

Laser-Assisted Green Synthesis of Silver Nanoparticles for Ferric Ion Detection

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ABSTRACT

In the present study, we report a rapid, single-step, room-temperature laser-assisted green synthesis method for the development of functional silver nanocomposites designed for Fe^{3+} ion detection. The nanocomposites were synthesized by irradiating silver precursor solutions containing natural biopolymer stabilizers, which simultaneously acted as reducing and capping agents. Under mild and environmentally benign conditions, laser-induced photoreduction converted Ag^+ ions into silver nanoparticles, while the stabilizing biomolecules underwent self-assembly to encapsulate the newly formed particles, producing uniformly dispersed AgNP-based nanocomposites. The entire process required no toxic chemicals, organic solvents, or specialized chemical reducing agents, relying solely on laser energy and green stabilizers to drive nanoparticle formation. Characterization of the

optimized nanocomposites confirmed the successful synthesis of fine, nearly spherical particles with a narrow size distribution and a robust biopolymer capping layer, enhancing colloidal stability. The nanocomposites enabled instrument-free visual detection of Fe^{3+} ions, where Fe^{3+} –biopolymer coordination triggered rapid nanoparticle aggregation with a distinct color change. The detection system demonstrated a visual detection limit of approximately 20 μM , while UV–Vis and SEM analyses further verified aggregate formation upon Fe^{3+} addition. Owing to their strong surface plasmon resonance response, excellent colloidal stability, and environmentally friendly synthesis route, the prepared nanocomposites exhibit significant potential for large-scale applications in environmental sensing, medical diagnostics, and water-quality monitoring. In addition, the AgNP-based nanocomposites displayed potent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, along with notable antifungal and antiviral properties, underscoring their multifunctional nature.

Introduction

Heavy metal ions have attracted the considerable attention of the research community because they play a pivotal role in many biological systems and have an extreme impact on the environment [1], [2], [3]. Among various important elements that play a vital role in the survival of living organisms, iron is considered an essential component for many biological systems [4]. Iron is an essential component for many cellular processes such as DNA synthesis, oxygen transportation, and cellular metabolism, etc. [5], [6]. So, many efforts have been done to develop innovative and accurate techniques, such as atomic absorbance spectroscopy, fluorescent, electrochemical analysis, high-performance liquid chromatography, and magnetic resonance imaging, etc. for iron detection [7], [8], [9], [10]. However, these complex techniques require very expensive measuring instruments, sample preparation, and a highly trained operator for the sample analysis. Thus, it has been a great challenge to develop practical, sensitive sensors for the detection of iron. [11], [12], [13]. Silver nanoparticles (AgNPs) have gained widespread attention due to their unique physicochemical properties, including strong surface plasmon resonance (SPR), high surface reactivity, and

broad-spectrum antimicrobial and antiviral activity.. However, conventional methods for synthesizing AgNPs often involve harsh chemical reducing agents, elevated temperatures, long reaction times, and toxic organic solvents, all of which raise concerns regarding biocompatibility, environmental safety, and large-scale applicability. These limitations have increasingly driven the development of green nanotechnology, where natural, environmentally friendly materials are utilized to produce nanomaterials in a safer and more sustainable manner. Laser-assisted synthesis has recently emerged as a modern adaptation of green nanoparticle production [11], [12], [13] . In this technique, high-energy laser irradiation induces rapid photoreduction of metal ions in aqueous media, eliminating the need for chemical reducers and enabling precise control over nucleation and growth. Among various applications of AgNPs, colorimetric metal-ion detection has become particularly important due to its simplicity, rapidity, and accessibility.

Iron(III) (Fe^{3+}) ions are essential for biological systems but can cause severe environmental and health-related problems at elevated levels, including oxidative stress, organ damage, and water contamination. Conventional Fe^{3+} detection techniques rely on sophisticated instrumentation such as ICP-MS or electrochemical analyzers which are costly, time-consuming, and unsuitable for on-site analysis[14]. AgNP-based sensors, however, offer a promising alternative: Fe^{3+} ions can induce aggregation of capped AgNPs through coordination chemistry, producing a distinct color change easily observable by the naked eye. This makes AgNP nanocomposites highly valuable for point-of-care and field-based detection systems [15] . The integration of laser-assisted synthesis with natural biopolymer stabilizers provides a powerful platform for producing functional silver nanocomposites capable of Fe^{3+} detection at room temperature. The biomolecule-rich capping layer improves aqueous stability, enhances Fe^{3+} selectivity, and enables visible detection without specialized equipment [16] . In addition to their sensing capabilities, these nanocomposites retain the inherent antimicrobial, antifungal, and antiviral properties of AgNPs, thereby offering multifunctional utility for medical, environmental, and analytical applications. In this study, we present the development and characterization of laser-assisted, green-synthesized AgNP-based nanocomposites for rapid Fe^{3+} ion detection. The work highlights the advantages

of a clean, chemical-free synthetic route, evaluates the structural and optical properties of the nanocomposites, examines their sensing performance, and demonstrates their significant antimicrobial and antiviral potential. This multifunctional approach offers a promising path toward sustainable nanomaterial development for real-world detection and biomedical applications.

Materials and Methods

Silver salt precursors and naturally derived stabilizing agents (such as plant polyphenols or biopolymers) were used to prepare the reaction mixture. Gallic Acid Monohydrate (Sigma, Merck) Natural Gallic Extract™. Silver nitrate (AgNO_3), sodium hydroxide (NaOH), sodium borohydride (NaBH_4) trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), and ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), were purchased from Sigma-Aldrich. Potassium, sodium and magnesium salts were obtained from Merck (Germany). Rubidium chloride (RbCl), manganese chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), were purchased from Sigma-Aldrich. All the chemicals were stored in the desiccator and used without any further purification. Ultrapure deionized water was used in all the synthesis processes.

Synthesis of Silver Nanoparticles by Laser-Assisted Green Approach

Khizra

The synthesis of silver nanoparticles (AgNPs) was carried out via a laser-assisted green approach in which laser irradiation interacted with an aqueous silver–biopolymer system. In this process, silver ions underwent rapid photoreduction under controlled laser energy, leading to nucleation and growth of nanoparticles. For the experimental setup, silver plates with 99.99% purity and glass substrates were used. The substrates were first sonicated in acetone and subsequently cleaned with ethanol and deionized water to remove surface contaminants. The silver targets were mounted on a holder positioned 0.5–1.5 cm from the glass substrate. Laser parameters, were adjusted at 400–450 nm range, a low optical power density below 50 mW cm^{-2} , and an exposure duration of 10–20 minutes was selected to ensure complete ion-to-metal conversion while minimizing unwanted photothermal effects. Throughout the process, ambient temperature (25–35 °C) and mildly alkaline conditions (pH 8–9) were maintained to support the photoreduction of silver

ions[17].

Formation of AgNP-Biopolymer Nanocomposites

Following laser irradiation, the reduction of Ag^+ ions (typically introduced at concentrations of 0.5–1.0 mM AgNO_3) resulted in the formation of silver nanoparticles that immediately became encapsulated by the surrounding biopolymer matrix, which was present at 0.1–0.5% w/v depending on viscosity and functional group density. The natural stabilizing molecules such as tannic acid 12 gram/L, gum Arabic 2 gram/L, chitosan 2 gram/L, formed a protective organic layer approximately 2–10 nm thick around each nanoparticle. This coating prevented particle aggregation, improved colloidal stability, and ensured uniform dispersibility across the medium.

Surface Characterizations: Arooj

UV-vis absorption spectra of the synthesized silver nanocomposites were carried out using a spectrophotometer (Model: JASCO V-770). A high-power thin-film X-ray diffractometer (D8 Advance) was used to determine the crystal structure of the silver nanocomposites. The sample was prepared at silicon wafer substrate and X-ray diffraction (XRD) measurements were determined using Cu-K radiation in a wide range of Bragg angles 2θ at a scanning speed of $2^\circ/\text{min}$. A Schottky Field Emission Scanning Electron Microscopy (FE-SEM, Model: JEOL JSM-7600F) was used to analyze the size distribution and surface morphology of the developed nanocomposites. A High-Resolution Transmission Electron Microscopy (HR-TEM, Model: JEM-3010), working at 200 kV was used to analyze the product at high magnification. For TEM analysis, the product was dispersed in the DI water and drop coated on the carbon copper grids. The elemental analysis of the product was analyzed using an energy dispersive spectrometer (EDS, Model: X-MAX 50). Zetasizer Malvern Instrument (Model: Nano-SZ) was used to determine the zeta-potential analysis of the aqueous sample. An X-ray Photoelectron Spectrometer (XPS, Model: ESCALAB250) was used to analyze the composition of the product. A Fourier Transform Infrared spectrophotometer (JASCO, FT/IR-4700) was used to further analyze the chemical structure of the product. For FTIR analysis, a powder sample of the product was prepared, and the spectrum was obtained in the range of

300–4,000 cm⁻¹ at the 4mm/min scanning speed[18].

Antipathogenic Test

Antibacterial Testing

The antibacterial effectiveness of all samples was detected by AATCC 147 (zone of inhibition test). This is a type of qualitative test method in which bacterial strains of Gram-positive *Staphylococcus aureus* (CCM 3953) and Gram-negative *Escherichia coli* (CCM 3954) were selected. Bacterial colonies of both strains were grown in the Luria-Bertani (LB) agar plates. Round-shaped fabric samples (1.5 × 1.5 cm²) containing various content of silver nanocomposites (100 mg, 200 mg, 300 mg, and 400 mg) were prepared by drop coating. Three samples of each fabric sample along with reference (control, C, without any silver) were placed in the LB agar plates. The LB agar plates were placed in the incubator at standard conditions (37 °C, 24 h) and the zone of inhibition was determined[19].

Antifungal Activity Assessments

The antibacterial properties of the treated cotton fabric were evaluated according to American Association of Textile Chemist and Colorists (AATCC) test method 100–2004.

Detection of Fe³⁺ ion

For the visual and optical detection of Fe³⁺ ion, the aqueous solution of Fe³⁺ was mixed with the aqueous solutions of silver nanocomposites (1 mL) to prepare the final concentration in the range of 1.0 μM to 25 μM. Under the same conditions, we also analyzed the selectivity of the synthesized silver nanocomposites for Fe³⁺ ions using other monovalent, divalent, or trivalent ions instead of Fe³⁺ ions, including K¹⁺, Na¹⁺, Rb¹⁺, Ag¹⁺, Mg²⁺, Cu²⁺, Mn²⁺, Ni²⁺, Cd²⁺, Co²⁺, Al³⁺, Cr³⁺, and Nd³⁺, at a final concentration of 25 μM. The characteristic yellow color of the silver nanocomposites is disappeared, indicating the successful detection of metal ions. The addition of Fe³⁺ ions results in the aggregation of silver nanocomposites. This aggregation of silver nanocomposites is due to the interaction between the metallic ions and nanocomposites, which was further investigated by the UV-vis spectra.

Results and Discussion

Physicochemical Characterization of Particles

The well-known technique for imaging materials is TEM. The technique was employed to visualize the size and shape of Conjugates of Silver NPs. The TEM image in Figure 3.1 a,b represents a heterogeneous form of the Silver NPs, with the sizes ranging from 900 nm. It was also observed that most of the Silver NPs were spherical in shape. In the analysis of silver NPs conjugates, formation of conjugates was clearly observed. Silver NPs composites were found to be irregular in shape and unevenly distributed. The Silver NPs were found to be increased in shape. Bio-organic layer of polymer was also observed. To transmission electron microscopy image also leads to the aggregation of the Silver NPs. Infact, the formation of irregular shapes and difference in sizes can be attributed to the hydrophobic organic peptoid oligomers interacting with each other (Pal, Bhattacharyya, Kar, Zarena, Bhunia, & Atreya, 2019). These hydrophilic interactions seem to mediate the arrangement of the AMP conjugates into spherical assemblies and increase their size as shown in Figure 3.1 In this case, the additional oligo meric chains served as templates that mediate the aggregation.

The TEM image in panel (a) shows well-defined, dark, spherical particles, confirming the successful formation of metallic silver nanoparticles with a dense electron-rich core. The particles appear uniform in shape, and no significant debris or impurities are visible, indicating a clean synthesis. In contrast, the size distribution profile in panel (b) reveals a dominant peak around 900–1000 nm, along with a smaller secondary peak, suggesting that although the primary nanoparticles are nanoscale in their dry state (as seen in TEM), they tend to form large aggregates when dispersed in solution. Because DLS measures the hydrodynamic diameter, which includes solvation layers and clustering effects, the apparent particle size becomes much larger than the true core size observed in TEM. Together, these results indicate that while the AgNPs themselves are nanoscale and spherical, they exhibit substantial aggregation in suspension, leading to significantly increased hydrodynamic size measurements.

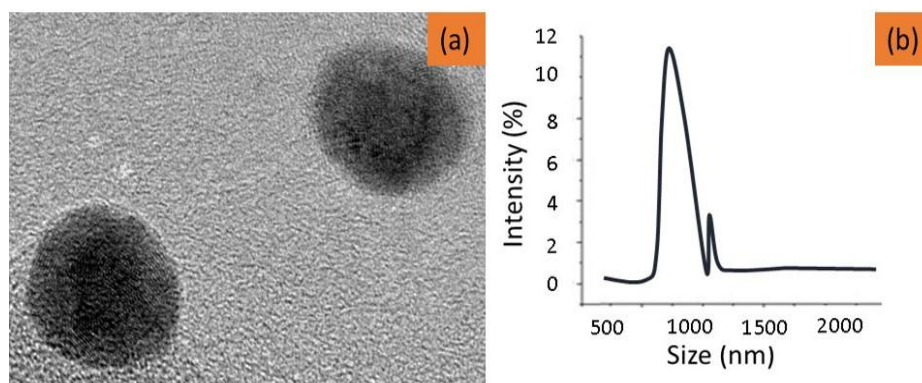


Figure 3.1: TEM Analysis and Size Distribution of Silver NPs

FTIR Analysis

FTIR spectrum of Silver NPs coated with polmer provided different absorption peaks which assigned the different functional group of protenic compounds. The absorption peaks at 3309 cm^{-1} , 486 cm^{-1} were assigned the O-H stretch of phenolic compounds. Actually, the primary protenic screening shows that the extract was rich in protenic peptides. To further demonstrate the successful conjugation of Silver NPs, the characteristic peaks at near 3100 cm^{-1} confirmed the presence of unsaturated C-H stretching of AMP. The obvious absorption peak at 3100 cm^{-1} can be assigned to the unsaturated C-H stretching vibration of biopolymer coated. (Da Costa, Cova, Ferreira, & Vitorino, 2015). Moreover, the decrease in intensities in peaks in Silver NPs coated by polymers spectra and displacement specially at 1636 cm^{-1} , reflects the interactions between the atoms of Ag, O and N. Also, this peak can be attributed to the imine group ($\text{C}=\text{N}$). The absorption bands located at approximately 3286 cm^{-1} which are found in the spectra of all analyzed samples can be associated with the $-\text{C}=\text{O}$ group as shown in Figure 2. The appearance in the spectrum of bio-composite of peaks at 678 cm^{-1} , 601 cm^{-1} , 563 cm^{-1} and 489 cm^{-1} revealed that the Silver NPs was successfully incorporated into films. FTIR spectrum of the silver nanoparticles coated with a biopolymer shows several characteristic absorption bands that confirm the presence of organic functional groups on the nanoparticle surface.

The broad peak around 3200 cm^{-1} corresponds to O-H and N-H stretching vibrations, indicating hydroxyl and amine groups typically found in biopolymers such as polysaccharides or proteins. The distinct band at 1650 cm^{-1} represents

C=O stretching of amide I or C=C vibrations, confirming the presence of protein- or polyphenol-based components involved in nanoparticle coating and stabilization. The strong absorption observed near $674\text{--}745\text{ cm}^{-1}$ is associated with C–H bending or out-of-plane vibrations of aromatic or carbohydrate groups, further supporting the presence of a biopolymeric matrix around the nanoparticles. These functional groups collectively indicate successful biopolymer coating of the silver nanoparticles, which provides both reduction of Ag^+ to Ag^0 and stabilization of the synthesized nanostructures by preventing agglomeration.

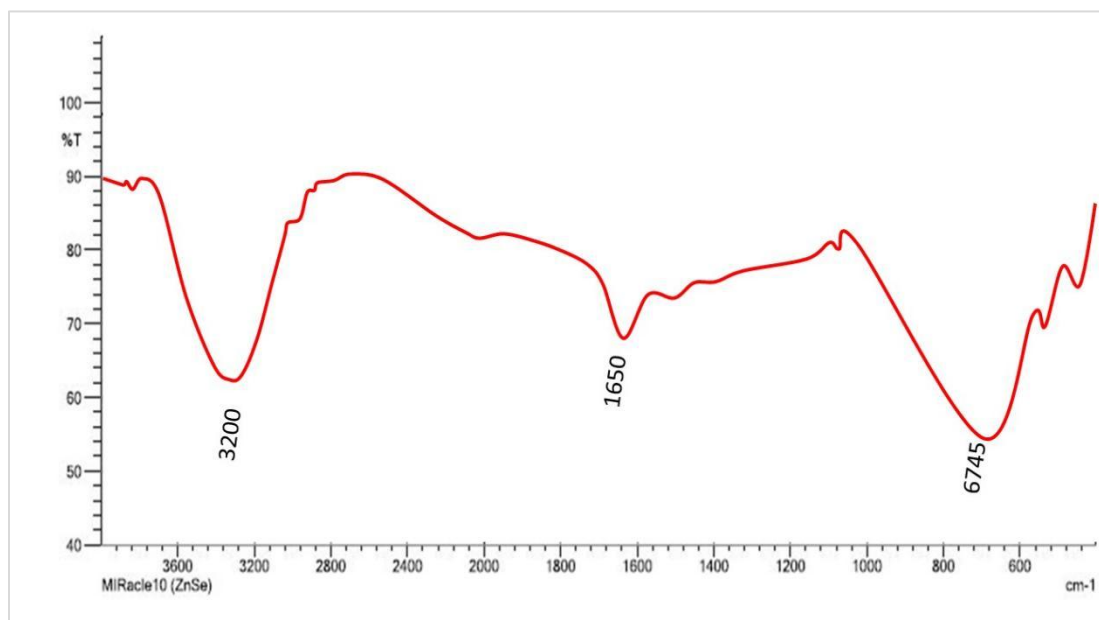


Figure 3.2: FTIR Spectra of Conjugates.

Antibacterial Activity of the Silver NPs Composites

The Silver NPs were coated over the white paper to observe the zone of inhibition antibacterial effect. The antibacterial activity of plain paper and paper coated with Silver NPs composites was tested both qualitatively and quantitatively using standard testing protocols.

Zone of Inhibition Test (Qualitative Measurements)

The AATCC-147 (disc-diffusion method) standard testing protocol was followed for the qualitative assessment of all treated samples. The antibacterial efficacy of all samples was evaluated against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria. The test was carried out three times and the mean value calculated for each sample is presented in Table 1. The obvious zones of inhibition around each

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c sample after 24 hours of incubation at 37 °C in the dark are depicted in Figures 3. All samples have shown significant zone of inhibition (ZOI) against both test microbes (Yun, Fan, & Li, 2013). However, it was observed that ZOI for dyed is higher with higher concentration.

The images in Figure (a) and (b) represent antibacterial activity tests, most likely using the agar well diffusion or disc diffusion method. In both plates, three distinct zones of clearance surround the sample spots, indicating that the material being tested—such as silver nanoparticles or silver–biopolymer composites—successfully inhibited bacterial growth. In panel (a), the inhibition zones appear more diffuse and spread across a larger area, suggesting strong antimicrobial activity or faster diffusion of the sample through the agar. In contrast, panel (b) shows more defined, uniform inhibition zones, indicating controlled diffusion and consistent antibacterial action. The clear areas around each spot confirm that the bacterial lawn was effectively suppressed in those regions due to the antimicrobial effect of the material. Overall, the plates demonstrate that the synthesized silver-based samples exhibit notable antibacterial properties, as evidenced by the formation of visible inhibition zones around each application point.

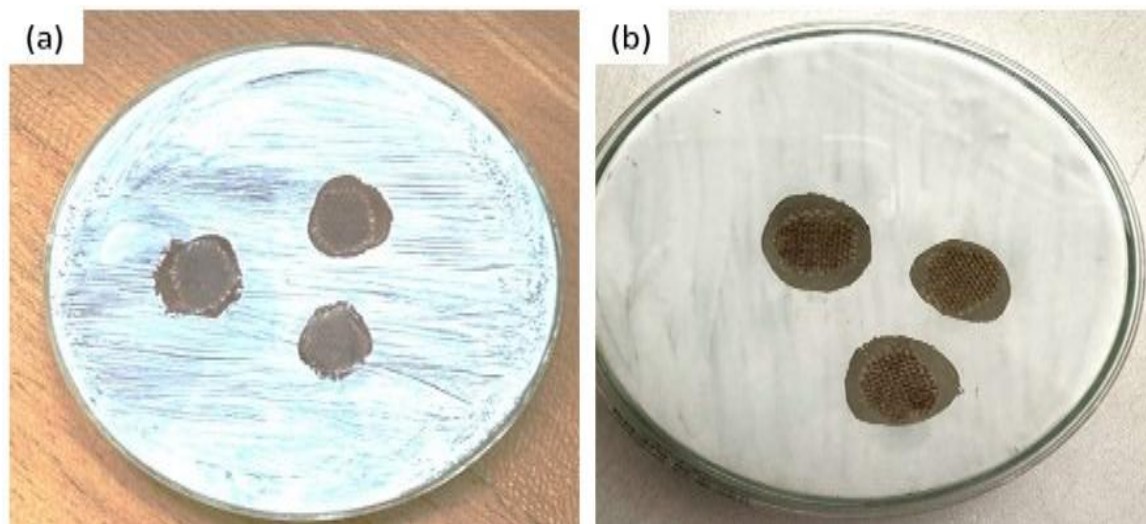


Figure 4.3: Zone of Inhibition of silver Coated Samples

Table 3.1: The Values of Zone of Inhibition for Developed Samples

Sample code	Concentration of AMP g/200ml	ZOI (mm)	
		<i>S. aureus</i>	<i>E. coli</i>

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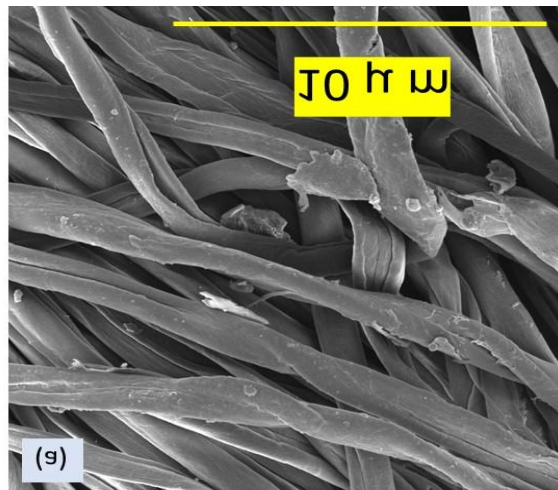
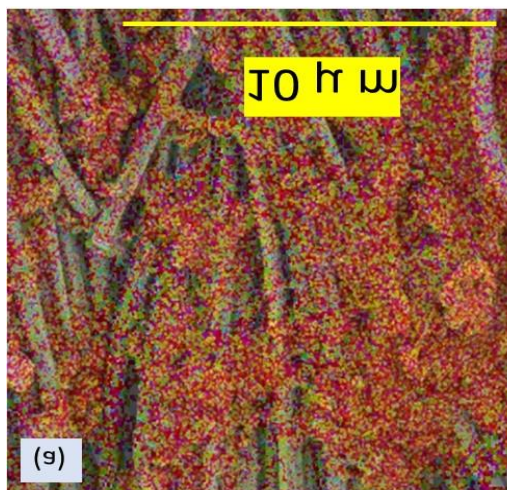
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1	F1	2	0	0
2	F2	4	5	2
3	F3	6	7	3

SEM Analysis of Silver NPs Composites

After the dip coatings with concentrations (2g/200ml, 4g/200ml and 6g/200ml all the samples were rinsed in ethanol solution. Subsequently, SEM analysis was performed. The purpose of rinsing was to stabilize the coating of Silver NPs composites on paper surfaces. The SEM micrographs analysis of all silver samples with different concentration was done. In comparison with all uncoated samples, the coating was clearly done on all samples. Secondly, the dense coating was found for more smooth, polished. The reason is more roughness, corrugation and micro fractures, will provide more active sites and surface area for the deposition of coatings. Scanning electron microscopy also revealed the homogeneous deposition of Silver NPs composites on all surfaces (Carmona-Ribeiro & Araújo, 2021). Moreover, with increase in concentration from the deposition of was found more uniform and denser. This further indicated the higher tendency of formation of coating network when concentration was increased as shown in Figure 4.



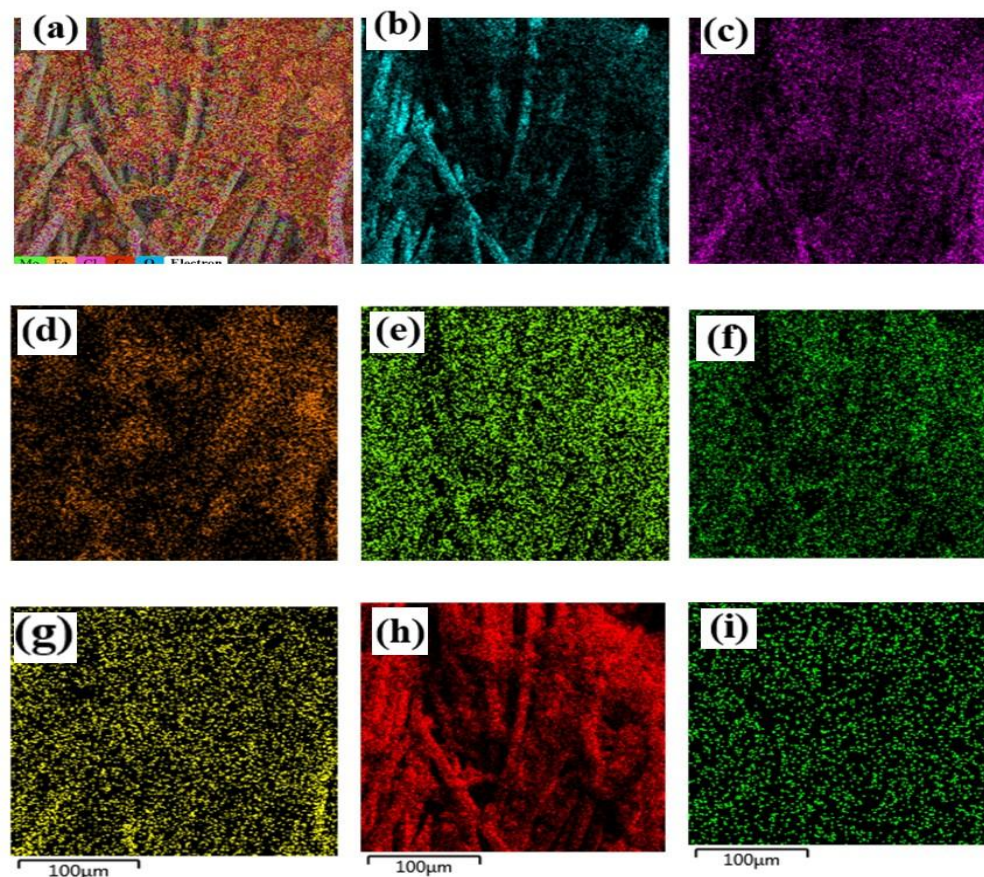


Figure 3.4: SEM Images of Coated with silver and Uncoated Samples.

Biofilm Inhibition – Quantitative Assay

The increase, emergence, and spread of drug-resistant bacteria with biofilm formation on cotton bandage's ability increased the survival chance of bacterial species. Due to the immediate requirement to identify novel antibacterial agents for the treatment of such bacterial infections forming biofilm, we conducted this study to introduce a biological approach to combat. *S. aureus* and *S. epidermidies* biofilm formation. The performance of biosynthesized Silver NPs composite in inhibition of biofilm formation was measured at the concentrations of 2, 4 and 5g/200ml against the standard *S. aureus* and *S. epidermidies*, same protocol were performed. The OD₆₀₀ (growth value) were found (Muszanska et al., 2014). The performance of Silver NPs composite with various concentrations toward the species of *S. aureus* and *S. epidermidies* in preventing the formation of biofilm by phenotypic assay was exhibited Figure 5

The performance of Silver NPs composite with various concentrations toward the species of *S. aureus* and *S.epidermidis* in inhibition of the biofilm growth percentage by phenotypic assay is exhibited in Figure 6 (Yu et al., 2021). Where the results are showing the Inhibition of biofilm growth $94.2\% \pm 1.2SD$ and $96.2\% \pm 1.3SD$ against *S.aureus* and *S.epidermidis* respectively.

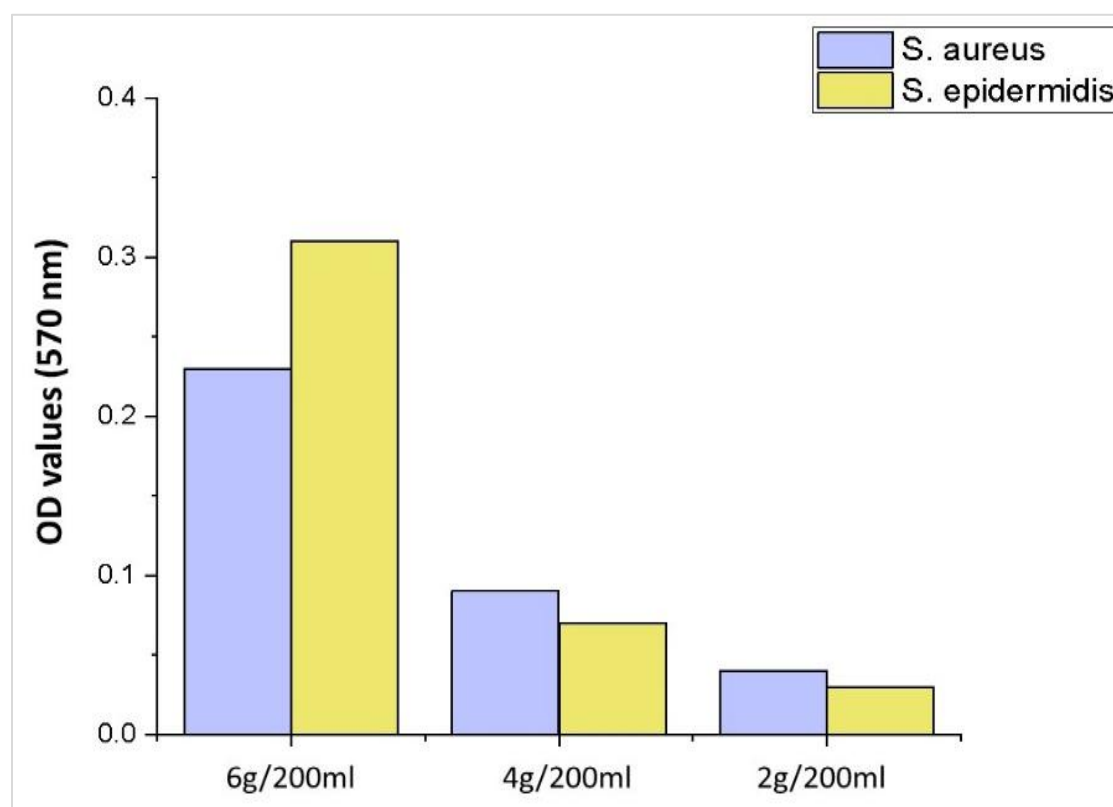


Figure 3.5: OD Values of Samples with Different Silver NPs composite Conc.

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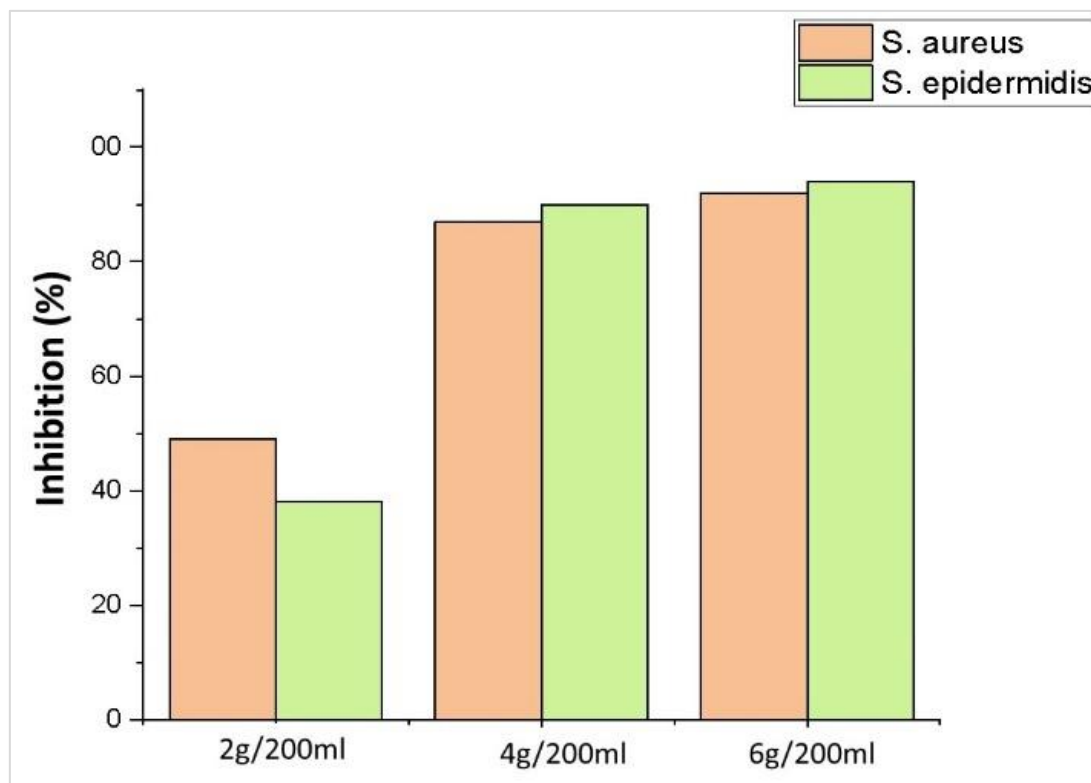
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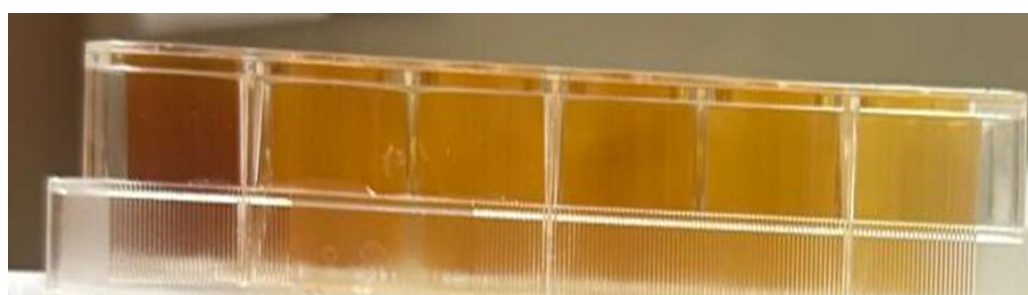
Figure 3.6: Biofilm Inhibition of Conjugates by Silver NPs Composite

3.3. Visual Detection of Ferric Ions

The synthesized silver nanocomposites were used as a sensor for the visual detection of Fe^{3+} ions. This detection method depends upon the aggregation of synthesized silver nanocomposites in an aqueous solution. Fe^{3+} ions coordinate with the biopolymer present on the surface of the nanocomposites and form aggregates. Upon addition of various concentrations of ferric ions into the solution under ambient conditions, the yellow color of the solution starts to turn transparent. This aggregation phenomenon of the silver nanocomposites in the presence of Fe^{3+} ions can also be observed by the naked eye, which eliminates the need for a specific instrument to detect Fe^{3+} ions. The minimum aqueous concentration of Fe^{3+} ions required for the visible color change by the naked eye was also recorded and analyzed.

We also evaluated the high selectivity of silver nanocomposites for Fe^{3+} ions using other monovalent, divalent, or trivalent ions instead of Fe^{3+} ions, including K^{1+} , Na^{1+} , Rb^{1+} , Ag^{1+} , Mg^{2+} , $^{+}$, at a final concentration of 20 μM and found that the

synthesized silver nanocomposites were highly selective toward Fe^{3+} ions (Figure 7). There was no visible color change in the nanocomposites even at the 100 μM concentration of ions. No visible color change or aggregation could be observed (except for silver ions) even after five (5) weeks, showing the long-term aqueous stability of the silver nanocomposites-based detection system. Thus, this is the first very simple method based on silver nanocomposites that can be conveniently used for visual detection of ferric ions with high sensitivity and selectivity.



Na^+ , K^+ , Rb^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} Fe^{2+} ,

Figure 3.7: A photograph of the fresh solutions containing mixtures of functionalized silver nanocomposites with different metal cations. The ion concentrations of Na^+ , K^+ , Rb^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , Fe^{2+} , were each 100 μM . The concentration of Fe^{3+} was 20 μM .

To assess the ion-selective sensing capability of the synthesized silver nanocomposites, we systematically evaluated their response toward a broad range of common mono-, di-, and divalent cations. Even after extended storage for up to five weeks, the solutions with Na^+ , K^+ , Rb^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} , and Fe^{2+} remained unchanged, confirming the long-term aqueous stability of the nanocomposite system. In contrast, the addition of Fe^{3+} ions at a significantly lower concentration (20 μM) resulted in an immediate and visually noticeable color change. This rapid response is attributed to the strong affinity of Fe^{3+} toward the surface functional groups (such as catechol, phenolic, or hydroxyl groups) present in the biopolymer shell, which induces cross-linking and nanoparticle aggregation.

Conclusion

The proof of layer reduction was confirmed by spectrum, where the conjugates of silver exhibited the specific absorption of at 395 nm. Size of conjugates and surface

morphology was justified by TEM, Zeta sizer and SEM analysis. The silver were found to be increased in shape and size (Qamer, Che-Hamzah, Misni, Joseph, Al-Haj, & Amin-Nordin, 2023). Furthermore, the appearance in the FTIR spectrum of silver composite of peaks at 678 cm^{-1} , 601 cm^{-1} , 563 cm^{-1} and 489 cm^{-1} revealed that the conjugate was successfully incorporated into films. Antimicrobial assessment of silver composite was performed by Resazurinmicro titre assay method (REMA) and minimum inhibition concentration was found. The biosynthesized silver nanoparticle (AgNP) composites demonstrated strong multifunctional performance, confirming their suitability for advanced antimicrobial and sensing applications such as smart bandages. The qualitative antibacterial assays (AATCC-147) revealed clear and concentration-dependent zones of inhibition against both *S. aureus* and *E. coli*, with the highest AgNP loading ($6\text{ g}/200\text{ mL}$) producing the most pronounced antimicrobial activity. SEM analysis confirmed that increasing AgNP composite concentration resulted in progressively denser, smoother, and more uniform coatings on the substrates, supporting enhanced antibacterial performance due to improved surface coverage and greater availability of active sites.. The AgNP–biopolymer network selectively aggregated in the presence of Fe^{3+} , producing a visible color change at concentrations as low as $20\text{ }\mu\text{M}$, while showing no response toward a wide range of interfering ions even at higher concentrations. This high selectivity and the long-term aqueous stability of the nanocomposites demonstrate their practicality for low-cost, instrument-free detection of Fe^{3+} . Overall, the results confirm that the developed silver nanocomposite system offers a robust combination of broad-spectrum antibacterial activity, strong anti-biofilm behavior, stable surface morphology, and selective Fe^{3+} sensing, making it an excellent candidate for integration into next-generation smart bandages and biomedical monitoring platforms.

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