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Chitosan-based Magnetic Iron Oxide Nanohybrids Enabling pH-Controlled Adsorption and Release of DNA

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Abstract

Efficient recovery of high-quality DNA is critical for molecular diagnostics, yet conventional extraction methods remain labor-intensive, time-consuming, and frequently affected by inhibitory substances. Herein, a pH-responsive magnetic DNA isolation platform was developed using chitosan-coated iron oxide nanoparticles (IONP@Chitosan). The chitosan-coated magnetic nanohybrids were synthesized via co-precipitation followed by surface coating with chitosan and characterized using FTIR, SEM-EDS, TGA, and DLS, confirming successful surface functionalization. Under acidic conditions, protonated amino groups (NH_3^+) facilitated electrostatic interaction with the negatively charged phosphate backbone of genomic DNA, whereas alkaline conditions enabled efficient DNA desorption. In binding experiments, IONP@Chitosan adsorbed >90% of genomic DNA with ~76% recovery during elution, substantially exceeding the performance of uncoated iron oxide nanoparticles (<40%

adsorption, ~8% recovery). From 1 mL bacterial cultures, the platform yielded 8.5 µg and 7.6 µg of genomic DNA from *Escherichia coli* and *Staphylococcus aureus*, respectively, with acceptable purity. DNA quality and integrity were validated by agarose gel electrophoresis, UV-visible spectroscopy, and successful PCR amplification of the *uidA* and *nuc* genes. Notably, DNA-nanoparticle complexes were directly compatible with PCR, enabling successful amplification without prior elution and supporting the feasibility of simplified molecular workflows. These findings suggest that chitosan-functionalized magnetic nanomaterials may serve as promising chaotropic salt-free platforms for genomic DNA isolation with potential utility in molecular diagnostic applications.

Keywords

Magnetic nanoparticles; Chitosan; DNA isolation; pH-responsive adsorption; PCR-compatible DNA.

1. Introduction

Efficient recovery of high-quality DNA is essential for molecular diagnostics, food safety monitoring, and veterinary surveillance, where rapid and accurate pathogen detection is critical [9], [14]. Molecular assays such as polymerase chain reaction (PCR) require inhibitor free DNA, and their performance depends strongly on the efficiency of the extraction step [16], [24]. Conventional DNA isolation methods, including phenol-chloroform extraction and silica-based purification, involve multiple steps, chaotropic reagents, and repeated centrifugation, limiting their suitability for rapid and field-based applications [19], [21].

Nanomaterial based approaches have emerged as promising alternatives due to their high surface area and tunable surface chemistry. Various systems, including gold nanoparticles, graphene oxide, and mesoporous silica, have been explored for DNA capture [11], [16]. However, many of these systems rely on chaotropic salts, involve complex synthesis, or require specialized instrumentation, restricting their routine applicability [12].

Magnetic iron oxide nanoparticles (IONPs) offer advantages such as superparamagnetism, biocompatibility, and rapid separation under an external magnetic field [8], [13]. Despite these benefits, uncoated IONPs exhibit aggregation, nonspecific interactions, and relatively low DNA binding efficiency, necessitating surface modification to improve performance [17], [7]. Various surface modification strategies have been explored to improve the performance of magnetic nanoparticles in DNA extraction. Silica coatings enhance stability but require chaotropic salts for DNA binding [12], while polyethylene glycol (PEG) improves dispersion

but may reduce electrostatic interactions [25]. Other modifiers such as carboxylated polymers and aminosilanes (e.g., APTES) introduce functional groups but increase synthesis complexity and may raise concerns regarding biocompatibility and cost [19], [8].

Among surface modifiers, chitosan is a biocompatible polysaccharide with protonatable amino groups that enable electrostatic interaction with negatively charged DNA under acidic conditions [23]. Additionally, chitosan improves nanoparticle stability and dispersion, enhancing their applicability in biological systems [8], [13]. Chitosan-coated magnetic nanoparticles have therefore gained attention as effective platforms for DNA extraction, particularly in complex samples [15].

The pH responsive behavior of chitosan enables reversible DNA adsorption under acidic conditions and release under mildly alkaline conditions. However, systematic comparisons between uncoated and chitosan-coated magnetic nanoparticles, particularly for bacterial genomic DNA, remain limited, and the mechanistic role of chitosan properties is not fully understood [10].

In this study, we developed chitosan-coated iron oxide nanoparticles (IONP@Chitosan) as a magnetically assisted platform for bacterial genomic DNA isolation from *Escherichia coli* and *Staphylococcus aureus*. The system was designed to enable DNA adsorption under acidic conditions and controlled release under alkaline conditions, offering a potentially salt-free alternative for genomic DNA isolation.

2. Materials and methods

2.1 Materials

All chemicals and reagents used in this study were of analytical grade and used as received unless otherwise stated. Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) were purchased from Sigma-Aldrich. Low molecular weight chitosan (catalogue no. 448869, batch no. 0000121422, Sigma-Aldrich) with a batch-specific degree of deacetylation of 83% and solution viscosity of 108 cP (1% w/v in 1% acetic acid at 25 °C) was used as received. Sodium chloride (NaCl), sodium hydroxide (NaOH), ethylenediaminetetraacetic acid (EDTA), proteinase K, sodium dodecyl sulfate (SDS), phenol, ethidium bromide (EtBr), Tris base (hydroxymethyl)aminomethane, and sodium acetate were sourced from Sigma-Aldrich. Absolute ethanol was procured from Sigma-Aldrich. Aqueous ammonia was from Thomas Baker, and agar from Oxoid. Acetic acid, hydrochloric acid (HCl), RNase, and 6× DNA loading dye were purchased from Thermo Fisher Scientific. Chloroform: isoamyl alcohol (24:1), isopropanol, Luria-Bertani (LB) medium, and mannitol salt agar

(MSA) were obtained from SRL (India). Eosin methylene blue (EMB) agar, glycine, and nuclease-free water were from HiMedia Laboratories. The PCR kit containing KAPA Taq DNA Polymerase was procured from KAPA Biosystems.

2.2 Methods

2.2.1 Synthesis of iron oxide nanoparticles (IONPs)

Iron oxide nanoparticles were synthesized using a modified chemical co-precipitation method as previously reported with slight modifications [11], [20]. Briefly, Ferric chloride hexahydrate (3.31 g, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and ferrous chloride tetrahydrate (1.21 g, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) were separately dissolved in 10 mL of deionized water under constant stirring at 600 rpm for 10 minutes at room temperature. The two solutions were then combined in a 250 mL conical flask and stirred for an additional 20 minutes to ensure homogeneity. Subsequently, 30 mL of 25% (v/v) aqueous ammonia was added dropwise to the reaction mixture under vigorous stirring at room temperature, maintaining the pH at approximately 10. The reaction was allowed for 30 min, during which the formation of a black precipitate indicated the generation of magnetite (Fe_3O_4) formation. The resulting nanoparticles were magnetically separated using a neodymium bar magnet and washed three to four times with deionized water, dried in a vacuum oven for 36 hours and stored in a vacuum desiccator until further use.

2.2.2 Synthesis of chitosan-coated iron oxide nanoparticles (IONP@Chitosan)

Chitosan-coated iron oxide nanoparticles were prepared by dispersing iron oxide nanoparticles in a chitosan solution under controlled conditions. Briefly, low-molecular-weight chitosan (20 mg) was dissolved in 100 mL of 1 M acetic acid, prepared by diluting 5.76 mL of glacial acetic acid with deionized water. The solution was stirred at 600 rpm at 40 °C for 24 h to ensure complete dissolution of chitosan and subsequently filtered to remove any undissolved particulates. Dried iron oxide nanoparticles (1 g) were then added to the chitosan solution and dispersed under continuous stirring at 600 rpm and 40 °C for 18 h to allow uniform coating. The resulting chitosan-coated iron oxide nanoparticles were magnetically separated using a neodymium bar magnet and washed three to four times with deionized water to remove excess chitosan and residual acetic acid. The purified nanoparticles were dried in a vacuum oven for 48 h and stored in a desiccator until further use. A schematic representation of the nanoparticle synthesis and coating process is shown in **Figure 1**.

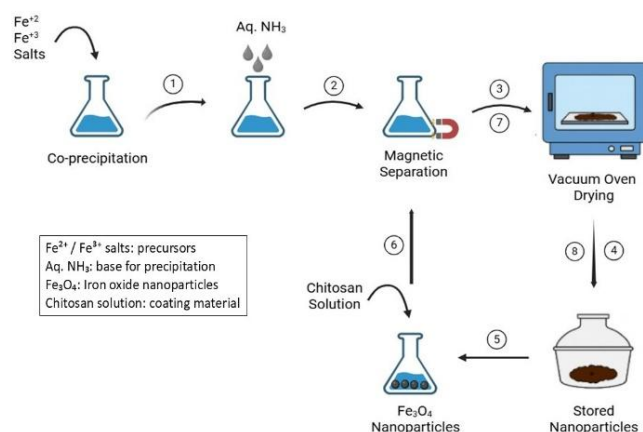


Figure 1. Graphical illustration of the synthesis and functionalization of iron oxide nanoparticles (IONPs). The magnetite (Fe₃O₄) nanoparticles were synthesized by co-precipitating the Fe²⁺ and Fe³⁺ salts in the presence of ammonia and subsequently magnetic separation and drying. Nanoparticles were then dispersed in a solution of chitosan to get the iron oxide nanoparticles coated by chitosan (IONP@Chitosan) to be used in downstream applications.

2.2.3 Physicochemical Characterization of Nanoparticles

The morphology and elemental composition of the synthesized nanoparticles were examined using a field emission scanning electron microscope (FEI Apreo LoVac, USA) equipped with an energy-dispersive X-ray spectroscopy (EDS) detector. Fourier-transform infrared (FTIR) spectroscopy (IR-Affinity 1, Shimadzu, Japan) was used to analyze surface functional groups and to confirm chitosan functionalization on the nanoparticle surface. FTIR spectra recorded in the range of 4000 - 400 cm⁻¹. The hydrodynamic diameter (Z-average), polydispersity index (PDI) and zeta potential of the nanoparticles were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). All measurements were performed in triplicate, and the results are reported as mean ± standard deviation (SD). Thermal stability and the relative organic content associated with chitosan coating were evaluated by thermogravimetric analysis (TGA) using a Shimadzu TGA-50 thermogravimetric analyzer under controlled heating conditions.

2.2.4 DNA Binding: Adsorption and Desorption Studies

Genomic DNA from *Escherichia coli* and *Staphylococcus aureus* was isolated using a modified phenol-chloroform method, as described previously [6]. Frozen bacterial stocks stored at -80 °C were thawed, and 10 µL of each culture was inoculated into 5 mL of Luria Bertani (LB)

broth. Cultures were incubated at 37 °C with shaking at 200 rpm for 12 to 16 h. Overnight cultures were then subsequently sub-cultured by transferring 100 µL into 5 mL of fresh LB broth and incubated at 37 °C for 5 to 6 h.

Bacterial cells were harvested by centrifugation at 5000 rpm for 5 minutes at room temperature. The supernatant was discarded, and the cell pellet was washed three times with NaCl-EDTA buffer (30 mM NaCl, 2 mM EDTA, pH 8.0). The pellet was resuspended in 300 µL NaCl-EDTA buffer supplemented with 500 µL of freshly prepared lysozyme solution (10 mg/mL) and 4 µL of RNase A solution (100 µg/mL), followed by incubation at 37 °C for 1 h with intermittent shaking. Subsequently, 50 µL of 10% sodium dodecyl sulphate (SDS) and 10 µL of proteinase K (20 mg/mL) were added, mixed gently, and incubated at 55 °C for 1 h.

DNA extraction was carried out sequentially using a Tris-saturated phenol (pH 8.0), phenol:chloroform (1:1), and chloroform: isoamyl alcohol (24:1), with centrifugation at 5000 rpm for 10 minutes at each step. The aqueous phase was collected, and genomic DNA was precipitated using two volumes of isopropanol and one-tenth volume of 0.3 M sodium acetate (pH 5.2). The DNA pellet was recovered by centrifugation at 5000 rpm for 10 min at 4 °C, washed with 70% ethanol, air-dried, and finally dissolved in 50 µL nuclease-free water. Nuclease-free water at neutral pH was used to avoid altering pH and ionic strength, which could influence subsequent adsorption or elution behaviour. The isolated DNA was used for adsorption-desorption experiments.

For DNA adsorption-desorption studies, 2 mg of either uncoated iron oxide nanoparticles (IONPs) or chitosan-coated iron oxide nanoparticles (IONP@Chitosan) were transferred into sterile glass tubes and washed three times with 100 µL nuclease-free water. After washing, 100 µL of binding buffer containing glycine-HCl-NaCl (pH 3.2) was added, and the suspension was vortexed for 40 s. Subsequently, 100 µL of isolated genomic DNA was added, and the mixture was gently inverted for 5 min to facilitate DNA adsorption. The nanoparticle-DNA complexes were magnetically separated using a neodymium bar magnet, and the supernatant containing unbound DNA was collected.

The nanoparticle pellet was washed with 100 µL nuclease-free water, vortexed briefly and separated magnetically. For DNA desorption, 200 µL of elution buffer containing Tris-EDTA and sodium chloride (pH 8.5) was added to the nanoparticle pellet followed by incubation at 60 °C with shaking at 250 rpm for 5 min. The eluted DNA was magnetically separated and collected. The schematic representation of the bacterial genomic DNA extraction and adsorption-desorption workflow is shown in **Figure 2**.

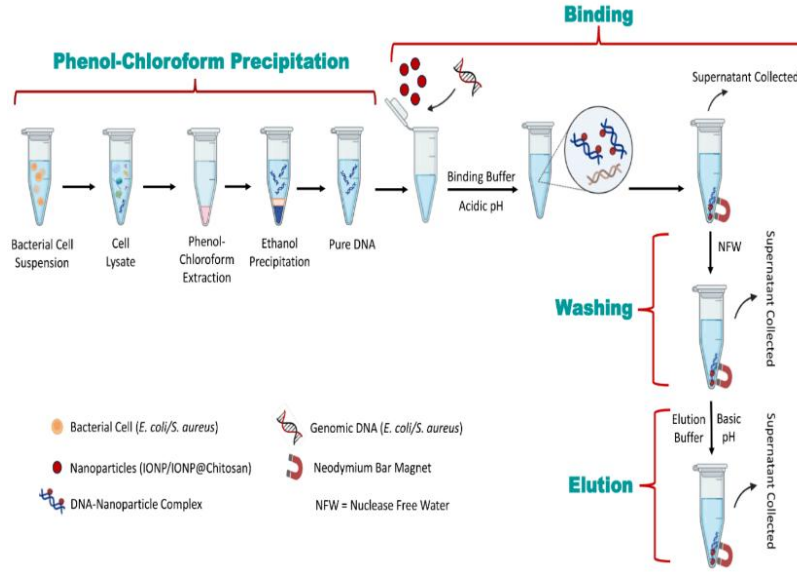


Figure 2. Flow chart of bacterial genomic DNA isolation and subsequent adsorption-desorption investigation of iron oxide nanoparticles (IONP) and chitosan-coated iron oxide nanoparticles (IONP@Chitosan). Purified genomic DNA was left to attach to nanoparticles under acidic conditions, washed to remove unbound DNA and then eluted under basic conditions to determine the efficiency of binding and release DNA.

The adsorption and desorption efficiency of IONP and IONP@Chitosan were calculated using the following equations (Zhou et al., 2012):

$$\text{Adsorption \%} = \left[\frac{C_o - C_f}{C_o} \right] \times 100 \quad (1)$$

The percentage of adsorbed DNA was determined using C_o , the initial DNA concentration, and C_f , the final DNA concentration after nanoparticle interaction, both measured in ng/ μ L.

$$\text{Desorption \%} = \left[\frac{C_f \times V_f}{C_o \times V_o} \right] \times 100 \quad (2)$$

Desorption efficiency was calculated using C_f , the final DNA concentration in the elution fraction, V_f , the elution fraction volume, C_o , the initial DNA concentration in the stock solution and V_o , the initial solution volume.

DNA concentration and purity in all fractions (binding, wash, and elution) were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA)

by measuring absorbance at 260 nm (A_{260}) and $A_{260/280}$ ratio. DNA integrity was further assessed by 0.8 % agarose gel electrophoresis using 5 μ L of each sample.

2.2.5 Magnetic Nanoparticle-Based DNA Extraction from Bacterial Isolates

The DNA extraction efficiency of uncoated iron oxide nanoparticles (IONP) and chitosan-coated iron oxide nanoparticles (IONP@Chitosan) was evaluated using *Escherichia coli* and *Staphylococcus aureus* cultures. Frozen bacterial stocks stored at -80 °C were thawed, and 10 μ L of each stock was inoculated into 5 mL of Luria-Bertani (LB) broth. Cultures were incubated at 37 °C with shaking at 200 rpm for 12-16 h. Subsequently, 100 μ L of the overnight culture was transferred into 1 mL of fresh LB broth and incubated at 37 °C with shaking at 200 rpm for 5-6 h to obtain actively growing cells.

Bacterial cells were harvested by centrifugation at 5000 rpm for 5 min at 25 °C, and the pellets were washed once with 500 μ L NaCl-EDTA buffer by centrifugation under the same conditions. The washed cells were resuspended in 200 μ L lysis buffer containing 10 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, 1% Triton X-100 (pH 8.0). For *S. aureus*, 10 μ L of lysozyme (20 mg/mL) was added, and the suspension was incubated at 37 °C for 10 min. Both *E. coli* and *S. aureus* samples were then treated with 10 μ L of 10% sodium dodecyl sulphate (SDS) and 5 μ L of proteinase K (20 mg/mL), followed by incubation at 56 °C for 10 min to obtain crude cell lysates suitable for nanoparticle-based DNA adsorption.

Nanoparticle-based extraction was performed by adding 2 mg of either IONP or IONP@Chitosan into a clean microcentrifuge tube followed by the addition of 600 μ L of binding buffer containing glycine-HCl-NaCl (pH 3.2). The nanoparticle suspension was vortexed for 60 s to ensure uniform dispersion. The bacterial lysate was then added and mixed gently by inversion for 5 min at room temperature to facilitate DNA adsorption. The nanoparticle-DNA complexes were magnetically separated using a neodymium bar magnet, washed with 200 μ L of 70% ethanol and air-dried for 5 min. DNA was eluted by resuspending the nanoparticle pellet in 50 μ L of elution buffer containing Tris-EDTA and sodium chloride (pH 8.5), followed by incubation at 60 °C for 5 min. After magnetic separation, the supernatant containing purified genomic DNA was collected and used for downstream analyses. A negative control was included by processing nuclease-free water with both IONP and IONP@Chitosan under identical conditions to confirm the absence of contaminating DNA from reagents or nanoparticles. A schematic illustration of bacterial genomic DNA isolation using IONP and IONP@Chitosan is shown in **Figure 3**.

DNA concentration and purity were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) by measuring $A_{260/280}$ and $A_{260/230}$ ratios. DNA integrity was assessed by 0.8 % agarose gel electrophoresis using 5 μ L of each sample.

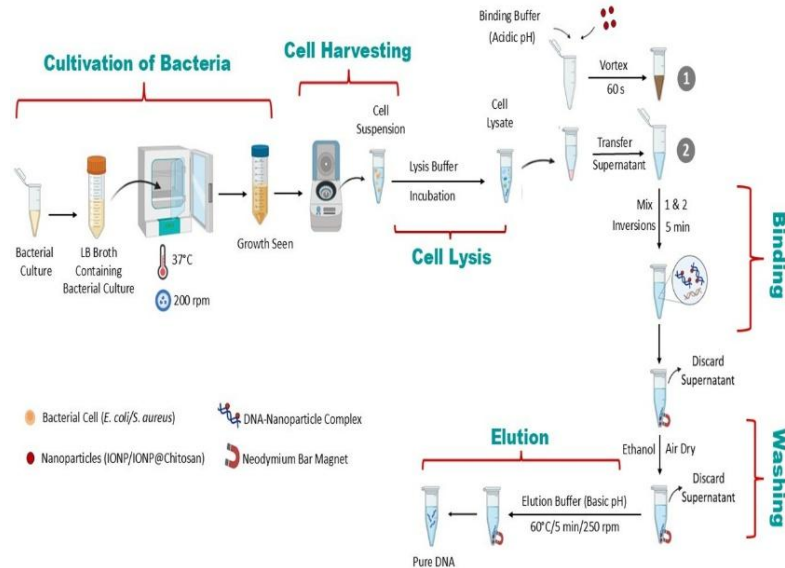


Figure 3. Graphical illustration of magnetic nanoparticle-based genomic DNA isolation procedure with iron oxide nanoparticles (IONP) and chitosan-coated iron oxide nanoparticles (IONP@Chitosan). Nanoparticles were incubated under acidic conditions with bacterial lysates in order to facilitate DNA binding and then followed washed and separated magnetically. Basic conditions were then used to elute genomic DNA to get purified DNA.

2.2.6 Molecular detection of *E. coli* and *S. aureus* by Polymerase chain reaction (PCR)

Conventional polymerase chain reaction (PCR) was employed to confirm the presence of bacterial genomic DNA by targeting the *uidA* gene specific to *Escherichia coli* and the *nuc* gene specific to *Staphylococcus aureus*. PCR amplification was carried out in a total reaction volume 20 μ L containing 10x PCR buffer (50 mM potassium chloride, 10 mM Tris-HCl, 0.1 percent Triton-X), 1 mM $MgCl_2$, 200 μ M of each deoxynucleotide triphosphate (dNTP), 0.2 μ M of each primer, and 1 U of Taq DNA polymerase. Template DNA isolated using iron oxide nanoparticles (IONPs) or chitosan-coated iron oxide nanoparticles (IONP@Chitosan) was added to each reaction. The primer sequences used for the amplification of *uidA* and *nuc* genes are listed in **Supplementary Information 1**. PCR amplification was performed using the following thermal cycling conditions: an initial denaturation at 95 $^{\circ}$ C for 5 min, followed by 30 cycles of denaturation at 95 $^{\circ}$ C for 15 s, annealing at 60 $^{\circ}$ C for 15 s, and extension at 72 $^{\circ}$ C for 15 s. A final extension was carried out at 72 $^{\circ}$ C for 4 min. The amplified PCR products

were analyzed by electrophoresis on a 2 % agarose gel using a 100 base pair DNA ladder as a molecular size marker. Gel images were visualized and documented using a Gel Doc imaging system (Bio-Rad).

3. Results

3.1 Morphological and Chemical Characterization of IONPs and IONP@Chitosan

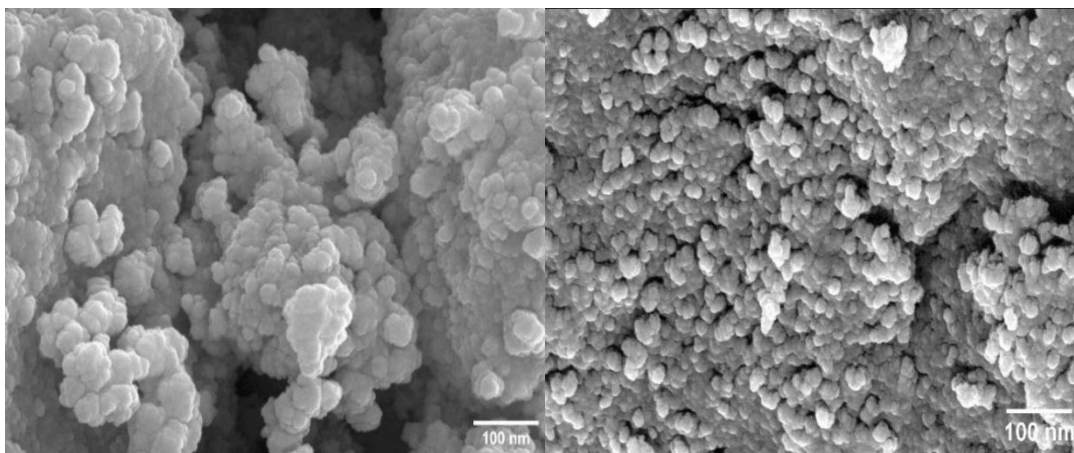
Scanning Electron Microscopy (SEM) was used to examine the morphology and surface features of uncoated iron oxide nanoparticles (IONPs) and chitosan-coated nanoparticles (IONP@Chitosan), as shown in **Figure 4(I)**. Uncoated IONPs appeared as irregular, densely aggregated clusters, consistent with magnetic dipole interactions and lack of surface stabilization, with an average diameter of 21.0 ± 5.2 nm ($n = 100$). In contrast, IONP@Chitosan showed more dispersed and discrete particles with a reduced mean diameter of 15.6 ± 3.9 nm ($n = 100$). This apparent size reduction reflects improved dispersion and decreased agglomeration following chitosan functionalization. The coating provides steric stabilization and surface charge modulation, resulting in enhanced colloidal stability and availability of surface amino groups for pH-responsive DNA adsorption and release.

Energy-dispersive X-ray spectroscopy (EDS) was used to analyze the elemental composition of the nanoparticles, with representative spectra shown in **Figure 4(II)** and quantitative data summarized in **Supplementary Information 2 and 3**. Uncoated IONPs primarily contained iron, oxygen, and carbon, with iron contributing 63.78 wt%. The carbon signal likely arises from residual organic species or sample preparation. In contrast, IONP@Chitosan exhibited increased carbon content and a distinct nitrogen peak (0.70 wt%), absent in uncoated IONPs. The presence of nitrogen confirms successful chitosan functionalization, corresponding to amino groups in the polysaccharide backbone. This coating contributes to surface charge modulation and supports the observed pH-responsive DNA adsorption and release behavior.

Fourier-Transform Infrared (FTIR) spectra of chitosan, IONPs and IONP@Chitosan are presented in **Figure 4(III)**. Chitosan exhibited characteristic polysaccharide bands, including O-H/N-H stretching ($3200\text{-}3500\text{ cm}^{-1}$), C-H stretching, amide II/N-H bending, and C-O-C/C-O vibrations, confirming its glucosamine backbone. Uncoated IONPs showed Fe-O stretching bands at 656 and 550 cm^{-1} , indicative of magnetite formation. The FTIR spectrum of IONP@Chitosan displayed features of both iron oxide and chitosan, with slight shifts in the O-H/N-H region, suggesting hydrogen bonding and interfacial interactions between chitosan and the nanoparticle surface. These results confirm successful surface functionalization and the

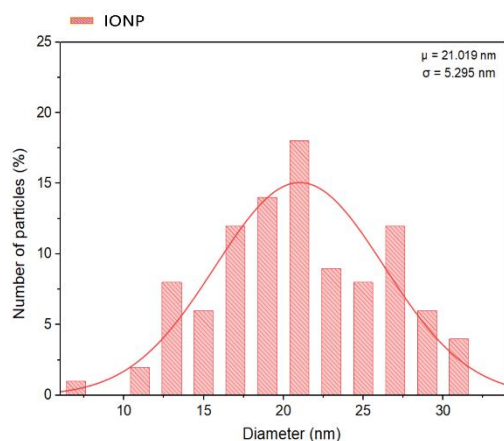
presence of protonable amino groups responsible for pH-responsive DNA adsorption and release.

I

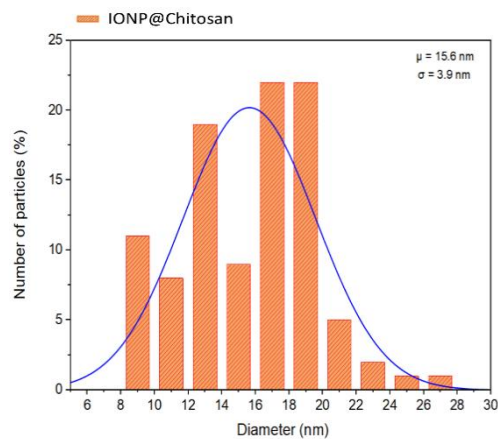


(a)

(b)

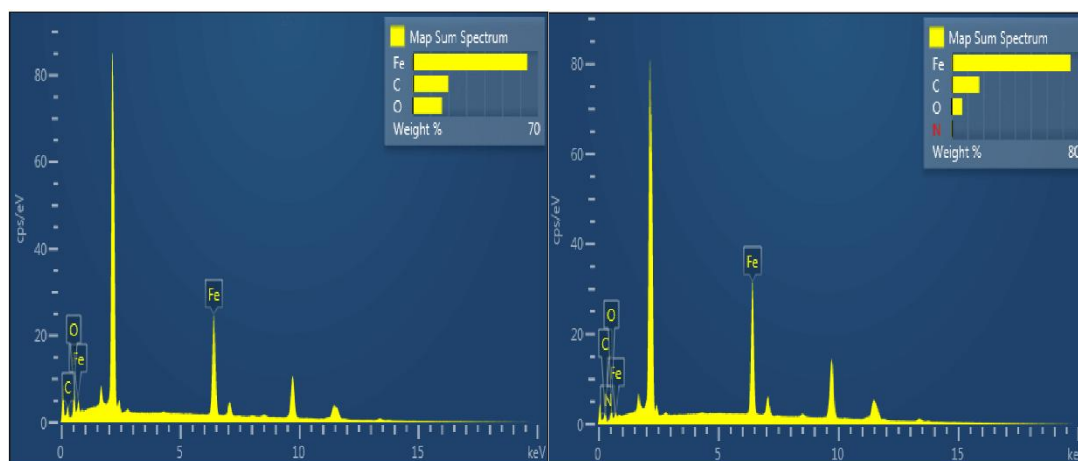


(c)



(d)

II



(a)

(b)

III

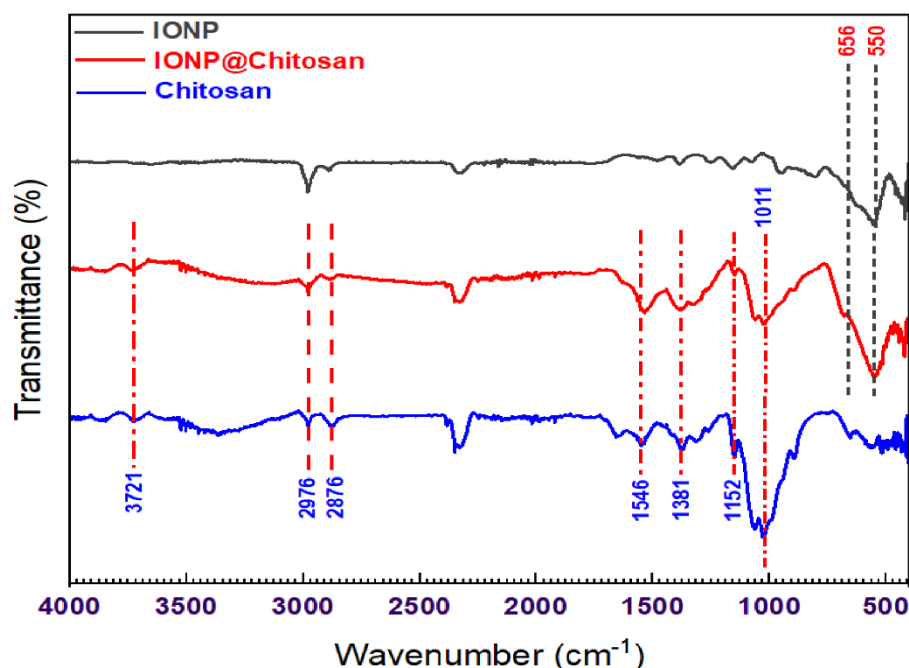


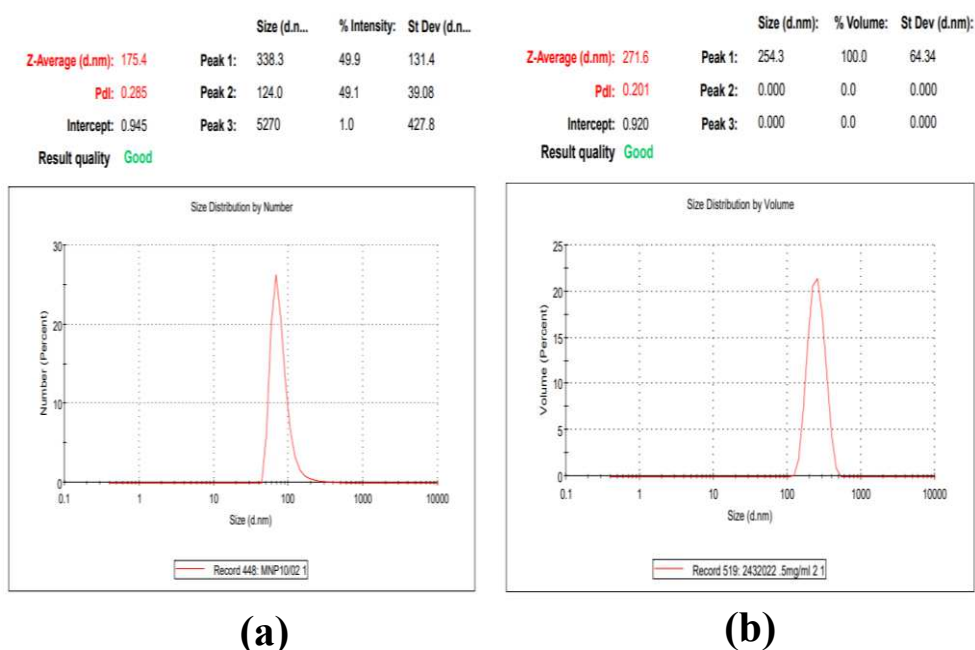
Figure 4. (I) SEM images of **(a)** uncoated iron oxide nanoparticles (IONPs) showing aggregation and **(b)** chitosan-coated nanoparticles (IONP@Chitosan) showing improved dispersion; **(c-d)** corresponding particle size distributions ($n = 100$) with mean diameters of 21.0 ± 5.2 nm and 15.6 ± 3.9 nm, respectively. **(II)** EDS spectra of **(a)** IONPs and **(b)** IONP@Chitosan indicating nitrogen presence in coated nanoparticles (**Supplementary Information 2 and 3**). **(III)** FTIR spectra of chitosan, IONP, and IONP@Chitosan confirming surface functionalization.

Dynamic light scattering (DLS) analysis was used to evaluate the hydrodynamic size and dispersion of the nanoparticles. Uncoated IONPs showed a Z-average diameter of 175.9 ± 7.0 nm ($n = 3$) with a PdI of 0.324 ± 0.040 , indicating moderate size heterogeneity and aggregation, with multiple size populations observed as shown in **Figure 5(I-a)**. In contrast, IONP@Chitosan exhibited a larger hydrodynamic diameter of 283.3 ± 10.2 nm ($n = 3$) with a PdI of 0.275 ± 0.064 , reflecting improved dispersion after surface modification as shown in **Figure 5(I-b)**. Detailed DLS measurements, including individual replicate values and corresponding mean $\pm$ SD for Z-average diameter and PDI, are provided in **Supplementary information 4**. The increase in size is attributed to chitosan coating and the associated hydration layer. Reduced aggregation in the coated nanoparticles suggests enhanced colloidal stability due to steric stabilization and surface charge modulation, which supports efficient pH-responsive DNA adsorption and release.

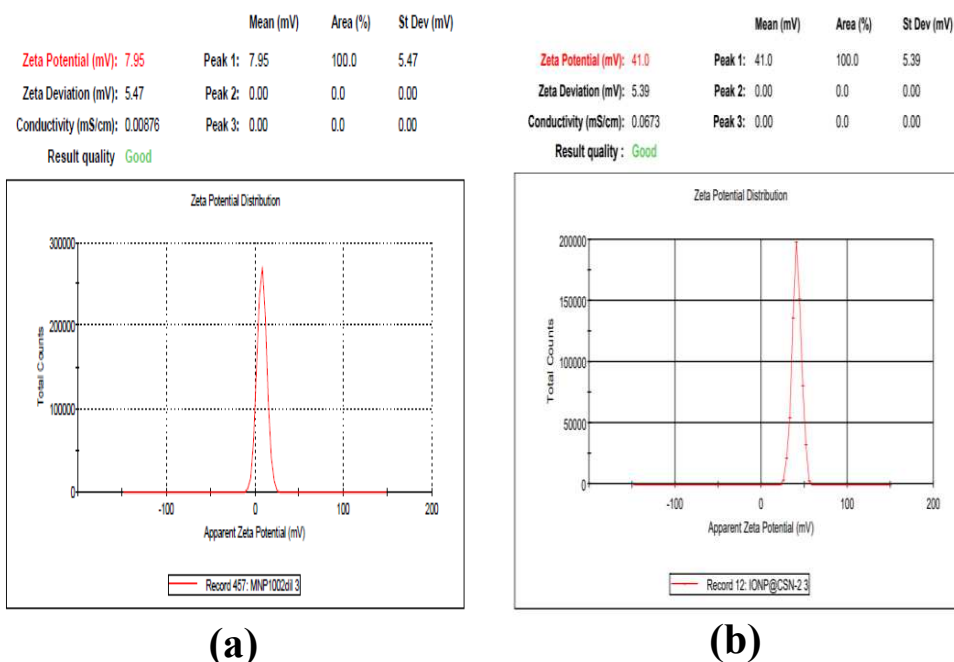
Zeta potential measurements were performed to evaluate surface charge characteristics and colloidal stability as shown in **Figure 5(II)**. Uncoated IONPs showed a low positive zeta potential of 7.51 ± 1.15 mV ($n = 3$), indicating limited electrostatic stabilization and a tendency toward aggregation. In contrast, IONP@Chitosan exhibited a substantially higher zeta potential of 38.9 ± 1.82 mV ($n = 3$), consistent with enhanced colloidal stability. Detailed zeta potential measurements, including individual replicate values and corresponding mean $\pm$ SD, are provided in **Supplementary information 5**. The increased positive surface charge is attributed to protonation of amino groups ($-\text{NH}_3^+$) in chitosan, enabling strong electrostatic interaction with negatively charged DNA. This also supports the pH-responsive adsorption and release behavior of the nanohybrid system.

Thermogravimetric analysis (TGA) was performed to evaluate the thermal stability and organic content of the nanoparticles as shown in **Figure 5(III)**. Uncoated IONPs showed a total weight loss of $\sim 12\%$ up to 900°C , with initial loss below 150°C attributed to adsorbed moisture, confirming the thermal stability of the iron oxide core. In contrast, IONP@Chitosan exhibited a higher weight loss of $\sim 30\%$, with a major reduction between 200 - 500°C corresponding to degradation of the chitosan coating. This additional mass loss confirms the presence of an organic polysaccharide layer and successful surface functionalization.

I



II



III

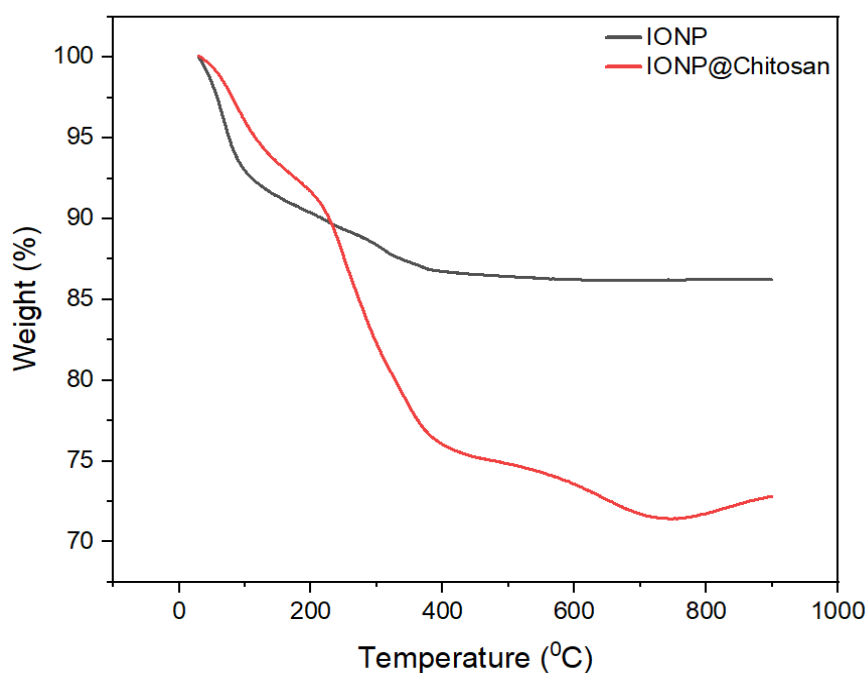


Figure 5. I. Dynamic light scattering (DLS) analysis of (a) IONP and (b) IONP@Chitosan showing hydrodynamic size distributions in aqueous suspension. Uncoated IONPs exhibited a Z-average diameter of 175.9 ± 7.0 nm ($n = 3$), whereas IONP@Chitosan displayed an increased Z-average diameter of 283.3 ± 10.2 nm ($n = 3$). **II. Zeta potential** distributions of (a) IONPs and (b) IONP@Chitosan. Bare IONPs exhibited a zeta potential of 7.51 ± 1.15 mV ($n = 3$), while IONP@Chitosan showed a significantly higher positive surface potential of 38.9 ± 1.82

mV (n = 3). **III. Thermogravimetric analysis (TGA) thermograms** of IONPs (black) and IONP@Chitosan (red), illustrating additional weight loss in the coated sample corresponding to thermal degradation of the chitosan polysaccharide layer.

3.2 Evaluation of DNA Adsorption and Desorption Efficiencies of IONP and IONP@Chitosan Using Bacterial Genomic DNA

The DNA adsorption and desorption performance of uncoated IONPs and IONP@Chitosan was evaluated using genomic DNA from *Escherichia coli* and *Staphylococcus aureus*.

For *E. coli*, uncoated IONPs showed limited performance with 39.4% adsorption and 4.1% desorption (4.1 µg DNA recovered). In contrast, IONP@Chitosan achieved 92.8% adsorption and 76.8% desorption, recovering 38.5 µg of DNA.

A similar trend was observed for *S. aureus*, where uncoated IONPs exhibited 36.3% adsorption and 8.4% desorption (3.6 µg DNA recovered), whereas IONP@Chitosan showed 90.5% adsorption and 75.9% desorption, yielding 31.5 µg of DNA.

The improved performance of IONP@Chitosan is attributed to the presence of protonable amino groups in chitosan, which promote electrostatic interactions with DNA under acidic conditions and facilitate its release under mildly alkaline conditions.

These results were further supported by agarose gel electrophoresis as shown in **Figure 6**, where strong DNA bands were observed for IONP@Chitosan (**lane e2**), compared with the faint bands obtained for uncoated IONPs (**lane e1**), consistent with the quantitative data presented in **Supplementary Information 6**.

DNA concentrations measured at different stages of the adsorption-desorption process are provided in **Supplementary Information 7**, further confirming the higher recovery achieved with IONP@Chitosan. In addition, UV absorbance profiles at 260 nm provided in **Supplementary Information 8 and 9** further support the enhanced DNA binding and release performance of IONP@Chitosan compared with uncoated IONPs for both bacterial species. Negative values observed in wash fractions are attributed to NanoDrop baseline artifacts and indicate DNA concentrations below the detection limits.

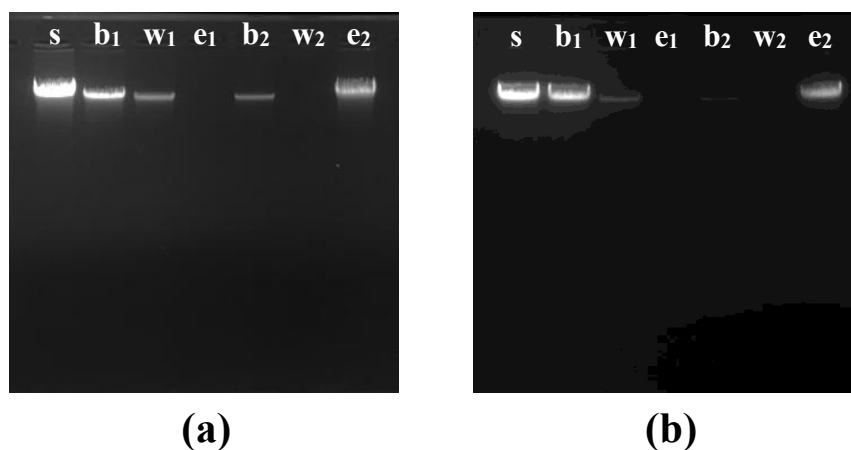


Figure 6. Agarose gel electrophoresis analysis of genomic DNA adsorption and desorption using (a) *E. coli* and (b) *S. aureus* DNA with uncoated iron oxide nanoparticles (IONPs) and chitosan-coated iron oxide nanoparticles (IONP@Chitosan). Lanes represent stock DNA (s), DNA remaining after adsorption (b₁, b₂), washing fractions (w₁, w₂), and elution fractions (e₁, e₂). The full-length uncropped gel images corresponding to **Figure 6(a) and **Figure 6(b)** are provided in **Supplementary information 14(a-b)**, respectively.**

3.3 Genomic DNA Isolation Using Nanoparticle-Based Methods

The performance of nanoparticle-based DNA isolation was evaluated using uncoated IONPs and IONP@Chitosan for *Escherichia coli* and *Staphylococcus aureus*. IONP@Chitosan consistently showed higher DNA yield and improved purity compared with uncoated IONPs, suggesting that chitosan functionalization contributed to improved DNA extraction performance.

Uncoated IONPs yielded low DNA concentrations (14.3 ng/μL for *E. coli* and 22.2 ng/μL for *S. aureus*) with variable purity ratios ($A_{260/280} = 1.46-2.70$; $A_{260/230} = 1.10-4.70$). In contrast, IONP@Chitosan yielded substantially higher DNA yields (170.9 ng/μL and 153.6 ng/μL) with purity ratios ($A_{260/280} = 1.86-1.95$; $A_{260/230} = 2.70$) consistent with good-quality genomic DNA. Detailed DNA concentration and purity measurements are provided in **Supplementary Information 10**.

Agarose gel electrophoresis results presented in **Figure 7** showed strong DNA bands for IONP@Chitosan (**lanes E₂ and S₂**) compared with faint bands obtained for uncoated IONPs (**lanes E₁ and S₁**), consistent with the quantitative data summarized in **Supplementary Information 10**.

UV-Visible spectroscopic analysis presented in **Supplementary Information 11** showed higher absorbance at 260 nm for DNA isolated using IONP@Chitosan than for DNA isolated

using uncoated IONPs in both bacterial species. Negative controls showed no detectable DNA, indicating the absence of contamination.

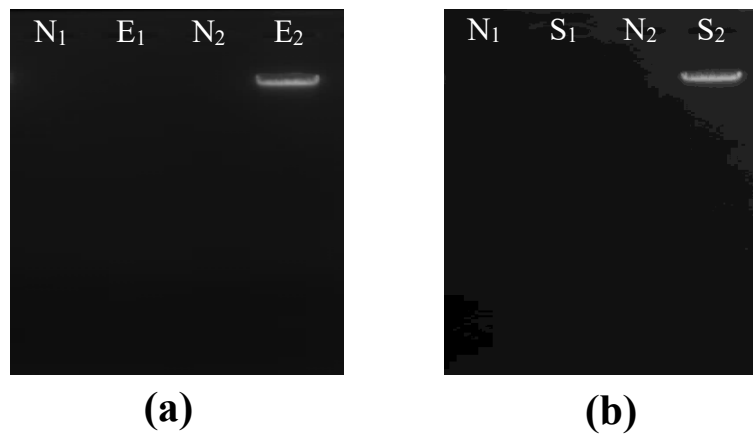


Figure 7. Agarose gel electrophoresis showing genomic DNA isolated from (a) *Escherichia coli* and (b) *Staphylococcus aureus* using uncoated iron oxide nanoparticles (IONPs) and chitosan-coated iron oxide nanoparticles (IONP@Chitosan). Lanes N₁ and N₂ represent negative controls. Lanes E₁ and S₁ correspond to DNA isolated using uncoated IONPs, whereas lanes E₂ and S₂ represent DNA isolated using IONP@Chitosan. The full-length uncropped gel images corresponding to **Figure 7(a) and **Figure 7(b)** are provided in **Supplementary information 14(c-d)**, respectively.**

3.4 DNA Quality and Amplification Using Polymerase Chain Reaction (PCR)

The quality of genomic DNA isolated using IONP@Chitosan was evaluated by PCR targeting species-specific markers for *Escherichia coli* and *Staphylococcus aureus*.

For *E. coli*, amplification of the uidA gene yielded a clear 200 bp product (**Figure 8-I**). Strong amplification was observed in samples isolated using IONP@Chitosan (E₂) and in DNA-nanoparticle complexes directly used for PCR (C₂), while no amplification was detected in negative controls (N₁, N₂).

Similarly, for *S. aureus*, amplification of the nuc gene yielded a specific 516 bp product (**Figure 8-II**). Clear bands were observed in IONP@Chitosan samples (S₂) and corresponding DNA-nanoparticle complexes (C₂), while no amplification was detected in the negative controls.

These findings indicate that DNA isolated using IONP@Chitosan was of sufficient quality for successful PCR amplification and suggest that the nanoparticle-based isolation method did not introduce detectable PCR inhibition under the conditions evaluated in this study.

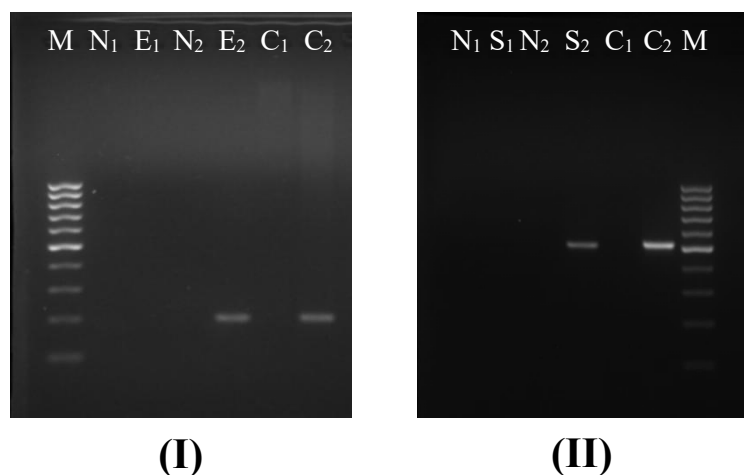


Figure 8. Agarose gel electrophoresis of PCR amplification products obtained from genomic DNA isolated using IONP and IONP@Chitosan. (I) Amplification of the *uidA* gene (200 bp) from *Escherichia coli*; **(II)** Amplification of the *nuc* gene (516 bp) from *Staphylococcus aureus*. Lane **M**: 100 bp DNA ladder; **N₁-N₂**: negative controls; **E₁/S₁**: IONP; **E₂/S₂**: IONP@Chitosan; **C₁-C₂**: DNA-nanoparticle complexes. Clear amplicons in IONP@Chitosan samples confirm PCR compatibility. The uncropped gel image corresponding to the PCR amplification results is provided in Supplementary Figure 17. The full-length uncropped gel images corresponding to **Figure 8(I)** and **Figure 8(II)** are provided in **Supplementary information 14(e-f)**, respectively.

Full-length uncropped agarose gel images corresponding to **Figures 6-8** are provided in the **Supplementary Information 14(a-f)**.

3.5 Discussion

In this study, chitosan-coated iron oxide nanoparticles (IONP@Chitosan) were developed as a magnetically assisted platform for bacterial genomic DNA isolation and evaluated against uncoated IONPs. Chitosan functionalization improved nanoparticle dispersion, surface charge, and DNA recovery, suggesting the potential utility of carbohydrate-based coatings in magnetic bioseparation systems.

Physicochemical characterization confirmed successful coating of iron oxide nanoparticles with chitosan. SEM and DLS analyses indicated reduced aggregation and improved colloidal stability following chitosan functionalization, while EDS and FTIR analysis verified the presence of nitrogen-containing functional groups and characteristic polysaccharide features. TGA further confirmed the incorporation of an organic coating layer without substantially

affecting the thermal stability of the iron oxide core. Collectively, these findings indicate that chitosan modifies the surface properties of iron oxide nanoparticles and may enhance their interaction with biomolecules.

Adsorption-desorption studies revealed limited DNA recovery using uncoated IONPs (<40% adsorption and <10% desorption), which is consistent with previous reports describing the relatively low affinity of bare iron oxide surfaces for nucleic acids [4], [26], [3]. In contrast, IONP@Chitosan achieved >90% adsorption DNA adsorption and ~76% desorption for both *Escherichia coli* and *Staphylococcus aureus* DNA adsorption.

The enhanced DNA recovery observed with IONP@Chitosan is likely associated with the presence of protonable amino groups in chitosan. Under acidic conditions (pH 3-4), protonation of amino groups promotes electrostatic interactions between the positively charged nanoparticle surface and the negatively charged phosphate backbone of DNA. Under mildly acidic conditions (pH 8-9), partial deprotonation reduces electrostatic attraction and facilitates DNA release [2], [8]. Hydrogen bonding between chitosan and DNA may also contribute to adsorption, although the present study did not directly investigate the relative contribution of individual interaction mechanisms [23], [17].

A schematic illustration of chitosan functionalization and DNA interaction is presented in **Supplementary Information 12**, highlighting the proposed electrostatic interaction between protonated amino groups and the DNA phosphate backbone.

Application of the developed system to bacterial cultures demonstrated that IONP@Chitosan yielded higher DNA concentration and improved purity compared to uncoated IONPs. Purity ratios ($A_{260/280} \approx 1.9$) and the presence of intact genomic DNA bands were consistent with the isolation of good-quality DNA, whereas uncoated IONPs produced lower DNA yields and more variable purity values. UV-visible spectroscopic analysis further supported improved DNA recovery using IONP@Chitosan. In addition, successful PCR amplification of the *uidA* and *nuc* genes indicated that the isolated DNA was suitable for the downstream molecular analysis evaluated in this study, while the absence of amplification in negative controls suggested that contamination was not detected under the experimental conditions employed.

Compared with conventional phenol-chloroform extraction, the IONP@Chitosan approach reduces processing steps, avoids the use of hazardous organic solvents, and enables magnetic separation of DNA-bound nanoparticles. Unlike silica-based systems, the developed method operates without chaotropic salts, which may offer practical advantages for cost-effective DNA isolation in laboratory settings.

Comparison with previously reported magnetic DNA adsorption systems, as presented in **Supplementary Information 13** indicates that IONP@Chitosan achieved comparable DNA adsorption and desorption efficiencies under relatively mild operating conditions and short incubation times. The observed performance may be associated with the increased surface charge and improved colloidal stability imparted by chitosan coating, which can increase the availability of binding sites for DNA interaction. Similar observations have been reported for other polymer-functionalized magnetic nanoparticle systems [5], [1].

The present study has several limitations. The performance of the developed nanoparticle system was evaluated using genomic DNA from laboratory cultures of *E. coli* and *S. aureus* under controlled experimental conditions. Its applicability to a broader range of bacterial species and more complex biological samples, remains to be investigated. Furthermore, long-term storage stability, batch-to-batch reproducibility, and scale-up production of IONP@Chitosan were not evaluated in the present study. Additional studies are therefore required to assess the robustness and practical applicability of the system under diverse sample conditions.

Overall, the results suggest that IONP@Chitosan represents a promising salt-free approach for bacterial genomic DNA isolation. The combination of magnetic separation and pH-responsive chitosan chemistry enabled efficient DNA capture and release while producing DNA suitable for PCR amplification under the conditions evaluated in this study.

3.6 Conclusion

Chitosan-coated iron oxide nanoparticles (IONP@Chitosan) were developed as a magnetically assisted, chaotropic salt-free platform for bacterial genomic DNA isolation. Surface functionalization improved DNA adsorption, recovery, and purity compared with uncoated nanoparticles, yielding DNA suitable for PCR amplification from *Escherichia coli* and *Staphylococcus aureus*. The observed performance is likely associated with the presence of protonable amino groups in chitosan, which facilitate pH-responsive and reversible electrostatic interactions with DNA. The simple workflow, rapid magnetic separation, and compatibility with PCR suggest that this system may represent a promising approach for bacterial genomic DNA isolation. Further studies are required to evaluate its performance across a wider range of bacterial species and complex sample matrices, as well as its scalability for practical applications.

Data Availability

The data supporting the findings of this study are available within the article and its Supplementary Information files. Additional data are available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethics Statement

This study did not involve human participants or animal experimentation. All bacterial strains used were handled in accordance with standard laboratory biosafety guidelines.

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