Morphological and Structural Characterization of *Spirulina*-Mediated Titanium Dioxide Nanoparticles

Abstract

In the present study, confirmation of *Spirulina platensis*-mediated TiO_2NPs formation was indicated by a noticeable shift in hue from white to light yellow. SEM analysis revealed the presence of spherically distributed nanoparticles with sizes between 90 and 150 nm, while TEM analysis further disclosed a hexagonal structure. EDAX analysis identified elemental composition, with titanium peaks appearing at 4.5 to 5 keV, carbon peaks at 0.5 keV, oxygen peaks at 0.4 to 1.0 keV, silica peaks at 1.7 to 2.0 keV, zinc peaks at 8.0 keV, copper peaks at 7 keV, potassium peaks at 4 keV, phosphorus peaks at 3 keV, and iron peaks at 6 keV. These comprehensive findings confirm the successful synthesis of well-defined *Spirulina platensis*-mediated TiO_2 nanoparticles and provide valuable insights into their structural and elemental characteristics.

Keywords: *Spirulina,* TiO_2 NPs, SEM and TEM with EDAX analysis

Introduction

Efforts to boost silk production, such as hybridization, artificial diets, and phyto-juvenoid use, lack significant results, urging novel approaches (Ni *et al.,* 2015). Nanotechnology's integration into sericulture opens new pathways for enhancing silk output, serving fields like drug delivery and electronic imaging (Sankar *et al.,* 2014). Emerging methods, like green-synthesized nanoparticles from sources like *Spirulina platensis*, offer eco-friendly alternatives, enriching silk gland parameters (Thangapandiya and Dharanipriya, 2019). Metal oxide semiconductors, notably TiO_2 nanoparticles, possess antibacterial properties and promote cocoon quality, suggesting their potential as feed additives (Li *et al.,* 2015). In summary, nanotechnology's adoption, especially via eco-friendly synthesis, and the use of metal oxide nanoparticles like TiO_2, show promise for advancing silk production sustainably in sericulture.

Materials and Methods

Collection of algae materials

Spirulina platensis dried powder was acquired from the India's R.K. Algae Centre is located in Mandapam, Tamil Nadu. The Botanical Survey of India in Coimbatore, Tamil Nadu, carried out the process of verifying the identity of algal species. (Plate 1).

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दिनांक/Date: 20th December 2021

पौधे प्रमाणीकरण प्रमाणपत्र / PLANT AUTHENTICATION CERTIFICATE

The micro-algal sample brought by you for authentication is identified as *Arthrospira platensis* Gomont - MICROCOLEACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

20/12/2021

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Plate 1. Confirmation of algal species (BSI, Coimbatore)

Preparation of aqueous extraction of *Spirulina platensis*

About 10g of finely chopped *Spirulina platensis* powder was heated for 45 minutes at 90°C in 100 milliliters of deionized water aqueous extract. Debris was then removed by passing a filter through the first Whatman filter paper. For the purposes of the following experiments, the clear green solution that subsequently cooled to a temperature of 4–8°C (Some et al., 2019).

Synthesis of titanium dioxide nanoparticles

Applying a water-based extract of *Spirulina* platensis as a capping agent and bio-reductant, 0.01 mM titanium dioxide was used to create *Spirulina*-mediated TiO2NPs in an environmentally friendly synthesis. Twenty milliliters of watery extract were combined with eighty milliliters of 0.01 M solution TiO2, that was stirred continuously in a hotplate magnetic stirrer for six hours at room temperature. a color shift that indicates TiO2NP production. To characterize the green synthesised *Spirulina*-mediated TiO2NPs, SEM and TEM with EDAX were in employment.

Scanning Electron Microscope

A scanning electron microscope (SEM) modelled after the Japanese Hitachi SU3500 was used to examine the morphology of titanium dioxide nanoparticles mediated by *Spirulina*. Samples were sputter-coated with gold palladium for 120 seconds prior to observation. After that, 10 kV of voltage was used to take the SEM images at various magnifications (Yedurkar et al., 2016).

Transmission Electron Microscopy with Energy Dispersive X-Ray Spectroscopy

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Characterizing the individual particles of the material was done using transmission electron microscopy, which has a resolution Tenfold greater than that of scanning electron microscopy. An small quantity of green synthesised TiO2NPs was sonicated for 10 minutes and then dispersed in dry ethanol for TEM analysis. In the prepared sample, On a grid covered in copper, a single drop of Spirulina-mediated TiO2NPs was carefully positioned. TEM pictures were taken with a Jeol JEM-2100 electron microscope (Japan). With EDAX, the generated TiO2NPs' elemental analysis was also completed.

Results and Discussions

Synthesis of TiO_2NPs using aqueous *Spirulina platensis* extract

After heating 10 g of finely chopped *Spirulina platensis* powder was heated to 90°C for 45 minutes in 100 milliliters of deionized water. The mixture was then filtered through Whatman filter paper No. 41 to remove impurities and yielded the aqueous extract of *Spirulina platensis*. The resulting transparent solution was kept at 4 to 8□. The *Spirulina platensis* mediated TiO_2NPs were prepared using Both titanium dioxide and *Spirulina platensis* aqueous extract are used as capping and bio-reductants. After adding 20 milliliters of *Spirulina* aqueous extract to 80 milliliters of 0.01 mM TiO2 solution, the mixture was stirred continuously at ambient temperature 200 rpm. The formation of green synthesised *Spirulina platensis* mediated TiO_2NPs was confirmed by the change of colour (pale yellow) within an hour. In the present investigation, the formation of *Spirulina platensis* mediated TiO_2NPs was verified by the shift in hue from white to pale yellow. This result corroborates Using the results of Gunasundari *et al*. (2014) who stated that the formation of *Spirulina platensis* mediated with various metal nanoparticles (Silver, Chromium, Zinc, Lead and Iron) were confirmed by the change of colours such as The hue of AgNps transformed from yellow to brown in the solution; the color of CrNPs changed changing from orange to green; the color of PbNPs changed blue instead of white; the color of ZnNPs became white instead of green; and the color of FeNPs changed from yellow to green.

Characterization of nanoparticles

The structural and particle size characterization of *Spirulina platensis* mediated TiO_2NPs were investigated by SEM and TEM with EDAX analysis. In the present experiment, S. platensis mediated TiO2NPs were characterized through SEM and TEM with EDAX analysis. The results are present here.

Scanning Electron Microscopy (SEM)

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The SEM investigation of *Spirulina platensis* mediated TiO_2NPs was spherical and irregularly distributed evenly with dimensions between 90 and 150 nm (Plate. 2). The When ultrasonic-assisted *Spirulina platensis* algal extract loaded green synthesized metal nanoparticles was applied, rougher and irregular spores developed on the surface of UASP. This suggested that the UASP material had effectively been coated with the metal nanoparticles (Ag, Cr, Pb, and Zn) (Gunasundari et al., 2017). According to Santhosh Kumar et al. (2014), *Psidium guajava*-mediated TiO2 NPs had a smooth, spherical shape with a range of physical morphologies and particle sizes visible in the SEM images. Miankushki (2012) who reported that SEM analysis of TiO_2NPs showed interconnected NPs are predominantly used for biological and electrochemical purpose. Suresh *et al.* (2015) stated that the existence of metal oxides in the sample creates agglomeration of TiO_2NPs.

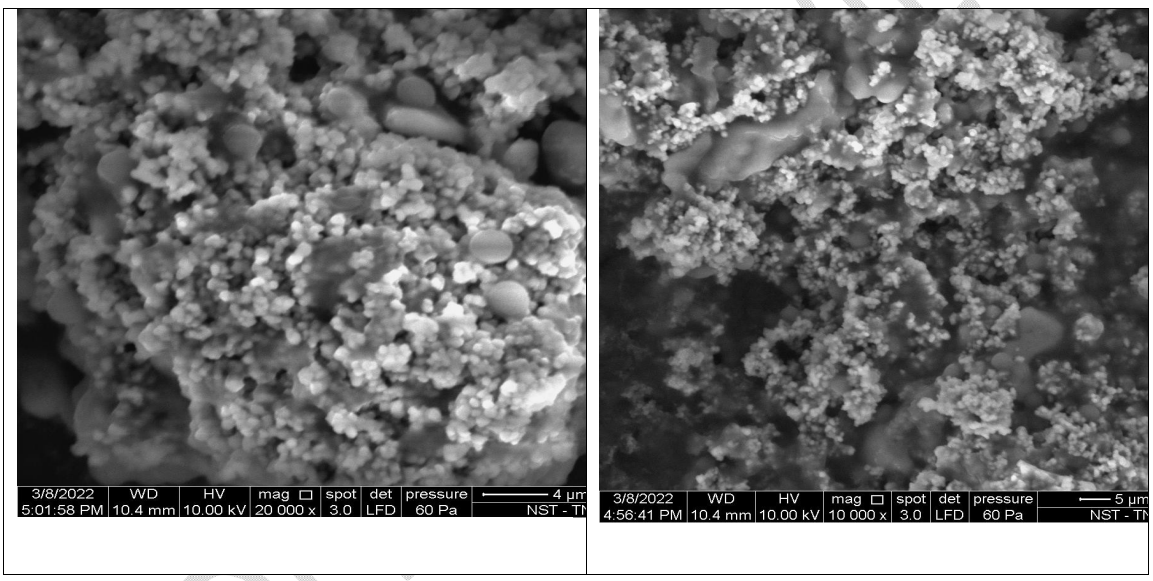
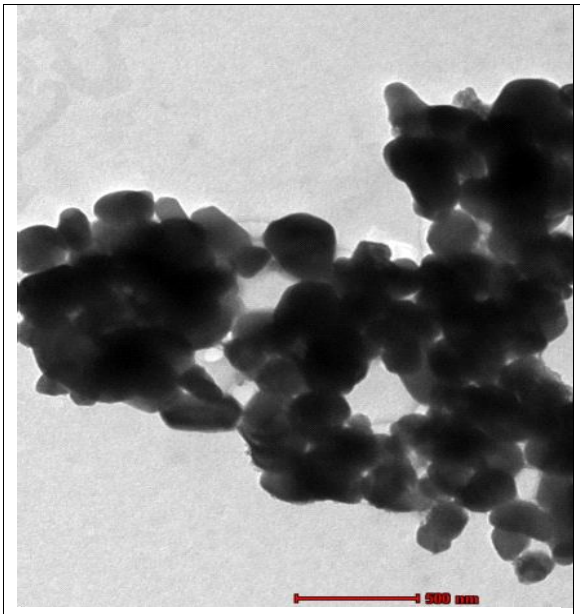
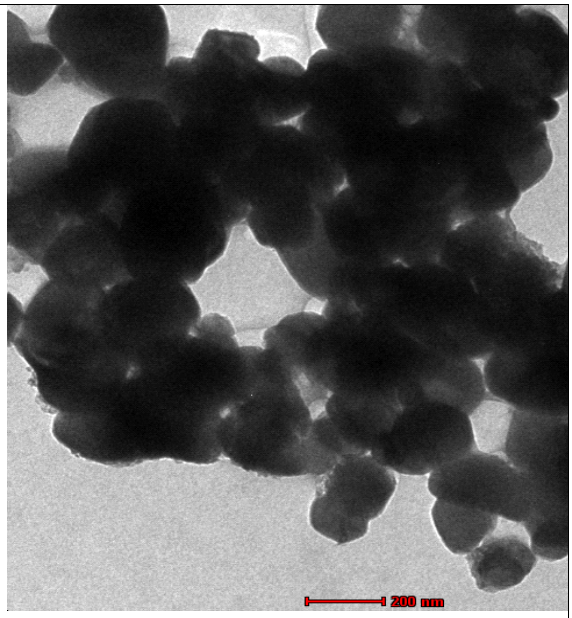


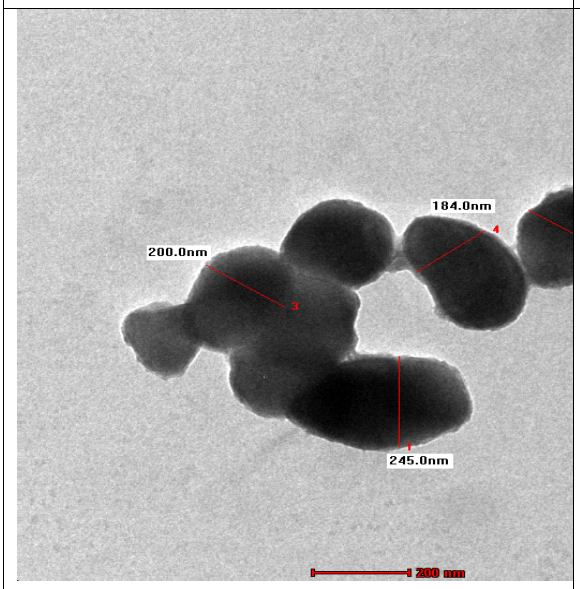
Plate 2. SEM images of *Spirulina platensis mediated* TiO_2NPs

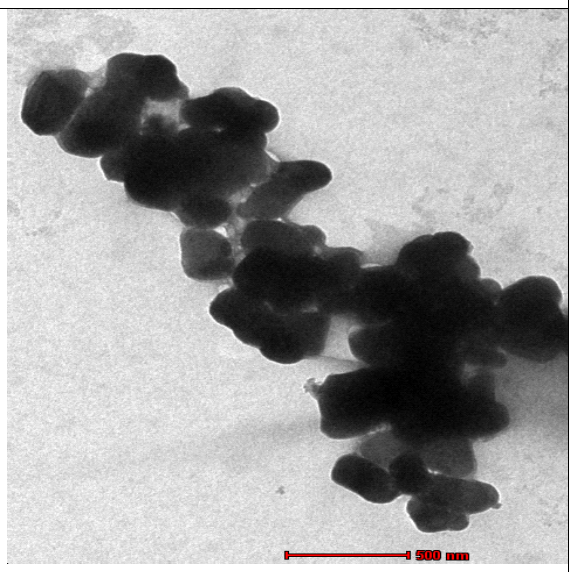
Transmission Electron Microscopy (TEM) with Energy Dispersive X-Ray Spectroscopy (EDAX)

TEM images were used to assess the size, shape, and crystallinity of *Spirulina platensis*-mediated TiO2NPs. The nanoparticles had a moderate degree of size variation and an uneven, hexagonal shape. The dimensions fell between 50 to 60 nm (Plate 3a)


500 nm


200 nm


184.0nm
200.0nm
245.0nm
200 nm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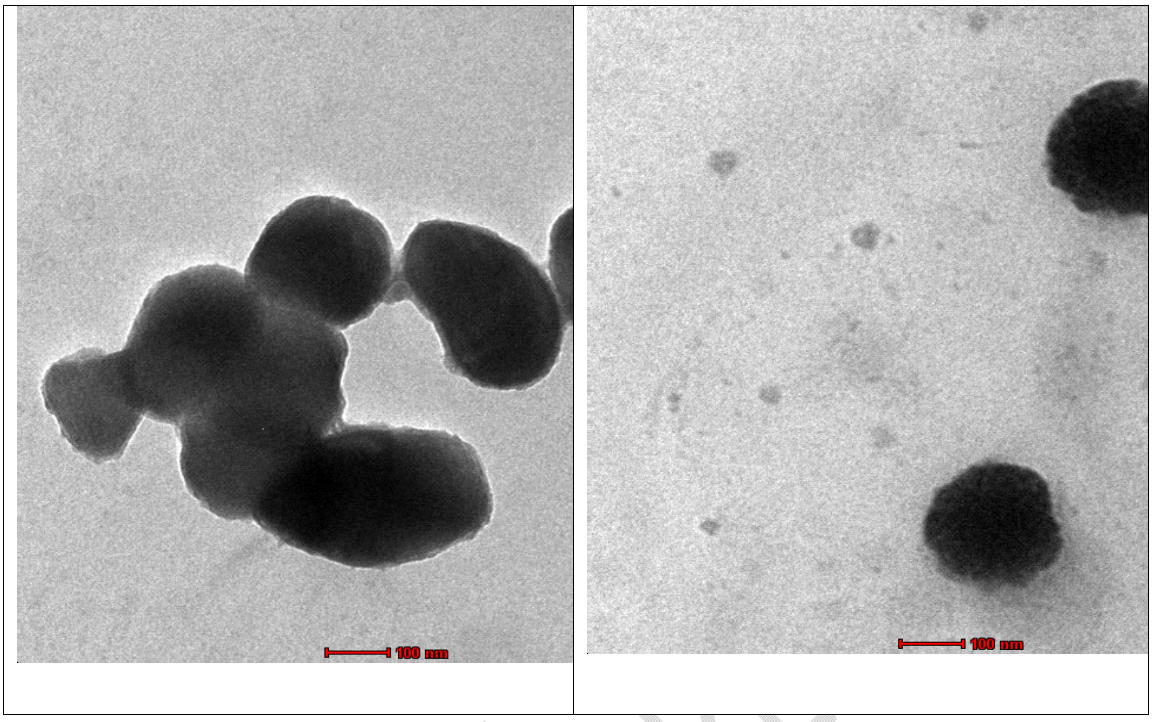

Plate 3a. TEM images of *Spirulina platensis mediated* TiO_2NPs

The analysis using energy dispersive X-ray spectroscopy (EDAX) showed the elemental composition, which was present in the *Spirulina platensis* mediated TiO_2NPs (Plate 3b). The titanium peaks appeared at 4.5 to 5 keV, carbon peaks at 0.5 keV, oxygen peaks at 0.4 to 1.0 keV, silica peaks at 1.7 to 2.0 keV, zinc peaks at 8.0 keV, copper peaks at 7 keV, potassium peaks at 4 keV, phosphorus peaks at 3 keV, iron peaks at 6 keV and various metal oxides were found and the existence of magnesium, carbon, and oxygen, zinc, phosphorus and potassium and chlorine indicated the existence of biomolecules deposited on the exterior of titanium nanoparticles. The particles were in the energy dispersive analysis, which demonstrated fact metallic and crystalline TiO_2NPs. The SEM and TEM analysis was applied for estimating the volume and morphological appearance of artificial NPs. In the present study, the obtained results of SEM and TEM with EDAX analysisthe, nanoparticles' sizes varied from 90 to 150 nm. whereas in TEM analysis it was found to be 50 to 60 nm and the EDAX analysis showed the elemental composition such as carbon, oxygen, magnesium, zinc, phosphorus and potassium and chlorine indicates biomolecules that have been adsorbed onto the surface of titanium nanoparticles. The particles were in the energy dispersive analysis, which demonstrated fact metallic and crystalline TiO_2NPs. Spherical shape and interconnected NPs were identified from *A. indica* leaf extract mediated TiO_2NPs (Sankar *et al.,* 2014). Hariharan *et al.* (2017) used TEM images to reveal the size, shape, and crystallinity of green synthesised TiO_2NPs. The nanoparticles varied in shape from irregular

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to hexagonal, and their sizes varied somewhat ranging from 13 to 34 nm. Based on the above information the synthesised *Spirulina platensis* mediated TiO_2NPs could be used for various biological purposes.

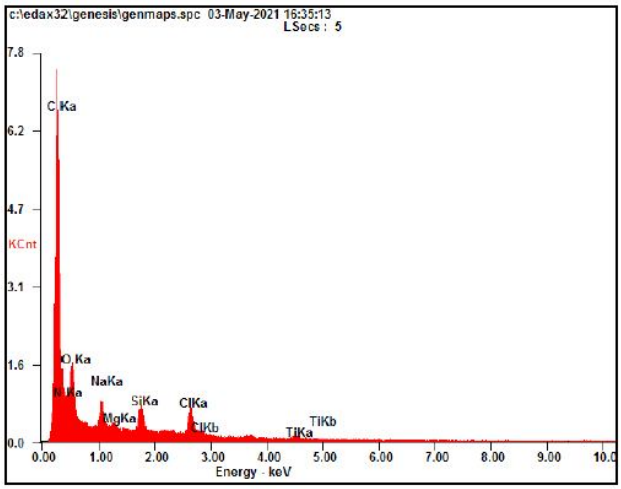

Plate 3b. TEM with EDAX element of *Spirulina platensis* mediated TiO_2NPs

Conclusions

The SEM analysis demonstrated the production of TiO2 nanoparticles (TiO2NPs), which are spherical and range in size from 90 to 150 nm, while TEM analysis revealed a hexagonal structure in *Spirulina platensis*-mediated TiO_2NPs. These findings underscore the effectiveness of *Spirulina platensis* as a bioresource for synthesizing well-defined and structured TiO_2 nanoparticles, holding promise for diverse applications in nanotechnology and highlighting the potential of environmentally friendly biogenic synthesis methods.

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