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Enhanced sensitivity and efficiency of detection of *Staphylococcus aureus* based on modified magnetic nanoparticles by photometric systems

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ABSTRACT

Staphylococcus aureus is an important infectious factor in the food industry and hospital infections. Many methods are used for detecting bacteria but they are mostly time-consuming, poorly sensitive. In this study, a nano-biosensor based on iron nanoparticles (MNPs) was designed to detect *S. aureus*. MNPs were synthesized and conjugated to Biosensors. Then *S. aureus* was lysed and nano-biosensor (MNP-TiO₂-AP-SMCC-Biosensors) was added to the lysed bacteria. After bonding the bacterial genome to the nano-biosensor, MNPs were separated by a magnet. Bacterial DNA was released from the surface of nano-biosensor and researched by Nano-drop spectrophotometry. The results of SEM and DLS revealed that the size of MNPs was 20–25 nm which increased to 38–43 nm after modification and addition of biosensors. The designed nano-biosensor was highly sensitive and specific for the detection of *S. aureus*. The limit of detection (LOD) was determined as 230 CFU mL⁻¹. There was an acceptable linear correlation between bacterial concentration and absorption at 3.7×10^2 – 3.7×10^7 whose linear diagram and regression was $Y = 0.242X + 2.08$ and $R^2 = .996$. Further, in the presence of other bacteria as a negative control, it was absolutely specific. The sensitivity of the designed nano-biosensor was investigated and compared through PCR.

Abbreviations: MNPs: iron nanoparticles; LOD: limit of detection; SMCC: sulfosuccinimidyl 4-N-maleimide methyl cyclohexane-1-carboxylate; DTT: dithiothreitol; LB-broth: Luria broth; TEOS: Tetraethoxysilane; APTES: 3-aminopropyltriethoxysilane; NHS: sulfo-NHS acetate; MNP-TiO₂-AP-SMCC-Biosensors: Nano-biosensors; DLS: dynamic light scattering; MNP-TiO₂-AP-SMCC-biosensors: nano-biosensor; HRP: horseradish peroxidase enzyme

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Introduction

Detection of pathogen microorganisms is very important in medical, health, and biologic fields. *Staphylococcus aureus* is one of the most ubiquitous pathogens in public healthcare worldwide. Different methods have been developed for detection of microorganisms, such as PCR, ELISA, SPR, and latex [1–5]. PCR is one of the most sensitive methods for detection but it is able to detect only 10^3 – 10^7 CFU mL⁻¹ [3,6,7]. In ELISA method, an expert person could detect microorganisms with greater specificity compared to PCR method, but ELIASA is also limited to detecting 10^4 – 10^7 CFU mL⁻¹ of microorganisms [8–10]. Latex method is easier and quicker but less sensitive compared with ELISA method. SPR is a more accurate technique but needs an expert person to conduct the process and is more expensive. Therefore, offering a simple, sensitive, and specific method for micro-organism detection and isolation is an important challenge of today [11–14].

Over the past decades, nano-biotechnology has made great progress and opened new doors in different fields. Due to their physical and chemical properties, nanoparticles have created many opportunities in the development of different sciences. Iron nanoparticle (MNPs) is one of the important metal nanoparticles employed in different research fields [15–18]. A variety of methods are used for iron nanoparticle production, including co-precipitation, hydrothermal sol-gel, micro-emulsion and some other methods with polymer utilized in the process of production. Co-precipitation is one of the commonest methods with sodium hydroxide and ammonia being the main components used in the production process [19–22]. The size of MNPs is dependent on the following factors: the proportion of iron salt, type of salt and alkaline substance, mixing rate, pH, presence of nitrogen, and the temperature. MNPs are rapidly oxidized in the presence of oxygen and water causing precipitation and reduction in magnetic properties of

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nanoparticles [23–25]. An effective technique to prevent this is creating a layer of particles such as silica, amine, carboxyl, thiol, gold, and silver on them and forming a core-layer structure [26–29]. This process is called capsulation and creates a core-shell structure that is resistant to the presence of oxygen and water. In this case, MNPs have a higher magnetic strength than their oxide, which can be used as a superparamagnetic nanoparticle. Therefore, the formation of a coating on these nanoparticles could be preventing of perception and oxidation of MNPs [30–32].

Modified magnetic nanoparticles have many usages in biology and medical fields, such as DNA extraction, purification, drug delivery, hyperthermal therapy, magnetic resonance imaging, enzyme activity assay, and separation of target cells. The amine form nanoparticles could be used for isolation and extraction of proteins and pathogenic bacteria by creating electrostatic, hydrogen and covalent bound [32–34]. Also, magnetic nanoparticles modified with small biomolecules such as cytosine, mannose, antibody, Aptamers, DNA biosensors, and antibiotics are used for targeting and extracting bacteria, fungi, and viruses. The efficiency and specificity of the nanoparticles can be enhanced through the design and selection of appropriate functional groups. In this study, in order to boost the sensitivity and efficiency of DNA separation, MNPs' surface was modified by an amine, SMCC and biosensor groups. Also, for elevating the specificity of detection of *S. aureus* target DNA, the MNPs were conjugated with DNA biosensors. The sensitivity of the nano-biosensor designed was investigated and compared through PCR and Nano-drop spectrophotometry.

Materials and methods

Materials

The standard bacteria of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 as positive controls were prepared from Pasteur Institute, Tehran, Iran. The *Escherichia coli* ATCC 25,922 *Pseudomonas aeruginosa* ATCC 27853, *Shigella dysenteriae* PTCC 1188, *Klebsiella pneumoniae* ATCC7881 and *Staphylococcus epidermidis* ATCC 12228 as negative controls were prepared from Musavi Hospital, Zanjan, Iran. All oligonucleotides were purchased from Bioneer Co. (Daejeon, South Korea). dithiothreitol (DTT), ethyl acetate; NaCl, Luria broth (LB-broth), tetraethoxysilane (TEOS) and 3-aminopropyltriethoxysilane (APTES), ferric chloride hexahydrated ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) were prepared from Merck Co. Germany. Sulfosuccinimidyl 4-N-maleimidomethyl cyclohexane-1-carboxylate (SMCC) and sulfo-NHS acetate were prepared from Thermo Co. (Waltham, MA).

Designing primers and biosensors

For the detection of *S. aureus*, primers and biosensors were designed for protein A as a target DNA. Gene bank database was used for defining the protein A DNA sequence and a small sequence of protein A was selected as a target DNA. Oligo software (Sina gen, Co, Iran) and DNASIS

Molecular Biology Insights, Inc., Cascade, CO) were used for designing forward, reverse primers, and biosensors. Primers and biosensors were synthesized by Bioneer Co. Korea. The primers and the biosensors sequence are presented in Table 1.

MNPs synthesis

MNPs were prepared by alkaline co-precipitation of ferric chloride hexahydrates ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and ferrous chloride tetrahydrated ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) in an aqueous solution. Briefly, 1.6 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.8 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ were mixed on a shaker at 80 °C. Ethanol and propanol (4 mL) were added to the mixture reaction. The reaction continued at 80 °C for 30–45 min. When the yellow colour of the solution changed to brown, hydroxide ammonia (NH_3OH , 25%, 15 mL) was added to the reaction mixture through dripping to regulate the pH on 8–9 [3,19,21]. When the formed black precipitated, the mixture reaction was shaken at 80 °C for 2–3 h, and then washed three times (Figure 1(A)).

MNP-TiO₂ synthesis

The MNPs prepared in Section “MNPs synthesis” were encapsulated by silica nanoparticles. Briefly, 2.5 g MNPs was added to 15 mL hydroxide ammonia (25%) dripping to regulate pH on 9–10. Then, 1.0 mL TEOS solution (98%) was added to the mixture reaction and shaken at 80 °C with 700 rpm for 1–2 h. The TEOS was bound to the surface of MNPs. The final production was MNPs-TiO₂ which was isolated from the reaction mixture with a magnet and washed with distilled water three times (Figure 1(A)) [3,19,21].

MNP-TiO₂-AP synthesis

The MNP-TiO₂ synthesized in Section “MNP-TiO₂ synthesis” was modified with APTES. For this purpose, hydroxide ammonia (25%, 15 mL) was added to the reaction mixture to regulate pH at 9–10. The APTES (0.5 mL, V/V 98%) was solved in ethanol (5 mL), after which the solution was added to the reaction mixture at 80 °C with 700 rpm for 3 h. Finally, MNP-TiO₂-AP was isolated with a magnet and washed with distilled water three times (Figure 1(A)) [3,19,21].

MNP-TiO₂-AP-SMCC synthesis

The MNP-TiO₂-AP synthesized in Section “MNP-TiO₂-AP synthesis” was mixed with 300 µg of SMCC at 5 mL of coupling buffer (0.1 M PBS, 0.2 M NaCl, pH 7.2) and mixed with 700 rpm at room temperature for 3–5 h. Afterwards, MNP-TiO₂-AP conjugated with SMCC was isolated with a magnet. The MNP-TiO₂-AP-SMCC was washed with coupling buffer three times. These nanoparticles could bind to the thiol agents of biosensor, enzymes, and proteins by a covalent band (Figure 1(A)) [3,19,21].

Table 1. Strand DNA and primers sequence.

Biosensors and primers	Sequence	T_m (°C)	CG (%)	Length (bp)
Primer forward	5'-CGAATCTCAAGCACCGAAAG-3'	57.2	50	20
Primer reverse	5'-CAGGCTTGTGTGCTTCC-3'	57.22	50	20
Strand DNA1	5'-(SH)GCCTAAGTTGAACGAAGAAC-3'	54.67	45	20
Strand DNA2	5'-CAGGCTTGTGTGCTTCC(SH)-3'	55	50	20

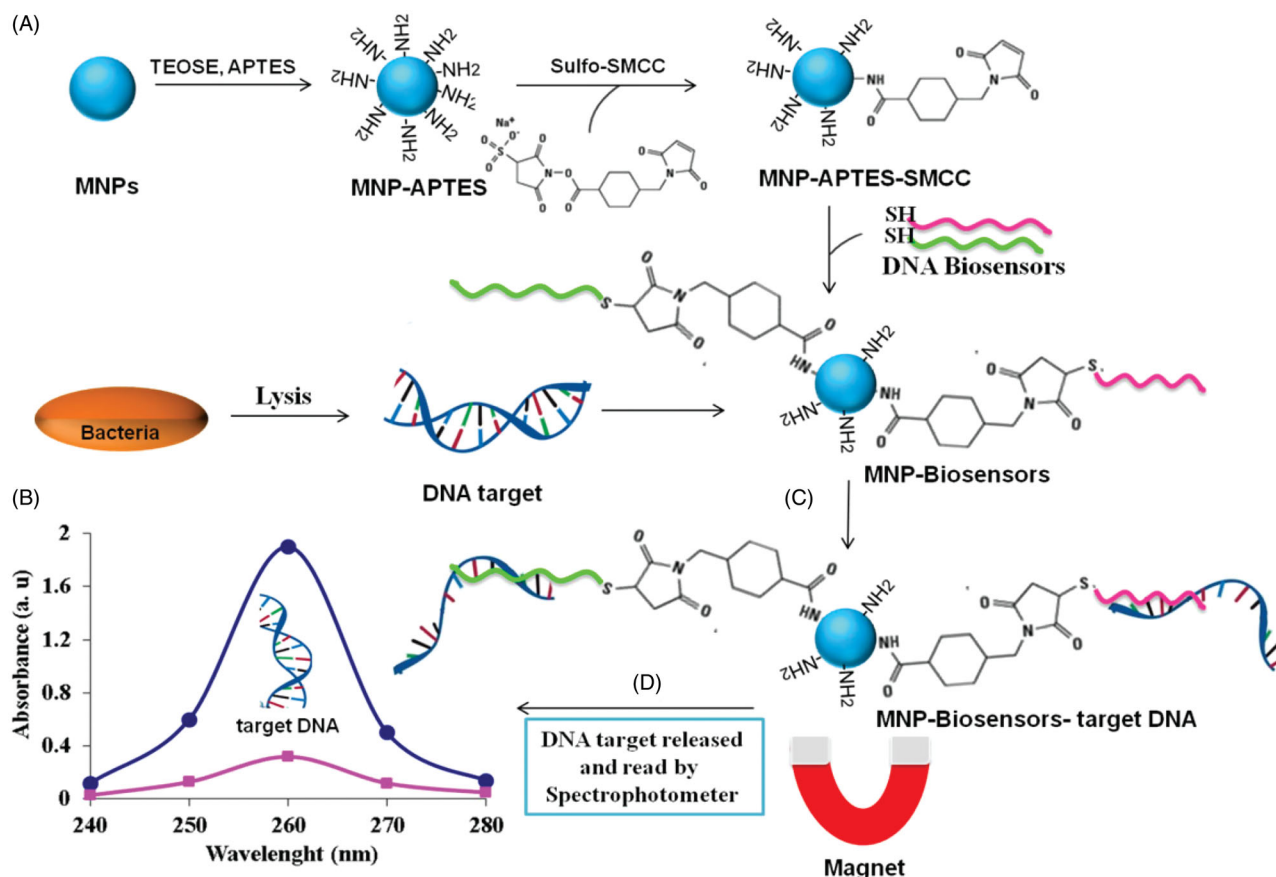


Figure 1. Schematic view of the designed nano-biosensor for detection of *S. aureus*, (A) biosynthesis and conjugation of nanoparticles, (B) preparation of bacteria target DNA, (C) conjugation of target DNA with nano-biosensor, and (D) release of bacteria target DNA of nano-biosensor and the concentration determined by Nanodrop spectrophotometry.

Functionalization of biosensor

Due to the presence of the thiol group on the biosensors, they form disulphide bonds with each other, reducing the efficiency of conjugation. Therefore, the disulphide bonds between biosensors must be broken [3,19,21]. For this purpose, $15 \mu\text{g mL}^{-1}$ of each biosensor was added to $50 \mu\text{L}$ dithiothreitol (DTT, 1 N) for 1 h at room temperature. Before that, conjugated biosensors must be removed from DTT of the environment because of inhibiting conjugation between biosensors and nanoparticles. Accordingly, $100 \mu\text{L}$ ethyl acetate was added to the reaction mixture to DTT dissolved in the organic phase and removed from the reaction mixture. Then, the reaction mixture was centrifuged at 14,000 rpm for 15 min, whereby the organic phase was isolated from the watery phase. This process was repeated three times to remove all DTT from the reaction mixture. Immediately, the biosensors in the watery phase must have been used as disulphide bonds could form again [3,19,21].

Functionalization of magnetic nanoparticles with biosensors

For the conjugation of biosensors, the activated biosensors ($15 \mu\text{g mL}^{-1}$) were added to 1 mL of MNP-TiO₂-AP-SMCC complex in the coupling buffer (0.1 M PBS, 0.2 M NaCl, pH 7.2) at 700 rpm, at room temperature, for 8–12 h. The product was isolated with a magnet and washed three times with the coupling buffer. The group agents of nanoparticles that were not conjugated with biosensors were deactivated with $10 \mu\text{g}$ of sulfo-NHS-acetate. The sulfo-NHS-acetate stopped the polymerization of nanoparticles via these group agents. To this end, sulfo NHS-acetate (10 mM, 5 mL) was added to the MNP-TiO₂-AP-SMCC-biosensors complex at room temperature, for 5 h. Then, the complex was isolated from the reaction mixture by a magnet and washed three times with passivation buffer (0.2 M Tris, pH 8.5). Finally, the MNP-TiO₂-AP-SMCC-biosensors complex was stored in a storage buffer (100 mM PBS, 0.2 M NaCl, pH 7.4) [3,19,21]. The efficiency of

conjugation was measured and researched by Nanodrop spectrophotometry (Figure 1(A)).

$$E.C = (C_T - C_S)/C_T \times 100$$

Where, E.C: Percentage of conjugation of biosensor, C_T : Primary concentration of the biosensors, C_S : Concentration of the biosensors at supernatant.

Detection of target DNA

Staphylococcus aureus was cultivated in LB-Broth, for 18 h. specifically; 1 mL of the culture media was transferred to a micro tub and centrifuged at 5000 g for 10 min. Then, the supernatant was isolated from precipitation. Protease K (10 μ L), lysozyme (10 μ L), and 200 μ L lyse solution (30 mM Tris-HCl, pH 7.0, 1 M NaCl, 4.5 M GuSCN, 20 mM EDTA, 1.5% Triton X-100) were added to the bacterial precipitation. The mixture was pipetted a few times and incubated at 55 °C for 30 min. Next, 30 mg of MNP-TiO₂-AP-SMCC-biosensors complex was added to 300 μ L bounding buffer (100 mM PBS buffer, 0.15 M NaCl, 0.1% SDS, pH 7.4) after which *S. aureus* bacteria were added to the solution. Bacterial DNA was released and bounded to the nano-biosensor (MNP-TiO₂-AP-SMCC-biosensors) at 55 °C. Then, 250 μ L isopropanol was added to the reaction mixture for 15-20 min. The MNP-nano-biosensor was bounded to target DNA isolated by a magnet. The nano-biosensor was washed three times with each of the following solutions: (WB1: 30 mM Tris-HCl, pH 7.0, 2.5 M GuSCN, 56% ethanol; WB2: 10 mM Tris-HCl, pH 7.0, 70% ethanol). Once the washing was finished, the remnant was left to be completely air-dried. Finally, 100 μ L releasing or elution buffer (Elution buffer: 10 mM Tris-HCl, pH 8.5, 1 mM EDTA) was added to the dried precipitant MNP-TiO₂-APTES-Biosensors-target DNA complex and incubated for 5 min at 65 °C. This process caused the release of target DNA from nano-biosensors [6,12,22]. The MNP-TiO₂-AP-Biosensors were isolated from target DNA free using a magnet. The concentration of extracted target DNA was assayed via Nanodrop spectrophotometry (Figure 1).

Sensitivity and specificity of nano-biosensor

In this study, for sensitivity assay, McFarland solution was used for different bacterial concentrations prepared ranging from 3.7×10^1 up to 3.7×10^8 . Briefly, each bacterial concentration was added to 30 mg of MNP-TiO₂-AP-SMCC-Biosensors according to the method mentioned in Section "Detection of target DNA". The target DNA extracted from each concentration was evaluated through Nano-drop spectrophotometry at 260 nm wavelength, PCR, and gel agarose electrophoresis. Finally, the sensitivity of the two methods was researched and compared with each other. Note that the PCR was performed in 50 μ L volume while containing forward and reverse primers (0.4 mM, 10 pm μ L⁻¹), PCR Buffer (10X, 5 μ L), MgCl₂ (1.5 mM), dNTP (0.2 mM), target DNA (2 μ L) and Taq DNA polymerase (2.5 U) under the following conditions: initial denaturation at 95 °C for 5 min, 30 cycles; secondary denaturation at 94 °C for 30 s; annealing at 54 °C for 30 s; the

extension temperature at 72 °C for 30 s; and the final incubation temperature at 72 °C for 5 min. Finally, the PCR product was electrophoresed on 2% agarose gel [2,22]

The specificity of the nano-biosensor under optimized condition (3.7×10^5 CFU mL⁻¹, 30 mg of MNP-TiO₂-AP-SMCC-Biosensors) was determined with different species of bacterial target DNA including *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 as positive controls, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. dysentery* PTCC 1188, *K. pneumoniae* ATCC 7881 and *S. epidermidis* ATCC 12228 as negative controls. The extracted target DNA was evaluated via Nano-drop spectrophotometry (Figure 1(C,D)).

Results and discussion

Characterization of MNPs

Microscopic characterization of MNPs

SEM images of MNPs, MNPs-biosensors, and MNP-biosensors-target DNA are shown in Figure 2. The results of this study revealed that the size of MNP was 20–25 nm (Figure 2(A)). After the modification of MNPs with TEOS, APTES, and SMCC, the size of MNPs increased from 20–25 nm to 30–35 nm and 35–43 nm, respectively (Figure 2(C)). The results also indicated that the structure of nanoparticles was spherical and semi-spherical (Figure 2(A,C)).

DLS

The size and potential of MNPs were analyzed by Dynamic Light Scattering (DLS). The results are shown in Figure 2. The size of MNPs was measured as 25 nm; after adding TEOS, APTES, and SMCC on the surface of MNPs, it changed to 32, 35, and 36 nm, respectively (Figure 2(B)). The size of MNP-TiO₂-AP-SMCC after conjugation with biosensors was changed from 36 nm to 38 nm up to 43 nm (Figure 2(B)). On the other hand, the potential of MNPs was –8 mV but after adding TEOS, APTES, and SMCC it changed from –8 mV to –21 mV. After adding biosensors, the potential was altered from –21 mV to –12 mV. Finally, when the MNPs were conjugated with target DNA, the potential changed from –12 mV to –16 mV, which is associated with oligonucleotides.

FTIR

FTIR technique was used to determine the characteristic of biochemical interactions between the macromolecules and the surface of MNPs at the wavelength 400–4000 cm⁻¹. Figure 2 shows the IR spectra of MNPs, MNP-TiO₂-AP, MNP-TiO₂-AP-SMCC-biosensor, and MNP-TiO₂-AP-SMCC-biosensor-DNA. The absorption peak within 568–580 cm⁻¹ wavelength is associated with the stretching vibration of Fe–O. The absorption peak at 1625 cm⁻¹ is related to the stretching vibration of O–H showing the presence of water molecules at the surface of MNPs. The absorption peak at wavelengths of 1009–1018 cm⁻¹ and 634 cm⁻¹ is related to Si–O–Fe and Si–O–Si, respectively, indicating the presence of TiO₂ bound on the surface of MNPs. Also, the absorption peak at wavelengths of 2830–2937 cm⁻¹ is related to CH₂ and CH₃ on the surface of APTES. Three bonds of 1560–1670 cm⁻¹ and

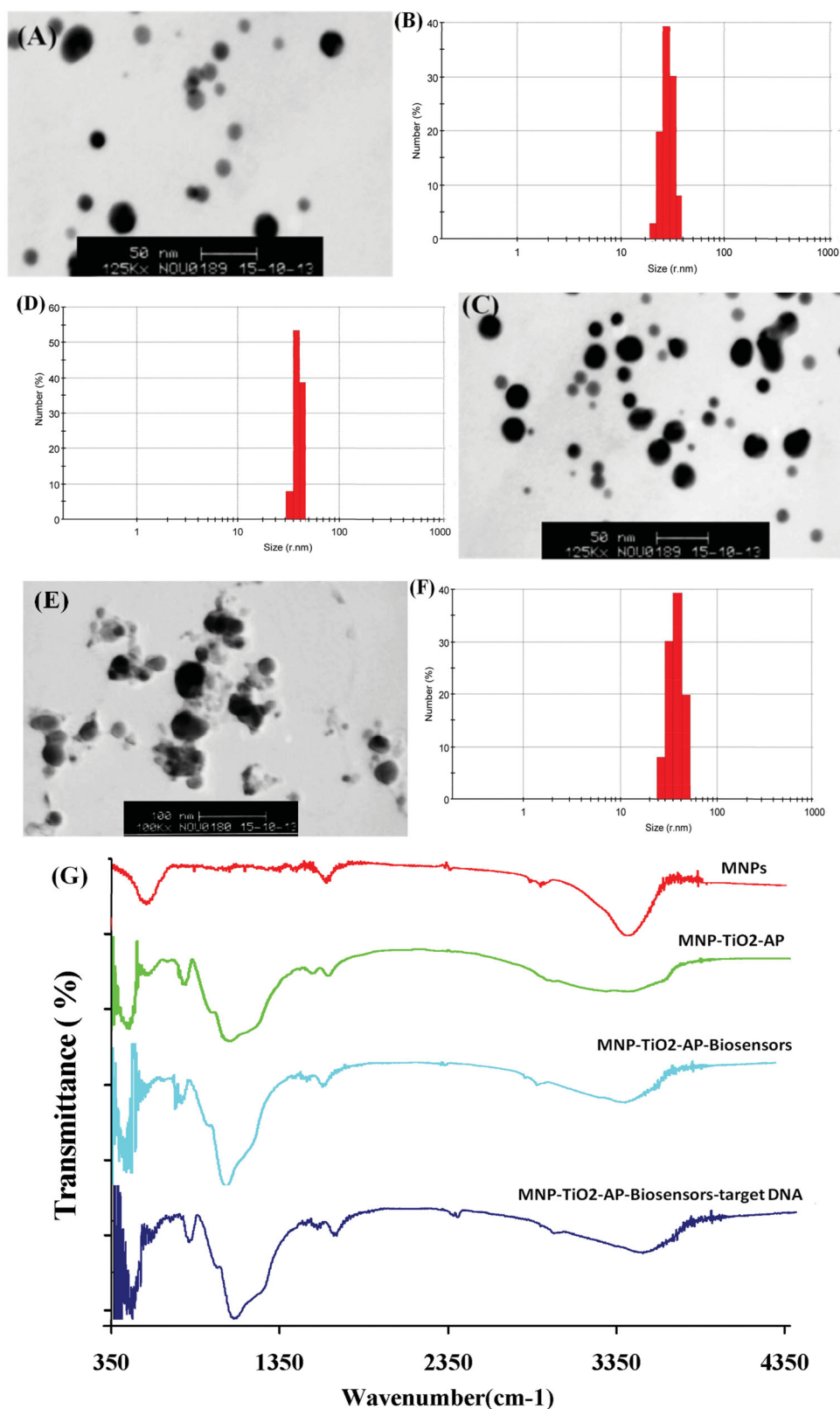


Figure 2. (A) SEM image of MNPs, (B) DLS of MNPs, (C) SEM image of MNP-TiO₂-AP-SMCC, (D) DLS of MNP-TiO₂-AP-SMCC, (E) SEM of MNP-TiO₂-AP-SMCC-biosensors-target DNA, (F) DLS of MNP-TiO₂-AP-SMCC-biosensors-target DNA, and (G) IR designed nano-biosensor.

3435 cm⁻¹ are related to O-H and free NH₂ on the surface of APTES. Finally, the absorption peak at 2387 cm⁻¹ is related to the presence of SH at the surface of SMCC and biosensors representing their connection to the surface of MNPs (Figure 2(C)).

Efficiency of conjugation

The efficiency of the conjugation of biosensors with MNPs complex was determined using Nanodrop spectrophotometry. For this purpose, the absorbance and concentration of

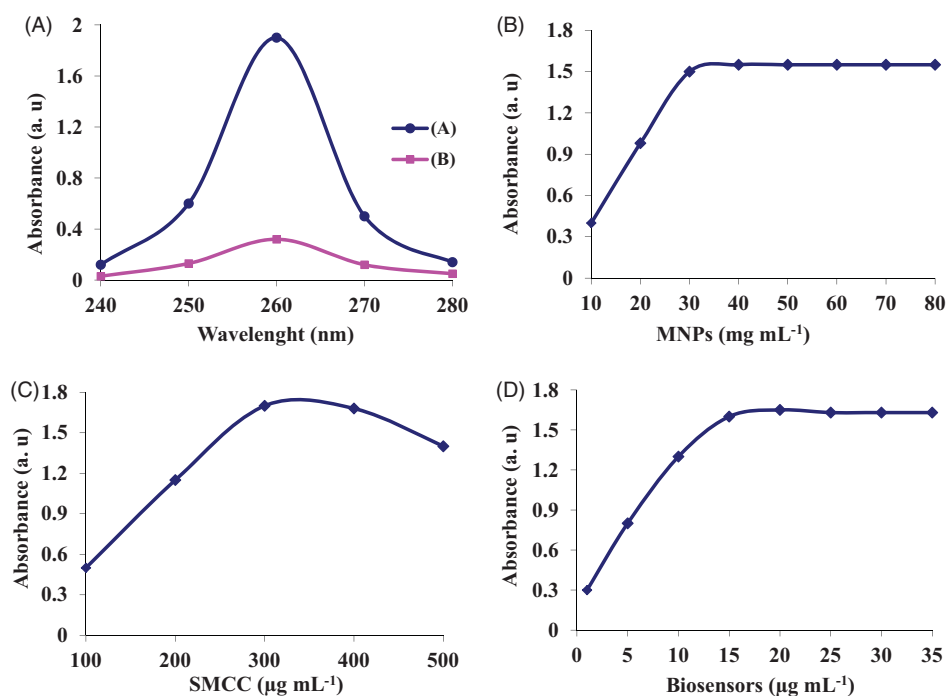


Figure 3. (A) Efficiency of biosensors conjugation, (B) optimization of MNPs concentration, (C) optimization of SMCC concentration, and (D) optimization of biosensors concentration.

biosensors before and after conjugation with nanoparticles were determined at 260 nm. According to the results, the efficiency of biosensors binding on the surface of MNPs was approximately determined as 83% (Figure 3(A)).

Optimization

MNPs concentration

For the detection of *S. aureus*, different concentrations of MNPs including 80, 60, 50, 40, 30, 20 and 10 mg mL⁻¹ were used. The results revealed that the optimum concentration for bacterial detection was 30 mg mL⁻¹ as seen in Figure 3(B). At higher concentrations of MNPs, no significant change of absorption was observed, while at concentrations less than 30 mg mL⁻¹, there was an increase in the absorption by elevating the concentration from 10 up to 30 mg mL⁻¹ (Figure 3(B)).

SMCC concentration

For the bonding of biosensors to MNPs, SMCC was used as a linker. For this purpose, different concentrations of SMCC (500, 400, 300, 200 and 100 μg mL⁻¹) were used. The biosensors under optimum conditions were conjugated with MNP-SMCC and the absorption of target DNA of each concentration was studied by Nano-drop spectrophotometry. The results of this study revealed that the optimum concentration of SMCC for conjugation of biosensors was 300 μg mL⁻¹ observed in Figure 3(C).

Biosensors' concentration

For the detection and extraction of bacterial target DNA, different concentrations of biosensors were used. Under

optimum conditions, concentrations of 1, 5, 10, 15, 20 and 25 nm of biosensors were used. The results showed that the optimal concentration of biosensors was 15 nm as displayed in Figure 3(D).

Sensitivity

To determine the sensitivity of this method, different concentrations of bacteria 3.7×10^1 – 3.7×10^8 CFU mL⁻¹ were used. Firstly, after lysis of the bacteria with the mentioned protocol, 30 mg mL⁻¹ nano-biosensor was added to the reaction mixture. Bacterial target DNA bonded to the nano-biosensor and extracted by a magnet. The quantity of the extracted target DNA was studied by Nano-drop spectrophotometry. The results revealed that the designed nano-biosensor was well able to detect *S. aureus* (Figure 4(A)). According to the results, there was a linear relationship between the bacterial target DNA concentration and absorbance at concentrations of 3.7×10^2 – 3.7×10^7 CFU mL⁻¹, as observed in Figure 4(B,C). At concentrations greater than 3.7×10^7 CFU mL⁻¹, no significant change occurred in the adsorption rate (Figure 4(B)). Linear equation and regression for detection of *S. aureus* were calculated as follows $Y = 0.242X + 2.08$ and $R^2 = .9957$. Limit of detection of this method was defined as 230 CFU mL⁻¹, suggesting its higher sensitivity compared to other reports CBDGFG. Also, PCR and gel agarose electrophoresis methods were used to compare and determine the sensitivity of the two methods. The results indicated PCR and gel agarose electrophoresis methods could detect bacterial target DNA at concentration of 3.7×10^3 – 3.7×10^8 CFU mL⁻¹ and 3.7×10^4 – 3.7×10^8 CFU mL⁻¹, respectively (Table 2); they have lower sensitivity compared to Nano-drop spectrophotometric method (Figure 4(D)).

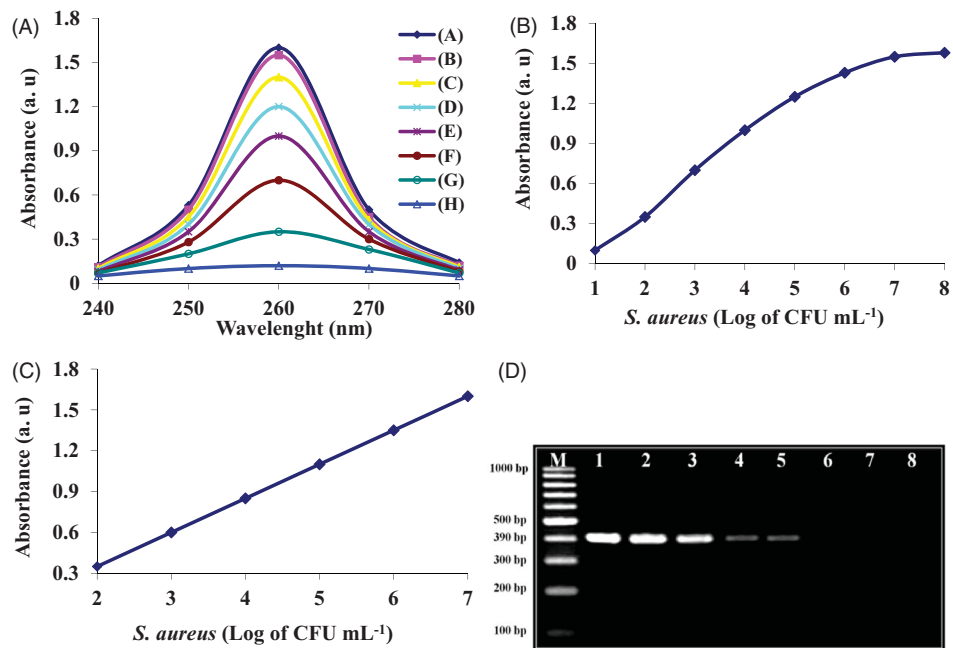


Figure 4. (A) Sensitivity of the designed nano-biosensor at different concentrations of bacteria 3.7×10^1 – 3.7×10^8 CFU mL⁻¹ (from A up to H), (B) linear relationship between different concentrations of bacteria and absorbance within 3.7×10^2 – 3.7×10^7 CFU mL⁻¹, (C) linear relationship between different concentrations of bacteria and absorbance within 3.7×10^2 – 3.7×10^7 CFU mL⁻¹, (D) sensitivity of PCR gel agarose electrophoresis at different concentrations of bacteria within 3.7×10^8 – 3.7×10^1 CFU mL⁻¹ (from 1 to 8), respectively, M: size marker 100 bp.

Table 2. Comparison of the detection limit of *S. aureus* using different methods.

Method	Detection limit	References
Silica nanoparticle-probe assay	682 CFU/mL	[35]
Colorimetric assay	4×10^4 CFU/mL	[36]
Loop-mediated isothermal amplification (LAMP)	6.4×10^3 CFU/mL	[37]
Quantum dots	1×10^2 CFU/mL	[27]
enzyme-linked immunosorbent assay (ELISA)	1×10^4 CFU/mL	[4–7]
PCR assay	1×10^3 CFU/mL	[27]
MNP-biosensors assay	230 CFU/mL	Our method

Specificity

To determine the specificity of this method, 30 mg mL⁻¹ of the designed nano-biosensor were added to the target DNA (3.7×10^5 CFU mL⁻¹) of *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 as positive controls. Further, 30 mg mL⁻¹ of the designed nano-biosensor was added to the target DNA of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. dysenteriae* PTCC 1188, *K. pneumoniae* ATCC7881, and *S. epidermidis* ATCC 12228 as negative controls. The results indicated that the nano-biosensor was specifically bound to *S. aureus* ATCC 25923 (Figure 5, curve A), *S. aureus* ATCC 29213 (Figure 5, curve B) target DNA, and increased the absorption rate as displayed in Figure 5. However, the nano-biosensor was not bound to negative control bacterial target DNA (Figure 5, curve from D up to H). Finally, there was a significant reduction in the absorption rate at 260 nm (Figure 5).

Conclusions

In this study, a nano-biosensor based on iron nanoparticles (MNPs) was successfully designed for detection of *S. aureus*. Firstly, MNPs were synthesised and then the designed biosensors were conjugated with MNPs. The designed nano-

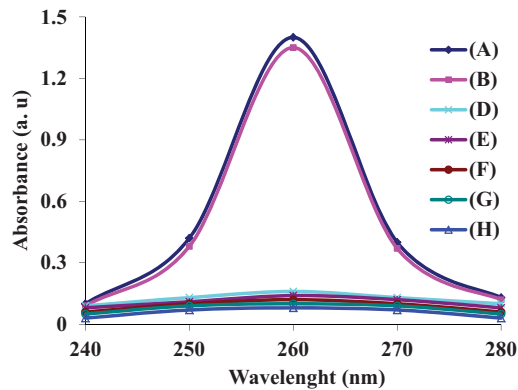


Figure 5. Specificity of the designed nano-biosensor, (Curve A) *S. aureus* ATCC 25923, (Curve B) *S. aureus* ATCC 29213, (Curve D) *Escherichia coli* ATCC 25922, (Curve E) *P. aeruginosa* ATCC 27853, (Curve F) *S. dysenteriae* PTCC 1188, (Curve G) *K. pneumoniae* ATCC7881, (Curve H) *S. epidermidis* ATCC 12228.

biosensor was added to bacterial target DNA. After bonding of target DNA to the nano-biosensor, they were extracted using a magnet and washed for three times. Bacterial target DNA was released using a release buffer. The absorption and the concentration of target DNA were studied by spectrophotometry at 260 nm wavelength. Sensitivity of this method was studied at different concentrations of bacteria and compared with PCR and gel agarose electrophoresis. Also, the specificity was proved using positive controls and negative bacteria. The results of the study revealed that the size of synthesised MNPs was 20–25 nm which after conjugation with biosensors increased to 38–43 nm. The potential of modified MNPs was -21 mV; after conjugation, it changed into -16 mV. The optimal concentrations of biosensors, MNPs, SMCC linker, and bacterial concentration were determined as 15 nm, 30 mg mL⁻¹, 300 μ g, and 3.7×10^2 – 3.7×10^7 , respectively. The minimum detection limit (LOD) was defined as 230

CFU mL⁻¹. The sensitivity of the designed nano-biosensor was determined and compared to PCR and electrophoresis methods at different bacterial concentrations. Finally, the results indicated that the nano-biosensor was sensitive more than PCR and electrophoresis methods and completely specific for *S. aureus* target DNA.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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