

# Synthesis, Characterization and Antibacterial Activity of TiO<sub>2</sub> and Cu-Doped TiO<sub>2</sub> Nanoparticles Using Chemical and Green Synthesis Methods

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## Abstract

Titanium dioxide (TiO<sub>2</sub>) nanoparticles and copper-doped TiO<sub>2</sub> nanomaterials were synthesized and investigated for their structural, morphological, and antibacterial properties. TiO<sub>2</sub> nanoparticles were prepared using both a chemical sol-gel route and a green synthesis approach employing *Aloe vera* leaf extract. Titanium tetra isopropoxide (TTIP) was used as the precursor, while *Aloe vera* extract acted as a natural reducing and stabilizing agent in the green synthesis process. The synthesized materials were subjected to structural and morphological characterization using X-ray diffraction (XRD) and scanning electron microscopy (SEM). XRD analysis confirmed the formation of crystalline anatase TiO<sub>2</sub>, with the characteristic diffraction peak at  $2\theta \approx 25.27^\circ$  corresponding to the (101) plane. The average crystallite size of the pristine TiO<sub>2</sub> sample was approximately 41.9 nm. Cu doping resulted in slight changes in diffraction peak intensity, broadening, and peak position while retaining the anatase TiO<sub>2</sub> structure. SEM analysis revealed a porous surface morphology, with pore sizes ranging approximately from 0.5 to 3  $\mu\text{m}$ . The antibacterial activity of the synthesized nanoparticles was evaluated against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* using the agar disc diffusion method. Pure TiO<sub>2</sub> exhibited inhibition zones of 12 mm and 18 mm against *E. coli* and *S. aureus*, respectively, whereas 25% Cu-doped TiO<sub>2</sub> showed significantly enhanced inhibition zones of 24 mm and 31 mm, respectively. These results demonstrate that Cu incorporation substantially improves the antibacterial performance of TiO<sub>2</sub> nanoparticles. The synthesized TiO<sub>2</sub> and Cu-doped TiO<sub>2</sub> nanomaterials therefore show promising potential for antibacterial applications in biomedical, surface-coating, and related antimicrobial technologies.

**Keywords:** TiO<sub>2</sub>; Cu; Nanoparticles; XRD; SEM; Antibacterial Activity

## 1. Introduction

Numerous organic and inorganic nanoparticles are available for use as antibacterial agents in medicine delivery systems, skin care products, and cosmetics. Because of their vast surface area and tiny size, nanoparticles in the 1–100 nm range are very effective. volume ratio that can make it more reactive [1]. Nanoparticles of oxides (ZnO, CuO, TiO<sub>2</sub>, AgO) and MnO) exhibit potent antimicrobial properties [2]. Antimicrobial properties are associated with certain substances that either eliminate or inhibit the growth of micro organisms in the surrounding region. Any material's nanoparticles exhibit distinct features from their bulk form, which can be altered for a variety of uses. Some applications use nanoparticles that prevent specific germs from growing uses including surface coating, sanitizer, and bacterial infection treatment, among others [3]. Nanoparticles have a lot of potential for use in biology as well.

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The body undergoes nanoscale biochemical reactions. As a result, it would be assumed that the domains of nanoscience and life science may work in tandem. Based on their chemical makeup, antibacterial agents can be classified as either inorganic or organic. The use of organic antibacterial agents has been restricted by a number of drawbacks, including their short lifespan, high decomposability, and low heat resistance. Consequently, inorganic antimicrobial in the market for antibacterial products, agents are becoming increasingly and more well-known [4, 5].

In the present paper, the obtained materials, TiO<sub>2</sub> nanoparticles and Cu doped TiO<sub>2</sub> prepared via chemical route method and Green synthesis method. The samples were characterized by XRD and SEM. The applications of nanoparticles as antibacterial and antifungal agent have been extensively investigated.

## 2. Experimental

### 2.1 Chemical Synthesis of Titanium Oxide (TiO<sub>2</sub>)

Titanium tetra iso propoxide [Ti(OCH(CH<sub>3</sub>)<sub>2</sub>)<sub>4</sub>], iso-propanol [(CH<sub>3</sub>)<sub>2</sub>CHOH] and nitric acid [HNO<sub>3</sub>] were used as received without any further purification. A 20 ml of solution Titanium tetra isopropoxide was added drop by drop into the 22 ml of solution containing 10 ml of iso-propanol and 12 ml deionised water under constant stirring at 80°C into the round bottom beaker. After 1 hour, concentrated HNO<sub>3</sub> (.8 ml) mixed with deionised water was added into the TTIP solution and keep it under constant stirring at 60 °C for 6 hours highly viscous sol gel was obtained. The prepared sol-gel was heated at 300 °C for 2 hours in the open atmosphere. After annealing, the TiO<sub>2</sub> nanocrystalline 2 g powder was obtained. Further preparation of TiO<sub>2</sub> film, the prepared powder was added in the ratio of 1:10 of the solution of iso-propanol. The obtained powder is kept in vacuum oven at 70 °C for 24 hours so as to get completely dried powder [6-7].

### 2.2 Green Synthesis Method

#### Preparation of Aloe Vera Extract

The aloe vera plant was collected from the Botanical Garden, Vidya Bharati Mahavidyalaya, Amravati. The plant's leaves were first selected and properly cleaned with tap water, then rinsed with distilled water to rid particles and then cut into pieces. After weighing 50 g of the leaves using an electronic balance, it was then put in a 250 ml beaker containing 100 ml of distilled water where it was heated and stirred for 2 hrs at 80 °C. It was allowed to settle down and then it was filtered with filter paper [7-8].

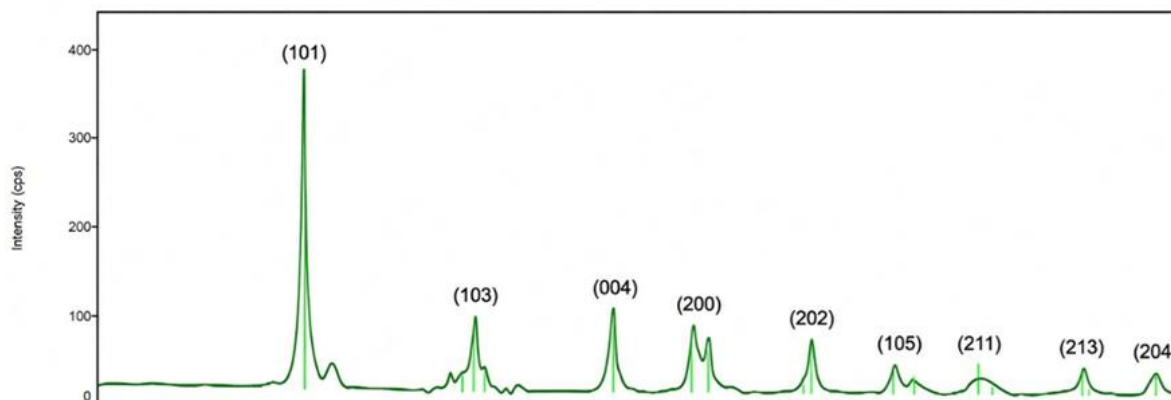
#### Green Synthesis of TiO<sub>2</sub> Nanoparticles

TiO<sub>2</sub> was synthesized using aloe vera extract as a reducing agent and titanium tetra isopropoxide (TTIP) as the precursor. 4 ml of TTIP was dissolved in 100 ml of deionized water. The aloe vera extract solution was added dropwise under constant stirring for 2 hours. The resultant mixture was filtered using filter paper. The residue was washed thoroughly using distilled water and ethanol to remove the impurity (un-dissolved species). The resulting material was dried in the oven overnight at a temperature of 100 °C and then calcined at 400 °C for 4 hours. The resultant material is Titanium Dioxide nanoparticles [9].

## 3. Results and Discussion

### 3.1 X-ray Diffraction (XRD)

Fig. 1 and 2 shows XRD pattern of pristine material TiO<sub>2</sub> and TiO<sub>2</sub> doped with 25% Cu nanocrystalline materials synthesized by chemical route method respectively.

**Fig. 1 XRD of TiO<sub>2</sub>**

The XRD graph of pristine titanium dioxide (TiO<sub>2</sub>) nanostructure synthesized by chemical route method calcined at 400 °C as shown in Fig. 1. The crystalline nature with 2θ peak lying at (101), (103), (004), (200), (202), (105), (211), (213) and (204) planes. The observed angle at 25.27° (101) represents the high crystalline nature of TiO<sub>2</sub> NPs. All the XRD peaks match well the standard structure of anatase titanium dioxide (TiO<sub>2</sub>) with lattice constants  $a = b = 3.7892$  nm and  $c = 9.5370$  nm [JCPDS card no. 5000223]. All the observed peaks correspond exactly to pure TiO<sub>2</sub> structure, this confirms the high purity of the sample obtained. The average crystalline size was found to be 41.9 nm calculated by Debye-Scherrer formula [6-9].

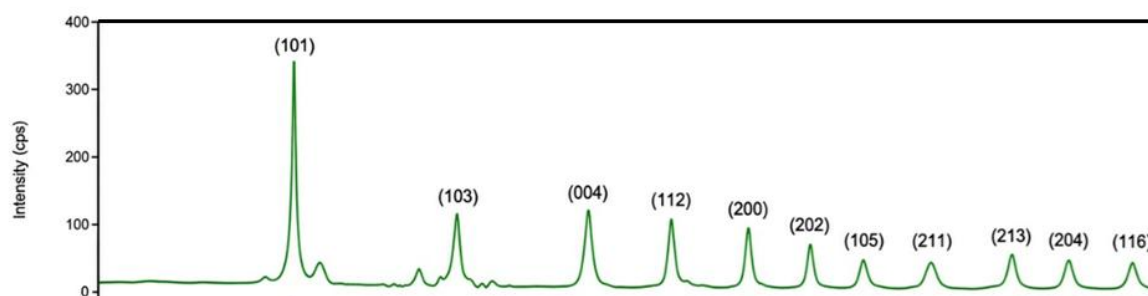
**Fig. 2. XRD of TiO<sub>2</sub> doped with 25% Cu**

Fig. 2. Shows the XRD patterns of TiO<sub>2</sub> nanostructures doped with 25% copper. The diffraction peaks correspond to crystalline planes at 2θ values of (101), (103), (004), (112), (200), (202), (105), (211), (213), (204), and (116), with the prominent peak at 25.27° (101) indicating the high crystallinity of the TiO<sub>2</sub> nanoparticles. Upon doping with varying amounts of copper, slight variations in peak intensity, minor broadening of diffraction lines, and small shifts in Bragg peak positions were observed. The diffraction patterns align well with the anatase phase of TiO<sub>2</sub>, characterized by lattice parameters  $a = b = 3.7892$  nm and  $c = 9.514$  nm, as referenced from JCPDS card no. 5000223. All detected peaks correspond exclusively to the anatase TiO<sub>2</sub> phase, confirming that the Cu-doped TiO<sub>2</sub> samples maintain a pure tetragonal anatase crystal structure.

### 3.2 Scanning Electron Microscopy (SEM)

The surface morphology of TiO<sub>2</sub> sample and 25% Cu-doped TiO<sub>2</sub> sample materials were studied by SEM and its picture is shown in the Fig. 3. and Fig. 4.

From SEM picture, it is observed that  $\text{TiO}_2$  is porous in nature and pore size varies from  $\sim 0.5$  to  $3\ \mu\text{m}$ . Due to small pores size, its surface area is more [11-17] and it shows more sensing nature. Some portion of SEM picture shows some rods with fine voids over them which helps to increase sensing properties [10].

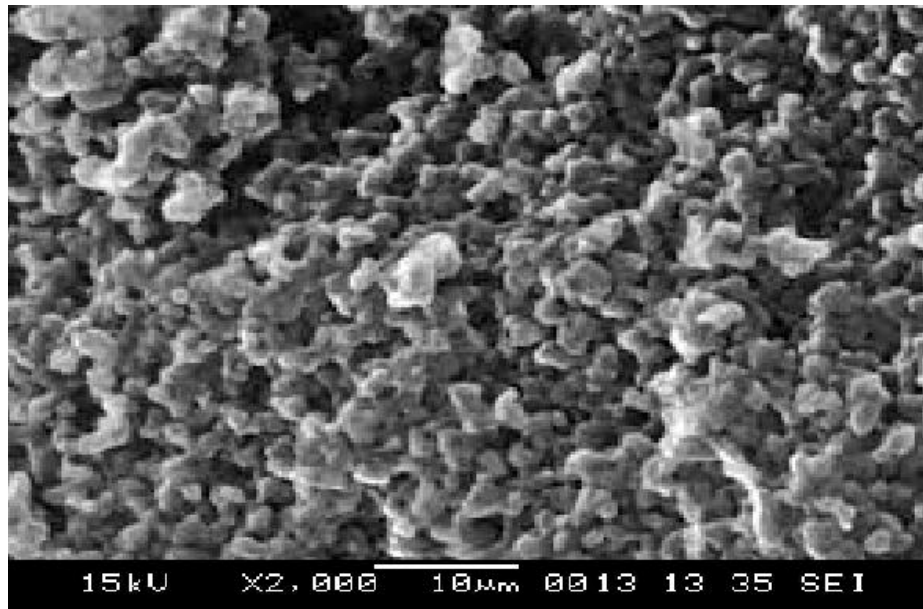


Fig. 3. SEM pictures of  $\text{TiO}_2$

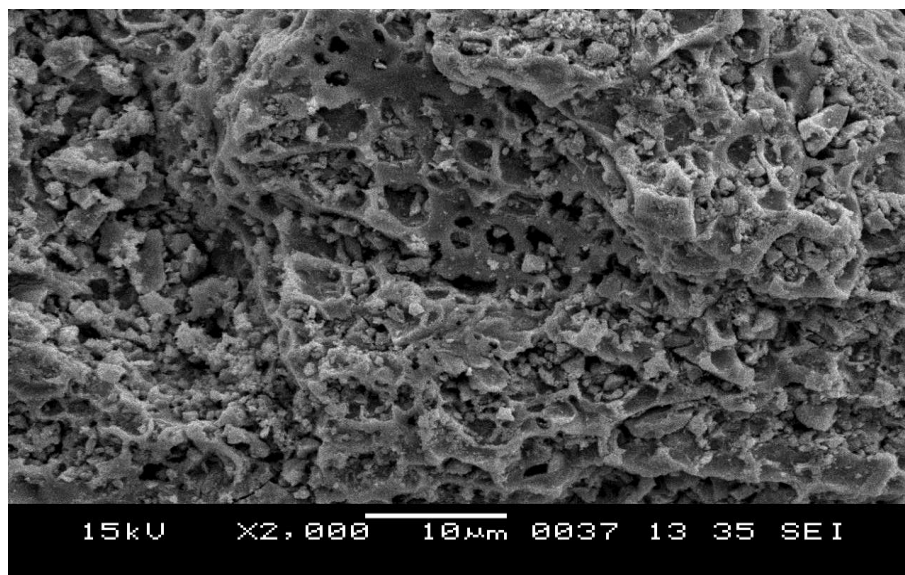


Fig. 4. SEM pictures of 25% Cu-doped  $\text{TiO}_2$  sample

The surface morphologies of pure  $\text{TiO}_2$  materials were studied by SEM and its picture which shows the average diameter and number of pores per inch were found to be  $622\ \text{nm}$  and  $92\text{nm}$  respectively .

### 3.3 Antibacterial Activity Test

The antibacterial effect against the gram negative bacteria *Escherichia coli* (NCBI-01578) and gram positive bacteria *Staphylococcus aureus* (MTCC-109) of pristine material TiO<sub>2</sub> and nanocrystalline materials tested by Agar disc diffusion assay method.

Antibacterial activity of the pure TiO<sub>2</sub> nanoparticle and TiO<sub>2</sub> nanoparticle doped with Cu nanoparticle at 25% doping concentrations were determined, using the disc diffusion assay method [11-24]. Approximately 25 mL of molten nutrient agar, after cooling, was poured into sterilized petri dishes. The plates were then left overnight at room temperature to check for any contamination. The bacterial test organisms *Staphylococcus aureus* and *Escherichia coli* were cultured in nutrient broth for 24 hours. A 100 µL aliquot of each bacterial culture was used to prepare bacterial lawns on the agar plates. Ofloxacin antibiotic served as a positive control for each bacterium to compare the antibacterial effect against TiO<sub>2</sub> nanoparticles. The plates containing bacteria and TiO<sub>2</sub> nanoparticle solutions were incubated at 37 °C. Antibacterial activity was evaluated by measuring the diameter of the Zone of Inhibition at multiple cross angles after 24 hours of incubation, as summarized in Table 4.5. The inhibition of bacterial growth by pure TiO<sub>2</sub> nanoparticles and Cu-doped TiO<sub>2</sub> nanoparticles with varying doping concentrations synthesized via the sol-gel method is illustrated in Figures 4.65 and 4.66. The Zone of Inhibition data for pure and Cu-doped TiO<sub>2</sub> nanoparticles against the tested pathogens are presented in Table 1.

**Table 1 Antibacterial ZOI of Synthesized TiO<sub>2</sub> Nanoparticles**

| Sample Name                         | Zone of Inhibition (mm) |           |
|-------------------------------------|-------------------------|-----------|
|                                     | E. Coli                 | S. Aureus |
| TiO <sub>2</sub>                    | 12                      | 18        |
| TiO <sub>2</sub> doped with Cu (25) | 24                      | 31        |

### 4. Conclusions:

In the present study, TiO<sub>2</sub> nanoparticles and 25% Cu-doped TiO<sub>2</sub> nanomaterials were successfully synthesized and investigated for their structural, morphological, and antibacterial properties. TiO<sub>2</sub> was prepared using a chemical sol-gel route, while *Aloe vera* extract was employed as a green synthesis medium for the preparation of TiO<sub>2</sub> nanoparticles. XRD analysis confirmed the formation of crystalline anatase TiO<sub>2</sub>, with the prominent diffraction peak at  $2\theta \approx 25.27^\circ$  corresponding to the (101) crystallographic plane. The average crystallite size of the pristine TiO<sub>2</sub> nanoparticles was found to be approximately 41.9 nm. The Cu-doped TiO<sub>2</sub> sample retained the anatase crystal structure, although slight changes in peak intensity, broadening, and peak position were observed following Cu incorporation.

SEM analysis revealed that the synthesized TiO<sub>2</sub> possesses a porous surface morphology, with pore sizes ranging from approximately 0.5 to 3 µm. Such a porous structure can provide a relatively large active surface area, which may contribute to the functional and antimicrobial properties of the material.

The antibacterial activity was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar disc diffusion method. Pure TiO<sub>2</sub> produced inhibition zones of 12 mm against *E. coli* and 18 mm against *S. aureus*. In comparison, 25% Cu-doped TiO<sub>2</sub> exhibited substantially larger inhibition zones of 24 mm and 31 mm, respectively. Thus, Cu doping significantly enhanced the antibacterial activity of TiO<sub>2</sub> nanoparticles against both Gram-negative and Gram-positive bacteria. The greater inhibition observed for Cu-doped TiO<sub>2</sub> indicates that copper incorporation can improve the antimicrobial effectiveness of TiO<sub>2</sub> while maintaining its anatase structure.

Overall, the findings demonstrate that TiO<sub>2</sub> and particularly Cu-doped TiO<sub>2</sub> nanomaterials possess promising antibacterial properties. The combination of nanoscale structure, porous morphology, and enhanced antibacterial activity makes these materials potential candidates for applications such as antimicrobial coatings, biomedical materials, and other technologies requiring inhibition of bacterial growth. The green synthesis approach using *Aloe vera* also provides a more environmentally friendly alternative for nanoparticle preparation [25–31].

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