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Research Article
Characterization and Eco-Friendly Synthesis of Silver and Iron Nanoparticles Using Microalgae Extracts: Implications for Nanobiotechnology

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Abstract

Background and Objective: The remarkable surface-to-volume ratio and efficient particle interaction capabilities of nanoparticles have garnered significant attention among researchers. Microalgal synthesis presents a sustainable and cost-effective approach to nanoparticle production, particularly noteworthy for its high metal uptake and ion reduction capabilities. This study focuses on the eco-friendly and straightforward synthesis of Silver (AgNPs) and Iron (FeNPs) nanoparticles by utilizing Spirulina (*Arthrospira platensis*) and *Chlorella pyrenoidosa* extract, devoid of any chemical reducing or capping agents. **Materials and Methods:** Following the mixing of 1 mM $AgNO_3$ and 1 mM iron oxide solution with the algal extract, the resulting filtrated solution underwent comprehensive characterization, including UV-visible absorption spectra analysis, observation of particle morphology, Zetasizer measurements and Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) analysis. **Results:** The UV-visible spectroscopy revealed a maximum absorbance peak at 430-440 nm, confirming the successful green synthesis of AgNPs and FeNPs, as indicated by the distinct color change from transparent to dark reddish-yellow and brown to reddish-brown, respectively. The SEM-EDX analysis further elucidated the spherical morphology of the nanoparticles, with an average diameter of 93.71 nm for AgNPs and 6198 nm for FeNPs. The Zeta potential measurements indicated average values of -56.68 mV for AgNPs and 29.73 mV for FeNPs, with conductivities of 0.1764 and 0.6786 mS/cm, respectively. **Conclusion:** The observed bioaccumulation of silver and iron nanoparticles within the algal extract underscores its potential as an environmentally friendly and cost-effective method for nanoparticle synthesis. These findings suggested a promising avenues for the application of silver and iron nanoparticles in the field of nanobiotechnology. Future research endeavors could focus on optimizing preparation conditions and controlling nanoparticle size to further enhance their utility and effectiveness.

Key words: Green synthesis, iron nanoparticles, silver nanoparticles, Spirulina platensis, *Chlorella vulgaris*

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nanobiotechnology has emerged as a promising field, celebrated for its eco-friendly, cost-effective and sustainable approach to silver nanoparticle synthesis. Over the past decade, nanoparticles have captivated scientific interest due to their extraordinary electronic, optical, mechanical, magnetic and chemical properties, distinct from those of bulk materials[1]. Nanotechnology, defined as the manipulation of materials at the atomic or molecular level, has revolutionized various industries by enabling precise control over material properties[2].

Nanoparticles, typically ranging in size from 1 to 100 nm, boast a remarkably high surface-to-volume ratio, rendering them ideal for an array of applications, including drug delivery, chemical sensing, cosmetics, antioxidants and beyond[3,4]. While conventional physicochemical methods have been employed for nanoparticle synthesis, they come with inherent drawbacks such as scalability issues, utilization of toxic chemicals, stringent equipment requirements and elevated costs[5,6]. In response, there has been a growing demand for green synthesis methods characterized by environmentally benign solvents, eco-friendly reducing and capping agents, high efficiency, low cost, non-toxicity and minimal environmental impact[7-10].

Biologically mediated synthesis of nanoparticles has emerged as a promising alternative, harnessing the power of natural products to facilitate nanoparticle formation[11]. Among these biological sources, microorganisms, plants and green algae have garnered considerable attention for their ability to synthesize nanoparticles effectively[11-17].

Spirulina platensis, a filamentous photoautotrophic cyanobacterium and *Chlorella vulgaris*, a eukaryotic green microalga, have emerged as promising candidates for nanoparticle synthesis due to their inherent properties and widespread availability[18-20]. Their ability to thrive under diverse environmental conditions and interact with various particulate materials positions them as valuable assets in the green synthesis of nanoparticles. While the potential applications of silver nanoparticles span diverse fields such as medicine, science and technology, including antibacterial agents, catheters, food containers, anticancer treatments, electronics and water treatment, among others, there remains a need for further exploration and optimization of microalgal nanoparticle production[16,21-30].

In this context, the present study aims to elucidate a green synthesis approach for silver and iron nanoparticles utilizing commercially available Spirulina and *Chlorella* extracts as algal sources. By optimizing the physicochemical characterization of these nanoparticles, the study shed light on their potential for diverse nano-biotechnological applications.

MATERIALS AND METHODS

Study area: The study was conducted at the Central Laboratory of the Biological Department, King Abdulaziz University, located in Jeddah, Saudi Arabia over 18 months from March, 2022 to November, 2023.

Preparation of microalgae extracts: To prepare the microalgae extracts, commercial super algae tablets containing Spirulina (*Arthrospira platensis*) and *Chlorella pyrenoidosa* extract were obtained from iHerb corporate, located in the USA (California, Kentucky), with the product code SFD-10075[31]. The aqueous extract of Spirulina and *Chlorella* was then prepared by mixing 2 g of crushed tablet powder with 100 mL of double-distilled sterile water. The mixture was allowed to stand at room temperature for 30 min to facilitate extraction. Subsequently, the resulting suspension was stored at 4°C for further analysis.

Synthesis of the Nanoparticles, Silver Nitrate ($AgNO_3$) and Iron Oxide (Fe_2O_3): The synthesis of Silver Nanoparticles (AgNPs) and Iron Oxide Nanoparticles (FeONPs) involved the preparation of 1 mM solutions of Silver Nitrate ($AgNO_3$) and Iron Oxide (Fe_2O_3), respectively. For the preparation of the silver nitrate solution, 0.042 grams of $AgNO_3$ (obtained from Sigma, CAS No: 7761-88-8) were dissolved in 250 mL of deionized distilled water.

To synthesize AgNPs, 45 mL of the 1 mM $AgNO_3$ solution were combined with 5 mL of the algal extract. The resulting mixture was subjected to a temperature of 90°C for 15 min. Subsequently, it was centrifuged at 5500 rpm for 30 min and then incubated for 48 hrs at room temperature. The formation of AgNPs was visually indicated by the color change from colorless to yellow[32].

For the preparation of the iron oxide solution, 0.031 g of Ferrous Chloride ($FeCl_2$) were dissolved in 250 mL of distilled water. Then, 0.067 g of Ferric Chloride ($FeCl_3$) were added to the solution to obtain the desired 1 mM iron oxide solution. The FeONPs were synthesized by adding 5 mL of algal extract to 34 mL of the 1 mM iron oxide solution. The mixture was heated to 90°C for 15 min, followed by centrifugation at 5500 rpm for 30 min. Subsequently, the mixture was incubated for 48 hrs at room temperature to allow for nanoparticle formation.

Nanoparticle characterization: The nanoparticle characterization involves several key analyses to confirm synthesis and determine their physical properties. Firstly, UV-visible absorption spectroscopy was performed to verify the synthesis of Silver Nanoparticles (AgNPs = $AgNO_3$) and Iron Oxide Nanoparticles (FeNPs = Fe_2O_3)[31]. Subsequently, the size and Zeta potential of the nanoparticles were determined using a Zetasizer system (Nano ZS, UK). This involved conducting experiments in triplicate using the algal extract to ensure robust results. These characterization techniques provide crucial insights into the optical properties, size distribution and surface charge of the nanoparticles, which are essential for understanding their behaviour and potential applications.

SEM-EDX analysis: The SEM-EDX (Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy) analysis was conducted to investigate the cellular accumulation of silver nanoparticles and iron oxide nanoparticles within the extract of *Arthrospira platensis* and *Chlorella pyrenoidosa*. The aim was to assess any morphological damage to the cells resulting from the treatment with AgNPs and FeNPs.

To prepare the samples for SEM-EDX analysis, a suspension containing 25 mL of AgNPs and FeNPs treated with algal extract was centrifuged at 5,000 rpm for 10 min. The resulting pellet was washed twice with 0.1×PBS (phosphate-buffered saline) and distilled water to remove any residual impurities. Subsequently, the washed pellet was freeze-dried to preserve the cellular structure and morphology[33].

The freeze-dried algal suspension was then subjected to SEM-EDX analysis using a JSM-6701F instrument from Joel, Japan. This analysis allowed for the visualization of the cellular structure and the identification of any accumulated nanoparticles within the cells. Additionally, the EDX component of the analysis enabled the elemental composition of the nanoparticles to be determined, providing valuable insights into their chemical properties.

RESULTS

Synthesis of $AgNO_3$ and Fe_2O_3 nanoparticles: The *in vitro* green synthesis of silver nanoparticles (SNPs) using the algal cell-free extract was monitored via UV-visible spectrophotometry. Upon addition of the algal extract to the silver nitrate solution, a distinct color change was observed, transforming the solution from transparent to dark reddish yellow. Similarly, the iron oxide solution changed from brown to reddish brown, indicating nanoparticle production.

These color changes result from the excitation of surface plasmon vibrations induced by the formation of nanoparticles within 24 hrs of the reaction. This characteristic color, primarily attributed to the deposited silver nanoparticles, serves as a visual indicator of successful synthesis. The UV-Vis absorption spectra of the nanoparticles formed in the reaction displayed absorbance maxima at 430-440 nm. Analysis of the absorption spectra revealed an exponential increase in the formation of silver nanoparticles within 48 hrs of the reaction.

Furthermore, when microalgal cells were incubated with deionized water (positive control), they retained their original color, while cells treated with silver nitrate solution exhibited a transition from bright green to dark brown. Notably, the algal extract remained intact and retained its original shape, similar to unexposed cells. In contrast, no observable change in color was noted for the negative control, consisting of silver nitrate and iron oxide solution, even after 7 days of incubation.

Nanoparticle characterization: The synthesized Iron Oxide Nanoparticles (Fe_2O_3) and Silver Nanoparticles ($AgNO_3$) were subjected to comprehensive characterization to elucidate their physical properties based on dynamic light scattering (DLS, Fig. 1 and 2).

The Z-average size, representing the mean hydrodynamic diameter, was determined to be 6197.67 nm for Fe_2O_3 nanoparticles and 93.71 nm for $AgNO_3$ nanoparticles (Fig. 1). The polydispersity index (PI), indicative of particle size distribution, was measured at 0.8765 for Fe_2O_3 and 0.607 for $AgNO_3$, suggesting a relatively broader size distribution for Fe_2O_3 nanoparticles compared to $AgNO_3$ nanoparticles (Fig. 1a, d).

The mean count rate, indicative of nanoparticle concentration, was found to be 198.77 kcps for Fe_2O_3 and 318.77 kcps for $AgNO_3$. Analysis of peak intensity revealed multiple peaks for both nanoparticles, with peak 1 mean by intensity ordered by area measured at 66.91 nm for Fe_2O_3 and 166.37 nm for $AgNO_3$. Additionally, peak 2 mean by intensity ordered by area was observed at 0 nm for Fe_2O_3 and 23.16 nm for $AgNO_3$, while peak 3 mean by intensity ordered by area was found to be 0 nm for Fe_2O_3 and 4914.33 nm for $AgNO_3$ (Fig. 1a, c).

The Zeta potential, a measure of nanoparticle surface charge, was determined to be 29.73 mV for Fe_2O_3 and -56.68 mV for $AgNO_3$. The conductivity of the suspensions was measured at 0.6786 mS/cm for Fe_2O_3 and 0.1764 mS/cm for $AgNO_3$. The wall Zeta potential, representing the potential of the particle surface relative to the solvent, was calculated to be 20.88 mV for Fe_2O_3 and -59.06 mV for $AgNO_3$ (Fig. 2a, d).

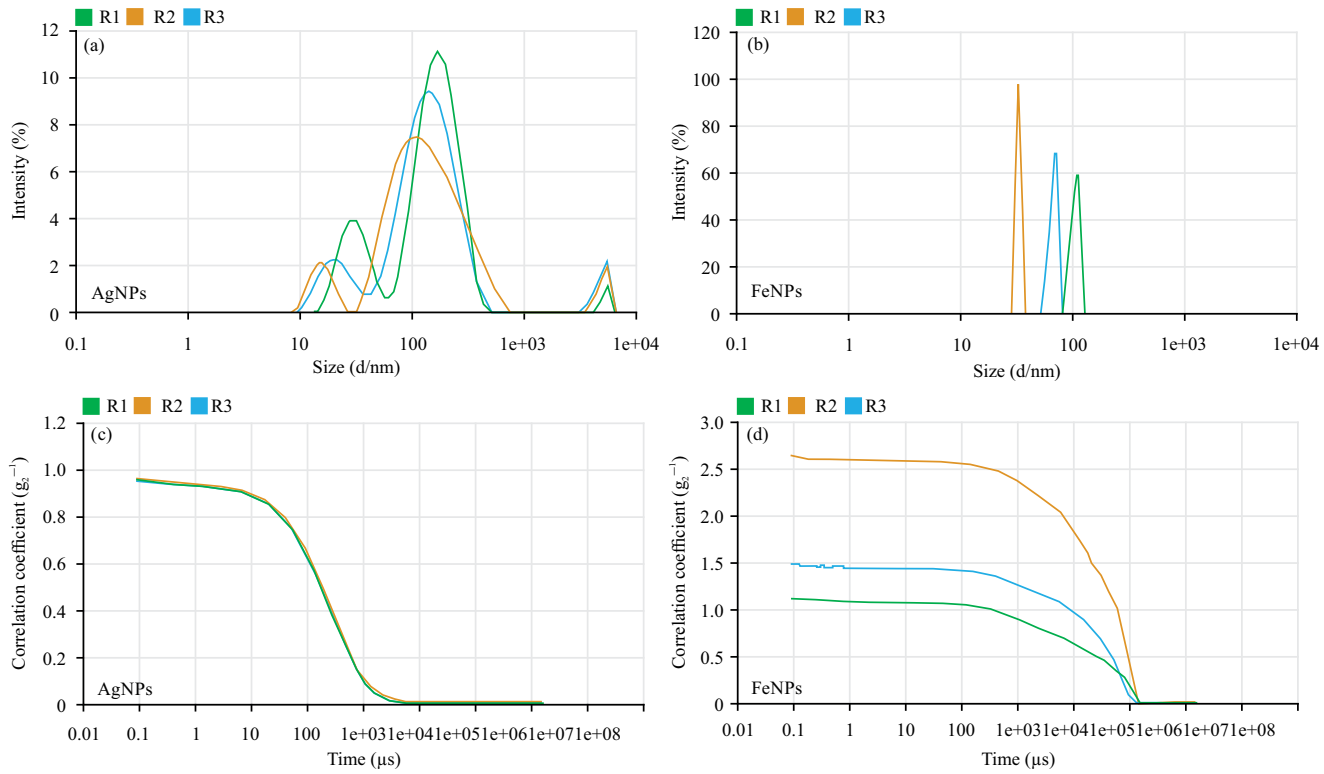

Fig. 1(a-d): Mean particle size distribution and correlation over time of, (a-b) AgNPs and (c-d) FeNPs (Fe_2O_3) biosynthesized using Spirulina and *Chlorella* extracts

The derived mean count rate, reference beam count rate and quality factor were also assessed. The Fe_2O_3 nanoparticles exhibited a derived mean count rate of 6445 kcps, while $AgNO_3$ nanoparticles showed a significantly lower value of 0.0005895 kcps. The reference beam count rate was measured at 3476 kcps for Fe_2O_3 and 3404 kcps for $AgNO_3$. Finally, the quality factor, reflecting the reliability of the measurement, was calculated to be 2.3057 for Fe_2O_3 and 4.3973 for $AgNO_3$ (Fig. 2).

SEM-EDX analysis: The Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) images depicted in (Fig. 3 and 4) illustrated the impact of treating cells with a cell-free algal extract of Spirulina and *Chlorella*, both without treatment (control) and with exposure to 100 µg/mL of AgNPs and FeNPs. The SEM images at various magnifications reveal nanoparticles evenly distributed throughout the cell structure without significant agglomeration. The AgNPs and FeNPs appear spherical, with dimensions ranging from 2-16 and 4-12 nm, respectively and average sizes of 4 nm and 6 nm for AgNPs and 3 and 8 nm for FeNPs. Elemental analysis via EDX confirms the presence of elemental silver and iron nanoparticles by sharp signals, with an optical absorption band at 3 KeV indicative of metallic silver and iron (Fig. 3 and 4).

Conversely, cells treated with AgNPs exhibit nanoparticle attachment on the cell surface, accompanied by agglomeration, fragmentation and distortion of the Spirulina and *Chlorella* extract. The presence of Ag in the EDX spectrum of AgNPs treated cells, absent in the control cells, confirms the accumulation of AgNPs in the algal suspension. Carbon and oxygen signals observed can be attributed to X-ray emission from the Spirulina and *Chlorella* cell wall. In the case of Silver Nanoparticles (AgNPs), varying weight percentages of carbon, oxygen and silver were observed across different scans. In Scan1, carbon, oxygen and silver constituted 33.69, 56.91 and 9.41% of the composition, respectively. These percentages shifted in Scan2 to 24.95% carbon, 71.74% oxygen and 3.31% silver and further changed in Scan3 to 39.49% carbon, 48.57% oxygen and 11.94% silver. This variability indicates fluctuations in the elemental composition of AgNPs across different measurements (Fig. 3).

On the other hand, Iron Oxide Nanoparticles (FeNPs) displayed distinct elemental compositions compared to AgNPs. In Scan1, carbon, oxygen and iron constituted 31.69,

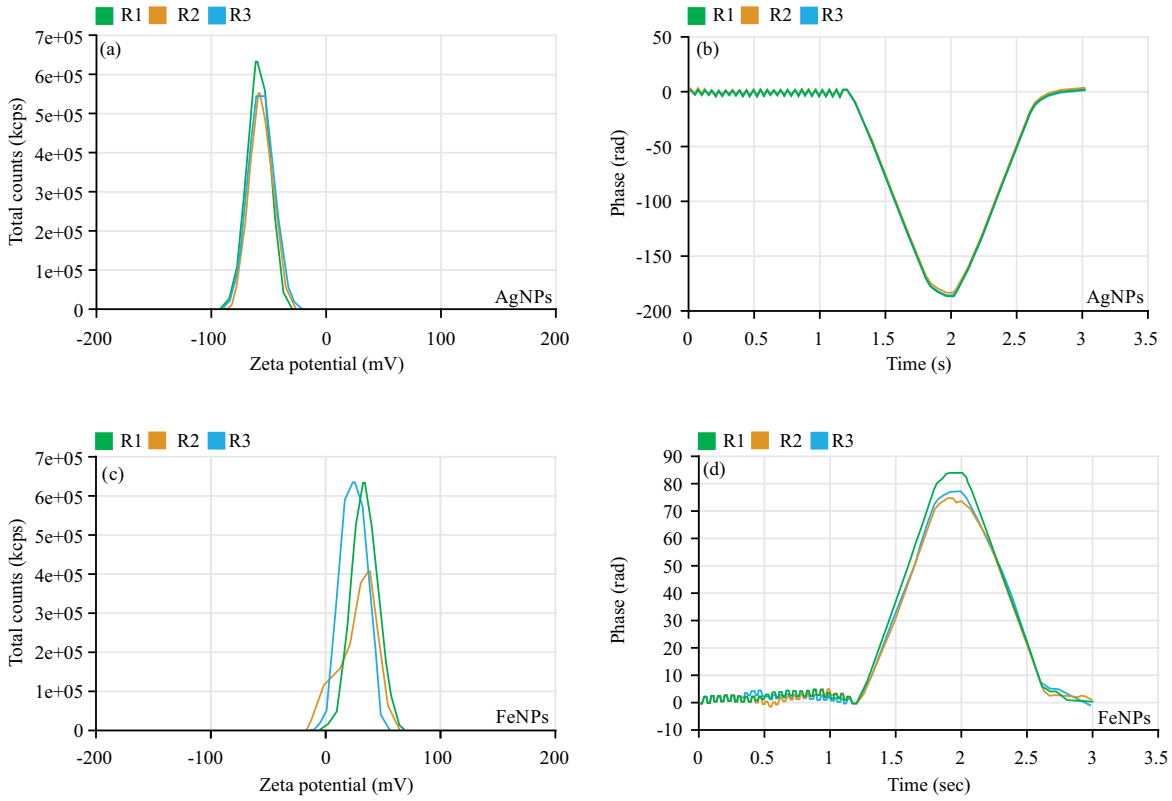

Fig. 2(a-d): Mean Zeta potential and phase over time of, (a-b) AgNPs and (c-d) FeNPs (Fe_2O_3) biosynthesized using Spirulina and *Chlorella* extracts

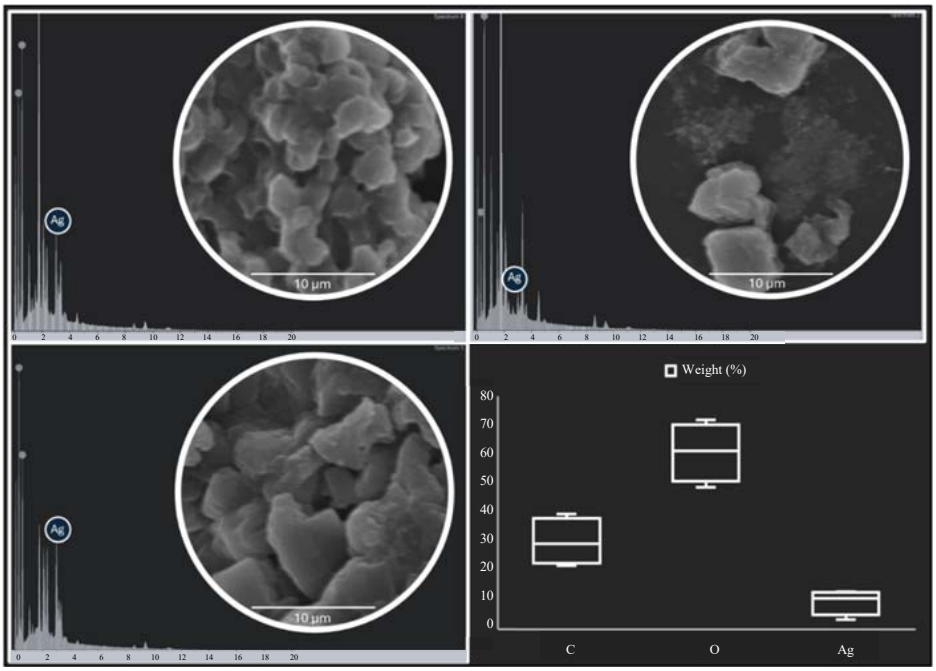

Fig. 3: Scanning Electron Microscopy (Sem-EDX) image for Ag nanoparticles synthesized using Spirulina and *Chlorella* extract
Boxplot shows the weight % for the carbon (C), oxygen (O) and silver (Ag) content collectively from three different scans

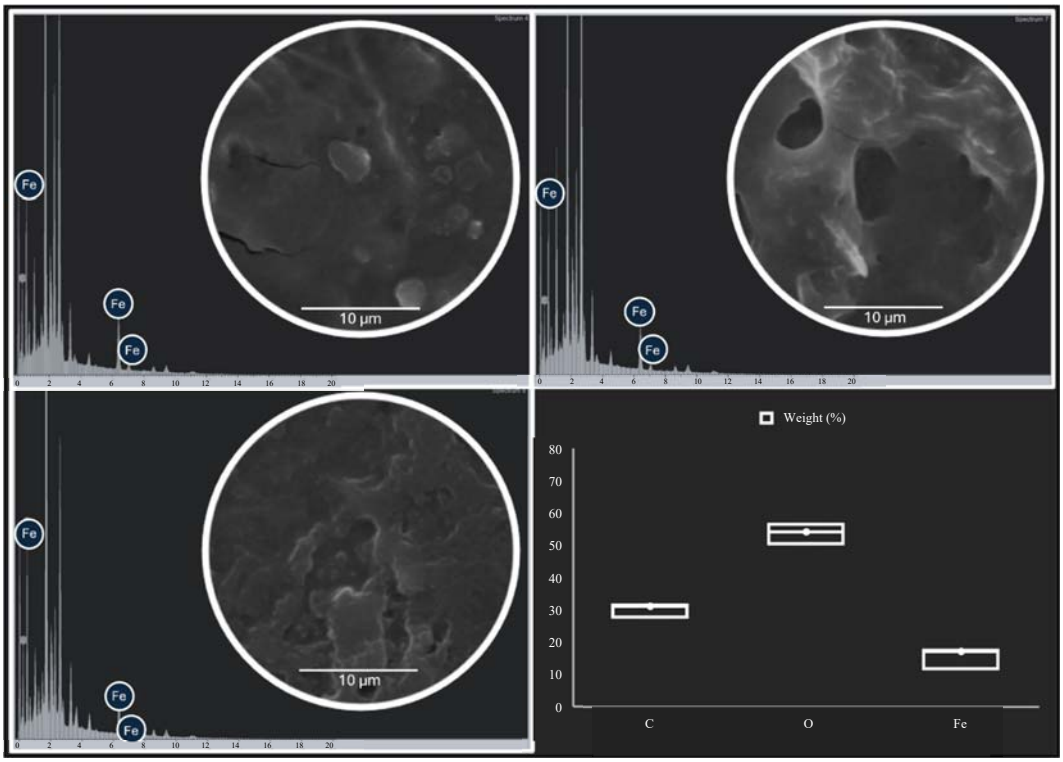

Fig. 4: Scanning Electron Microscopy (Sem-EDX) image for Fe nanoparticles synthesized using Spirulina and *Chlorella* extract
Boxplot shows the weight % for the carbon (C), oxygen (O) and iron (Fe) content collectively from three different scans

50.83 and 17.48% of the composition, respectively. These percentages altered in Scan2 to 28.11% carbon, 54.44% oxygen and 17.44% iron and further shifted in Scan3 to 31.19% carbon, 56.85% oxygen and 11.97% iron. The predominant presence of iron in FeNPs across all scans highlights the consistency in elemental composition compared to AgNPs (Fig. 4).

Comparative details between $AgNO_3$ and Fe_2O_3 nanoparticles: When comparing the properties of Silver Nanoparticles ($AgNO_3$) and Iron Oxide Nanoparticles (Fe_2O_3), it becomes evident that $AgNO_3$ nanoparticles exhibit several superior parameters. Firstly, $AgNO_3$ nanoparticles demonstrate a significantly smaller Z-average size of 93.71 nm compared to the much larger size of 6197.67 nm for Fe_2O_3 nanoparticles. This indicates that $AgNO_3$ nanoparticles are smaller and potentially more suitable for certain applications requiring smaller particle sizes. Additionally, $AgNO_3$ nanoparticles exhibit a lower polydispersity index (PI) of 0.607 compared to Fe_2O_3 nanoparticles with a PI of 0.8765, suggesting a more uniform size distribution among $AgNO_3$ nanoparticles. In terms of stability, $AgNO_3$ nanoparticles show a negative Zeta potential of -56.68 mV, indicating greater stability in suspension compared to the positive Zeta potential of 29.73 mV for Fe_2O_3 nanoparticles. Furthermore, $AgNO_3$ nanoparticles have a higher mean count rate of 318.77 kcps compared to Fe_2O_3 nanoparticles, which have a mean count rate of 198.77 kcps, suggesting a higher concentration of $AgNO_3$ nanoparticles in the sample. However, it's important to note that Fe_2O_3 nanoparticles exhibit a significantly higher derived mean count rate of 6445 kcps compared to $AgNO_3$ nanoparticles, indicating a higher intensity of Fe_2O_3 nanoparticles in the sample. Finally, $AgNO_3$ nanoparticles have a higher quality factor of 4.3973 compared to Fe_2O_3 nanoparticles, which have a quality factor of 2.3057, indicating greater reliability of the measurement for $AgNO_3$ nanoparticles. These findings collectively suggest that $AgNO_3$ nanoparticles possess superior properties in terms of size, size distribution, Zeta potential and mean count rate compared to Fe_2O_3 nanoparticles, making them potentially more suitable for various applications requiring nanoparticles with these characteristics.

DISCUSSION

Recent years have witnessed a surge in interest in green nanoparticle synthesis, driven by the unique properties of nanomaterials and their burgeoning applications in various

industrial and commercial sectors[34]. Both intracellular and extracellular synthesis of inorganic metal nanoparticles have been explored extensively across numerous microorganisms[35]. Among these, photoautotrophic organisms like prokaryotic cyanobacteria, eukaryotic algae, diatoms and components of higher plants have demonstrated the ability to biosynthesize metallic nanoparticles[36-38]. The current study investigated the synthesis and characterization of Silver Nanoparticles (AgNPs) and Iron Oxide Nanoparticles (FeNPs) using an algal cell-free extract. Upon adding the extract to silver nitrate and iron oxide solutions, distinct color changes indicated successful nanoparticle production within 24 hrs, with AgNPs displaying absorbance maxima at 430-440 nm. Dynamic light scattering (DLS) analysis revealed AgNPs' smaller size and more uniform distribution compared to FeNPs. The SEM-EDX images confirmed the presence of elemental nanoparticles, with AgNPs showing attachment on the cell surface. Comparative analysis favored AgNPs, highlighting their smaller size, uniform distribution, stability and higher concentration compared to FeNPs. These results suggested that the potential of AgNPs synthesized with algal extract for various applications, warranting further investigation.

In this study extracts from eukaryotic microalgae were utilized, specifically Spirulina and *Chlorella*, for the synthesis of stable silver and iron nanoparticles. Spirulina and *Chlorella* are autotrophic microscopic organisms characterized by high growth rates and biomass productivity, requiring minimal resources such as sunlight, atmospheric CO_2 and inexpensive mineral salts, rendering them suitable candidates for nanomaterial biosynthesis[39]. Khanna *et al.*[4] highlighted the pharmaceutical and nutraceutical potential of Spirulina, which serves as a rich source of pharmacologically active natural products and nutraceuticals.

Silver Nanoparticles (AgNPs) are known for their distinct reddish-yellow color in aqueous solutions, attributed to the excitation of surface plasmon vibrations[40]. The reduction of silver ions to AgNPs, accompanied by a color change, was monitored using UV-Vis spectroscopy, providing insights into the conversion reaction kinetics. The green synthesis of AgNPs and FeNPs using Spirulina and *Chlorella* extract resulted in clear yellowish-brown solutions, confirming the production of nanoparticles within 45 min and reaching the exponential phase within 48 hrs[41].

Furthermore, the bioactivity of AgNPs is size-dependent, favoring smaller particle sizes due to the increased surface area-to-volume ratio, which enhances contact surface area and bioavailability[42,43]. Zeta potential analysis revealed the stability of biosynthesized AgNPs and FeNPs, with net charges averaging -56.68 and -29.73 mV, respectively, indicating sufficient electrostatic repulsion for stability in solution[44]. Notably, previous studies have reported similar particle size and Zeta potential values for AgNPs biosynthesized from Spirulina microalgae, corroborating present findings[41].

The SEM-EDX analysis was employed to investigate the cellular accumulation of AgNPs and FeNPs in Spirulina and *Chlorella* extract, elucidating morphological changes induced by nanoparticle treatment. Similar observations were reported in studies involving *Streptomyces platensis* treated with Zinc Oxide Nanoparticles (ZnONPs), indicating potential physical stress on photosynthetic microbes due to NP accumulation[45,46]. The identification of elemental silver and iron via EDX analysis further confirmed the presence of AgNPs and FeNPs in the biomass, with strong signals observed at 7 keV. Quantitative analysis revealed mean percentages of silver and iron, providing valuable insights into the elemental composition of the synthesized nanoparticles[47]. The synthesized silver and iron nanoparticles hold promise for various applications, including but not limited to antibacterial agents, drug delivery systems, catalysis and environmental remediation.

CONCLUSION

The demonstrated the potential of utilizing eukaryotic microalgae, specifically Spirulina and *Chlorella*, for the green synthesis of stable silver and iron nanoparticles. Through a combination of UV-Vis spectroscopy, Zeta potential analysis and SEM-EDX characterization, we confirmed the successful biosynthesis of AgNPs and FeNPs using microalgal extracts. The obtained nanoparticles exhibited favorable properties such as size-dependent bioactivity and stability, as evidenced by their Zeta potential values. Additionally, SEM-EDX analysis provided insights into the cellular accumulation of nanoparticles in Spirulina and *Chlorella* extract, highlighting potential morphological changes induced by nanoparticle treatment. Overall, this research contributes to the growing body of knowledge on green nanoparticle synthesis methods and underscores the potential of microalgae as sustainable and eco-friendly sources for nanomaterial production. Further studies could explore additional applications and optimization strategies for these bio-synthesized nanoparticles in various fields. The successful synthesis of silver and iron nanoparticles using microalgae extracts opens up avenues for diverse applications in fields such as medicine, environmental science and nanotechnology. Further research and development

efforts are warranted to explore and optimize the nanoparticles' potential in antibacterial treatments, drug delivery, catalytic processes and environmental remediation strategies.

SIGNIFICANCE STATEMENT

The objective of this study was to explore the eco-friendly synthesis of silver and iron nanoparticles using microalgae extracts. Current research aimed to contribute to the field of nanobiotechnology by providing a sustainable and low-cost method for nanoparticle synthesis. The main results revealed successful synthesis of nanoparticles with distinct properties, showcasing the potential of microalgae as bio-reducing agents.

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