Antimicrobial Activity of Synthesized TiO_2 Nanoparticles

Sabriye Pişkin, Arzu Palantöken, and Müge Sari Yılmaz

***Abstract*—**Nanosized materials are attracting a great deal in biological and pharmaceutical applications. Especially, metal oxide nanoparticles are known to possess antibacterial properties. TiO_2 nanoparticles, have become a new generation of advanced materials due to their optical, dielectric, and photo-catalytic characteristics from size quantization. These properties have been applied in removing bacteria and harmful organic materials from water and air.

In this work, we investigated antimicrobial activity of synthesized TiO_2 nanoparticles by ultrasound method. The TiO_2 nanoparticles were characterized by X-ray diffraction, nano sizer and atomic force microscopy analyses. The antibacterial effect of these obtained nanoparticles was examined on *E. coli* (ATCC 8739), *Staph.aureus* (ATCC 6538), *P.aeruginosa* (ATCC 9027), *C.albicans* (ATCC 36232) and *B.subtilis* (ATCC 6633). The synthesized TiO_2 nanoparticles were found to be effective against these bacterias. Based on this study, we may conclude that the synthesized by ultrasound method TiO_2 nanoparticles can be a good inorganic antimicrobial agents.

***Keywords*—** Antimicrobial activity, TiO_2 Nanoparticles, Minimum inhibitory concentration, *Escherichia coli, Staphylococcus aureus.*

I. Introduction

NANO as a word means one-billionth of a physical quantity. One nanometer is a unit of length equal to one billionth of a meter. Nanoscale materials have very different new properties which differ from materials in molecular form. Nanomaterials have a much greater surface area to volume ratio than their conventional forms. The large surface area affects the interaction of the elements and nanoparticles. That is quite different than the larger size of the electrical, mechanical, optical and magnetic characteristics of atomic and molecular sizes (1-100 nm), functional materials, devices, systems subject to the production of the branch of science is called nanotechnology [1].

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level [2].

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It can prove to be a boon for human health care, because nanoscience and nanotechnologies have a huge potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger, lighter materials [3]. Nanomaterials may also used for special medical purposes such as to produce novel drug delivery systems, to enhance the performance of medical devices, or to produce diagnostic imaging materials [4].

An antimicrobial refers to a substance that kills or inhibits the growth of microorganisms [5]. Antimicrobial agents are highly relevant for a host of industrial applications in environmental, food, synthetic textiles, packaging, health care, and medical care products. Metal oxide nanoparticles (NPs) are known to possess strong antimicrobial properties [6]. In addition to industrial uses, such as catalysts, these are also used in products familiar to ordinary consumers such as sunscreens, cosmetics, pharmaceutical products, and fuel additives [7].

Titanium dioxide (TiO_2) is a photo catalyst and widely utilized as a self-cleaning and self disinfecting material for surface coating in many applications. It has a more helpful role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect [8]. They are used frequently in cosmetics, pharmaceutical, paint and paper industry. However, the small particle size of nanoparticles may have different chemical, magnetic optical and structural features, so they may have more differential toxicity profiles than normal-sized TiO_2.

II. Experimental

A. Materials and methods

All reagents used in the synthesis of TiO_2 and antibacterial studies were analytical grade and employed without any further treatments. Distilled water was used for all synthesis and antimicrobial activity processes. *Esherichia coli* (ATCC 8739), *Stapyloccus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 36232) *and Bacillus subtilis* (ATCC 6633) were obtained from American Type Culture Collection. All the media and chemicals were purchased from Oxoid Microbiology Products and Difco Laboratories.

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In the present work, TiO_2 NPs were prepared by a sonochemical method according to previous study [9].

B. Characterization of Synthesized TiO_2 Nanoparticles

The X-ray diffraction (XRD) patterns of nanoparticles were carried out with a diffractometer (Philips PANalytical X'Pert Pro) using Cu Kα radiation at 45 kV and 40 mA. Size characterization of the 0.1 % w/v TiO_2 NPs sample dispersed in 10 mM NaCl was made on a ZetaSizer Nano ZS at 25°C. Particle size distribution obtained from Zetasizer Nano ZS. Size and surface topography of the TiO_2 nanoparticles were investigated using AFM Shimadzu Scanning Prop Microscope SPM-9600 and high resolution surface images were produced.

C. Antibacterial Activity

1. Well diffusion technique

Antimicrobial activity of synthesized nanoparticles were investigated by well diffusion method against five bacterial strains, *Esherichia coli* (ATCC 8739), *Stapyloccus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 36232) and *Bacillus subtilis* (ATCC 6633). The obtained bacterial cultures were maintained on nutrient agar slants that contained peptone, 5.0; meat extract, 1.0; yeast extract 2.0; sodium chloride, 5.0; and agar 15.0 g per liter of distilled water. The test bacterial suspensions (50 µl) containing 10^4 cells ml^{-1} were spread on Nutrient Agar plates. 25 µl TiO_2 NPs suspension (25 µg) was added in the tested wells. The samples were initially incubated for 15 min at 4°C (allow diffusion) and later on at 37°C for 24 h for the bacterial cultures.

2. Minimum inhibitory concentration (MIC)

The micro dilution method for estimation of MIC values was carried out to evaluate the antimicrobial activity. The MIC values were determined on 96-well micro dilution plates according to published protocols [10].

III. Results and Discussion

A. X-ray Diffraction Analysis

The XRD pattern of the synthesized sample is illustrated in Figure 1. The characteristic peaks of TiO_2 were observed at 3.52 Å, 1.89 Å, and 1.67 Å (PDF: 01-084-1286) from the XRD spectrum. According to analysis result, the crystal structure of synthesized TiO_2 was anatase-powder.

The mean particle diameter of the nanoparticles was calculated from the XRD pattern using the Scherrer's equation:

$$D = \frac{0.9.\lambda}{\beta.\cos\theta} \quad (1)$$

Where 0.9 is the Scherrer's constant, λ is the Cu-Kα radiation wavelength and is peak width at half-height and θ is the incidence angle of the X-ray. The crystallite size was found to be 33.56 nm for TiO_2.

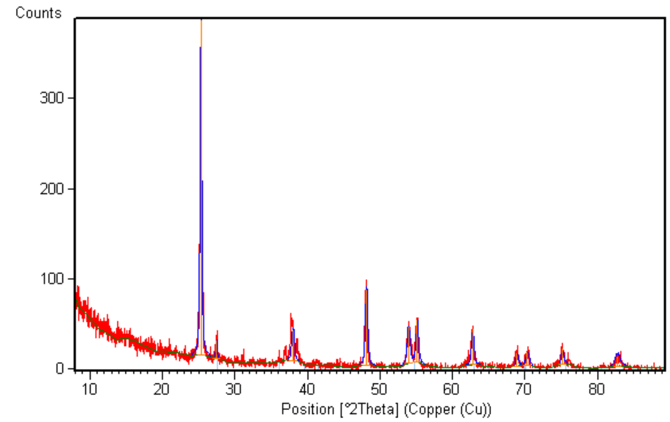


Fig. 1 XRD pattern of TiO_2 NPs

A. Nano-Sizer Analysis

Titanium dioxide nanoparticles samples were prepared in 10 mM NaCl [11]. Size characterization of the 0.1 % w/v TiO_2 NPs sample dispersed in 10 mM NaCl was made on a ZetaSizer Nano ZS at 25°C. Fig. 2 is the intensity particle size distribution obtained from Zetasizer Nano ZS. The z-average diameter was measured 460 nm.

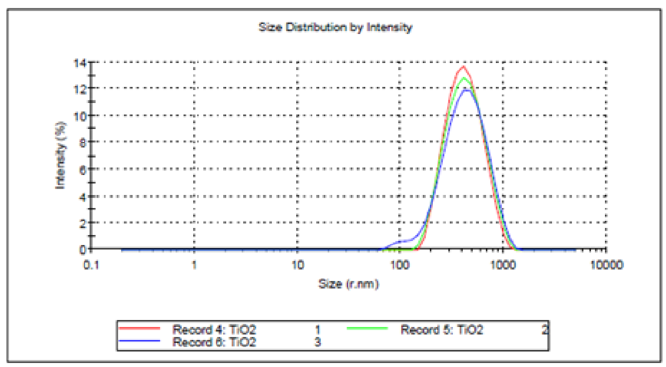
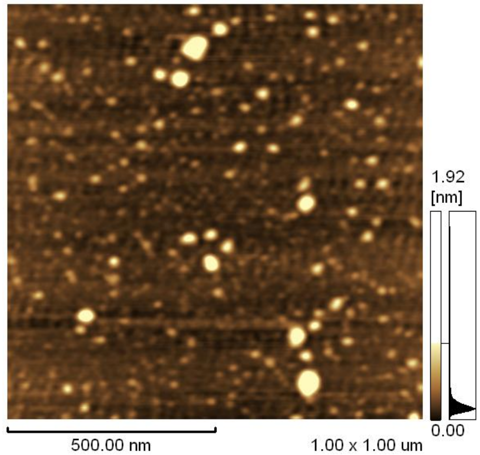


Fig. 2 Particle size distribution of the 0.1 % w/v TiO_2 NPs dispersed in 10 mM NaCl measured on a Zetasizer Nano ZS

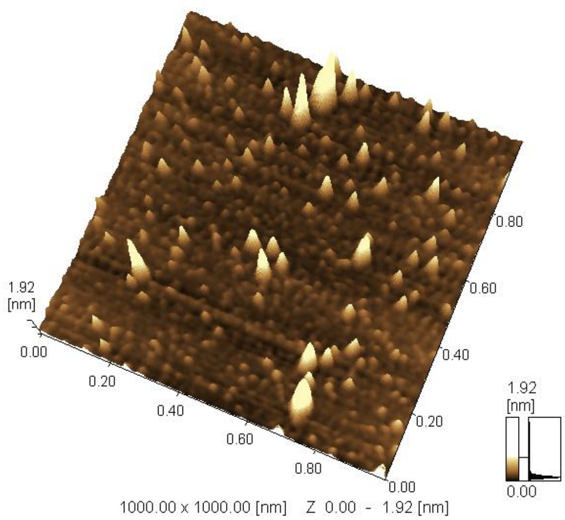
Different from this in dry form, the average size of the 0.1 % w/v synthesized TiO_2 NPs dispersed in 10 mM NaCl was in the range of 350-500 nm, which was much larger than the expected sizes. Although AFM micrograph of this nanoparticles show that this was nano-sized particles, its primary nanoparticles aggregated rapidly in solutions. Considerable particle aggregation was observed for NP resulting in the formation of flocs.

B. AFM Studies

The synthesized TiO_2 NPs were characterized by AFM for its detail size, morphology and agglomeration. Characterization of the synthesized nanoparticles using AFM offered a three-dimensional visualization. The uneven surface morphology was explained by the presence of both individual and agglomerated nanoparticles (Fig.3).



(a)



(b)

Fig. 3 TEM micrograph of TiO_2 NPs showing: (a) increased surface view and (b) 3D view

C. Well Diffusion and Minimum Inhibitory Concentration

Antimicrobial activity of TiO_2 nanoparticles were investigated by well diffusion method against five bacterial strains, *E. coli*, *S. aureus*., *Ps.aureginosa*, *C.albicans* and *B.subtilis*. The results of zone inhibition method has been described from the Fig. 4, it is seen that TiO_2 NPs shows good inhibition zone around the films. Figure X shows the activity against (a) *E.coli*, (b) *S.aureus*., (c) *P.aeruginosa*, (d) *C.albicans* and (e) *B.subtilis*.

TABLE I
ZONE OF INHIBITION AND MIC OF SYNTHESIZED TiO_2 NANOPARTICLES AGAINST VARIOUS MICROORGANISMS

Microorganism	Synthesized TiO_2 NPs	
	Zone of inhibition (mm)	MIC ($\mu g.ml^{-1}$)
E.coli	31	9.7 µg /ml
S.aureus.	29	19.5 µg /ml
P.aeruginosa	15	19.0 µg /ml
C.albicans	25	9.7 µg /ml
B.subtilis	15	19.5 µg/ml

The MIC observed in the present study for synthesized TiO_2 NPs were 9.7 µg /ml for *E. coli*, 19.5 µg /ml for *S.aureus*., 19.0 µg /ml, *P.aeruginosa*, 9.7 µg /ml for *C.albicans* and 19.5 µg/ml for *B.subtilis* (Table 1). The bactericidal effect of TiO_2 generally has been attributed to the decomposition of bacterial outer membranes by reactive oxygen species (ROS), primarily hydroxyl radicals (OH), which leads to phospholipid peroxidation and ultimately cell death [12].

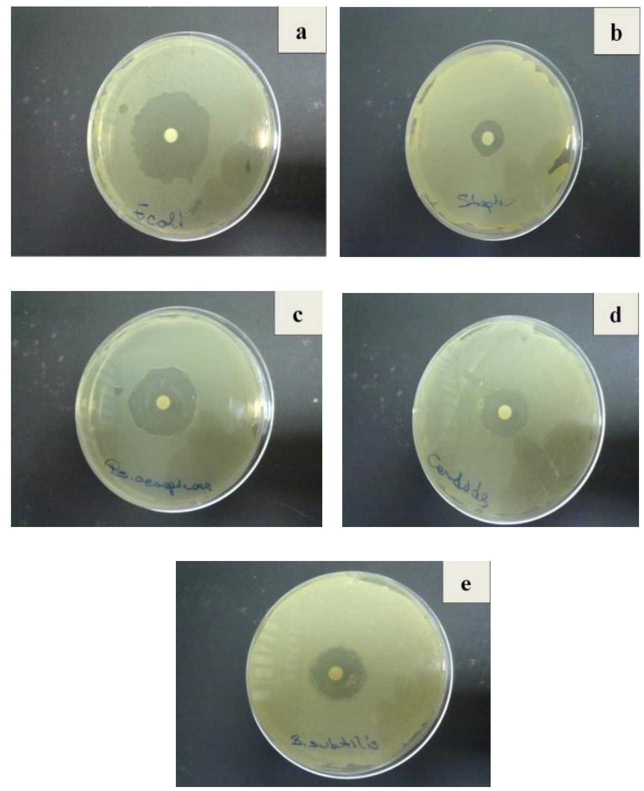


Fig. 4 Well diffusion assay for TiO_2 nanoparticles against (a) *E.coli*, (b) *S.aureus*., (c) *P.aeruginosa*, (d) *C.albicans* and (e) *B.subtilis.*

IV. CONCLUSION

In this work we report the characterization and antimicrobial activity of TiO_2 nanoparticles which were prepared by a sonochemical method according to previous study [4]. The crystallite sizes were calculated using Scherrer's formula applied to the major intense peaks and found to be in the range of 33.56 nm.

The MIC observed in the present study for synthesized TiO_2 NPs were 9.7 µg /ml for E. coli, 19.5 µg /ml for *S.aureus*., 19.0 µg /ml, *P.aeruginosa*, 9.7 µg /ml for *C.albicans* and 19.5 µg/ml for *B.subtilis*. This method is considered as an innovative approach for the synthesis of TiO_2 NPs possessing antibacterial activity.

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