. Hannah, S.L. Kendrick, N.P. Tucker, G. MacGregor, P. Connolly,  
27      Identification and characterisation of *Staphylococcus aureus* on low cost screen printed  
28      carbon electrodes using impedance spectroscopy, *Biosens. Bioelectron.* 110 (2018) 65–  
29      70.
- 30      [14]  M.L. Del Giallo, D.O. Ariksoysal, G. Marrazza, M. Mascini, M. Ozsoz, Disposable  
31      Electrochemical Enzyme Amplified Genosensor for Salmonella Bacteria Detection,  
32      *Anal. Lett.* 38 (2007) 2509–2523.
- 33      [15]  E.B. Settingington, E.C. Alocilja, Electrochemical biosensor for rapid and sensitive  
34      detection of magnetically extracted bacterial pathogens, *Biosensors*. 2 (2012) 15–31.
- 35      [16]  T.T.N. Do, T. Van Phi, T.P. Nguy, P. Wagner, K. Eersels, M.C. Vestergaard, L.T.N.  
36      Truong, Anisotropic In Situ-Coated AuNPs on Screen-Printed Carbon Surface for  
37      Enhanced Prostate-Specific Antigen Impedimetric Aptasensor, *J. Electron. Mater.* 46  
38      (2017) 3542–3552.
- 39      [17]  B. Yang, D. Bin, J. Wang, S. Li, P. Yang, C. Wang, Y. Shiraishi, Y. Du, *Journal of*  
40      *Colloid and Interface Science* Electrochemical synthesis of gold nanoparticles  
41      decorated flower-like graphene for high sensitivity detection of nitrite, 488 (2017) 135–  
42      141.
- 43      [18]  Y. Zhang, X. Li, D. Li, Q. Wei, A laccase based biosensor on AuNPs-MoS<sub>2</sub> modified  
44      glassy carbon electrode for catechol detection, *Colloids Surfaces B Biointerfaces*. 186  
45      (2020) 110683.

- 1 [19] R. Karthik, Y.S. Hou, S.M. Chen, A. Elangovan, M. Ganesan, P. Muthukrishnan, Eco-  
2 friendly synthesis of Ag-NPs using *Cerasus serrulata* plant extract - Its catalytic,  
3 electrochemical reduction of 4-NPh and antibacterial activity, *J. Ind. Eng. Chem.* 37  
4 (2016) 330-339
- 5 [20] A.M. Noor, P. Rameshkumar, N. Yusoff, H.N. Ming, M.S. Sajab, Microwave synthesis  
6 of reduced graphene oxide decorated with silver nanoparticles for electrochemical  
7 determination of 4-nitrophenol, *Ceram. Int.* 42 (2016) 18813-18820.
- 8 [21] H. Zhao, C. Zhou, Y. Teng, C. Chen, M. Lan, Novel Pt nanowires modified screen-  
9 printed gold electrode by electrodeposited method, *Appl. Surf. Sci.* 257 (2011) 3793-  
10 3797.
- 11 [22] E.B. Settingington, E.C. Alocilja, Electrochemical biosensor for rapid and sensitive  
12 detection of magnetically extracted bacterial pathogens, *Biosensors.* 2 (2012) 15-31.
- 13 [23] M. Xu, R. Wang, Y. Li, An electrochemical biosensor for rapid detection of *E. coli*  
14 O157:H7 with highly efficient bi-functional glucose oxidase-polydopamine  
15 nanocomposites and Prussian blue modified screen-printed interdigitated electrodes,  
16 *Analyst.* 141 (2016) 5441-5449.
- 17 [24] S. Cinti, F. Arduini, D. Moscone, G. Palleschi, A.J. Killard, Development of a  
18 hydrogen peroxide sensor based on screen-printed electrodes modified with inkjet-  
19 printed prussian blue nanoparticles, *Sensors (Switzerland).* 14 (2014) 14222-14234.
- 20 [25] S. Viswanathan, C. Rani, J.A.A. Ho, Electrochemical immunosensor for multiplexed  
21 detection of food-borne pathogens using nanocrystal bioconjugates and MWCNT  
22 screen-printed electrode, *Talanta.* 94 (2012) 315-319.
- 23 [26] P.D. Tam, C.X. Thang, Label-free electrochemical immunosensor based on cerium  
24 oxide nanowires for *Vibrio cholerae* O1 detection, *Mater. Sci. Eng. C.* (2016).
- 25 [27] D. Zhang, Q. Liu, Biosensors and bioelectronics on smartphone for portable  
26 biochemical detection, *Biosens. Bioelectron.* 75 (2016) 273-284.
- 27 [28] S.X. Lee, H.N. Lim, I. Ibrahim, A. Jamil, A. Pandikumar, N.M. Huang, Horseradish  
28 peroxidase-labeled silver/reduced graphene oxide thin film-modified screen-printed  
29 electrode for detection of carcinoembryonic antigen, *Biosens. Bioelectron.* 89 (2017)  
30 673-680.
- 31 [29] F. Qu, H. Lu, M. Yang, C. Deng, Electrochemical immunosensor based on electron  
32 transfer mediated by graphene oxide initiated silver enhancement, *Biosens.*  
33 *Bioelectron.* 26 (2011) 4810-4814.
- 34 [30] Y. Song, Y. Luo, C. Zhu, H. Li, D. Du, Y. Lin, Recent advances in electrochemical  
35 biosensors based on graphene two-dimensional nanomaterials, *Biosens. Bioelectron.* 76  
36 (2016) 195-212.
- 37 [31] T. Hong, P. Nguyen, M. Tonezzer, T. Thanh, L. Dang, Q.K. Vu, Q.H. Tran, D.H.  
38 Nguyen, V.H. Nguyen, Stable Electrochemical Measurements of Platinum Screen-  
39 Printed Electrodes Modified with Vertical ZnO Nanorods for Bacterial Detection, *J.*  
40 *Nanomater.* 2019 (2019) 2341268.
- 41 [32] Y. Li, S. Xu, H.J. Schluesener, Gold nanoparticle based biosensors, *Gold Bull.* 43  
42 (2010) 29-41.
- 43 [33] D. Zhao, Y. Liu, Q. Zhang, Y. Zhang, W. Zhang, Q. Duan, Z. Yuan, R. Zhang, S. Sang,  
44 Surface stress-based biosensor with stable conductive AuNPs network for biomolecules

- 1 detection, *Appl. Surf. Sci.* 491 (2019) 443–450.
- 2 [34] X. Cao, Y. Ye, S. Liu, Gold nanoparticle-based signal amplification for biosensing,  
3 *Anal. Biochem.* 417 (2011) 1–16.
- 4 [35] J.M. Pingarrón, P. Yáñez-Sedeño, A. González-Cortés, Gold nanoparticle-based  
5 electrochemical biosensors, *Electrochim. Acta.* 53 (2008) 5848–5866.
- 6 [36] K.M. Craft, J.M. Nguyen, L.J. Berg, S.D. Townsend, Methicillin-resistant:  
7 *Staphylococcus aureus* (MRSA): Antibiotic-resistance and the biofilm phenotype,  
8 *Medchemcomm.* 10 (2019) 1231–1241.
- 9 [37] J. Su, X. Liu, H. Cui, Y. Li, D. Chen, Y. Li, G. Yu, Rapid and simple detection of  
10 methicillin-resistance *staphylococcus aureus* by orfX loop-mediated isothermal  
11 amplification assay, *BMC Biotechnol.* 14 (2014) 1–8.
- 12 [38] J. Hulme, Recent advances in the detection of methicillin resistant *Staphylococcus*  
13 *aureus* (MRSA), *Biochip J.* 11 (2017) 89–100.
- 14 [39] N. Hiremath, R. Guntupalli, V. Vodyanoy, B.A. Chin, M.-K. Park, Detection of  
15 methicillin-resistant *Staphylococcus aureus* using novel lytic phage-based  
16 magnetoelastic biosensors Nitilaksha, *Sensors Actuators B Chem.* 2210 (2015) 129–  
17 136.
- 18 [40] N. Hiremath, B.A. Chin, M.-K. Park, Effect of Competing Foodborne Pathogens on the  
19 Selectivity and Binding Kinetics of a Lytic Phage for Methicillin-Resistant  
20 *Staphylococcus aureus* Detection, *J. OfThe Electrochem. Soc.* 164 (2017) B142–B146.
- 21 [41] M. Jesús, M.-C. Estévez, A. Fernández-Gavela, J.J. González-López, A.B. González-  
22 Guerrero, L.M. Lechuga, Label-free detection of nosocomial bacteria using a  
23 nanophotonic interferometric biosensor, *Analyst.* 145 (2020) 497–506.
- 24 [42] C.J. Huang, P.H. Chiu, Y.H. Wang, K.L. Chen, J.J. Linn, C.F. Yang, Electrochemically  
25 controlling the size of gold nanoparticles, *J. Electrochem. Soc.* 153 (2006) 193–198.
- 26 [43] S.K. Bhatia, L.C. Shriver-Lake, K.J. Prior, J.H. Georger, J.M. Calvert, R. Bredehorst,  
27 F.S. Ligler, Use of thiol-terminal silanes and heterobifunctional crosslinkers for  
28 immobilization of antibodies on silica surfaces, *Anal. Biochem.* 178 (1989) 408–413.
- 29 [44] W. Shen, S. Li, M.-K. Park, Z. Zhang, Z. Cheng, V.A. Petrenko, B.A. Chin, Blocking  
30 Agent Optimization for Nonspecific Binding on Phage Based Magnetoelastic  
31 Biosensors, *J. OfThe Electrochem. Soc.* 159 (2012) B818–B823.
- 32 [45] R. Ahirwar, S. Bariar, A. Balakrishnan, P. Nahar, BSA blocking in enzyme-linked  
33 immunosorbent assays is a non-mandatory step: a perspective study on mechanism of  
34 BSA blocking in common ELISA protocols, *RSC Adv.* 3 (2015) 100077.
- 35 [46] M. Xu, R. Wang, Y. Li, Rapid detection of *Escherichia coli* O157:H7 and *Salmonella*  
36 *Typhimurium* in foods using an electrochemical immunosensor based on screen-printed  
37 interdigitated microelectrode and immunomagnetic separation, *Talanta.* 148 (2016)  
38 200–208.
- 39 [47] J. Kampeera, P. Pasakon, C. Karuwan, N. Arunrut, A. Sappat, S. Sirithammajak, N.  
40 Dechokiattawan, T. Sumranwanich, P. Chaivisuthangkura, P. Ounjai, S.  
41 Chankhamhaengdecha, A. Wisitsoraat, A. Tuantranont, W. Kiatpathomchai, Point-of-  
42 care rapid detection of *Vibrio parahaemolyticus* in seafood using loop-mediated  
43 isothermal amplification and graphene-based screen-printed electrochemical sensor,  
44 *Biosens. Bioelectron.* 132 (2019) 271–278.

- 1 [48] Y.H. Lin, S.H. Chen, Y.C. Chuang, Y.C. Lu, T.Y. Shen, C.A. Chang, C.S. Lin,  
2 Disposable amperometric immunosensing strips fabricated by Au nanoparticles-  
3 modified screen-printed carbon electrodes for the detection of foodborne pathogen  
4 *Escherichia coli* O157:H7, *Biosens. Bioelectron.* 23 (2008) 1832–1837.  
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Journal Pre-proof

## 1 Captions for Figures

2 **Figure 1.** Preparation of AuNP-modified carbon SPEs for biosensing applications.

3 **Figure 2.** TEM images of AuNPs of different sizes: (A) 15, and (B) 25 nm. SEM images of  
4 the SPE modified with AuNPs. (F) EDX spectra of the SPEs modified with AuNPs.

5 **Figure 3.** Electrochemical behaviour of SPEs before and after modification with AuNPs of  
6 different sizes: (A) CV, (B) EIS, and (C) DPV responses.

7 **Figure 4.** Electrochemical stability of 15 nm AuNP-modified SPEs: (A) DPV responses in the  
8 range of 0.10–0.35 V (inset: calibration curve of the anode current corresponding to the  
9 increase in  $E_{\text{pulse}}$ ) and (B) 25 CV cycles at the scan rate of  $50 \text{ mV s}^{-1}$  (inset: stable redox  
10 current).

11 **Figure 5.** (A) SEM image of *MRSA*. (B) CV cycles of SPEs, including (a) bare SPE, (b)  
12 SPE/AuNPs-15 nm, (c) SPE/AuNPs/NHS, (d) SPE/AuNPs/NHS/Ab, (e)  
13 SPE/AuNPs/NHS/Ab/BSA, and (f) SPE/AuNPs/NHS/Ab/*MRSA*.

14 **Figure 6.** (A) Nyquist plots of 15 nm AuNP-modified SPEs before and after  
15 functionalization; and (B) for the detection of *MRSA* at different concentrations (inset:  
16 equivalent circuit); (C) Calibration curve of  $\Delta R_{\text{ct}}$  vs different *MRSA* concentrations ( $n=5$ ); and  
17 (D) Nyquist plots for the control test with *E. coli* O157:H7.

18

## 19 Caption for Tables

20 **Table 1.** Performance comparison of different type of modified SPEs for bacterial detection

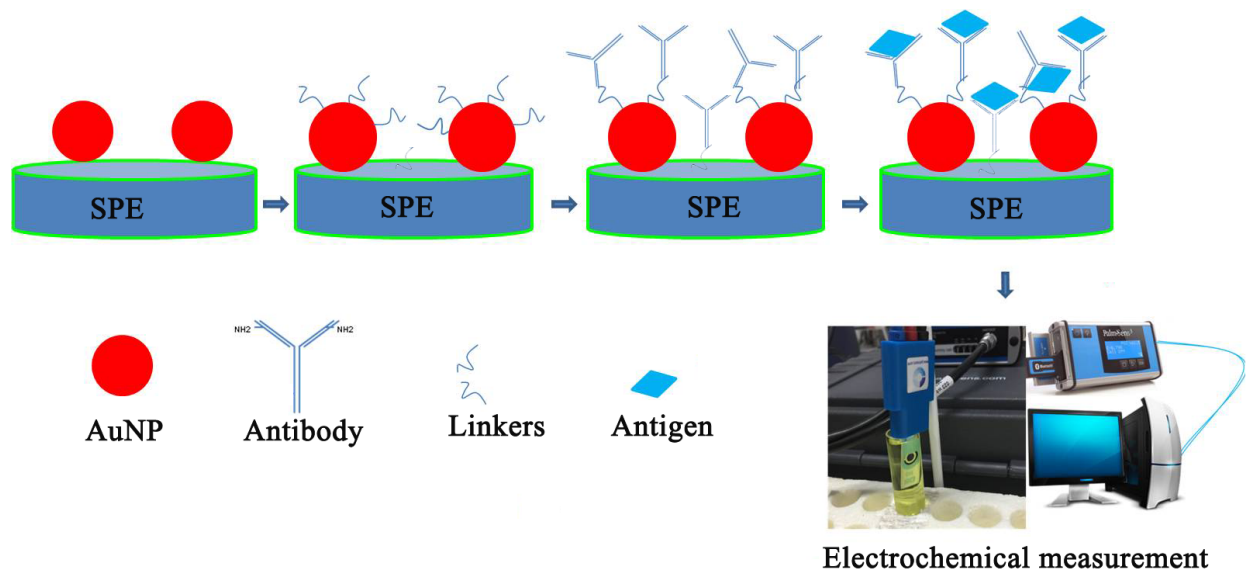
Table 1.

Type of modified SPEs	Pathogenic bacteria	Remarks	Ref.
Carbon SPEs	<i>Staphylococcus aureus</i>	- Detection from a starting concentration of $1.8 \times 10^6$ CFU/ml within 30 min	[13]
13 nm AuNPs-modified SPEs	<i>Escherichia coli</i> O157:H7	-Detection in the range of $10^2$ to $10^7$ CFU/ml; -LODs were approximately 6 CFU/strip in PBS buffer and 50CFU/strip in milk	[48]
Bi-functional glucose oxidase-polydopamine nanocomposites and Prussian blue modified screen-printed interdigitated electrodes	<i>Escherichia coli</i> O157:H7	-Detection in the range from $10^2$ to $10^6$ CFU/ml in the pure culture within 1 h; -LoD was $10^2$ CFU/ml.	[23]
Carbon SPEs	<i>Bacillus cereus</i> (as a surrogate for <i>B. anthracis</i> ) and <i>Escherichia coli</i> O157:H7	-The use of magnetic/polyaniline core/shell nano-particle (c/sNP) for sample extraction; - Detection in the range of 1 to $10^2$ CFU/ml, with LoDs were 40 CFU/ml and 6 CFU/ml, respectively	[22]
Gold screen-printed interdigitated microelectrodes	<i>Escherichia coli</i> O157:H7 and <i>Salmonella Typhimurium</i>	- The use of magnetic beads (MBs) for sample separation -Detection in the range of $10^2$ - $10^6$ CFU/ml in the pure culture samples - LODs were $2.05 \times 10^3$ CFU/g ( <i>E.coli</i> ) in ground beef, and $1.04 \times 10^3$ CFU/ml ( <i>Salmonella</i> ) in chicken rinse water	[46]

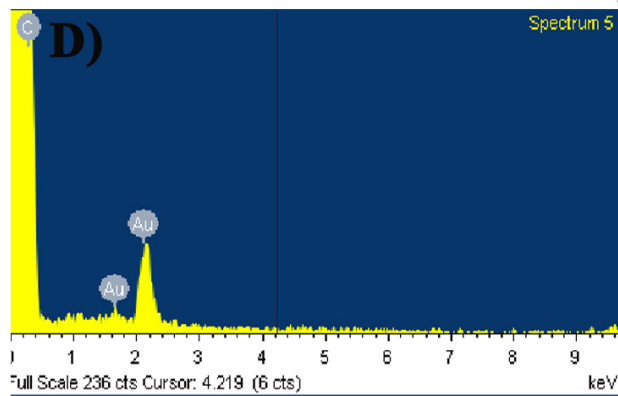
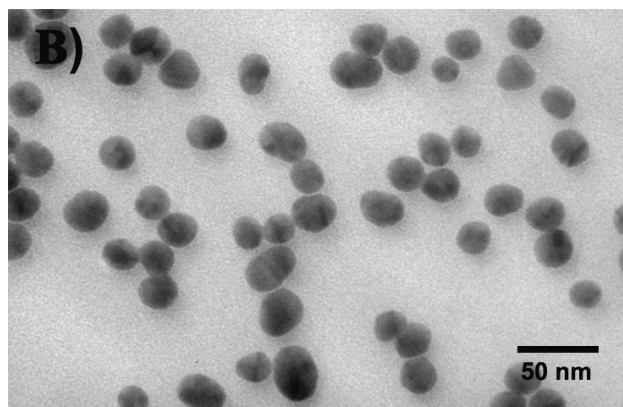
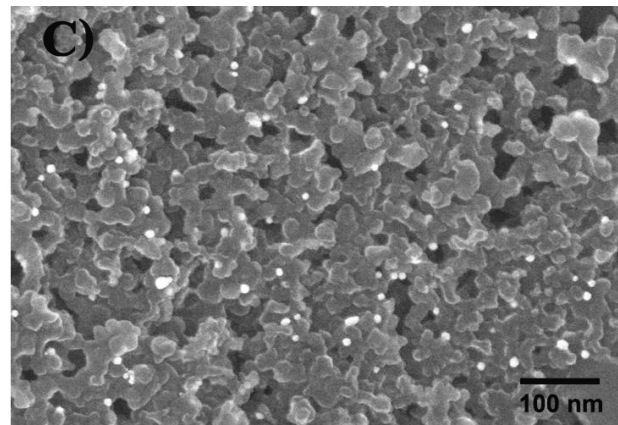
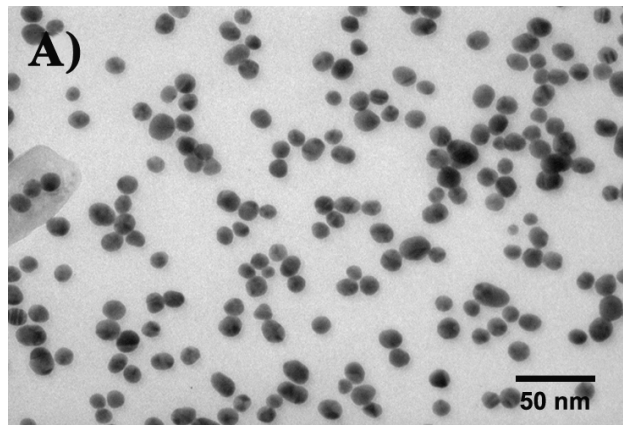
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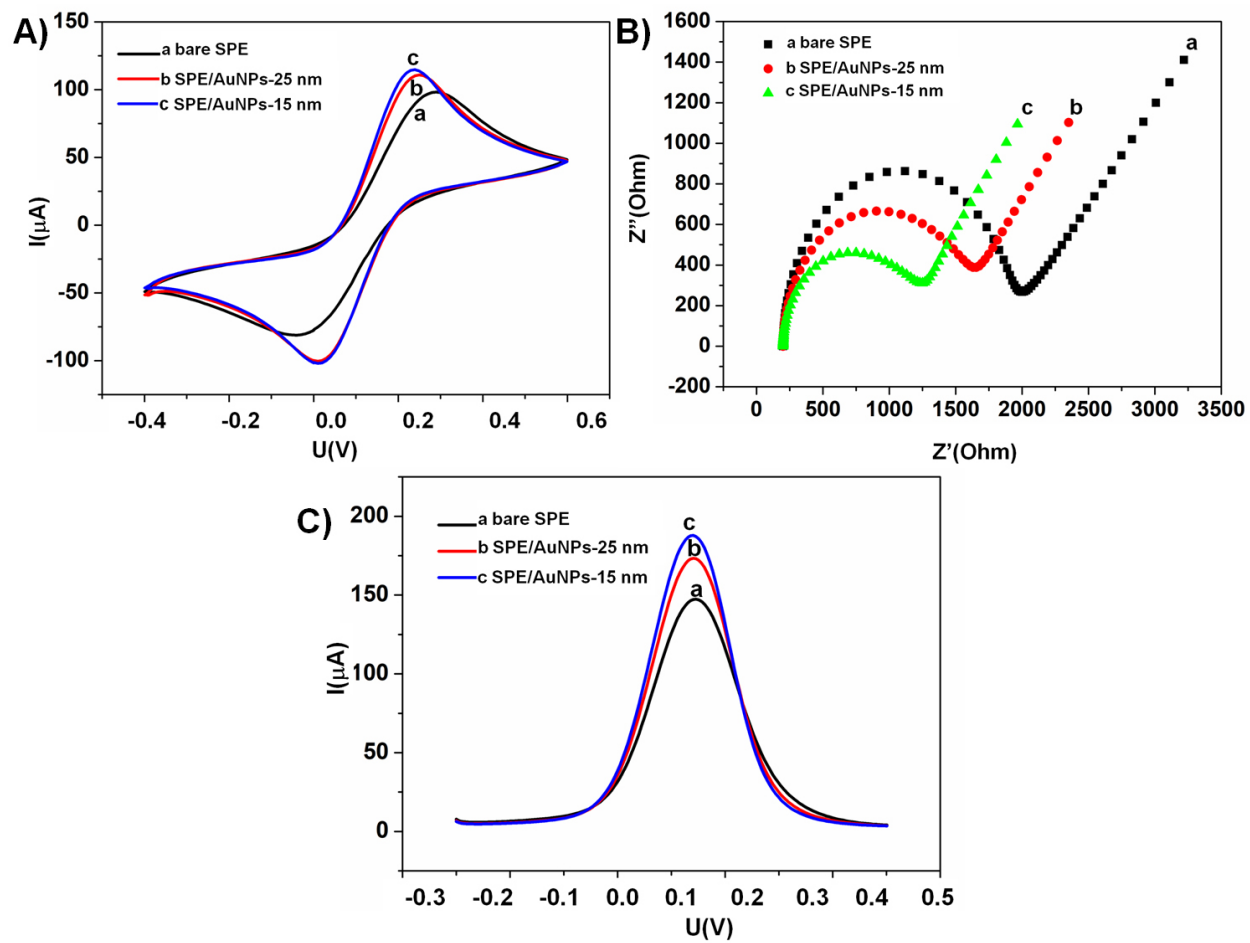
Screen-printed graphene electrodes	<i>Vibrio parahaemolyticus</i>	- Combining with loop-mediated isothermal amplification (LAMP) - LoD was 0.3 CFU per 25 g of raw seafood within 45 min	[47]
15 nm AuNPs –modified SPEs	Methicillin-resistant <i>Staphylococcus aureus</i>	-Detection the range of $10-10^6$ CFU/ml after 30 min of incubation -LoD was about 13 CFU/ml	<b>This study</b>

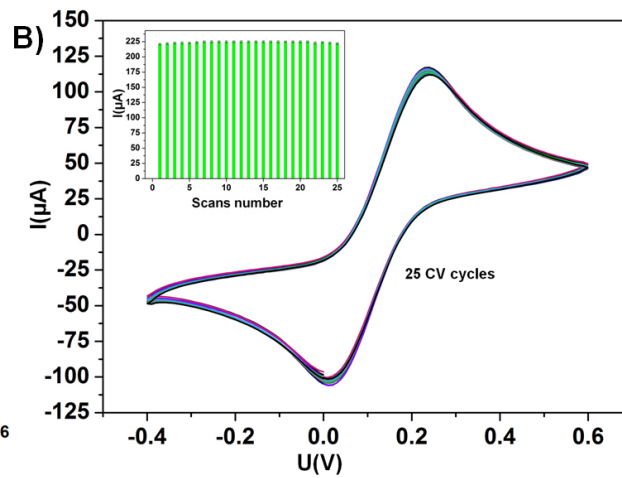
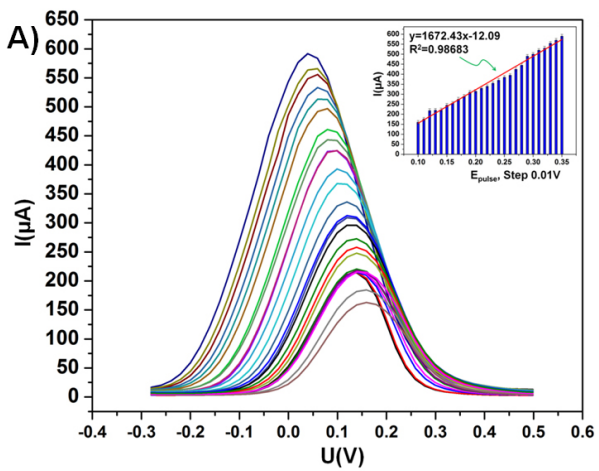
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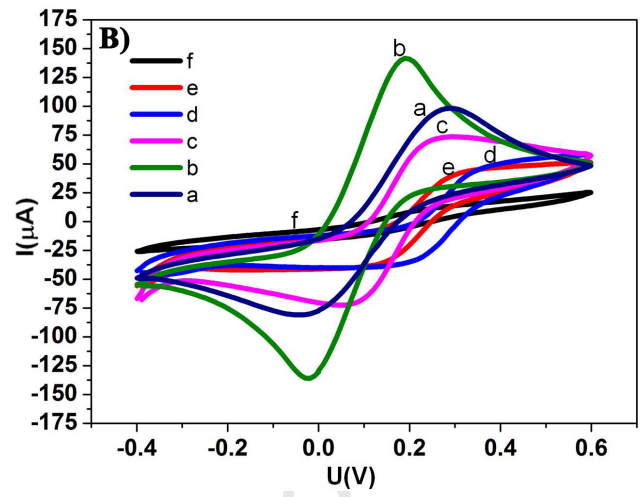
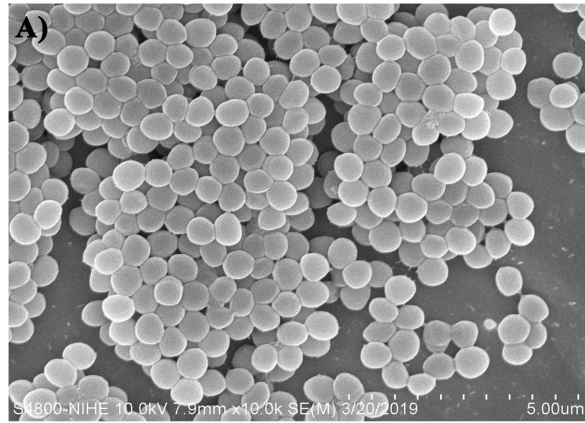


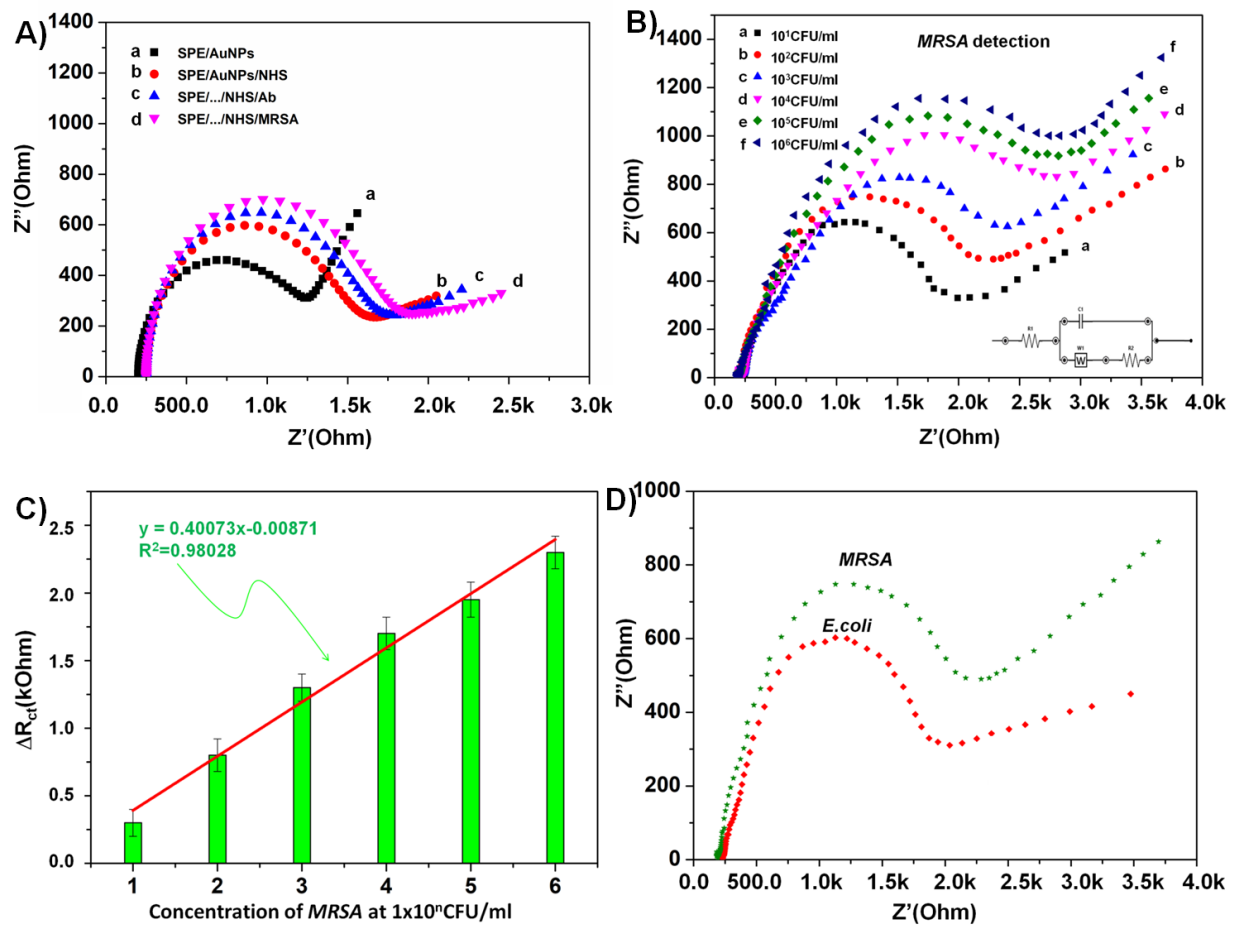












- The carbon SPEs are modified with AuNPs of different sizes to investigate their electrochemical stability.
- The electrochemical behaviour of modified SPEs strongly depends on the size of AuNPs and keep stable after 25 CV cycles.
- The AuNPs-modified carbon SPEs can detect Methicillin-resistant *Staphylococcus aureus* with the LoD of 13 CFU/ml.