

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.810.268>

Bio-Synthesis and Characterization of Titanium Dioxide Nanoparticles (TiO₂) Using *Azadirachta indica* Leaf (Neem Leaf) Extract

Vidya Kulkarni¹, Vijayakumar Palled^{2*}, Sharanagouda Hiregoudar³,
K. V. Prakash², Devanand Maski² and Sushilendra¹

¹Department of Farm Machinery and Power Engineering, ²Department of Renewable Energy Engineering, ³Centre for Nano Technology, Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur-584104, Karnataka, India

*Corresponding author

ABSTRACT

The green synthesis of nanoparticles is an eco-friendly approach which is inexpensive and non-hazardous. Numerous plant extracts are used in the synthesis of nanoparticles from neem leaf, lemongrass, aloe vera, moringa leaves, etc. The present study was aimed at the bio-synthesis and characterization of Titanium dioxide nanoparticles (TiO₂) using *Azadirachta indica* leaf (neem leaf) extract. Characterization was carried out using Zetasizer, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). The average size of nanoparticles measured by Zetasizer was 56.13 nm. XRD confirmed the anatase crystalline structure of synthesized Titanium dioxide nanoparticles. SEM analysis showed that the morphology of synthesized nanoparticles was spherical and results of AFM indicated that the nanoparticles are smooth. The study inferred that Titanium dioxide nanoparticles could be synthesized using neem leaf extract which provides a novel replacement for chemical synthesis.

Keywords

Bio-synthesis,
Morphology, Neem
leaf extract,
Titanium dioxide
nanoparticles

Article Info

Accepted:
17 September 2019
Available Online:
10 October 2019

Introduction

Nanotechnology is the branch of science which deals with matter having at least one dimension sized from 1-100 nm. Materials

reduced to nano scale can show different properties compared to what they exhibit on macro scale. Generally, properties of nanoparticles depend on size, shape, composition morphology and crystalline

phase. Titanium dioxide nanoparticles possess interesting optical, dielectric, antimicrobial, antibacterial, chemical and catalytic properties which lead to industrial applications such as pigment, fillers, catalyst support and photo catalyst.

Traditionally, nano metal oxides are synthesized using chemical methods such as sol-gel technique and electrochemical technique, reduction etc. but these methods are costly, toxic, high pressure, high energy requirement and potentially hazardous (Sundrarajan and Gowri, 2011). Hence, developing a reliable biosynthetic approach has added much importance, as it is a bottom up approach where the main reaction occurring is reduction/oxidation. The plant phyto-chemicals with antioxidant or reducing properties are usually responsible for the preparation of metal and metal oxide nanoparticles (Kim *et al.*, 2013). Among the various biosynthetic approaches, the use of plant extract has advantages such as easily available, safe to handle and possess a broad viability of metabolites.

The main phytochemicals responsible for the reduction of nanoparticles are terpenoids, flavones, ketones, aldehydes, amides (Sivaranjani and Philominathan, 2015). The biological process eliminates the elaborate process of maintaining self cultures and can also be easily scaled up for large scale production of nanoparticles (Veeraswamy *et al.*, 2011).

In this paper, process of biosynthesis of Titanium dioxide using neem leaf extract has been explained. Characterization of TiO₂ nanoparticles was carried out to determine parameters like particle size using zetasizer, morphology by Scanning Electron Microscope (SEM), crystallinity by X-Ray Diffraction (XRD) and material structural characteristics by Atomic Force Microscope (AFM).

Materials and Methods

The biosynthesis of Titanium dioxide nanoparticles using *A. indica* leaf extract was carried out as explained below.

Preparation of *Azadirachta indica* leaf extract

Synthesis of Titanium dioxide nanoparticles was carried out at the Centre for Nanotechnology, College of Agricultural Engineering, University of Agricultural Sciences Raichur. Fresh neem leaves were collected from campus of University of Agricultural Sciences Raichur, were washed with distilled water to remove dust and dirt and shade dried for 7 days under normal atmospheric conditions. Dried leaves were cut into fine pieces, grinded to get the finest powder. 15 g of dried leaves were mixed with 150 ml of ethanol and extracted under reflux conditions at 50° C using soxhlet apparatus (M/s Tarsons, 6090, Kolkata, India). After one hour the ethanolic leaf extract was obtained by filtering the mixture through Whatman No. 1 filter paper and stored at 4° C for further use (Patidar and Jain, 2017)

Biosynthesis of Titanium dioxide nanoparticles using *Azadirachta indica* leaf extract

The process flowchart for biosynthesis of nanoparticles is presented in Plate 1 and Figure 1. For synthesis of Titanium dioxide nanoparticles, 0.5 M Titanium isopropoxide was dissolved in the mixture of 10 mL of ethanol and 40 mL of distilled water and homogenized for 15 min using homogenizer (M/s Tarsons, 6090, Kolkata, India). Ten mL of ethanolic leaf extract was added to 50 mL of homogenized mixture. The mixture was heated (24 hours) using magnetic stirrer (M/s Tarsons, 6090, Kolkata, India) (50°C) until colour changed. Upon heating, the chemical

reaction took place resulted in colour change in the reactants from white to brown and the mixture was taken off from the magnetic stirrer and cooled. The appearance of brown colour indicated the formation of Titanium dioxide nanoparticles. The formed Titanium dioxide nanoparticles were acquired by centrifugation at 5000 rpm for 15 minutes using high speed centrifuge (M/s Tarsons, 6090, Kolkata, India). Separated Titanium dioxide nanoparticles were dried to calcination using muffle furnace at 550 °C for five hours. The calcinated Titanium dioxide nanopowder was used for its characterization (Patidar and Jain, 2017).

Characterization of standard and biosynthesized Titanium dioxide nanoparticles

Characterization of standard and biosynthesized titanium dioxide nanoparticles using standard procedure is explained below

Particle size analysis using Zetasizer

Zetasizer (ZETA Sizer, nano383, Malvern, England) was used in the study as dynamic light scattering apparatus to measure average particle size (nm). A pinch of TiO₂ Nanoparticles were dissolved in 5 mL of ethanol and sonicated using ultra sonicator at 50 °C for 15 min. After sonication the solution is filled in cuvette up to 3/4th of volume and placed in the dynamic light scattering chamber. During analysis, the required settings were made. The average particle diameter (nm) was recorded from size distribution by intensity graph.

Surface morphology analysis using Scanning Electron Microscopy

The SEM image of the TiO₂ nanoparticles surface was obtained by scanning it with a high energy beam of electrons in vacuum

chamber. When the beam of electrons strikes the surface of the specimen and interacts with atoms of sample, it produces signals in the form of secondary electrons and back scattered electrons. These signals contain information about sample's surface morphology (Haq *et al.*, 2014).

Magnification can be adjusted from about 1 to 30,000 times to get clear morphology of Titanium dioxide nanoparticles at the accelerating voltage of 5 to 30 kV with working distance at 10 mm (Joseph *et al.*, 2016).

Phase identification using X-Ray Diffraction

X-Ray diffraction (XRD analysis) is a unique method in determination of crystallinity of a compound. Crystalline nature of the Titanium dioxide nanoparticles was measured on X-ray diffraction instrument (M/s Rigaku, Ultima 4, Tokyo, Japan) operated at 30 kV and 100 mA. Spectrum was recorded by CuK α radiation with wavelength of 1.5406 Å in the 2 θ range of 20-80°.

Surface analysis using atomic force microscopy

Atomic force microscope provides a 3D profile of the surface on a nanoparticles by measuring forces between a sharp probe (< 10 nm) and surface at very short distance (0.20-10 nm probe sample separation). The probe is supported and placed at the end of a flexible cantilever.

The AFM tip “gently” touches the surface and records the small force between the probe and the surface. The amount of force between the probe and sample is dependent on the spring constant, stiffness of the cantilever and the distance between the probe and the sample surface. This force can be described using Hooke's law (Hong *et al.*, 2017).

Results and Discussion

Synthesized nanoparticles were characterized using zetasizer, XRD, SEM and AFM.

Particle Size Analysis of standard and biosynthesized nanoparticles using Zetasizer

The characterization of standard and biosynthesized Titanium dioxide nanoparticles was done in terms of average particle diameter from the intensity distribution analysis by using zetasizer. The size distribution histogram of zetasizer indicated that, the size of standard and biosynthesized Titanium dioxide nanoparticles was 99 nm and 56.13 nm respectively.

Figure 2 and 3 show the dynamic light scattering (DLS) pattern of the suspension of biosynthesized Titanium dioxide nanoparticles (56.13 nm) using *A. indica* root extract and standard titanium dioxide nanoparticles. The obtained results were in accordance with the reviewed reports of Sankar *et al.*, (2015) who reported that the size of biologically synthesized Titanium dioxide nanoparticles was 124 nm and Hiremath *et al.*, (2014) who presented a histogram of biologically synthesized Titanium dioxide nanoparticles in which approximately 90% of the particles were within 100 nm.

Surface morphology analysis of standard and biosynthesized nanoparticles using SEM

The SEM image of standard and biosynthesized Titanium dioxide nanoparticles were in spherical shapes (Fig. 4 and 5). This might be due to the availability of different quantity and nature of capping agents present in the leaf extract (Srirangam and Rao, 2017). Similar results were obtained by Sundrarajan and Gowri (2011) with the average size was ranging between 100-150 nm with

interparticle distance and shape was uniformed spherical.

Phase identification of standard and biosynthesized nanoparticles using XRD

The crystalline nature of Titanium dioxide nanoparticles was confirmed from the X-Ray diffraction analysis. Figure 6 and 7 shows typical XRD pattern of standard and biosynthesized Titanium dioxide nanoparticles. XRD pattern showed five distinct diffraction peaks at 25.3°, 37.7°, 48.7°, 54.0° and 62.7° that were corresponding to (101) (004) (200) (105) and (204) reflection planes of biosynthesized Titanium dioxide nanoparticles respectively.

The highest peak was observed at 25.3° (101) reflection. The XRD study confirmed that, the resultant nanoparticles were face centred tetrahedral in nature with anatase crystalline structure. The obtained results are in close agreement with results reported by Ganapathi *et al.*, (2015). Our findings were also in correlation with Patidar and Jain (2017).

Surface analysis of standard and biosynthesized nanoparticles using AFM

Surface strength of Titanium dioxide nanoparticles were studied using atomic force microscope (AFM). AFM micrographs with a scanning area of 10 × 10 µm of Titanium dioxide nanoparticles in 2D and 3D images of standard and biosynthesized Titanium dioxide nanoparticles sample (Fig. 8 and 9) showed spherical particles with different sizes. Height and width of standard titanium dioxide was 79 nm and 157 nm respectively whereas for biosynthesized titanium dioxide nanoparticles 2.45 and 2.20 µm were recorded respectively. Jalill *et al.*, (2016) quoted that the Titanium dioxide nanoparticles were distributed in granularity volume when synthesized using *Curcuma Longa* plant extract.

Fig.1 Process flow chart for biosynthesis of Titanium dioxide nanoparticles using neem (*Azadirachta indica*) leaf extract

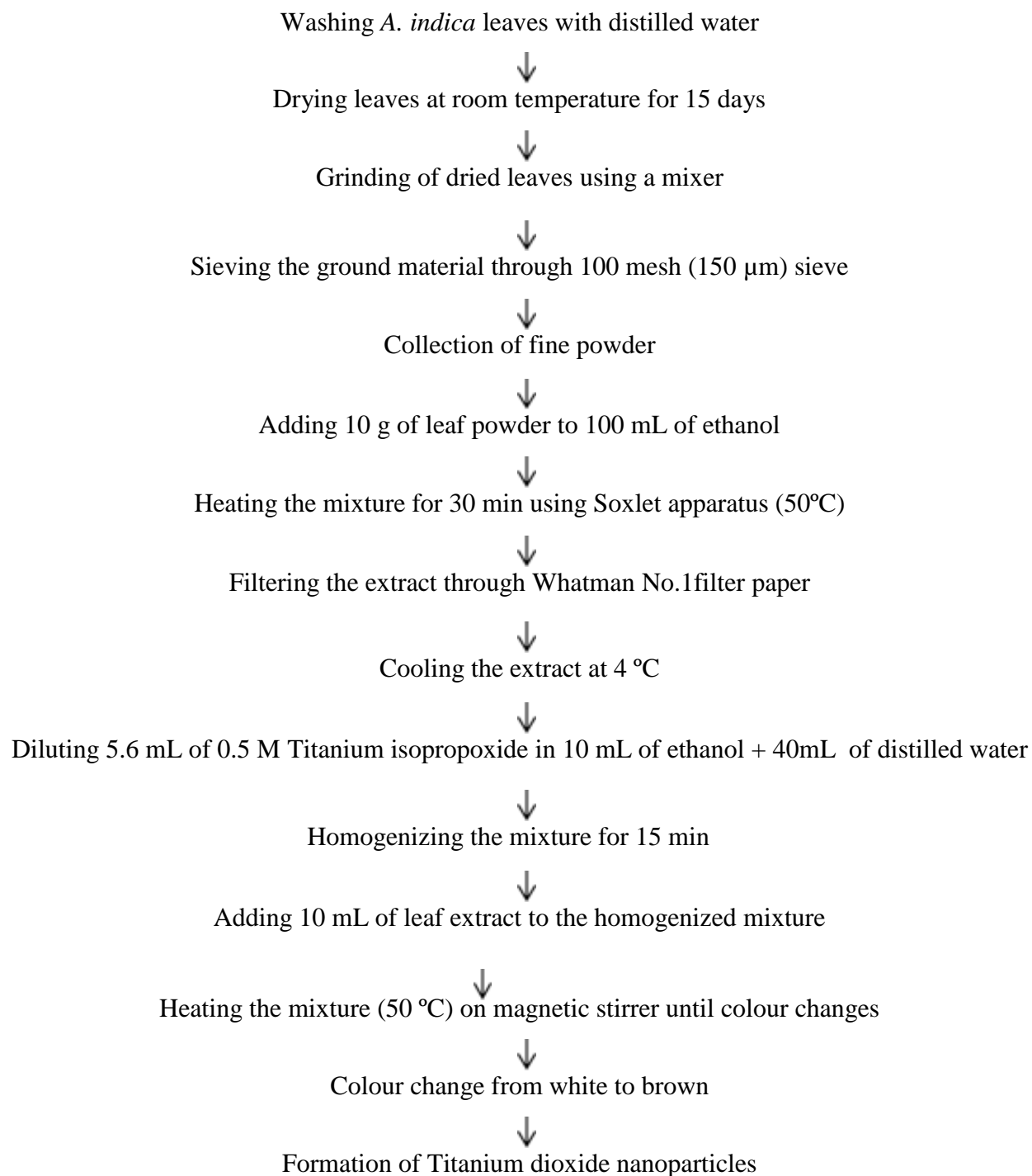


Fig.2 size distribution of standard TiO₂ nanoparticles

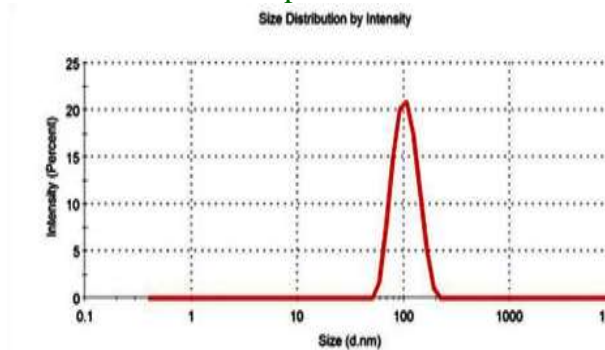


Fig.3 size distribution of biosynthesized TiO₂ nanoparticles

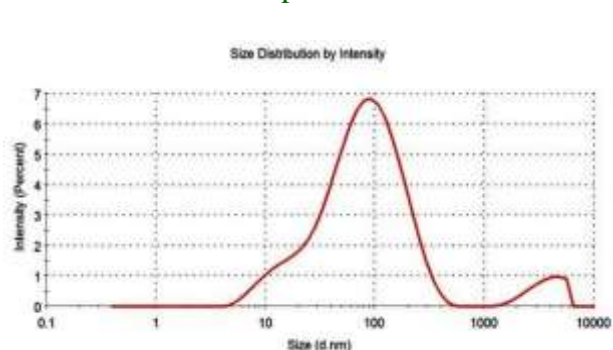


Fig.4 SEM image of standard TiO₂ nanoparticles

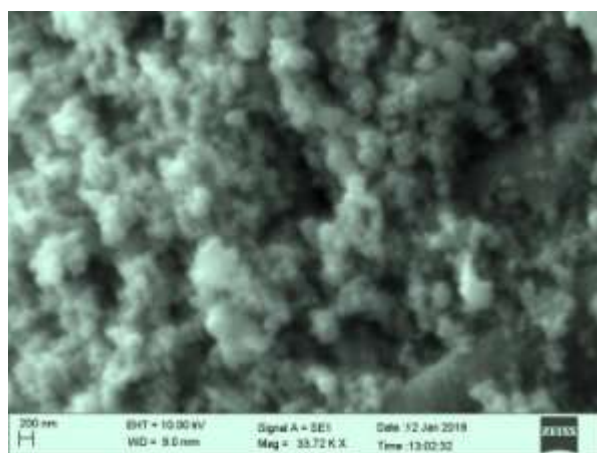


Fig.5 SEM image of biosynthesized TiO₂ nanoparticles

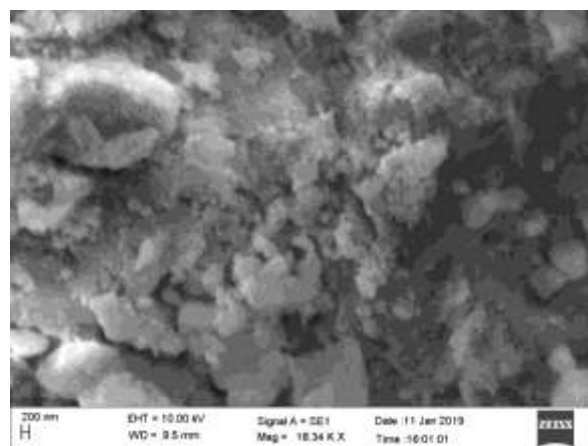


Fig.6 XRD image of standard TiO₂ nanoparticles

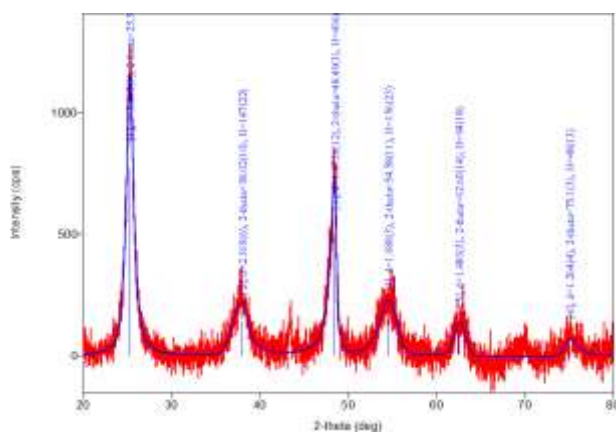


Fig.7 XRD image of biosynthesized nanopartic

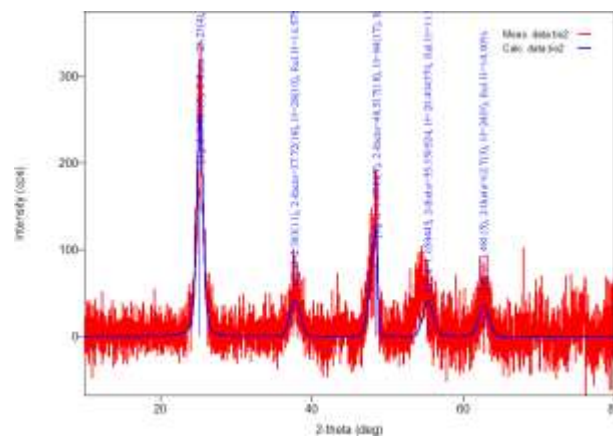
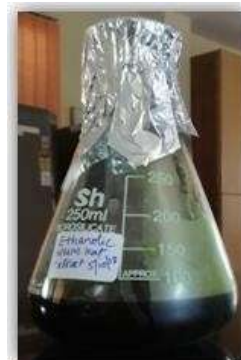


Plate.1 Biosynthesis of TiO₂ nanoparticles



Dried leaves



aqueous leaf extract



Reduced TiO₂ nanoparticles



Oven dried nanoparticles



Calcinated TiO₂ nanoparticles

Fig.8 AFM image and profile of standard titanium dioxide nanoparticles

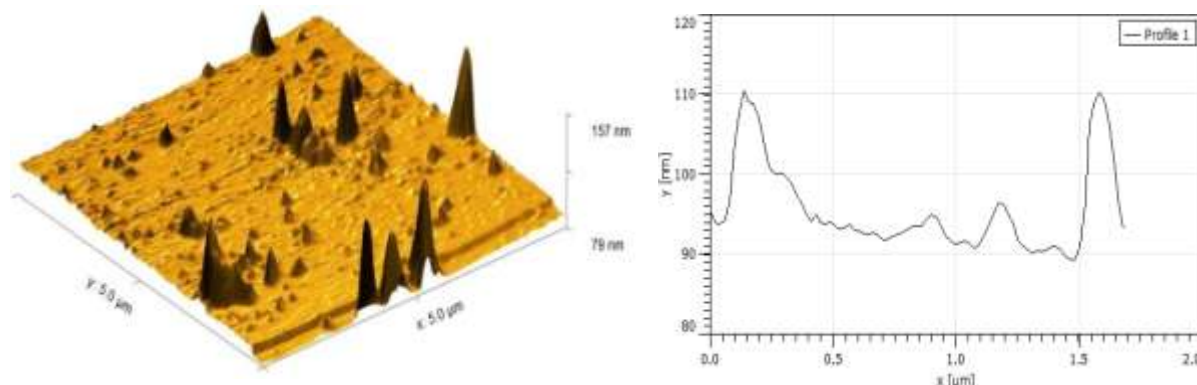
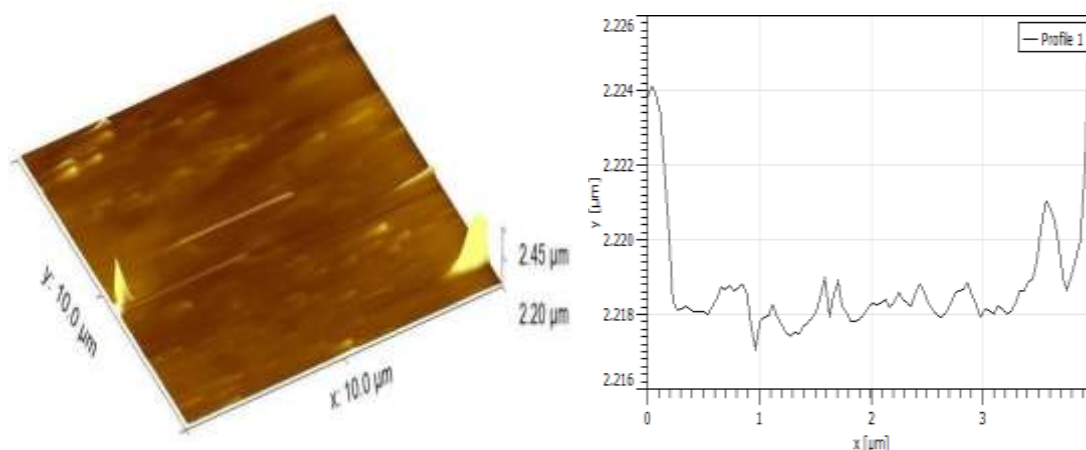


Fig.9 AFM image and profile of biosynthesized Titanium dioxide nanoparticles



Biosynthesis of nanoparticles proves to be a novel approach when compared with various approaches.

Titanium dioxide nanoparticles were synthesized using ecofriendly, non-toxic and cost effective approach.

Green synthesis of nanoparticles resulted in the size of 56.13 nm when analyzed using Zetasizer, XRD confirmed the anatase crystalline structure, SEM analysed revealed that the synthesized nanoparticles were spherical in shape, AFM analysis indicated the morphology of synthesized nanoparticles.

References

- Ganapathi, K., Ashok, C. H., Venkateswara Rao, K., Chakra, S. CH. and Tambur, P., 2015, Green Synthesis of TiO₂ Nanoparticles Using Aloe Vera Extract, *International Journal of Advanced Research in Physical Science*. 2(1A): 28-34.
- Haq, I. U., Akhtar, K. and Malik, A., 2014, Effect of experimental variables on the extraction of silica from the rice husk ash. *Journal of the Chemical Society of Pakistan*, 36(3): 382-387.
- Hiremath, S., Vidya, Antonyraj, L. A. M., Chandrarabha, M. N. and Seemashri,

- S., 2014, Green synthesis of TiO₂ nanoparticles by using neem leaf extract. *International Review of Applied Biotechnology and Biochemistry*. 2(1): 11-17.
- Hong, R., Ji, J., Tao, C., Zhang, D. and Zhang, D., 2017, Fabrication of Au/grapheme oxide/Ag sandwich structure thin film and its tunable energetics and tailorable optical properties. *Material Sciences*. 4(1): 223-230.
- Jalill, A., Raghad, D.H., Nuaman, R.S. and Abd, A.N., 2016, Biological synthesis of Titanium Dioxide nanoparticles by Curcuma longa plant extract and study its biological properties. *World Scientific News*. 49(2): 204-22.
- Joseph, A. T., Prakash, P. and Narvi, S. S., 2016, Phytofabrication and characterization of copper nanoparticles using *Allium sativum* and its antibacterial activity. *International Journal of Scientific Engineering and Technology*, 4(2): 463-472.
- Kim, H.J., Kim, D.J., Karthick, S.N., Hemalatha, K.V., Raj, C.J., Ok, S. and Choe, Y., 2013, Curcumin dye extracted from Curcuma longa L. used as sensitizers for efficient dye-sensitized solar cells. *International Journal of electrochemical science*. 8(6): 8320- 8328.
- Patidar and jain, 2017, Green Synthesis of TiO₂ Nanoparticles Using *Moringa oleifera* Leaf Extract. *International Research Journal of Engineering and Technology*. 4(3): 470-473.
- Sankar, R., Rizwana, K., Shivashangari, K. S. and Ravikumar, V., 2015, Ultra-rapid photocatalytic activity of *Azadirachta indica* engineered colloidal Titanium dioxide nanoparticles. *Applied Nanoscience*. 5(6): 731-736.
- Sivaranjani, V. and Philominathan, P., 2015, Synthesize of Titanium dioxide nanoparticles using *Moringa oleifera* leaves and evaluation of wound healing activity. *Wound Medicine*.
- Srirangam, G.M. and Rao, K.P., 2017, Synthesis and characterization of silver nanoparticles from the leaf extract of *Malachra capitata* (L.). *Journal of Chemistry*. 10(1): 46-53.
- Sundarajan, M. and Gowri, S., 2011, Green synthesis of Titanium dioxide nanoparticles by *Nyctanthes arbortristis* leaves extract. *Chalcogenide Lett*. 8(8): 447-451.
- Vanaja, M., Rajeshkumar, S., Paulkumar, K., Gnanajobitha, G., Malarkodi, C. and Annadurai, G., 2013, Phytosynthesis and characterization of silver nanoparticles using stem extract of *Coleus aromaticus*. *International Journal of Materials and Biomaterials Applications*. 3(1): 1-4.
- Veeraswamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N. and Dhanaraj, S. A., 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of Saudi Chemical Society*. 15(4): 113-120.

How to cite this article:

Vidya Kulkarni, Vijayakumar Palled, Sharanagouda Hiregoudar, K. V. Prakash, Devanand Maski and Sushilendra. 2019. Bio-Synthesis and Characterization of Titanium Dioxide Nanoparticles (TiO₂) Using *Azadirachta indica* Leaf (Neem Leaf) Extract. *Int.J.Curr.Microbiol.App.Sci*. 8(10): 2309-2317. doi: <https://doi.org/10.20546/ijcmas.2019.810.268>