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Rapid phytosynthesis of nano-sized titanium using leaf extract of *Azadirachta indica*

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Abstract: *Azadirachta indica* plant mediated biosynthesis of titanium nanoparticles was developed. Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), field emission scanning electron microscopy and energy dispersive spectrometry (FESEM–EDS), and transmission electron microscopy (TEM) were used to characterize the titanium nanoparticles. The results of the FTIR study confirmed that the presence of terpenoids, flavonoids and proteins was considered to be responsible for the formation and stabilization of titanium nanoparticles. XRD studies indicated that the titanium nanoparticles were crystalline in nature with mixture of rutile and anatase phases. Transmission electron micrograph analysis showed that the titanium nanoparticles were spherical in shape and the size ranged from 15 to 42 nm.
Keywords : *Azadirachta indica*, Titanium nanoparticles, FE-SEM, TEM, Phytosynthesis.

1. Introduction

Titanium nanoparticles are considered as an important nanomaterialdue to their photocatalytic properties,chemical stability and non-toxicity. Titanium dioxide nanoparticles are used in cosmetics industry[1], solar cells[2], electrochemical devices[3], pollution control[4] andantibacterial coatings[5]. Hence, the synthesis of titanium nanoparticles is gaining significant interest. In practice, the physical, chemical and biological approaches are employed for the synthesis of nanomaterials. Particularly, the biological methods like plant and microbial mediated process for the synthesis of nanoparticles are showing significant interest because of their less environmental consequences and energy intensive process. In biological methods using microorganisms, the titanium nanoparticles have been synthesized by either intra or extracellularlybybacteria[6,7], fungi[8] and yeast[6]. The microbial mediated synthesis of nanoparticles requires maintenance of organisms and optimum parameters such as temperature, pH and other factors for microbial growth. In order to develop the simple green synthesis, the plant extract mediated process was considered as a suitable method for the synthesis of titanium nanoparticles.

In recent years, the plant-mediated biological synthesis of nanoparticles is gaining importance due to its cost effectiveness and ecofriendliness. Few studies have been reported for the biosynthesis of TiO_2nanoparticles using plants such as *Catharanthus roseus*[9]*Ecliptaprostrata*[10] and *Nyctanthes Arbor-Tristis*[11].*Azadirachtaindica* plant mediated process has been developed for the synthesis of silver[12, 13]Au, Ag, bimetallic Au core–Ag shell nanoparticles[14]and TiO_2 nanoparticles from titanium isopropoxide[15]. *Azadirachta indica* is commonly available plant and therefore it was chosen for the present study. To the best of our knowledge, there is no report for the synthesis of titanium nanoparticles from titanium dioxide using *Azadirachta indica* plant extract.

The objective of the present study was to synthesize titanium nanoparticles from titanium dioxide using *Azadirachta indica* leaf extract. The synthesized titanium nanoparticles were characterized by using FTIR, XRD, FE-SEM, EDS, and TEM.

2. Materials and methods

All glassware's were washed with distilled water and dried in hot air oven before use. Fresh leaves of *Azadirachta indica* (Neem) were collected from Bharathidasan Institute of Technology campus, Anna University, Tiruchirappalli, India.

2.1. Preparation of *Azadirachta indica*leaf broth

Twenty grams of *Azadirachta indica* (Neem) leaves were thoroughly washed with distilled water and finely cut leaves were taken in 250mL Erlenmeyer flask. One hundred milliliters of sterile double distilled water was added and kept in a boiling water bath for 5 min. The obtained leaf extract was filtered using Whatman No.1 filter paper (pore size 25μm) and stored at 4 ^{0}C for further use.

2.2. Synthesis of titanium nanoparticles

Ten milliliters of *Azadirachta indi*ca leaf broth was added to 90 mL of 5 mM aqueous TiO_2 for the synthesis of titanium nanoparticles. The obtained titanium nanoparticles were purified by repeated centrifugation at 11,000 rpm for 15 min using double distilled water, followed by re-dispersion of the pellet in deionized water.

2.4. FTIR and XRD analysis of titanium nanoparticles

The synthesized titanium nanoparticles were analyzed by Fourier transform infrared (FTIR) spectroscopy (FTIR; Perkin Elmer, Spectrum RX1) to identify the responsible biochemical compounds involved in the phytosynthesis. The FTIR spectra were recorded in the range of 400–4000 cm^{-1}for the *Azadirachta indi*ca leaf broth and biosynthesized titanium nanoparticles.

X-ray diffraction (XRD) analysis of the powdered titanium nanoparticles was analyzed by using X-ray diffractometer (XRD; RigakuUltima III). XRD measurements were carried out in the diffraction angles (2θ) from 20^o to 80^o.

2.5. FESEM-EDS analysis of titanium nanoparticles

The morphology, size and elemental analysis of the titanium nanoparticles were analyzed by field emission scanning electron microscope (FESEM; Carl Zeiss Sigma), coupled with energy-dispersive X-ray spectroscopy (EDS; Oxford Instruments). The biosynthesized nanoparticles were dried at 37^{o}Cand the images were captured by FE-SEM at50.00 KX magnification.

2.5. TEM analysis of titanium nanoparticles

Transmission electron microscopy (TEM) analysis (TEM; Philips Technai-10) was performed for characterizing the size and shape of the biosynthesized titanium nanoparticles. A drop of the titanium nanoparticles solution was loaded on carbon-coated copper grids, and dried in vacuum before analysis.

3. Results and discussion

3.1 FTIR analysis of biosynthesized titanium nanoparticles and leaf extract

FTIR spectroscopy was done to find out the compounds responsible for the formation and stabilization of titanium nanoparticles. Fig. 1 shows the FTIR spectra of *Azadirachta indica* leaf extract and synthesized titanium nanoparticles. The prominent peaks present in the FTIR spectrum of *Azadirachta indica* leaf brothshowed at 3400.31, 2073.56, 1637.73 and 657.92 cm^{-1}. The peak at 3400.31 is assigned to the O–H stretching of alcohols and phenolic compounds. The absorption band at 2073.56 corresponds to the vibrations of C=C group. The peak at 1637.73 can be assigned to theC=O stretching of amide I or C=C groups of aromatic rings,and the band at 657.92 may be assigned tothe C–H bending. The results indicated that the presence of terpenoids, flavonoids and proteins in *Azadirachta indica* leaf extracts was believed to be responsible for the formation and stabilization of titanium nanoparticles. Similar results have been reported by Prathna et al.[13]and Shankar et al.[14].

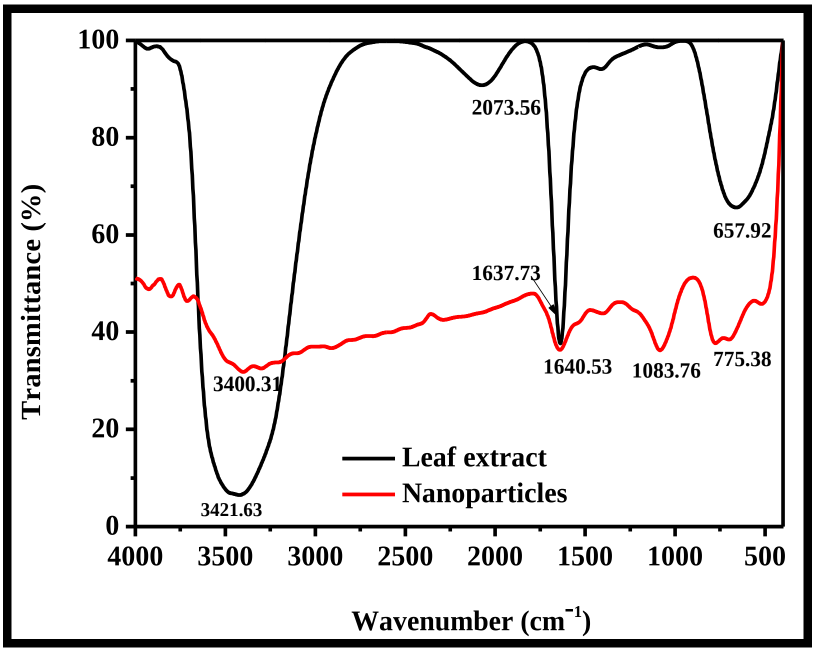

Fig. 1 FTIR spectra of titanium nanoparticles and *Azadirachta indica* leaf extract

The FTIR spectrum of biosynthesized titanium nanoparticles showed peaks at 3421.63, 1640.53, 1083.76 and 775.38.The peak at3421.63 corresponds to the O–H stretching of alcohols and phenolic compounds, 1637.73 can be assigned to the C=O stretching of amide I or to C=C groups of aromatic rings. The absorption band at 1083.76 denotes the C–O vibrations of carboxylic acids, alcohols. Similar observation has been reported by Yilmaz et al.[16]. The peak at 775 corresponds to the Ti-O-Ti stretching vibration of titanium nanoparticles. This result is consistent with other study reported by Vasconcelos et al.[17].The results revealed that the presence of O–H stretching and C=C group are indicative of the terpenoid compounds from *Azadirachta indica* leaf extract. Similarly, Tripathy et al. reported that the O-H stretching, C=C group in aqueous extract of *Azadirachta indica* (Neem) leaves due to the presence of terpenoid compounds[18]. The results of the present study suggest that the presence of terpenoids, flavonoids and proteinsin the *Azadirachta indica* leaf broth acted as both reducing and capping agents for the biosynthesis of titanium nanoparticles.

3.2 XRD analysis of titanium nanoparticles

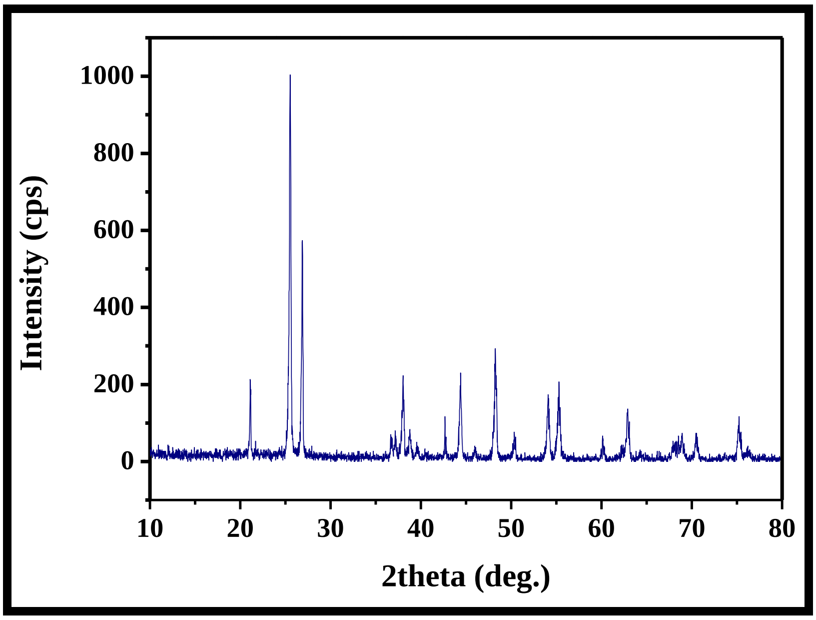

Fig. 2 X-ray diffraction pattern of titanium nanoparticles

The crystallinity nature and phase purity of the biosynthesized titanium nanoparticles were confirmed by X-ray diffraction analysis (Fig. 2). The XRD patterns of the biosynthesized titanium nanoparticles were observed at 25.5, 26.5, 38.5, 44.0, 48.2, 54.4, 55.4, 62.0, 69.0 and 76.5°. The observed patterns at 25.5, 38.5, 48.2 and 55.4°correspond to the anatase structure of the titanium nanoparticles and these results confirmed using Joint Committee on Powder Diffraction Standards (JCPDS)No. 21–1272.The XRD patterns at 26.5, 44.0, 54.0, 62.0, 69.0 and 76.5° correspond to the rutile structure of titanium nanoparticles and these results confirmed using JCPDS (No. 21–1276). It was found that the mixture of rutile and anatase phases was present in the biosynthesized titanium nanoparticles.

3.3FESEM and EDX analysis of titanium nanoparticles

The results of FESEM (Fig. 3 a) revealed that the biosynthesized titanium nanoparticles were in spherical shape and their size was ranged from 25 to 87 nm. The energy dispersive X- ray spectroscopy (EDS, Fig. 3b) analysis showed the strong titanium (Ti) peaks at 4.4 to 5 keV, silica (Si) at 1.8 keV, oxygen (O) at 0.6 keV and carbon (C) at 0.2 keV. The Si peak was observed due to glass substrate used for film preparation or detector of EDS. The C peak indicated the presence of biomolecules on the surface of titanium nanoparticles. Similar results have been reported by Wang et al.[19]

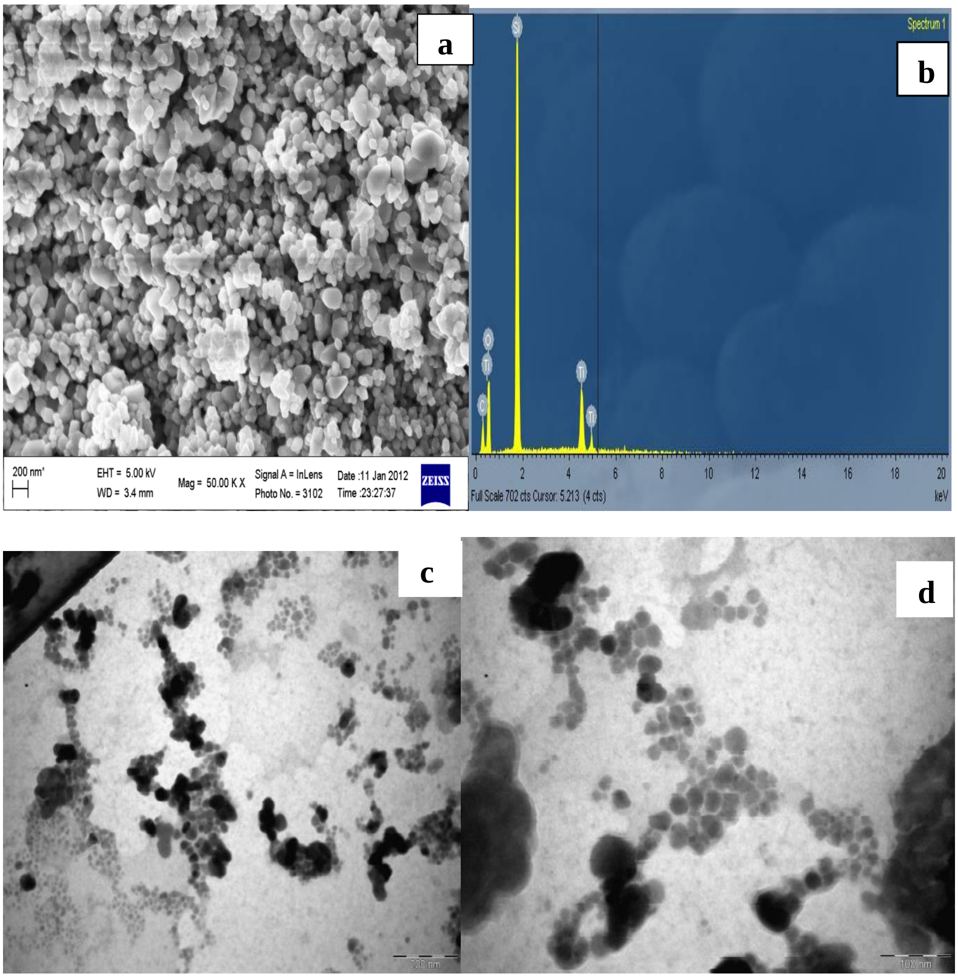

Fig. 3(a) FESEM image of titanium nanoparticles,(b) EDAX spectrum of titanium nanoparticles, (c and d)TEM images of titanium nanoparticles at different scale bar (200 nm and 100 nm)

3.4TEM analysis of titanium nanoparticles

Fig. 3 (c, d) shows the TEM images of biosynthesized titanium nanoparticles, The TEM images exhibit that the most of the titanium nanoparticles were in spherical shape with smooth surfaces and the size of the nanoparticles was ranged from 15 to 45 nm. The size of the titanium nanoparticles in FESEM analysis was higher than that of the TEM analysis. The reason for the size variations may be due to inconsistent calibration of magnification, secondary electron emission increase and sample preparations, as reported by Tuoriniemi et al.[20]

4. Conclusions

The rapid green synthesis for synthesizing titanium nanoparticles was developed. The presence of terpenoids, flavonoids and proteins were identified as the responsible biomolecules for formations of titanium nanoparticles. The *Azadirachta indica* leaf extract mediated synthesis for titanium nanoparticles is the simple, inexpensive and environmental friendly process. Furthermore, this process can be extended for the synthesis of other metal nanoparticles.

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