A novel electrochemical immunosensor based on Fe₃O₄@graphene nanocomposite modified glassy carbon electrode for rapid detection of *Salmonella* in milk

Kaiwen Feng, Ting Li, Cuizhu Ye, Xiaoyu Gao, Xianglin Yue, Shuangyan Ding, Qiuling Dong, Mingqi Yang, Ganhui Huang, and Jinsheng Zhang*

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, P. R. China

ABSTRACT

Foods contaminated by foodborne pathogens have always been a great threat to human life. Herein, we constructed an electrochemical immunosensor for *Salmonella* detection by using a Fe₃O₄@graphene modified electrode. Because of the excellent electrical conductivity and mechanical stability of graphene and the large specific surface area of Fe₃O₄, the Fe₃O₄@graphene nanocomposite exhibits an excellent electrical signal, which greatly increased the sensitivity of the immunosensor. Gold nanoparticles were deposited on Fe₃O₄@graphene nanocomposite by electrochemical technology for the immobilization of the antibody. Cyclic voltammetry was selected to electrochemically characterize the construction process of immunosensors. The microstructure and morphology of related nanocomposites were analyzed by scanning electron microscopy. Under optimized experimental conditions, a good linear relationship was achieved in the *Salmonella* concentration range of 2.4×10^2 to 2.4×10^7 cfu/mL, and the limit of detection of the immunosensor was 2.4×10^2 cfu/mL. Additionally, the constructed immunosensor exhibited acceptable selectivity, reproducibility, and stability and provides a new reference for detecting pathogenic bacteria in milk.

Key words: *Salmonella*, milk, immunosensor, Fe₃O₄@graphene nanocomposite, gold nanoparticles

INTRODUCTION

Numerous deaths are caused by accidental consumption of food contaminated by foodborne pathogens such as *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7 (Dong et al., 2013; Man et al., 2021).

According to official World Health Organization data, about 325,000 hospitalizations and 5,000 deaths are caused by *Salmonella* every year (Zhu et al., 2014). At present, more than 2,500 serotypes of *Salmonella* have been identified (Gast, 2007). Among them, *Salmonella* Anatum is worldwide in distribution and is also a common cause of food poisoning in humans and animals (Hartmann and West, 1995; Hassan et al., 2017). Normally, after eating *Salmonella* contaminated food, humans, especially children, may experience symptoms such as vomiting, headache, and diarrhea (Ni et al., 2018). With the continuous expansion of the global population, the demand for high-nutrient foods such as milk has also increased rapidly (Yang et al., 2021). However, raw milk is easily contaminated by *Salmonella* (Marathe et al., 2012). Therefore, to ensure human health, it is very important to develop a fast and efficient method for detecting *Salmonella* in milk.

At present, the methods used in the detection of foodborne pathogens are mainly divided into 2 categories; one is traditional culture plating, and the other is instrumental analysis technology (Ye et al., 2019). Although traditional methods are reliable, it usually takes 4 to 7 d to get the test result (Abdulai et al., 2014; Yu et al., 2017). Instrument analysis technology mainly includes surface plasmon resonance (SPR) biosensors (Chen et al., 2017), PCR (Mondal et al., 2018), and electrochemical sensors (Adkins et al., 2017; Soares et al., 2020; Huang et al., 2021). The SPR biosensors detect quickly but their application is limited by poor sensitivity (Wang et al., 2015). The PCR method has great advantages in rapidity, selectivity, and sensitivity, but the complicated nucleic acid extraction operation and expensive equipment limit its further application (Zhang et al., 2016). In this regard, electrochemical sensors are attracting increasing attention due to the advantages of fast response, good sensitivity, easy operation, and lower experimental costs (Güner et al., 2017; Sun et al., 2019). However, weak current signal cannot meet the requirements of electrochemical sensors for

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*Corresponding author: zhangjinsheng@ncu.edu.cn

low detection limits (Zhang et al., 2018a). Therefore, amplifying the electrical signal of the sensor is a key issue to improve the detection performance (Wang et al., 2017).

Research in recent decades has shown that the application of carbon materials, polymer materials, nano-hybrid materials, and metal nanoparticles has greatly improved the performance of electrochemical sensors (Kerman et al., 2008; Gao et al., 2019b). Among them, ferroferric oxide (Fe_3O_4) formed by Fe^{3+} and Fe^{2+} valence metals is widely used in the field of electrochemical sensors due to its unique advantages such as easy manufacturing, low cost, large specific surface area, and excellent biocompatibility (Gao et al., 2013; Li et al., 2013; Deshmukh et al., 2020). However, Fe_3O_4 nanoparticles have limitations such as weak electrical conductivity, poor mechanical stability, and weak electrocatalytic ability, resulting in undesirable linear range and low detection sensitivity (Madhuvilakku et al., 2017). On the other hand, in 2-dimensional materials, benefiting from its unique physical and chemical properties, graphene has a wide range of applications in the sensor field (Novoselov et al., 2004). Graphene is a new type of material with a 2-dimensional honeycomb structure composed of sp^2 -bonded carbon atoms. It has a series of properties that other materials cannot match, including large surface area, excellent electrical conductivity, and high mechanical strength (Zhang et al., 2015; Hampitak et al., 2020). Therefore, the combination of Fe_3O_4 and graphene can overcome the inherent limitations of metal oxides and provide a platform for the detection of *Salmonella*. As noble metal nanomaterials, gold (Au), palladium (Pd), silver (Ag), and so on are widely used in biosensors due to their excellent conductivity, biocompatibility, and catalytic activity (Solanki et al., 2011; Gao et al., 2019a). Existing studies have shown that gold nanoparticles (**AuNPs**) can form strong Au-NH_2 bonds with amino groups on antibody (**Ab**; Haji-Hashemi et al., 2018; Fan et al., 2019). In addition, antibody linked to AuNPs can maintain excellent biological activity (Mani et al., 2009). Chitosan (**CS**), a natural polysaccharide polymer, is extensively applied in the construction of electrochemical immunosensors due to its good biocompatibility and film-forming properties (Güner et al., 2017; Zeng et al., 2021). In addition, the presence of a large number of amino functional groups on the surface of CS can provide favorable factors for the immobilization of AuNPs (Li et al., 2021).

Therefore, this paper took *Salmonella* Anatum as the research model and a sensitive immunosensor was prepared to detect *Salmonella* by using electrodes modified with Fe_3O_4 @graphene nanocomposite. First, the Fe_3O_4

and graphene were mixed in the CS solution, and after ultrasound, a uniformly mixed solution was formed, which was adopted to modify glassy carbon electrode (**GCE**). Next, AuNPs were deposited onto Fe_3O_4 @graphene nanocomposite using an electrodeposition technology and then the monoclonal antibody was immobilized on AuNPs through the stable Au-NH_2 bond. Finally, *Salmonella* was captured by the immunosensor through the antigen-antibody reaction (Mitov et al., 2003; Pakkanen et al., 2015). Compared with bare GCE, the resulting Fe_3O_4 @graphene nanocomposite exhibit a larger current response and better electron transfer rate. The prepared immunosensor exhibited good selectivity, acceptable repeatability, and stability for *Salmonella* detection, and provided a good reference for detecting foodborne pathogens.

MATERIALS AND METHODS

Chemicals and Materials

Monoclonal antibody was obtained from Alpha Lifetech. The monoclonal antibody is specific to the following *Salmonella* species: *Salmonella* Typhi A, *Salmonella* Agona, *Salmonella typhimurium*, *Salmonella* Paratyphi B, *Salmonella* Thompson, *Salmonella* Blockley, *Salmonella* Kentucky (C3), *Salmonella enteritidis*, *Salmonella* Typhi, *Salmonella* Dublin, *Salmonella* Anatum, and *Salmonella arizonae*. Graphene was supplied by Tanfeng Graphene Technology Co. Ltd. Carboxylated Fe_3O_4 nanoparticles were supplied by Allrunnano Technology Co. Ltd. The $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$, BSA, and ethanol were supplied by Macklin Biochemical Co. Ltd. Chitosan was obtained from Sigma-Aldrich. The Al_2O_3 powder (300 and 50 nm) was supplied by Xianren Instrument Co. Ltd. The PBS (10 mM, pH 7.4) was obtained from Suolaibao Technology Co. Ltd. Deionized water was used in this experiment. Pasteurized milks were supplied by a supermarket.

Apparatus

All electrochemical operations are carried out on an electrochemical workstation (CHI660E). The classic 3-electrode system was adopted, with 3 mm GCE as the working electrode, a saturated calomel electrode as a reference, and the platinum electrode as an auxiliary electrode. The particle size of Fe_3O_4 was obtained by the Zetasizer Nano ZS90 (Malvern). The microstructure and elemental composition of related materials are obtained by using electron microscopy (JSM-6701F, JEOL Ltd.).

Bacteria and Culture Methods

Listeria monocytogenes (ATCC 19115), *E. coli* O157:H7 (CMCC 44102), *Salmonella* Anatum (ATCC 9270), *Staphylococcus aureus* (CMCC 2603), *Pseudomonas aeruginosa* (CMCC 11997), *Shigella sonnei* (Jiangxi CDC isolates), and *Proteus vulgaris* (CMCC 49027) was used in this experiment. These strains were cultured in a shaker containing Luria-Bertani liquid medium at 37°C overnight before use. The cultured bacteria were diluted into 10-fold gradient concentration suspension using 0.01 M PBS. After 18 h of incubation at 37°C, the number of bacteria was obtained by the plate counting

method. After the experiment, all of the strains were put into an autoclave at 121°C and inactivated for 30 min.

Preparation of Fe_3O_4 @graphene Nanocomposite

Ten milligrams of Fe_3O_4 and 5 mg of graphene were added to 5 mL of CS solution (0.2%). After 90 min of ultrasonic treatment at 60 W, a uniformly dispersed Fe_3O_4 @graphene nanocomposite was obtained. The synthesis process of Fe_3O_4 @graphene was depicted in Figure 1A. Before use, the dispersed nanocomposite was stored in a refrigerator at 4°C.

Figure 1. Schematic illustration for (A) synthesis of the Fe_3O_4 @graphene nanocomposite and (B) the construction process of the electrochemical immunosensor. DPV = differential pulse voltammetry. I = current (μA); E = potential (V).

Fabrication of the Immunosensor

Initially, GCE was polished to a smooth surface with Al_2O_3 powder (300 and 50 nm) in sequence, then sonicate at 60 W for 180 s in ethanol and ultra-pure water, consecutively. Finally, the GCE was completely rinsed using deionized water and dried at 37°C . Then $5\ \mu\text{L}$ of Fe_3O_4 @graphene composite was drawn and dropped on the GCE and dried at a constant temperature of 37°C . Next, the obtained electrode was sunk in $0.01\ \text{M}$ HAuCl_4 solution and AuNPs were deposited on the electrode through a constant potential for 30 s ($-0.2\ \text{V}$). After thorough washing with deionized water, $10\ \mu\text{L}$ of $25\ \mu\text{g/mL}$ Ab was incubated on AuNPs/ Fe_3O_4 @graphene/GCE for 90 min at 37°C . Afterward, to eliminate nonspecific absorption, 1% BSA (wt/wt; $10\ \mu\text{L}$) was pipetted onto the modified electrode (Ab/AuNPs/ Fe_3O_4 @graphene/GCE) and incubated for 120 min at 37°C . After carefully rinsing the electrode 3 times with PBS, the obtained electrode was marked as BSA/Ab/AuNPs/ Fe_3O_4 @graphene/GCE. Then, $10\ \mu\text{L}$ of different gradient concentrations of *Salmonella* was added to the obtained electrode and incubated in an incubator for 40 min at 37°C . Finally, the electrode was thoroughly washed of unbound *Salmonella* with PBS. The detailed construction steps of the sensor are depicted in Figure 1B.

Electrochemical Procedures

All electrochemical measurements were tested in PBS containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ($5\ \text{mM}$) as probe. Dif-

ferential pulse voltammetry (DPV) measurement was studied in a potential of 0.2 to $0.6\ \text{V}$ and amplitude of $50\ \text{mV}$. Cyclic voltammetry analysis was taken at potential from -0.2 to $0.6\ \text{V}$ at $100\ \text{mV/s}$ to characterize the whole process of electrochemical immunosensor construction. ΔI is the difference in DPV peak current, calculated using the following equation:

$$\Delta I = I_0 - I_1,$$

where I_0 is the DPV peak current response of immunosensor before incubation with the corresponding concentration and I_1 is the DPV peak current response after incubation with the corresponding concentration.

Pretreatment of Samples

First, $0.01\ \text{M}$ PBS was used to dilute the sterilized milk to 1:10. Then the diluted milk was sonicated for 30 min at 60 W. After centrifugation at $6,000 \times g$ at room temperature for 10 min using an ultrafiltration centrifuge tube (Millipore $0.5\ \text{mL}/50\ \text{kDa}$), the supernatant in the centrifuge tube was obtained. Finally, the processed milk was spiked with *Salmonella*.

RESULTS AND DISCUSSION

Characterization of Nanocomposite

The microstructure and morphology of Fe_3O_4 , graphene, Fe_3O_4 @graphene, and AuNPs/ Fe_3O_4 @graphene were observed by scanning electron microscopy. As

Figure 2. Scanning electron microscopy of Fe_3O_4 (A); hydrodynamic diameter of Fe_3O_4 (B); close-up of the surface of Fe_3O_4 (C); scanning electron microscopy images of graphene (D), Fe_3O_4 @graphene (E), and gold nanoparticles (AuNPs)/ Fe_3O_4 @graphene (F); and energy dispersive spectroscopy of Fe_3O_4 @graphene (E) and AuNPs/ Fe_3O_4 @graphene (F).

Figure 3. Cyclic voltammetry curves (A) of the bare glassy carbon electrode (GCE; a), Fe₃O₄@graphene/GCE (b), AuNPs/Fe₃O₄@graphene/GCE (c); cyclic voltammetry curves (B) of Ab/AuNPs/Fe₃O₄@graphene/GCE (d), BSA/Ab/AuNPs/Fe₃O₄@graphene/GCE (e), and *Salmonella*/BSA/Ab/AuNPs/Fe₃O₄@graphene/GCE (f). AuNPs = gold nanoparticles; Ab = antibody.

shown in Figure 2a and b, the shape of Fe₃O₄ was an approximate sphere with a particle size of about 180 nm. Further magnification showed that a very rough and rugged surface can be clearly observed (Figure 2c). Benefiting from this structure and shape, the surface area of Fe₃O₄ was greatly increased, and it provides abundant sites for the subsequent deposition of AuNPs, which plays a unique role in reducing the detection limit of the sensor (Gao et al., 2013). Graphene exhibited a typical thin fold-like sheet structure (Figure 2d), which provided many useful binding sites for the loading of

Fe₃O₄ (Jia et al., 2018). The morphology of Fe₃O₄@graphene was exhibited in Figure 2e. As shown in the picture, Fe₃O₄ was evenly distributed on the graphene, which implies a good mixing state of graphene and Fe₃O₄. After AuNPs were deposited on the electrode modified by Fe₃O₄@graphene, we can see directly that the AuNPs are arranged on the Fe₃O₄@graphene in an orderly manner (Figure 2f). To investigate the element composition, energy dispersive spectroscopy (EDS) was selected to conduct further analyze the elemental composition of related materials. The EDS images of

Figure 4. (A) Cyclic voltammetry analysis of the constructed sensor at different scan rates: 10, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 350 mV s⁻¹. (B) Linear relationship between the scan rates and redox peak currents. Potential vs. SCE means relative to the potential of saturated calomel electrode (SCE). V^{1/2} = square root of scan rate.

Fe_3O_4 @graphene was carried out on a silicon wafer and images of AuNPs/ Fe_3O_4 @graphene were carried out with GCE. The EDS images of the analyzed area in Figure 2e mainly contained elements such as C, O, and Fe, which illustrated the successful modification of Fe_3O_4 @graphene. Similarly, EDS of the analyzed area in Figure 2f confirmed that in addition to the existence of C, O, and Fe, the Au element also appears. This result also clearly showed that AuNPs were successfully electrodeposited on the Fe_3O_4 @graphene.

Electrochemical Characterization of Electrode Modification

The electrochemical behaviors of bare GCE, Fe_3O_4 @graphene, and AuNPs/ Fe_3O_4 @graphene were measured by cyclic voltammetry. Figure 3 was the cyclic voltam-

metry response of different electrodes in PBS buffer containing $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (5 mM). In Figure 3a, curve a is the cyclic voltammetry response of the bare GCE. The redox peak formed by oxidation reaction and reduction reaction of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in the electrolyte solution can be clearly seen (Sun et al., 2019). As expected, the cyclic voltammetry response current of the electrode modified by the Fe_3O_4 @graphene significantly increased compared with bare GCE (curve b). This can be attributed to the good electrical conductivity and mechanical stability of graphene and the large specific surface area of Fe_3O_4 . When AuNPs were modified on the Fe_3O_4 @graphene, the cyclic voltammetry response current was further increased, which benefits from the excellent electrical conductivity of AuNPs (curve c).

The construction steps of the immunosensor were further tested by cyclic voltammetry. As depicted in

Figure 5. Effects of (A) deposition time of gold nanoparticles (AuNPs), (B) incubation time of antibody, (C) incubation time of antigen, (D) incubation temperature of antigen. Error bar = SD ($n = 3$). ΔI = the difference of differential pulse voltammetry peak current.

Figure 6. (A) Differential pulse voltammetry responses of the immunosensor after incubation with different concentrations of *Salmonella* in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (pH = 7.4). (a) 0, (b) 2.4×10^2 , (c) 2.4×10^3 , (d) 2.4×10^4 , (e) 2.4×10^5 , (f) 2.4×10^6 , and (g) 2.4×10^7 cfu/mL. (B) The logarithmic calibration (lgC) plot for *Salmonella* measurements in the range of 2.4×10^2 to 2.4×10^7 cfu/mL. Error bar = SD (n = 3). ΔI = the difference of differential pulse voltammetry peak current. SCE = saturated calomel electrode.

Figure 3b, after Ab was immobilized on AuNPs/ Fe_3O_4 @graphene/GCE, the cyclic voltammetry response current was significantly reduced, which was attributed to the protein hindering the electron transfer on the electrode surface (curve d). Similarly, after the BSA was attached to the electrode surface, the cyclic voltammetry response current further drops (curve e). Finally, when

the modified electrode was incubated with *Salmonella*, the cyclic voltammetry response current dropped again owing to Ab-*Salmonella* immunocomplexes formed on the electrode (curve f), which serves as a mass transfer barrier to limit the transfer of electrons to the GCE surface (Huang et al., 2010). These results indicated that every step of the construction of the sensor is successful and the sensor can be used to detect *Salmonella*.

To thoroughly analyze the recognition mechanism of the sensor, it is critical to investigate the relationship between the response current of the cyclic voltammetry curve and scan rates. As shown in Figure 4a, as the scan rate continues to increase, the cyclic voltammetry response current also increases accordingly. Figure 4b exhibited the oxidation peak and reduction peak currents were proportional to the square root of the scan rate ($v^{1/2}$) with regression equation of $I_{pa} (\mu\text{A}) = 13.54v^{1/2} + 13.63$ ($R^2 = 0.998$) and $I_{pc} (\mu\text{A}) = -12.98v^{1/2} - 17.26$ ($R^2 = 0.993$); I_{pa} = the anodic peak current and I_{pc} = the cathodic peak current. The above findings clearly indicated the redox reaction on electrode was a diffusion-controlled process.

Optimization of Experimental Parameters

To obtain the best analysis performance of the sensor, the several key experimental conditions, such as the electrodeposition time of AuNPs, Ab incubation time, the incubation time of *Salmonella*, and the temperature of antigen-antibody reaction were optimized. The experimental results obtained were as follows.

Figure 7. Selectivity of the prepared immunosensor to 10^6 cfu/mL *Salmonella*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella sonnei*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7. ΔI = difference of differential pulse voltammetry peak current.

Electrodeposition Time of AuNPs

For electrodeposition time of AuNPs, in Figure 5a, ΔI continued to increase from 5 to 30 s. This is because large amounts of AuNPs were deposited on the Fe_3O_4 @graphene, which further makes a large number of antibodies immobilized on AuNPs. When the deposition time exceeds 30s, due to the steric hindrance, the effective binding sites of the antibody and the AuNPs were reduced, which leads to a decrease of ΔI . Consequently, 30 s was selected as the most suitable electrodeposition time.

Ab Incubation Time

The effect of Ab incubation time is shown in Figure 5b. Initially, ΔI increased rapidly with the increase of incubation time. When the incubation time was extended to 90 min, ΔI reached a peak. After the incubation time was further increased, there was no significant change in ΔI . Therefore, 90 min was adopted as the optimal incubation time.

Incubation Time of *Salmonella*

The incubation time of *Salmonella* is a nonnegligible factor for sensor analysis performance. Therefore, the influence of incubation time from 10 to 80 min was evaluated. In Figure 5c, before 60 min, ΔI kept increasing and reached the maximum at 60 min. As time increased to 80 min, ΔI slowly decreased. For better analysis performance of the immunosensor, 60 min was adopted in subsequent experiments.

Temperature of the Antigen-Antibody Reaction

The antigen-antibody reaction is greatly affected by temperature, so different reaction temperatures from 4 to 45°C were investigated. The result can be clearly observed in Figure 5d. When the temperature was less than 37°C, as the incubation temperature increased, ΔI also increased rapidly. However, too high temperature greatly reduced the activity of the antibody, which eventually led to a decrease in ΔI . Taken together, 37°C for 60 min was adopted for the following experiment.

Analytical Performance

Under optimized conditions, *Salmonella* in different concentrations was adopted for DPV measurement to verify the detection capability of the sensor. The relationship between ΔI and the concentration of *Salmonella* is depicted in Figure 6. As the concentration of *Salmonella* increases, the peak current gradually decreases (Figure 6a). The ΔI exhibited a linear correlation with the logarithm of the *Salmonella* concentration in the range of 2.4×10^2 to 2.4×10^7 cfu/mL (Figure 6b). The regression equation was $\Delta I (\mu\text{A}) = 6.499x - 11.038$ ($R^2 = 0.993$). The detection limit obtained by the immunosensor was 2.4×10^2 cfu/mL.

Selectivity, Stability, and Repeatability

To ensure the feasibility of the constructed immunosensor, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella sonnei*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *E. coli* O157:H7 were selected as nontarget bacteria to confirm the selectivity of the sensor. Under the same conditions, the prepared immunosensors were used to incubate ultra-pure water (black), *Salmonella*, and interfering bacteria. As shown in Figure 7, ΔI of *Salmonella* is much stronger than that of other interfering bacteria. This result implies that the constructed immunosensor has excellent selectivity.

Stability is achieved by placing the prepared immunosensor in the refrigerator (4°C). *Salmonella* detection experiments (10^6 cfu/mL) were carried out after storage for 1 and 2 wk, respectively. The response current retained 94.3% and 89.1% of the initial current, which suggest that the prepared immunosensor presented a acceptable stability.

Repeatability was also investigated by using 5 parallel electrodes to detect the same concentration of *Salmonella* (10^6 cfu/mL) under exactly the same conditions. The relative standard deviation was 2.37%, which shows good repeatability.

Real Sample Analysis

The constructed immunosensor was used to detect *Salmonella* in the milk sample to determine the actual detection ability of the immunosensor. Different con-

Table 1. Determination of *Salmonella* in real samples by using the prepared immunosensor (n = 3)

Sample	Plate count (cfu/mL)	Detected (cfu/mL)	Recovery (%)	RSD ¹ (%)
1	1.3×10^3	1.18×10^3	90.8	6.12
2	2.4×10^4	2.69×10^4	112.1	5.6
3	2.3×10^6	2.36×10^6	102.6	6.07

¹RSD = relative standard deviation.

Table 2. Comparison of detection performance between the prepared immunosensor and other methods

Methods	Detection time (h)	Linear range (cfu/mL)	LOD ¹ (cfu/mL)	Reference
Colorimetric immunoassay	5	3×10^3 to 3×10^6	3×10^3	Luo et al., 2014
ELISA	8	10^5 to 10^8	5.4×10^5	Feng et al., 2013
Photothermal effect of magnetic nanomaterials	1.5	300 to 10^3	300	Zhang et al., 2018b
NMR ² biosensor	1.5	10^3 to 10^7	10^3	Yang et al., 2021
Fluorescence biosensor	2	0 to 10^6	10^3	Kim et al., 2015
Impedance biosensor	3	1.9×10^3 to 1.9×10^6	1.9×10^3	Bhardwaj et al., 2017
Electrochemical immunosensor	1.5	2.4×10^2 to 2.4×10^7	2.4×10^2	This work

¹LOD = limit of detection.²NMR = nuclear magnetic resonance.

centrations of *Salmonella* were added to the pretreated milk with final bacterial concentration 1.2×10^3 , 2.4×10^4 , and 2.4×10^6 cfu/mL. Then, the above method was adopted to test milk samples. Each test sample has 3 parallel groups, and the plate technology method was used for comparative analysis. From Table 1, we can see the constructed immunosensor shows an acceptable result.

Comparison with Other Methods

At present, many detection methods for foodborne pathogens have been reported, but they often have certain limitations such as requiring complex experimental procedures and expensive equipment, and being time consuming. In this situation, electrochemical immunosensors have received extensive attention due to their easy operation, low cost, fast response, and sensitive detection (Yang et al., 2019). We compare the immunosensor we prepared with the methods reported in the literature (Feng et al., 2013; Luo et al., 2014; Kim et al., 2015; Bhardwaj et al., 2017; Zhang et al., 2018b; Yang et al., 2021). From Table 2, we can clearly see that the immunosensor we constructed has a faster detection time and better detection limit than other detection methods.

CONCLUSIONS

A sensitive immunosensor was successfully constructed based on Fe_3O_4 @graphene nanocomposite to detect *Salmonella*. Compared with bare GCE, the electrode modified by Fe_3O_4 @graphene nanocomposite exhibits better electrical conductivity. Additionally, the introduction of AuNPs can amplify current signals and serve as a carrier for immobilizing antibody. With the above advantages, the developed signal amplification method demonstrated excellent sensitivity, acceptable stability, and repeatability. Moreover, by replacing the corresponding antibodies, this method can be easily

used to detect other pathogenic bacteria. These advantages indicate that the constructed immunosensor has great application prospects in clinical diagnosis and food safety.

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ORCIDS

Ting Li <https://orcid.org/0000-0003-4118-8671>
 Cuizhu Ye <https://orcid.org/0000-0002-6425-1920>
 Ganhui Huang <https://orcid.org/0000-0003-0814-3754>
 Jinsheng Zhang <https://orcid.org/0000-0002-6616-0301>