Simple and eco-friendly magnetite preparation, its characterization and DNA extraction application

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Abstract
Magnetite nanoparticles in this study were synthesized using a simple and eco-friendly method. The synthesized particles were characterized using a Scanning electron microscope (SEM), Energy dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD) and Vibrating sample magnetometry (VSM). The ability of the particles to interact with DNA was evaluated using Infrared spectrophotometry (IR) and tested in DNA extraction procedures for several microbial species. Results showed that synthesized magnetite exhibited an agglomerated structure with nanoscale dimensions. Fe and O elements, detected through EDS analysis, confirmed the formation of iron oxide during the synthesis process. Magnetite characteristics were demonstrated in the XRD analysis, with the highest intensity at 2θ value 35.45°, interplanar spacing 2.530 Å and crystallite size of 17.422 nm.

The magnetic property of the synthesized magnetite was identified as ferromagnetic. The interaction between the magnetite and DNA was evident at 944.63, 1103.79, 1124.97 and 1161.37 cm^{-1} in the IR spectra. The produced magnetite was successfully applied for microbial DNA extraction from a laboratory consortium including E. coli, P. aeruginosa, S. aureus, S. paratyphi and M. tuberculosis. All extracted DNA samples were successfully amplified using a 16S rRNA primer pair. This demonstrated ability to extract microbial DNA makes the synthesized magnetite suitable for environmental monitoring applications including microbial identification and contamination detection.

Keywords: Bacteria, DNA Extraction, Iron oxide, Magnetic, Magnetite.

Introduction

Magnetite has many uses in various fields of science and technology. Magnetite can be used as an environmental remediation agent[4,8] as well as a material for DNA extraction[5]. Therefore, its synthesis and preparation methods have become a widely explored topic among researchers.

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In general, coprecipitation is the simplest method for magnetite preparation. It involves mixing aqueous iron salt (Fe^{2+} and Fe^{3+}) solutions under basic conditions, in an inert atmosphere up to temperature of 150°C[1,2,6,11]. The purpose of this research is to produce magnetite with a simple method under ambient conditions without the the use of inert gas. The synthesized magnetite was further analyzed using SEM, EDS, XRD and VSM. Its interaction with DNA was examined using IR spectrophotometry and its ability to extract microbial DNA was also evaluated.

Material and Methods

Material: $FeSO_4$, $FeCl_3$, Guanidine thiocyanate, Tris, EDTA, NaOH (Merck), Isopropanol and Ethanol (Bratachem); Triton-X (Vivantis), SDS (BASF), PEG 6000 (Clariant) and NaCl (Dominion Salt) were used. The microbial cultures used were *Eschericia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella paratyphi* ATCC 9150, *Mycobacterium tuberculosis* ATCC 25177 (Microbiologics). Mastermix (KODC), 27F forward primer and 907R reverse primer were used as the primers pair for 16S rRNA.

Magnetite Synthesis and Characterization: $FeSO_4$ and $FeCl_3$ with a molarity ratio of 1:2 were mixed in 100 mL water. While stirring, a 3 M NaOH solution was added dropwise until the pH of the mixture reached 8-10. The solution was then allowed to settle and the supernatant was decanted. The precipitate was washed with water twice, then dried in an oven at 60 - 100 °C for two hours. The dried magnetite was used for characterization. Appearance, morphology and size were analyzed using SEM. Elemental detection was performed using EDS in conjunction with SEM. Crystal structure analysis was conducted using XRD. Measurement of magnetite's magnetic properties was carried out using VSM.

DNA Interaction and Extraction: The interaction between magnetite and DNA was analyzed using IR spectrophotometry. The analysis was performed in the range of 200 to 4000 cm^{-1} on magnetite, DNA solution, a mixture of DNA and magnetite in binding buffer and a mixture of DNA and magnetite in elution buffer. DNA extraction was carried out following the general procedure which involved lysing bacterial cells with 10 mM Tris, 1 mM EDTA, 0.6% SDS and 0.2% Triton X. The lysate was then mixed with magnetite with 200 µl of binding buffer for about 3 minutes. Magnetite and supernatant were separated using a magnetic table for 3 minutes and the supernatant was decanted. The

next step was involved washing with 70% ethanol three times, followed by elution using elution buffer. Each eluted DNA sample was visualized via gel electrophoresis and amplified using primer pairs 27F and 907R, according to the protocol[9].

Results and Discussion

Magnetite nanoparticles have potential applications in various fields of science. Their easy preparation and the fact that they do not require high temperatures, make them energy-efficient. Preparing them under ambient conditions is particularly beneficial. The appearance, shape and size of the resulting magnetite are shown in figure 1. The magnetite obtained tended to aggregate, indicating the presence of magnetic dipole-dipole interactions[10]. Magnetite exhibited a spherical shape with a size less than 1 µm, classifying it as nanoscale, although the precise size could not be determined from the SEM images.

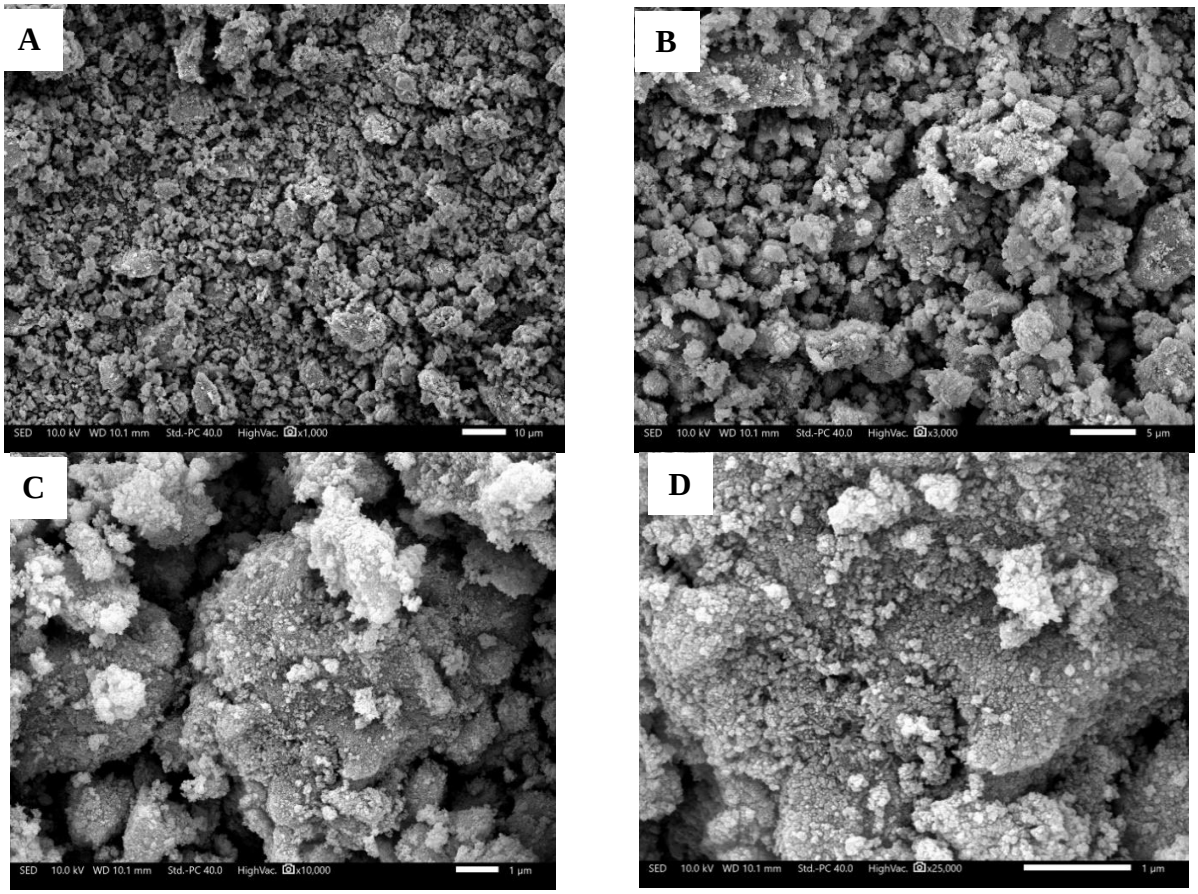
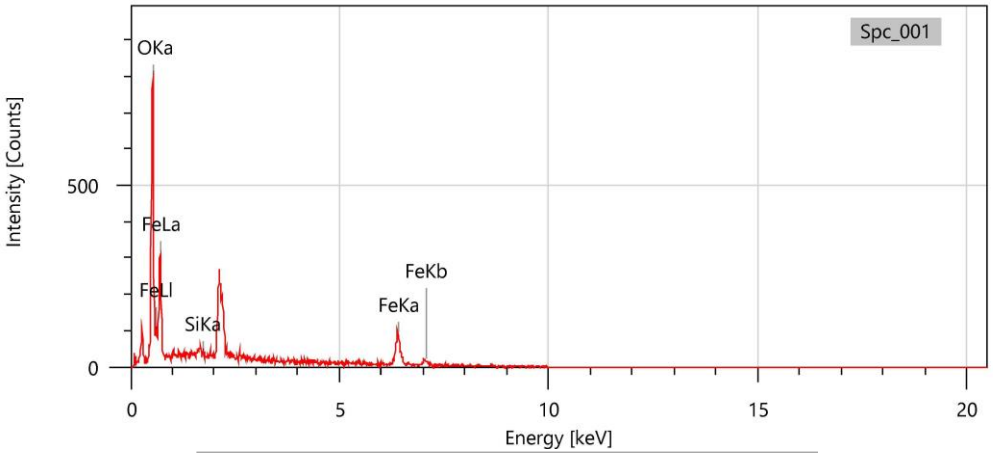


Figure 1: SEM Image of Magnetite. (A) 1000× magnification. (B) 3000× magnification. (C) 10,000× magnification. (D) 30,000× magnification.



Element	Line	Mass%	Atom%
O	K	20.00±0.27	46.59±0.64
Si	K	nd	nd
Fe	K	80.00±2.92	53.41±1.95
Total		100.00	100.00

Figure 2: EDS Analysis of the Produced Magnetite.

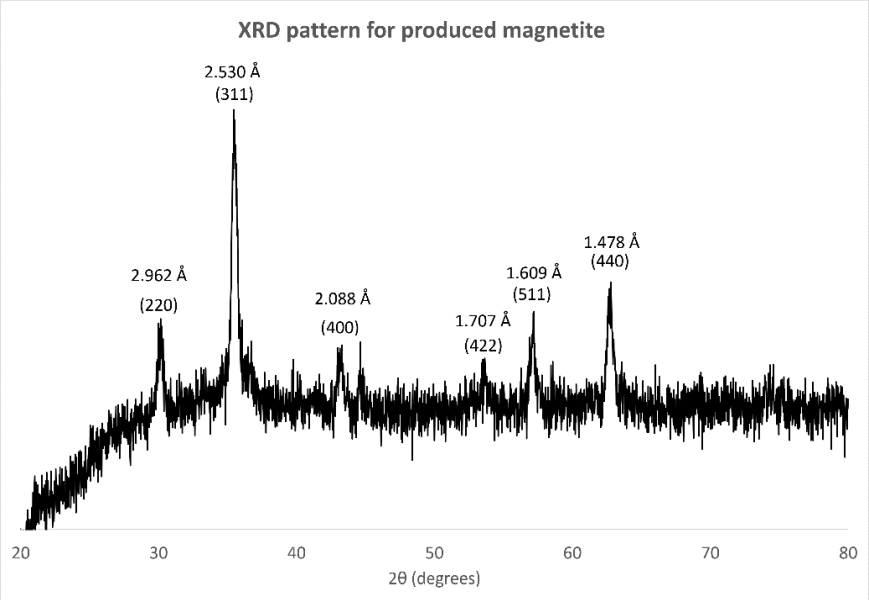


Figure 3: XRD Pattern of the Produced Magnetite.

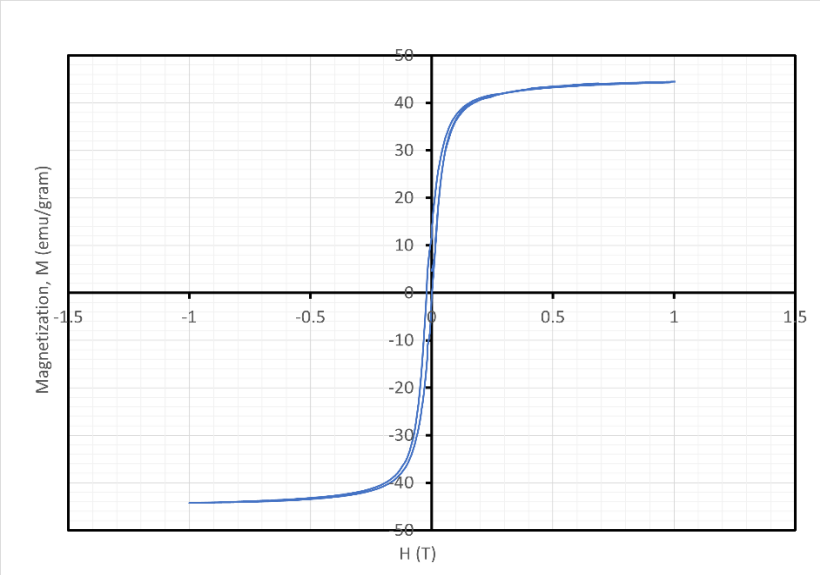


Figure 4: VSM Graph of the Produced Magnetite.

Elemental composition of the magnetite is presented in figure 2, based on EDS analysis. Magnetite, or iron oxide, has a chemical formula Fe_3O_4. The EDS analysis revealed that only two elements were detected, oxygen (O) and iron (Fe), with their respective mass percentages being 20.00 ± 0.27% and 80.00 ± 2.92 %.

XRD pattern was recorded using CuKα radiation at 1.541 Å. As shown in figure 3, the XRD analysis revealed six characteristic peaks at 30.15º, 35.45º, 43.29º, 53.65º, 57.21º and 62.81º corresponding to Fe_3O_4 crystal planes at (220), (311), (400), (422), (511) and (440) respectively. The XRD pattern showed the highest intensity at 2θ: 35.45º and the d-value, calculated using Bragg's law, was found to be 2.53 Å. Crystallite size, calculated using Scherrer's law with a shape factor (K) of 0.94 for a spherical crystal in a cubic system, yielded a crystallite size of 17.422 nm for the magnetite. VSM graph, shown in figure 4, indicates that the produced magnetite had a saturation magnetization of 44.442 emu/gram. The magnetization curve exhibited a very small hysteresis loop, with a coercivity (H_c) value of 0.021 T and a magnetization retentivity (M_r) value at 11.762 emu/gram. The produced magnetite retained residual magnetism after the removal of the magnetic field, indicating that it is ferromagnetic. This ferromagnetic property explains the aggregation observed in the SEM image in figure 1.

The interaction between DNA and magnetite was analyzed using IR spectrophotometry. The analysis was conducted by comparing the spectra of DNA solution, magnetite, magnetite + DNA in binding buffer and magnetite + DNA in elution buffer. As shown in figure 5, the interaction between DNA and magnetite was observed, indicated by vibration peaks at wavenumbers 944.63, 1103.79, 1124.97 and 1161.37 cm^{-1}.

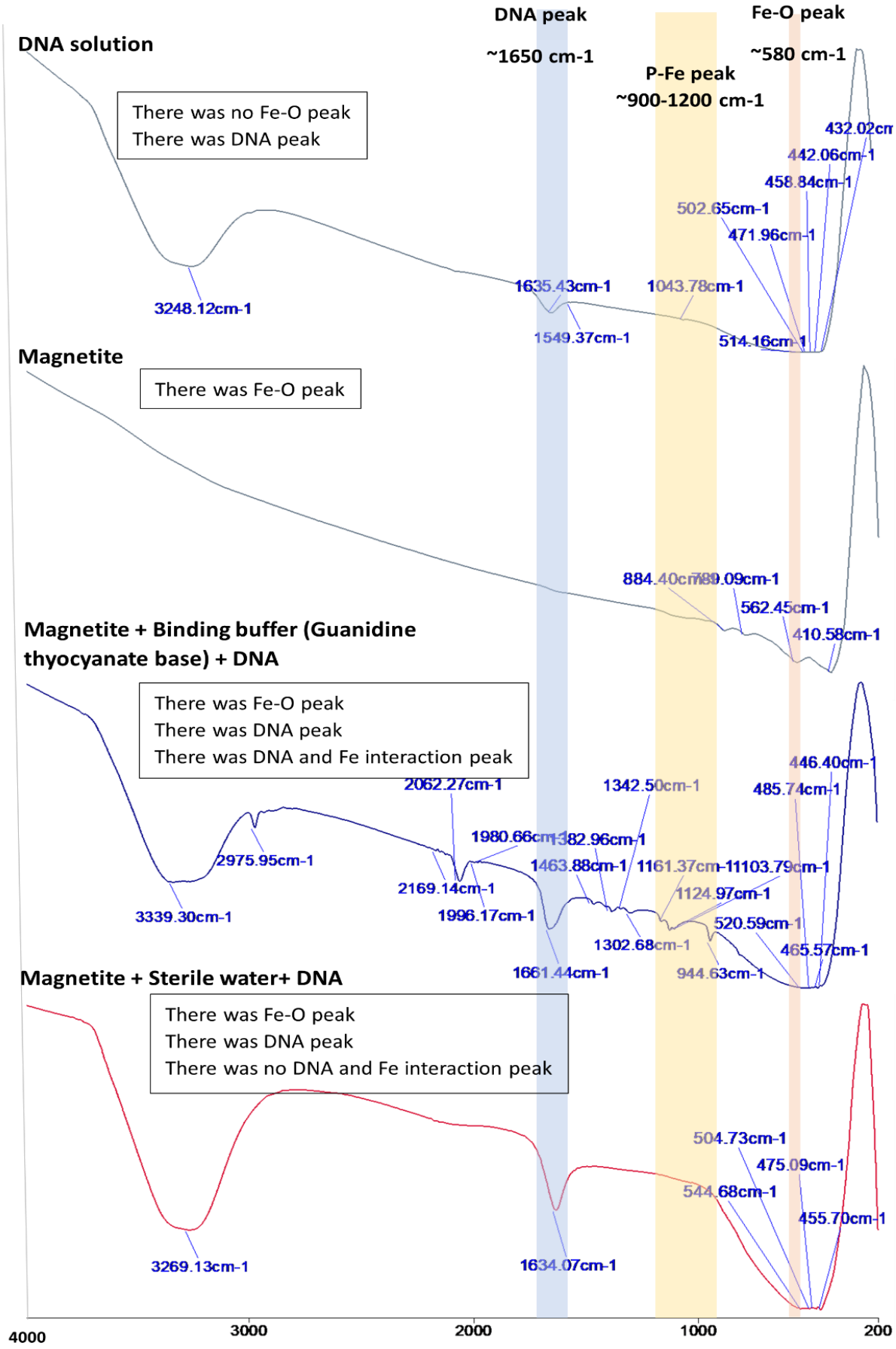


Figure 5: IR Spectra for DNA solution, Magnetite, Magnetite + DNA in Binding Buffer and Magnetite + DNA in Sterile Water (Elution Buffer).

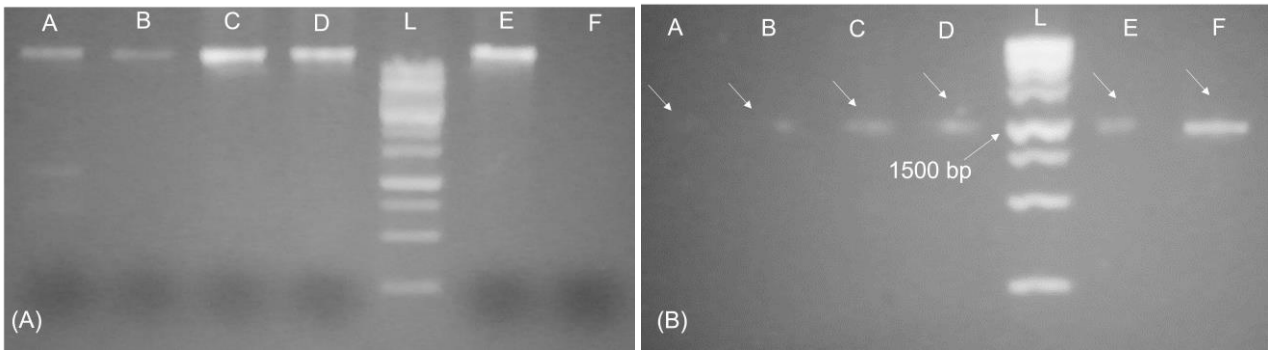


Figure 6: Electrophoresis visualization of (A) extracted DNA from several microbial cultures. (B) PCR amplification results using 27F and 907R 16S primers, with the amplicon size around 1500 bp. A). Environmental bacteria; B). *E. coli*; C). *P. aeruginosa*; D). *S. aureus*; E). *S. paratyphi*; F). *M. tuberculosis*; and L). Ladder

These peaks detected between 900 cm^{-1} and 1200 cm^{-1} correspond to the phosphate group of DNA that adsorbs to the surface of iron[13]. The peak at these wavenumbers was not detected when magnetite + DNA was dissolved in the elution buffer. This observation is favorable, as the elution buffer effectively detaches the bond between magnetite and DNA during the DNA extraction process. Analysis using other binding buffers was also performed. The use of NaCl-based binding buffer was analyzed by IR spectrophotometry (data not shown). In the IR spectra of magnetite + DNA in NaCl-based binding buffer, no peaks were observed in the wavenumber range between 900 cm^{-1} and 1200 cm^{-1}, indicating that the interaction between DNA and magnetite was not detected.

The only peaks detected were the DNA peak[12] at approximately 1650 cm^{-1} and the Fe-O peak for magnetite[3] at approximately 620 cm^{-1}, 580 cm^{-1} and 440 cm^{-1}. These peaks were also observed in figure 5, corresponding to the DNA solution and magnetite. The salt in the binding buffer likely precipitated the DNA, which helped to facilitate its adsorption to the magnetite surface. Different salts may have varying effects on DNA and magnetite inetraction. For instance, the use of guanidine thiocyanate might affect the elution process due to the formation of stronger bonds compared to NaCl[7]. Based on these observations, for further studies, we would continue using a NaCl-based binding buffer. Further analysis of binding buffer would be an interesting topic, especially regarding its influence on the elution process.

The fabricated magnetite was used for DNA extraction for several microbial cultures and a bacterial consortium grown from our laboratory environment. The whole genome electrophoresis visualization is shown in figure 6(A). DNA bands without smearing were observed for environmental bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella paratyphi*, indicating intact and high-quality DNA. These results suggest successful DNA extraction. However, no bands were detected for *Mycobacterium tuberculosis.* This could be due to the small number of cultures as *Mycobacterium tuberculosis* has a slow growth rate. Despite the absence of bands in electrophoresis, we believe the DNA extraction for *Mycobacterium tuberculosis* was successful and would proceed with it in subsequent steps.

Following DNA extraction, 16S rRNA gene amplification was performed to confirm the purity of the extracted DNA and to rule out the presence of PCR inhibitors. The results of the amplification can be seen in figure 6(B). Amplified 16S rRNA gene bands, approximately 1500 bp in size, were observed in every well, including *Mycobacterium tuberculosis*. This demonstrates that our magnetite-based system can effectively extract DNA for amplification.

Conclusion

We successfully prepared a magnetite capable of being used for DNA extraction. The magnetite exhibited a spherical shape and nanoscale size. Crystallite size, calculated from XRD data, was 17.422 nm. Its magnetic properties were found to be ferromagnetic. Our magnetite showed evidence of interaction with DNA using specific binding buffer. This magnetite can be employed to extract DNA from variouse microorganisms including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Mycobacterium tuberculosis*.

The extracted DNA could be amplified via PCR using 16S rRNA primer pair. Its ability to extract microbial DNA makes it a valuable tool for environmental monitoring including microbial molecular identification and contamination detection.

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