Antimicrobial Activity of Synthesized TiO$_2$ Nanoparticles

Sabriye Pişkin, Arzu Palantöken, and Muge Sari Yilmaz

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 bacteria. Based on this study, we may conclude that the synthesized by ultrasound method TiO$_2$ nanoparticles can be a good inorganic antimicrobial agent.

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].

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. Escherichia coli (ATCC 8739), Staphylococcus 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.
In the present work, TiO\textsubscript{2} NPs were prepared by a sonochemical method according to previous study [9].

B. Characterization of Synthesized TiO\textsubscript{2} Nanoparticles

The X-ray diffraction (XRD) patterns of nanoparticles were carried out with a diffractometer (Philips PANalytical X’Pert Pro) using Cu K\textalpha{} radiation at 45 kV and 40 mA. Size characterization of the 0.1 % w/v TiO\textsubscript{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\textsubscript{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, \textit{Esherichia coli} (ATCC 8739), \textit{Stapyloccus aureus} (ATCC 6538), \textit{Pseudomonas aeruginosa} (ATCC 9027), \textit{Candida albicans} (ATCC 36232) and \textit{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 \textit{E}. \textit{coli} cells ml\textsuperscript{-1} were spread on Nutrient Agar plates. 25 µl TiO\textsubscript{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\textsubscript{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\textsubscript{2} was anatase-powder. The mean particle diameter of the nanoparticles was calculated from the XRD pattern using the Scherrer’s equation:

\[ D = \frac{0.9 \lambda}{\beta \cdot \cos \theta} \]  

(1)

Where 0.9 is the Scherrer’s constant, \( \lambda \) is the Cu-K\textalpha{} radiation wavelength and is peak width at half-height and \( \theta \) is the incidence angle of the X-ray. The crystallite size was found to be 33.56 nm for TiO\textsubscript{2}.

Different from this in dry form, the average size of the 0.1 % w/v synthesized TiO\textsubscript{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 agregated rapidly in solutions. Considerable particle aggregation was observed for NP resulting in the formation of flocs.

B. AFM Studies

The synthesized TiO\textsubscript{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).
Antimicrobial activity of TiO$_2$ nanoparticles were investigated by well diffusion method against five bacterial strains, E. coli, S. aureus, P. aeruginosa, C. albicans and B. subtilis. The results of zone inhibition method have 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**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Synthesized TiO$_2$ NPs</th>
<th>Zone of inhibition (mm)</th>
<th>MIC ($\mu$g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>31</td>
<td></td>
<td>9.7 $\mu$g/ml</td>
</tr>
<tr>
<td>S. aureus</td>
<td>29</td>
<td></td>
<td>19.5 $\mu$g/ml</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>15</td>
<td></td>
<td>19.0 $\mu$g/ml</td>
</tr>
<tr>
<td>C. albicans</td>
<td>25</td>
<td></td>
<td>9.7 $\mu$g/ml</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>15</td>
<td></td>
<td>19.5 $\mu$g/ml</td>
</tr>
</tbody>
</table>

The MIC observed in the present study for synthesized TiO$_2$ NPs were 9.7 $\mu$g/ml for E. coli, 19.5 $\mu$g/ml for S. aureus, 19.0 $\mu$g/ml, P. aeruginosa, 9.7 $\mu$g/ml for C. albicans and 19.5 $\mu$g/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].

**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 $\mu$g/ml for E. coli, 19.5 $\mu$g/ml for S. aureus, 19.0 $\mu$g/ml, P. aeruginosa, 9.7 $\mu$g/ml for C. albicans and 19.5 $\mu$g/ml for B. subtilis. This method is considered as an innovative approach for the synthesis of TiO$_2$ NPs possessing antibacterial activity.

**REFERENCES**


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