Investigation of photocatalytic effects and extraction of genomic DNA from *Staphylococcus aureus* through $Fe_3O_4/SiO_2/TiO_2$ magnetic nanoparticles

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Abstract: *Staphylococcus aureus* has been considered as one of the main pathogens that cause various diseases. Therefore, access to fast and reliable DNA-based methods is crucial for the detection and identification of this bacterium. DNA extraction and purification are fundamental primary steps in almost all molecular biology studies. Therefore, the purpose of this work is utilising $Fe_3O_4/SiO_2/TiO_2$ magnetic nanoparticles to extract genomic DNA of *Staphylococcus aureus*. This paper contains extracting genomic DNA from standard strain of *Staphylococcus aureus* ATCC 25923 using $Fe_3O_4/SiO_2/TiO_2$ nanostructures. The quality of extracted DNA was evaluated after electrophoresis on gel agarose, also DNA purity and concentrations were measured by a NanoDrop spectrophotometer. The concentration of genomic DNA extracted by Fe_3O_4/SiO_2 magnetic nanoparticles from *Staphylococcus aureus* strain ATCC 25923 was 131.635 ng/µL. Also, A260/280 and A260/230 values of mentioned DNA were ranged 1.7 to 1.8 and 2 to 2.2 respectively. The obtained results showed that the DNA extracted by the synthesised magnetic nanoparticles has an acceptable concentration and purity for subsequent molecular biology studies in this bacterium.

Keywords: $Fe_3O_4/SiO_2/TiO_2$; DNA extraction; *Staphylococcus aureus*; genomic DNA magnetic nanoparticles.

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1 Introduction

Pathogenic bacteria cause mortality and significant complications for humans. Of these bacteria, *Staphylococcus aureus* (*S. aureus*) is believed to be one of the major pathogens which are able to make various types of infections (Gao et al., 2018; Hosseini et al., 2020). Recently, the important role of *S. aureus* in causing nosocomial and community acquired infections has led to increased researches on this bacterium using exact molecular methods (Holden et al., 2004; Bahador et al., 2013). DNA extraction and purification are fundamental primary steps in almost all molecular biology studies (Sosa-Acosta et al., 2018). DNA analysis today plays an important role in molecular biology (Sosa-Acosta et al., 2018). It is important to find a suitable DNA isolation system to successfully complete the subsequent experiments (Bahador et al., 2013; Niemirowicz et al., 2012; Neshani et al., 2019). Genomic DNA extraction of bacterial cells with appropriate quality, concentration, and purity is one of the procedures considered by genetic scientists and researchers in the field of molecular biology (Cheng and Jiang, 2006; Martínez-González et al., 2017). There are several key factors to consider when choosing the right method for DNA extraction, including their rapidity and cost-effectiveness (Sato et al., 2001; Intorasoot et al., 2009). Extraction of genomic DNA by traditional methods such as phenol and chloroform besides being toxic is very time consuming (Gong and Li, 2014; Patramool et al., 2013). In recent years using magnetic nanoparticles, due to their unique properties to remove DNA from various cells, has received much attention (Bagban et al., 2018). Magnetic nanoparticles are affected by the

magnetic field; also have a specific affinity for reversible binding to DNA, additionally, their small size creates more surface area for binding to biomolecules (Bagban et al., 2018).

One of the applications of magnetic nanoparticles is their use as a carrier for the accumulation of biologically active substances including enzymes, antibodies and DNA (McBain et al., 2007; Chen et al., 2012). Although uncoated magnetic nanoparticles have been used for isolation of DNA from various sources of biological materials, it has been shown that coated nanoparticles have greater recovery of DNA in comparison to them (Rahnama et al., 2016). On the other hand magnetic photocatalysts are widely used in wastewater treatment (Rahimi-Nasrabadi et al., 2015, 2016; Peymani-Motlagh et al., 2019a, 2019b; Sobhani-Nasab et al., 2019). Given the high non-toxicity and optical stability of titanate, this photocatalysts is commonly used in different industries (Ghaemifar et al., 2020; Gandomi et al., 2020; Marsooli et al., 2020a, 2020b; Amin Marsooli et al., 2020; Rahimi-Nasrabadi et al., 2019; Sobhani-Nasab et al., 2019). In recent years, many studies have focused on producing composite of magnetic nanoparticles and of titanate nanostructures (Sobhani-Nasab et al., 2015; Kooshki et al., 2019). In previous studies, magnetic nanoparticles have been used to extract genomic DNA from micro-organisms, including fungi (Sobhani-Nasab et al., 2015; Kooshki et al., 2019). Although there are limited studies in the field of using magnetic nanoparticles to extract nucleic acid from Gram-positive bacteria such as *S. aureus*. In comparison with time-consuming and unsafe techniques such as phenol-chloroform DNA extraction method, we used coated magnetic nanoparticles to achieve a fast, affordable, accurate and safe technique for DNA isolation. For this purpose, the present research aimed to use magnetic $Fe_3O_4/SiO_2/TiO_2$ nanoparticles for extraction of genomic DNA of *S. aureus*.

2 Materials and methods

In this study, in order to extract genomic DNA of *S. aureus*, standard strain ATCC 25923 was purchased from the microbial collection in Pasteur Institute, Tehran, Iran.

2.1 Synthesise of Fe_3O_4 nanoparticles

To fabricate Fe_3O_4, we used previous work (Figure A1) (Wei et al., 2012). The reagents of analytic grade (NaOH, ferric chloride hexahydrate, and ferrous chloride tetrahydrate) were utilised as a precursor. To synthesise these nanostructures, we performed the following procedures in order. At first, $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ with a molar ratio of 2:1 was dissolved in distilled water at 50°C, and then sodium hydroxide solution (1 mol. L^{-1}) was added into the above-mentioned solution dropwise under consistent mechanical stirring for 30 minutes to reach final pH of 11. Secondly, the precipitate was stirred at 50°C for 20 minutes, cooled in room temperature, and prior to be frequently washed with deionised water until achieving pH of 7, we separated particles through a magnet. Eventually, products were dried at 90°C in vacuum for 1 hour.

2.2 Synthesise of Fe_3O_4/SiO_2 nanostructures

The Fe_3O_4/SiO_2 nanostructures was obtained through modifying method by means of hydrolysis of $Si(OC_2H_5)_4$ in the presence of iron oxide nanostructures based on the preceding paper (Abbas et al., 2014). To do so, ultrasonic wave was used to disperse 0.1 g of as-synthesised iron oxide in 30 mL of water. Next, 5 mL of NH_4OH solution (32%) along with 80 mL of ethanol were added to the mixture. Afterward, 0.7 mL of tetraethyl orthosilicate was added dropwise into the mixed iron (III) oxide nanostructures under sonochemistry at 25°C, which took half day. To separate obtained precipitate, we used an external magnet, and it washed with purified water many times.

2.3 Synthesise of $Fe_3O_4/SiO_2/TiO_2$ nanostructures

First, 0.1 g of prepared Fe_3O_4/SiO_2 was dispersed in 30 mL of water under ultrasound. Subsequent, 0.35 mL of tetra-n-butyl titanate was added dropwise to the mixed Fe_3O_4/SiO_2 nanostructures under specific kind of irradiation already mentioned at 25°C temperature under stirring. To separate obtained precipitate, we used an external magnet, and it washed with purified water many times. Finally, the collected precipitate was, once more, washed with water and calcined in a vacuum oven at 500°C for two hours.

We suggest the reaction process of $Fe_3O_4/SiO_2/TiO_2$ nanoparticles as follows:

1 $2Fe\ (N_O3)_3.9H2O + H2O \rightarrow 2Fe^{3+} + 18NO_3^- + 19H_2O.$

2 $Fe\ (NO_3)_2.9H_2O + H_2O \rightarrow Fe^{2+} + 6NO_3^- + 10H_2O.$

3 $Fe^{2+} + 2Fe^{3+} + 8NaOH + 6NO_3^- + H_2O \rightarrow Fe\ (OH)_2 + 2Fe\ (OH)_3 + NaNO_3.$

4 $Fe\ (OH)_2 + 2Fe\ (OH)_3 + \Delta T \rightarrow Fe_3O_4 + 4H_2O.$

5 $H_2O + Si\ (OC_2H_5)_4 \rightarrow C_2H_5OH + Si\ (OH)_4.$

6 $Si(OH)_4 + Si(OH)_4 \rightarrow (OH)_3Si - O - Si(OH)_3 + H_2O.$

7 $(OH)_{3Si} - O - Si\ (OH)_3 + \Delta T \rightarrow SiO_2 + H_2O.$

8 $H_2O + Ti\ (OC_4H_9)_4 \rightarrow C_4H_9OH + Ti(OH)_4.$

9 $Ti(OH)_4 + Ti(OH)_4 \rightarrow (OH)_3Ti - O - Ti(OH)_3 + H_2O.$

10 $(OH)_3Ti - O - Ti(OH)_3 + \Delta T \rightarrow TiO_2 + H_2O.$

11 $Fe(NO_3)_3.9H_2O + Fe(NO_3)_2.9H_2O + NaOH + Si(OC_2H_5)_4 + Ti(OC_4H_9)_4 \rightarrow C_2H_5OH + NaNO_3 + C_4H_9OH + Fe_3O_4/SiO_2/TiO_2 + H_2O.$

2.4 Photocatalytic evaluation

To study Rhodamine B (RhB) degradation, we looked at the photoactivity of $Fe_3O_4/SiO_2/TiO_2$ nanocomposites. Therefore, photocatalytic decomposition was carried out in a 100 mL beaker containing 50 ml of 2×10^{-5} M Rhodamine B and 0.03 g of the $Fe_3O_4/SiO_2/TiO_2$ nanocomposites at 25°C. The aeration of the suspensions was performed to balance the photocatalyst surface and the dye molecules for half an hour. Then, the mixture was placed into the photoreactor which the vessel was 20 cm away from the visible source of 400 W Osram lamps. Aliquots of the mixture were removed at

definite interval of times during the irradiation. We pursued centrifugation of the samples by a UV-Vis spectrometer (RhB at 554 nm). The photocatalytic degradation, in terms of percentage, was evaluated with the following formula [equation (1)]:

$$\text{Degradation rate}(\%) = 100(C_0 - C_t) / C_0 \quad (1)$$

where C_0 and C_t are in respective the absorbance value of solution at 0 and t minute.

2.5 Genomic DNA extraction using Fe_3O_4/SiO_2 magnetic nanoparticles

S. aureus standard strain ATCC 25923 was cultured on Luria-Bertani (LB) agar (Merck, Germany) and incubated for one day at 37°C. The grown colonies were then transferred to Tryptic Soy Broth (TSB) (Merck, Germany) culture medium. After 24 hours of incubation at 37°C, 3 mL of the bacterial culture in the TSB culture medium was transferred to 2 mL sterile micro tubes and centrifuged at 10,000 rpm for five minutes. Then 500 µL of suspension buffer (2,000 µL EDTA 0.5 M and 5,000 µL Tris-HCl 1 M) was added to resulted pellet. After mixing the tube, lysis buffer (70 µL SDS 10%, 28 µL NaOH 0.5 M, lysozyme and 100 µL distilled water) was added and incubated at 37°C for one hour. Following the addition of 10 µL proteinase K enzyme (10 mg/M), and 60 minutes incubation at 55°C, centrifuging was done (10,000 rpm for four minutes at 4°C). The resulting supernatant was transferred to 200 µL of binding buffer including (1.25 µL of NaCl 1M, polyethylene glycol 60% and distilled water). After adding 0.8 mg Fe_3O_4/SiO_2 magnetic nanoparticles and ambient temperature incubation, the magnetic particles were distributed with the assistance of a magnetic rack. Then, 200 µL of chilled 70% ethanol was used to wash it and centrifuging done (10,000 rpm for ten minutes). At this stage the supernatant was thrown away and the tubes were placed at room temperature to dry. In order to precipitate the nanoparticles, 50 µL of TE buffer (pH:8) was added to tube and following incubation at 55°C for ten minutes, centrifugation was carried out for one minute at 5,000 rpm. Magnetic nanoparticles separation supported by magnetic rack and the resulting supernatant containing extracted DNA was transferred to a new clean tube and stored at –20°C.

2.6 The effect of different doses of magnetic nanoparticles

The effect of using various amounts of magnetic nanoparticles including (0.2 mg, 0.4 mg, 0.8 mg and 1 mg) on genomic DNA extraction was investigated.

2.7 The effect of different amounts of polyethylene glycol

The effect of using different percentages of polyethylene glycol including (20%, 40%, 50%, 60%, 70%, and 80% w/w) on genomic DNA extraction was investigated.

2.8 Evaluation of the quality of obtained DNA

The quality of extracted DNA was evaluated after electrophoresis on gel agarose 0.8%. Also, the concentrations and purity of DNA were evaluated by a NanoDrop spectrophotometer 2000 (Thermo Fisher Scientific, USA). The absorbance ratios of

260/280 nm were measured using spectrophotometers. A 260/280 ratio of ~1.7 was considered as 'pure' for DNA.

2.9 Characterisation of magnetic nanoparticles

Philips X'pert Pro MPD with a graphite-filtered Cu Kα (k = 0.154 nm) radiation applied for XRD analysis nanostructures. The shape and dimension in the synthesise of nanostructures were accomplished by means of transmission electron microscopy (TEM) and scanning electron microscope (SEM). Nanostructures sonicated for ten minutes. Then, 10 μL of sonicated nanostructures was dropped on a Cu grid and imaging was accomplished at an accelerating voltage of 200 kV. During SEM imaging, energy-dispersive X-ray spectroscopy (EDX) was done for the elemental analysis of the Fe_3O_4/SiO_2. The FTIR analysis of nanostructures was acquired employing a Nicolet Magna-550 spectrometer and KBr pellets with scan speed of 65 spectra/s at 16 cm^{-1}.

3 Results

The X-ray diffraction graph of the Fe_3O_4 nanostructures with space group of Fd-3m and pure phase cubic (JCPDS75-0449) has been shown in Figure 1. Moreover, the XRD pattern of $Fe_3O_4/SiO_2/TiO_2$ nanostructures demonstrates that it consists of Fe_3O_4 (JCPDS card No. 01-1111), SiO_2 (JCPDS card No. 14-0676) and TiO_2 (JCPDS card No. 14-0676). Therefore, the XRD graphs of the $Fe_3O_4/SiO_2/TiO_2$ nanostructures (Figure 2) the additional diffraction peaks are indexed to planes (110), (221), (111) and (000) at two hours = 33.6, 29.0, 37.1, and 61.1 for SiO_2 and TiO_2, which are in agreement with the standard card SiO_2 and TiO_2 (Figure 1 and Figure 2).

Figure 1 XRD pattern of Fe_3O_4 nanostructures (see online version for colours)

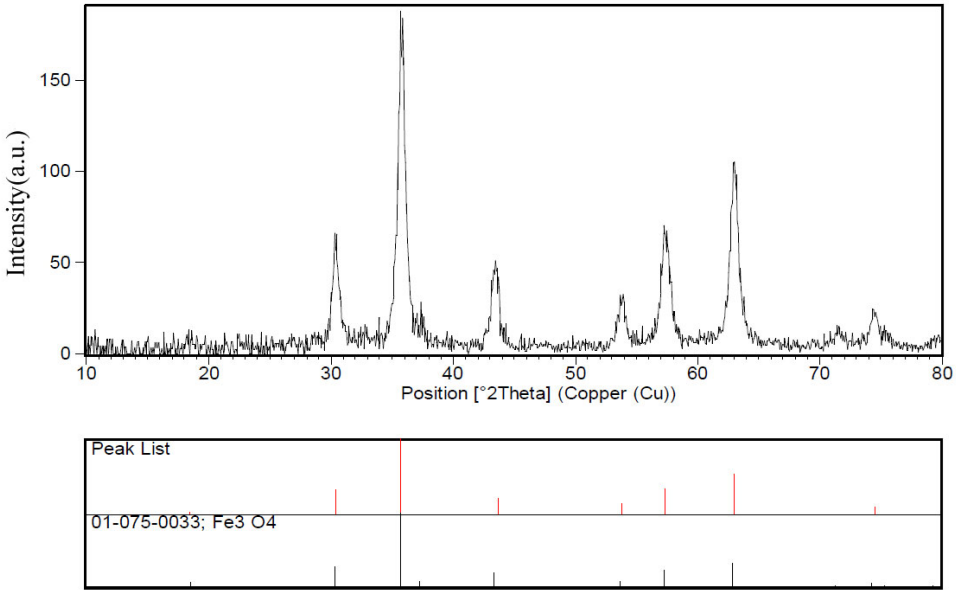


Figure 2 XRD pattern of $Fe_3O_4/SiO_2/TiO_2$ nanostructures (see online version for colours)

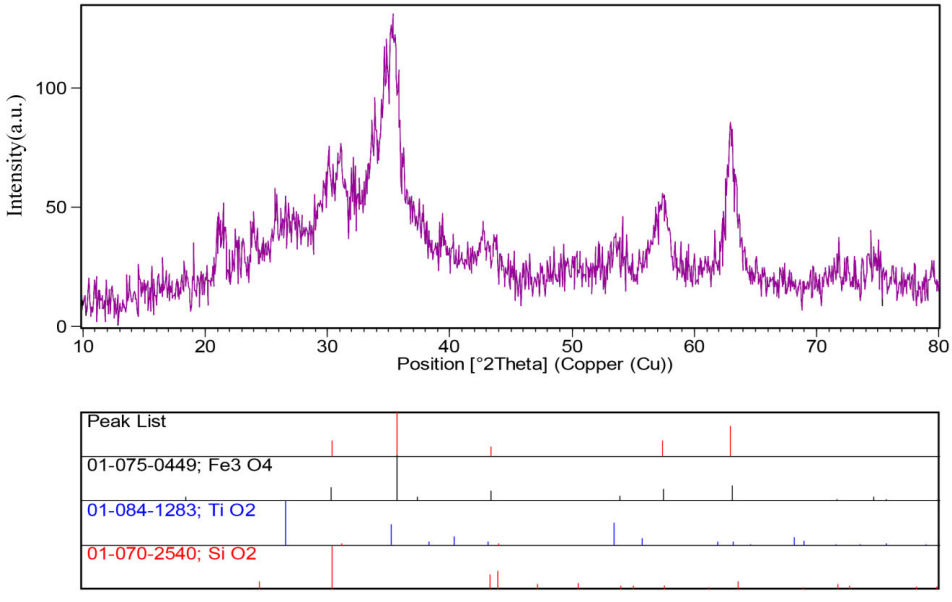
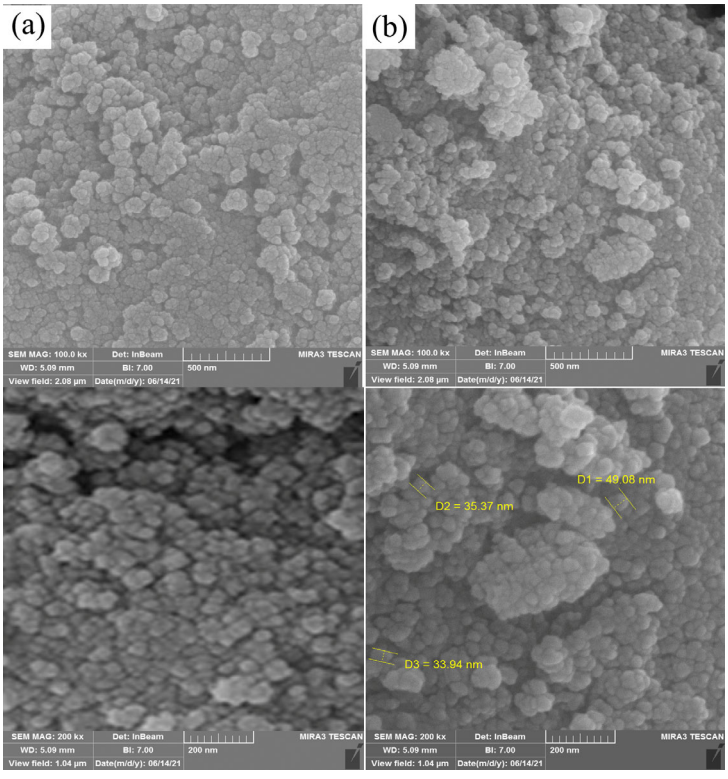
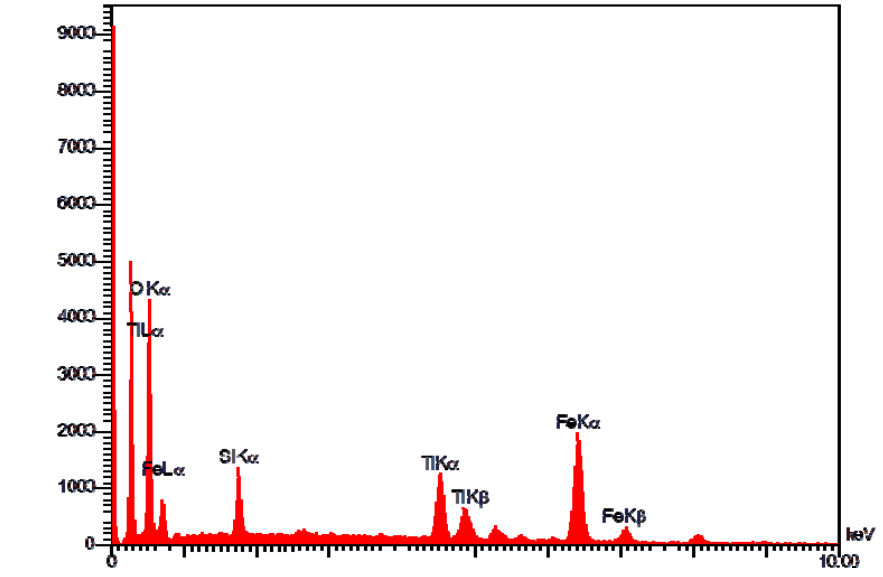


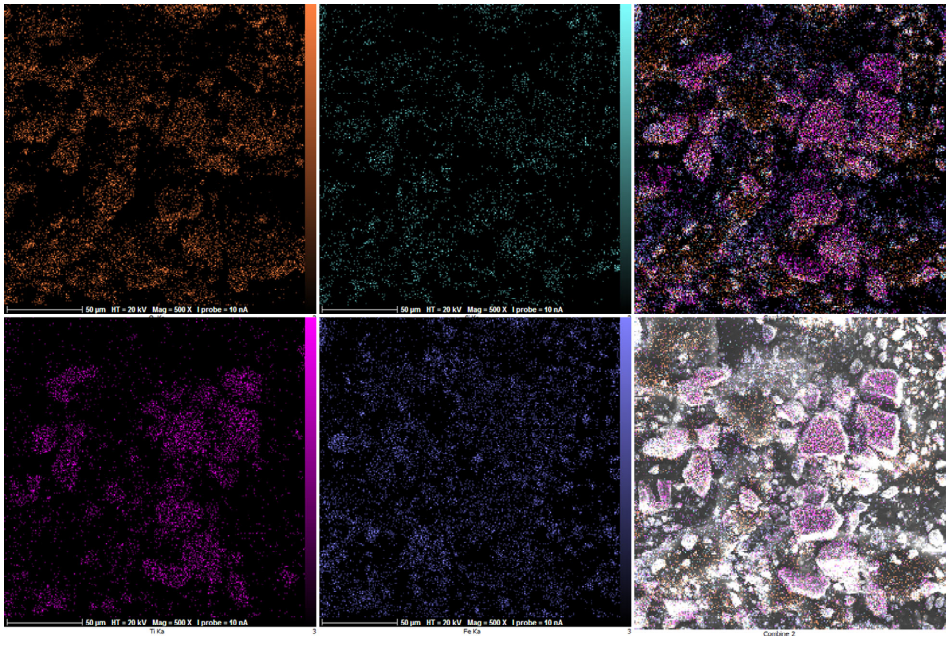
Figure 3 SEM image of (a) Fe_3O_4, (b) $Fe_3O_4/SiO_2/TiO_2$ nanostructures (see online version for colours)



The SEM images of Fe_3O_4 and $Fe_3O_4/SiO_2/TiO_2$ at high and low magnification show in Figures 3(a) and 3(b). Moreover, both samples were found to have the same morphology as well as constant distribution.

Figure 4 EDS spectra of optimum sample $Fe_3O_4/SiO_2/TiO_2$ nanostructures (see online version for colours)





EDS pattern of $Fe_3O_4/SiO_2/TiO_2$ nanoparticles synthesised is depicted in Figure 4. Fe, O, Si, and Ti lines are detectable in this figure. For throughly investigation of the shape and dimension of Fe_3O_4 and $Fe_3O_4/SiO_2/TiO_2$, we used TEM analysis.

The respective sizes of the sphere-like Fe_3O_4 and $Fe_3O_4/SiO_2/TiO_2$ nanostructures were found to be 38–44 and 78–81 nm in Figures 5(a) and 5(b), respectively.

Figure 5 TEM image of (a) Fe_3O_4, (b) $Fe_3O_4/SiO_2/TiO_2$ nanostructures

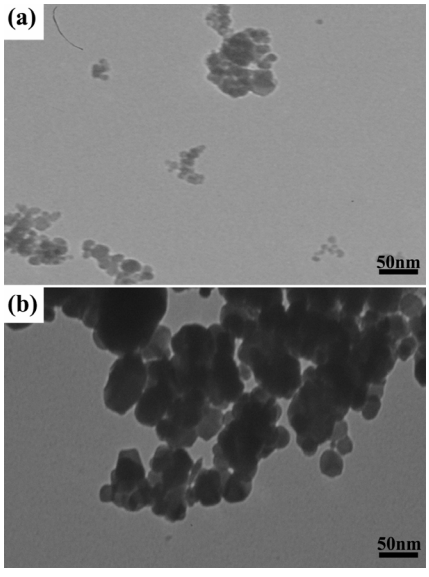
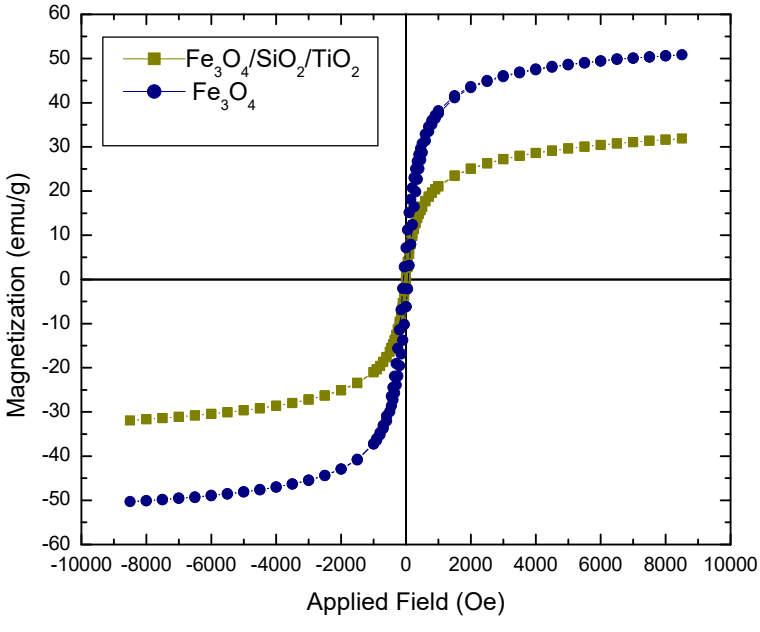
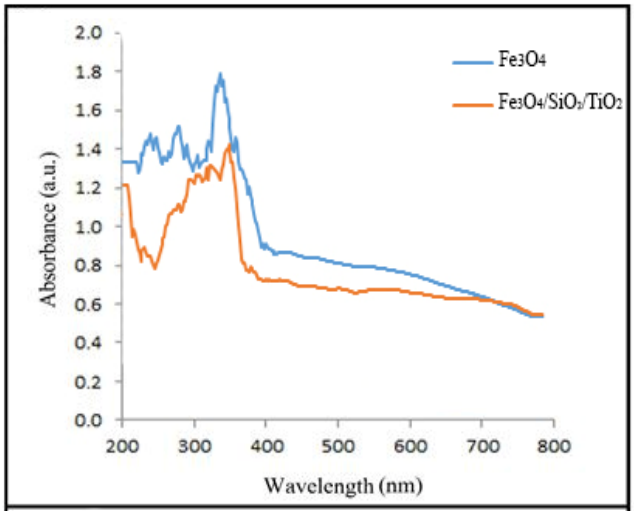


Figure 6 VSM curves of (a) Fe_3O_4, (b) $Fe_3O_4/SiO_2/TiO_2$ nanostructures (see online version for colours)

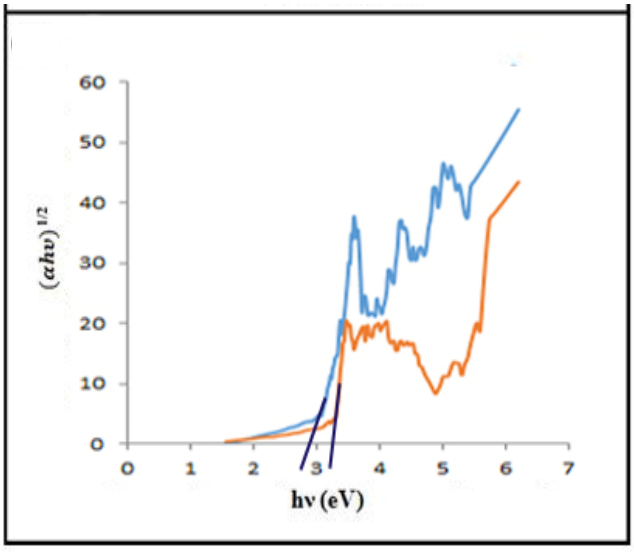


The magnetic behaviour of the nanocomposites was explored with the help of VSM at 60°C. The resulting magnetisation curves for Fe_3O_4 nanoparticles, $Fe_3O_4/SiO_2/TiO_2$ nanocomposites are represented in Figure 6. This displays that the saturation magnetisation (Ms) values to be 30.5, and 50.2 emu g^{-1} respectively. The tests also indicated that the synthesised photocatalyst samples have a typical superparamagnetic behaviour. The reduction in the saturation magnetisation of pure Fe_3O_4 nanoparticles in the other samples is originated from the fact that the further layers have been formed on these magnetic cores.

Figure 7 (a) UV-vis diffuse reflectance spectrum and (b) Tauc plot pattern for Fe_3O_4 and $Fe_3O_4/SiO_2/TiO_2$ nanostructures (see online version for colours)



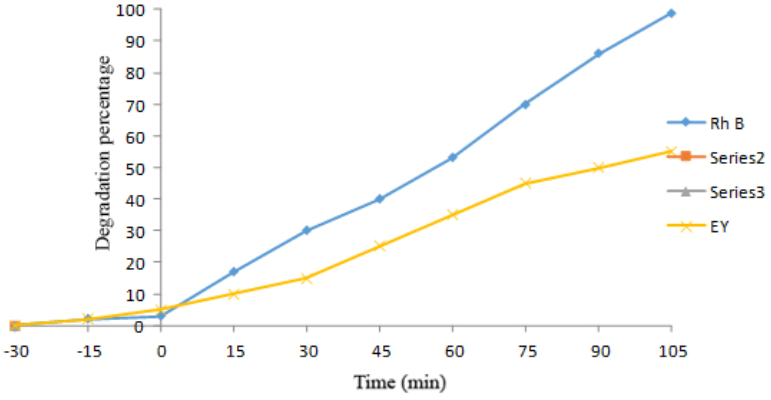
(a)



(b)

Figure 7 shows the diffuse reflective UV-vis spectrum of Fe_3O_4 nanoparticles, $Fe_3O_4/SiO_2/TiO_2$. The UV-vis spectra represents that the pure Fe_3O_4 nanoparticles has substantial absorption in the ultraviolet spectrum (200–400 nm) which is related to the, e.g., also, the $Fe_3O_4/SiO_2/TiO_2$ demonstrates absorption region from 403 to 423 nm, visible region, compared to that of Fe_3O_4, 394 nm. Our findings revealed that the Es values of $Fe_3O_4/SiO_2/TiO_2$ were between 2.9 Ev, which is substantially smaller than that of the pure Fe_3O_4, 3.2 eV. In addition, it was shown that visible light absorption could be improved with combined SiO2/TiO2 in Fe_3O_4 heterostructures.

Figure 8 $Fe_3O_4/SiO_2/TiO_2$ nanostructures on decomposition of Rh B (cationic) and Ey (anionic) (see online version for colours)



$Fe_3O_4/SiO_2/TiO_2$ nanocomposites photocatalysts displays degradation of two varying dyes as organic pollutant including methylene blue (MB), and Eosin Yellow (EY) under visible irradiation (Figure 8). The $Fe_3O_4/SiO_2/TiO_2$ nanocomposites photocatalysts efficiencies of MB, MV and MO were found to be 99%, 85%, and 79% for the 105 min, respectively.

4 Discussion

In comparison with time-consuming and unsafe techniques such as phenol-chloroform DNA extraction method, we used coated magnetic nanoparticles to achieve a fast, affordable, accurate and safe technique for DNA isolation. To the best of our knowledge, in the present study, for the first time in Iran, magnetic nanoparticles were used to extract genomic DNA from *S. aureus*. In a study by Min et al, has been reported that an increase in the amount of nanoparticles is reduced the yielded DNA using dimercaptosuccinic acid coated magnetic nanoparticles (DMSA-MNPS) (McBain et al., 2007). However, Rahnama et al, consistent with the present study has shown that by increasing the amount of magnetic nanoparticles from 0.05 to 0.2 mg, the concentration of extracted DNA plasmid increases (Bagban et al., 2018).

Despite the importance of PEG function in the extraction of DNA using magnetic nanoparticles, there are few studies in the literature for comparing the results. In our study, maximum recoveries of extracted DNA were obtained using concentration of 60% of PEG. At this concentration of PEG, the extracted DNA was of good-quality. The

260/280 ratios of genomic DNA extracted from *S. aureus* using Fe_3O_4/SiO_2 nanoparticles in this study were in the ranges of 1.71 to 1.85. In addition, the concentration of yields DNA was 131.635 ng/μL which indicates the extracted DNA could be suitable to be used in a method like convenient PCR. Bagban and colleagues has been used magnetic nanoparticles to extract genomic DNA from dried saliva and semen samples and showed that quality of DNA by magnetic nanoparticles-based method was much better than other methods such as kit-based method (Intorasoot et al., 2009). So that the 260/280 ratios of extracted DNA by magnetic nanoparticles-based method for the saliva and semen samples ranged 1.74–1.99 and 1.63–2.0 respectively (Intorasoot et al., 2009).

Based on the results obtained in this study and comparing with conventional methods, it can be concluded that genomic extraction using Fe_3O_4/SiO_2 magnetic nanoparticles has some advantages including:

1 Compared to some DNA extraction techniques, the current method has fewer steps.

2 Due to non-use of hazardous substances, it is a safe method compared to other techniques such as phenol-chloroform protocol.

3 The genomic DNA isolated by this procedure has enough quality to be appropriate for subsequent molecular biology techniques such as polymerase chain reaction.

5 Conclusions

In the present study, in order to achieve a fast, affordable, accurate and safe technique for DNA extraction from *S. aureus*, coated magnetic nanoparticles were used. For this purpose, the $Fe_3O_4/SiO_2/TiO_2$ magnetic nanoparticles were synthesised through modifying method using raw materials including $Si(OC_2H_5)_4$, NaOH, Tetra-n-butyl titanate, Ferric chloride hexahydrate, and Ferrous chloride tetrahydrate. The obtained results showed that the DNA extracted by the synthesised magnetic nanoparticles has an acceptable concentration and purity for subsequent molecular biology studies in this bacterium.

Acknowledgements

Farzaneh Firoozeh and Hadiseh Rostami contributed equally to this work.

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Appendix

Figure A1 Test steps (see online version for colours)

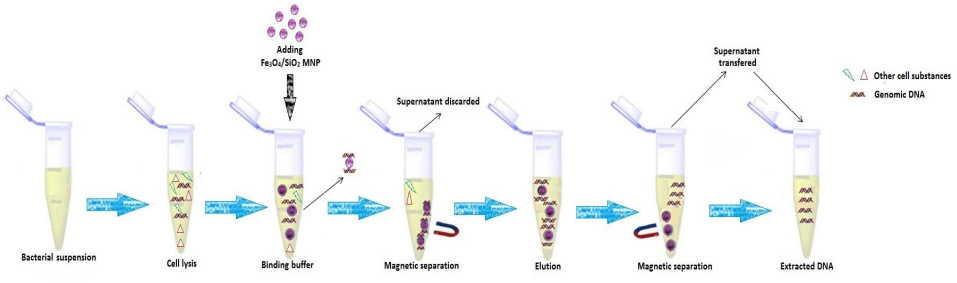

Table 1 Genomic DNA extracted from *Staphylococcus aureus* strain ATCC 25923 using different doses of magnetic nanoparticles

Factors	*Fe_3O_4/SiO_2 (mg)*			
	0.2	*0.4*	*0.6*	*1*
260/280 ratio	1.74	1.78	1.85	1.82
DNA concentration (ng/µL)	20.72	48.18	60.11	107.37

Table 2 Genomic DNA extracted from *Staphylococcus aureus* strain ATCC 25923 using different percentages of polyethylene glycol (PEG)

Factors	*PEG (w/w %)*					
	20	*40*	*50*	*60*	*70*	*80*
260/280 ratio	1.71	1.83	1.78	1.81	1.85	1.85
DNA concentration (ng/µL)	17.6	52.9	53.1	82.4	73.6	23.8