# Synthesis and characterization of $TiO_2$ nanoparticles and investigation of their *in vitro* action against *Staphylococcus aureus* (PTCC 1189)

ALI ABDOLAHZADEH ZIABARI[a,*], SAJIHE BAHREKAZEMI[b], MOHAMMAD FAEZI GHASEMI[b], KHOSRO ISSAZADEH[b]
[a]*Nano Research Lab, Lahijan Branch, Islamic Azad University, P.O. Box1616, Lahijan, Iran*
[b]*Department of Microbiology, Lahijan Branch, Islamic Azad University, Lahijan, Iran*

Titanium dioxide ($TiO_2$) nanopowders in different concentrations were successfully synthesized via sol-gel route. The products were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). The samples were polycrystalline in nature with anatase, rutile or a mixture of anatase and rutile phases. The diameter of nanoparticles was estimated as being: 28.70, 31.05, and 32.94 nm for 0.1, 0.3, and 1.0 molar concentration, respectively. The sharp peaks in FTIR spectrum determined the purity of $TiO_2$ nanopowders. Antibacterial action of the products against *Staphylococcus aureus* (PTCC 1189) was evaluated by colony counting method. The photo catalytic property of $TiO_2$ nanopowders was investigated following inactivation of *Staphylococcus aureus* (PTCC 1189) by irradiation with a UV-B lamp. The $TiO_2$ nanopowders have a main antibacterial effect on *S. aureus* (PTCC 1189) at concentration of 1 mg/mL. Increasing the molarity from 0.1 to 1.0 M has decreased the viable cell concentration of *S. aureus* (PTCC 1189) from 95.21 % to 6.73 % after 3 hours.

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*Keywords*: *Staphylococcus aureus* (PTCC 1189), $TiO_2$ nanopowder, Sol-gel, Antibacterial activity

## 1. Introduction

Nanoparticles (NPs) normally ranging in dimension from 1 to 100 nm have unique properties as compared to their bulk equivalent [1]. Currently, the metallic NPs are completely studied and extensively investigated as potential antimicrobial agents. As a metal oxide, titanium dioxide ($TiO_2$), also known as titania is a very important photo catalyst due to its high refractive index, light absorption, non-toxicity, chemical stability, and relatively low-cost production [2]. $TiO_2$ nanoparticles have attracted attention in the fields of environmental purification, solar energy cells, photocatalysis, gas sensors, photo electrodes and electronic devices. It has been widely used as a pigment in paints, ointments, toothpaste, etc. [3, 4]. The first report on antibacterial properties of anatase $TiO_2$ originating from oxidation of bacteria was presented in 1985. After that, anatase $TiO_2$ as an antibacterial photo catalyst has caught increasing attention in inactivation of bacteria, molds, viruses, and even cancer cells [5]. The antibactericidal properties of $TiO_2$ are due to the high redox potential of the surface species formed by photo-excitation affording the bacteria nano-selective oxidative attack [6]. The type and the source of $TiO_2$ play a significant role in bacterial inactivation because the rate of formation of reactive oxygen species (ROS) is a function of: particle size, isoelectric point, crystalline phase, aggregate size in suspension, and other nanostructural parameters [7]. In general, antimicrobial activity of $TiO_2$ strongly depends on the characteristic of this oxide. $TiO_2$ nanoparticles with photo catalytic properties can be synthesized by a variety of routes including sol-gel, hydrothermal method, solvothermal process, plasma evaporation, etc. [8, 9]. In this study, three different molarities of $TiO_2$ nanopowders were synthesized by the sol-gel route and the antibacterial action of $TiO_2$ nanopowders against *S. aureus* (PTCC 1189) was estimated.

## 2. Materials and methods

### 2.1 Preparation of $TiO_2$ nanopowder

In this work, $TiO_2$ nanopowders were prepared by sol-gel technique. To prepare the samples, 1, 3 and 10 mL of tetra-n-butyl-orthotitanate (Merck, 99 %) was slowly added (drop-wise) to 30 mL ethanol (Merck, 99.8 %) under vigorous stirring at room temperature. Then, 0.75 mL acetylacetone was added to the above prepared mixture. Thereupon, 0.2 mL of HCl solution was added. Finally, a transparent yellowish mixture was formed. The as-prepared solution was dried at 60 °C for 24-48 h, leading to an amorphous gel. The obtained powder was then annealed at 700 °C for 2 h leading to a white crystalline $TiO_2$ nanopowder.

### 2.2 Products characterization

Analysis of the crystalline structure was performed by X-ray diffraction at room temperature in the $4° < 2\theta < 94°$ range (Bruker $D_8$ Advance, $CuK_\alpha$ radiation) and SEM by a VEGAII TESCAN instrument. The size distribution of NPs was measured by TEM (CM120, Philips, Netherlands). FTIR studies in cell with $CaF_2$ windows, where samples were located, were carried out by a FTIR spectrophotometer model RS/1 from UNICAM. Water reference spectrum was always subtracted from every spectrum in the region between 1800 and 1000 $cm^{-1}$.

### 2.3 Antibacterial test

The antibacterial activity of $TiO_2$ nanopowders against *Staphylococcus aureus* (PTCC 1189) bacteria was studied. Before the microbiological experiment, all glassware and samples were sterilized by autoclaving at 120 °C for 30 min. One colony of culture from *S. aureus* (PTCC 1189) bacteria was inoculated into nutrient broth and then incubated under aerobic conditions at 37 °C for 15-17 h. During the stationary growth phase, bacterial cells were harvested by centrifugation at 10,000 rpm for 15 min. The bacterial pellets were subsequently washed twice with 10 mL sterile phosphate buffered saline (PBS) at pH = 7.2. The final pellets were resuspended with PBS in sterile tubes and standardized using a 0.5 Mc Farland apparatus which corresponds to a cell density of $1.5\times10^8$ colony forming units (CFU) per mL. The disinfection process was accomplished at following parameters: initial cell density of about $1.5\times10^8$ CFU/mL, $TiO_2$ nanopowder concentration of 1 mg/mL. The major fraction of irradiation for 3 h with a UV-B lamp (Philips) was performed at 280-315 nm, the lamp being situated sidewise at a distance 45 cm from the reaction vessels. $TiO_2$ nanopowder with concentration of 1 mg/mL was added into cell suspension with a density of about $1.5\times10^8$ CFU/mL and then incubated at 37 °C for 3 h. Then, 1 mg from any experimental tubes was withdrawn and serial dilutions were prepared before plating. The number of viable cells was determined by pour plate method as following: 100 μL from any dilution were streaked in duplicate on nutrient agar and poured in Petri dishes with an agar depth of 5 mm. After overnight incubation at 37 °C, the colonies on the plates were counted and the results were reported to 1 mL.

## 3. Results and discussion

XRD analysis was taken out to find the crystalline phase and structure of the produced particles. Fig. 1 (a, b, c) shows the XRD patterns of the samples in $2\theta$ range from 4 to 74 degrees. The crystallite diameter ($D$) has been calculated from the full width at half maximum (FWHM) and using the Scherrer's formula as follows:

$$D = \frac{0.89\lambda}{\beta\, cos\theta} \quad (1)$$

where, 0.89 is the shape factor, $\lambda$ is the X-ray wavelength, $\beta$ is the line broadening at FWHM in radians, and $\theta$ is the Bragg angle. Using different amount of the starting materials causes different crystallite size. The average crystallite diameters of 0.1, 0.3, and 1 M $TiO_2$ was determined about 28.70, 31.05, and 32.94 nm, respectively. Meanwhile, The prepared $TiO_2$ nanopowder at 0.1 M shows only anatase phase, but at 0.3 and 1.0 M, both anatase and rutile phases were observed.

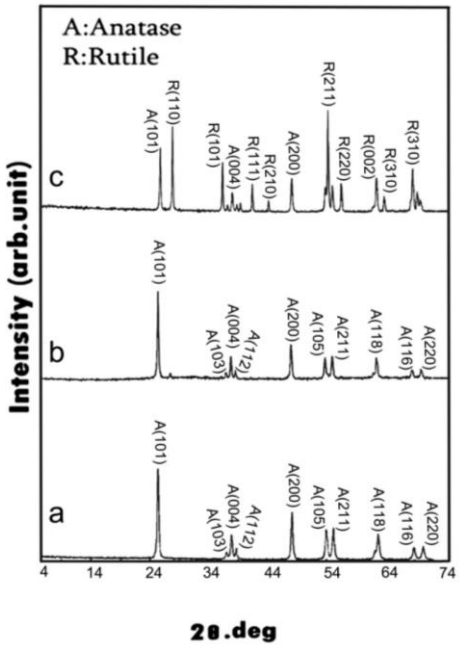


*Fig. 1. The XRD pattern of (a) 0.1 M, (b) 0.3 M and (c) 1 M $TiO_2$ nanopowders*

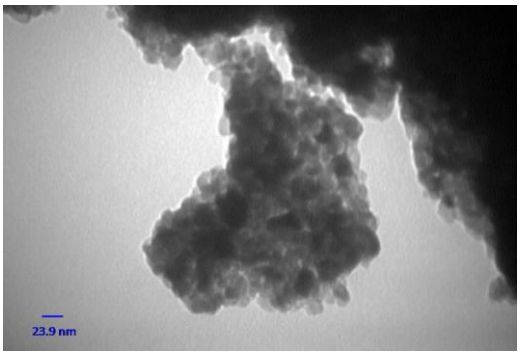


*Fig. 2. Representative TEM image of $TiO_2$ nanoparticles from 0.1 M solution*

The size and morphology of the prepared $TiO_2$ nanopowders were analyzed by TEM and presented in Fig. 2. The image revealed that the products consist of spherical particles with average size of 20-30 nm which is in good agreement with XRD results. The SEM micrographs (Fig. 3) show the morphology of $TiO_2$

nanopowders implying that the powders are consistent of spherical building units. The SEM micrographs of *S. aureus* (PTCC 1189) before and after being damaged by $TiO_2$ nanopowders have been illustrated in Fig. 3c (control) and Fig. 3d (treated with $TiO_2$ nanopowder).

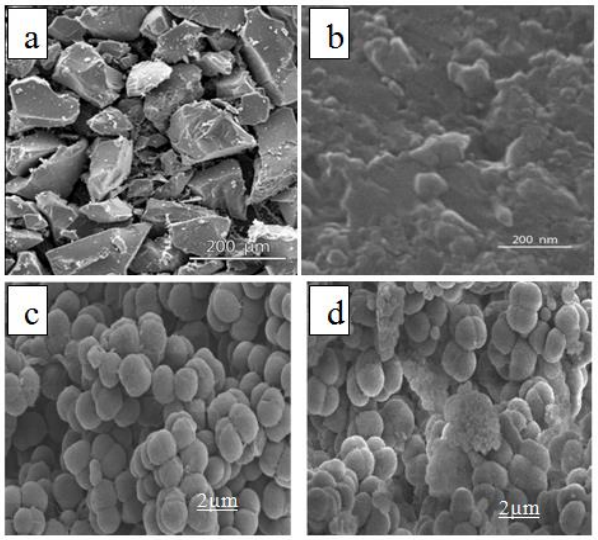


*Fig. 3. SEM micrograph of 1 M concentration of $TiO_2$ nanopowders at different magnifications (a and b) and the micrograph of S. aureus (PTCC 1189) before (c) and after being damaged by $TiO_2$ nanopowders (d)*

The FTIR spectra of the synthesized nanopowders are shown in Fig. 4 in the wavenumber range of 400-4000 $cm^{-1}$ which identifies the chemical bonds as well as functional groups in the compound. The broad peak appearing between 3200 and 4000 $cm^{-1}$ is assigned to the stretching vibrations of the OH groups. The sharp bands around 1600 $cm^{-1}$ are attributed to the bending vibrations of surface adsorbed water. Also, the stressed bending of C=O were detected around 1,570 $cm^{-1}$ and 450-525 $cm^{-1}$. This stressed swing is related to titanium atoms.

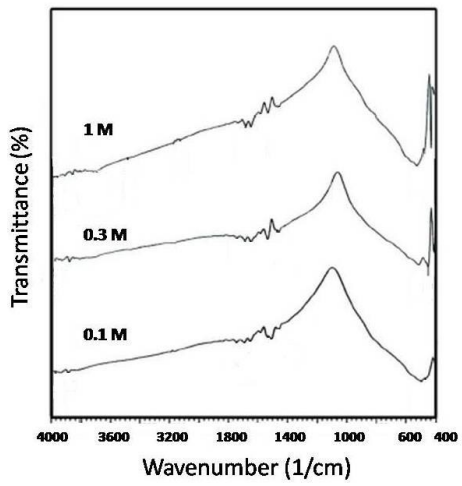


*Fig. 4. FTIR spectra for: (a) 0.1 M, (b) 0.3 M, and (c) 1 M $TiO_2$ nanopowders*

Antibacterial activity of the synthesized $TiO_2$ nanopowders against *S. aureus* (PTCC 1189) bacteria was investigated and was shown in Fig. 5. It is seen that the number of viable bacteria is decreasing in the presence of $TiO_2$ nanopowders. It is observed that *Staphylococcus aureus* exhibits a minimal susceptibility at 0.1 M of $TiO_2$. In all done experiments it was observed that the efficiency of the process reduced when the bacterial suspensions were not continuously stirred on a magnetic stirrer. The results showed that $TiO_2$ nanopowder has considerable antibacterial effect against *S. aureus* (PTCC 1189) at concentration of 1 mg/mL. By increasing the nanopowder molarity from 0.1 M to 1.0 M, the viable cell concentration of *S. aureus* decreased from 95.2 % to 6.7 % after 3 hours.

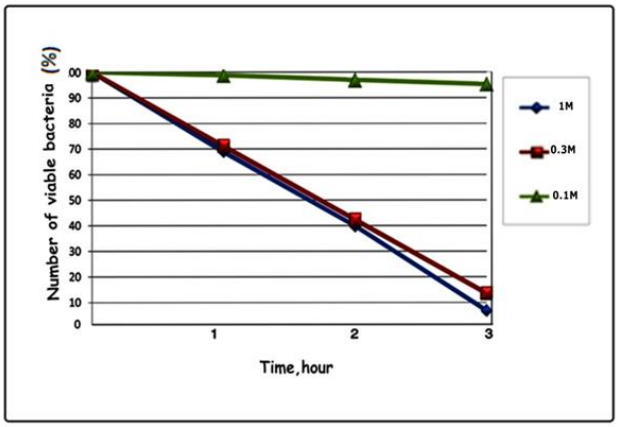


*Fig. 5. The effect of $TiO_2$ nanopowders towards survival of S. aureus (PTCC 1189) during photo catalytic process*

Irradiation of TiO photo catalyst with light of particular wavelength causes the formation of ROS initiating a cascade of redox reactions which can mineralize a species of organic compounds [10, 11]. Generation of ROS causes oxidative damages to living organisms suggesting that the cell membrane is the primary site of ROS attack. The cell membrane damage leads directly to the leakage of minerals, proteins, and genetic materials causing the cell death [12]. It is also observed that the efficiency of the process was reduced when the bacterial suspensions were not continuously stirred on a magnetic stirrer. So, the bacterial suspensions were stirred manually with adding $TiO_2$ powder every 2 minutes. As a result, the bactericidal action of $TiO_2$ nanopowder depends on the value of dissolved molecular oxygen and suitable $TiO_2$ cell contact (both of them are increased during magnetic stirring).

## 4. Conclusion

$TiO_2$ nanoparticles were synthesized by a simple sol-gel technique. The $TiO_2$ nano structures were composed of anatase and rutile phases. The average diameters of nanoparticles were estimated as being 28.70, 31.05, and 32.94 nm for 0.1, 0.3 and 1 M nanopowder concentrations,

respectively. It was revealed that the $TiO_2$ nanopowder at concentration of 1 mg/mL had strong antibacterial activity against *S. aureus* (PTCC 1189) bacteria under UV-B irradiation. In brief, higher molarity of $TiO_2$ nanopowders showed a great antibacterial activity as compared to low molarity of nanopowders.

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*Corresponding author: ali.abd.ziabari@gmail.com