In vitro anti-microbial activity of titanium dioxide nanoparticles

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Abstract: Titanium dioxide (TiO_2) has wide applications in various fields including cosmetics, pharmaceuticals, textile and waste water treatment due to its many properties such as photocatalytic activity and stability. In the present study, the synthesis of TiO_2 nanoparticles (NPs) was achieved by hydrolysis condensation method where a liquid phase of TiO_2 was obtained. TiO_2 NPs were characterized by X-ray Diffraction (XRD), UV-Visible spectrometry, Scanning Electron Microscopy (SEM). The photocatalytic activity of TiO_2 NPs was examined by monitoring the degradation of methylene blue dye in water when treated with TiO_2 NPs. TiO_2 NPs were found to be highly photocatalytic achieving 90% degradation ratio after 80 min. In this study antimicrobial activity test was carried out for TiO_2 NPs against selected Gram-positive/negative bacteria both in the presence or absence of UV exposure. TiO_2 NPs expressed a significant effect on microbial growth only on high concentrations exceeding 1 mg/ml.

Key Words: titanium dioxide, nanoparticles, antimicrobial activity, ultraviolet

INTRODUCTION

In recent years, microorganisms acquired resistance to the commonly used antibiotics, and thus diseases caused by these strains were getting troublesome to treat. Titanium dioxide (TiO_2) nanoparticles (NPs) are significantly known for their non-toxic property, high functionality toward biomaterials and chemical stability (Kaseem et al. 2019). TiO_2 has three crystal structures: anatase, rutile and brookite which have different characteristics and applications. Commonly used TiO_2 are considered as one of semiconductor metals which can also be utilized as a photocatalytic antimicrobial material (Kang et al. 2019).

According to what is known from band energy theory, the disconnected band structure of semiconductors is constituted of low energy valence bands filled with electrons, high-energy conduction bands, and band gaps. The values of band gap of TiO_2 are 3.0 and 3.2 eV for rutile and anatase phases. There are two characters to understand the mechanism of TiO_2 NPs: firstly, ability to degrade biopolymeric compounds (such as polysaccharides and proteins) and secondly, alteration of the surface properties of the objects to hydrophilic state when TiO_2 is on the surface. TiO_2 is more likely to oxidize when it is exposed to ultraviolet (UV), particularly in a wavelength lower than 385 nm. As a result, TiO_2 can produce reactive oxygen species (ROS) (Azizi-Lalabadi et al. 2019). The production of ROS (*e.g.*, hydrogen peroxides, superoxide radicals, and hydroxyl radicals) is initiated immediately after UV exposure (Kang et al. 2019). These active species destroy the outer membrane of the bacteria, namely phospholipids, proteins, and lipopolysaccharides, and finally damage the bacteria (Nosaka and Nosaka, 2017). Despite all these features, there are still some limitations; for instance, ROS are highly reactive and have very short lifetimes (2 μs for $^1O_2$, 200 μs for $OH^{\bullet}$, and from 5 s to hundreds of seconds for $O_2^{\bullet-}$), and their composition and concentration change almost constantly during photo catalysis (Wang et al. 2014).

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were supplied by Sigma-Aldrich (St. Louis, MO, USA), in ACS purity, used without further purification.

Synthesis of TiO_2

TiO_2 biphasic NPs were synthesized via hydrolysis condensation method and were obtained as liquid phase of TiO_2 dispersion.

Dynamic light scattering (DLS)

Particle size distribution was performed with the Zeta sizer Nano (Malvern Instruments, Malvern, UK). Size distribution was determined by the DLS measured three times.

X-Ray diffraction (XRD)

XRD analysis was carried out using an X-ray diffractometer (SmartLab, Rigaku, Japan). The air-dried NPs were coated onto an XRD grid and diffracted intensities were documented from 20 to 90 of 2θ angles with a scan speed of 2°/min at 40 kV and 30 mA.

Scanning electron microscopy (SEM)

NPs powder was coated onto a coverslip and the morphology of the synthesized NP powder was studied using SEM imaging (ZEISS Sigma Scanning Electron Microscope, 10 kV accelerating voltage).

Photocatalytic activity

Photocatalytic activity was studied by a modified method (Periyat et al. 2008). Two millilitres of 25 mg/ml TiO_2 NP solution was dispersed in 50 ml of 1.0×10^{-5} M methylene blue solution. Prior to the UV exposure, the suspension was kept in the dark for 30 min. Then the suspension was exposed to 312 mJ/s of UV radiation (GS Gene Linker UV Chamber, Bio-Rad). Photo degradation was monitored at 5 min time intervals by withdrawing 3 ml aliquots of the TiO_2 NPs added to methylene blue solution. Absorbance was taken using a UV-Visible spectrophotometer. Percentage of photo degradation was plotted against exposure time.

In vitro bacterial culture

In vitro antibacterial effect of TiO_2 NPs was evaluated by minimum inhibitory concentration (MIC) and disc diffusion method. *Staphylococcus aureus* (CCM 4223) and *Escherichia coli* (CCM 3954) (Czech Collection of Microorganisms, Brno, Czech Republic) were cultured on Muller-Hinton (MH) agar (Oxoid, Hampshire, UK) overnight at 37 °C.

Effect of UV irradiation on antimicrobial potential of TiO_2 NPs

To evaluate the effect of UV light exposure on antimicrobial activity of TiO_2 NPs, separate stocks were prepared using 10 mg TiO_2. NPs were dissolved in distilled ACS water and exposed to UV light (UV-C light – wavelength 254 nm) for 0, 30, 60, and 100 min for usage in antibacterial assay. UV transparent glass vials were used for that purpose, whereas for the purpose of disc diffusion method, different approaches were carried out, as every dish was irradiated separately via exposure of UV light for 10, 15, 20 and 30 min.

Antibiotic susceptibility test (Disk Diffusion method)

Antibiotic susceptibility test was carried out by disc diffusion method. MH agar media was poured in 90 mm Petri dishes and incubated at 37 °C overnight to check sterility. The bacterial suspension was adjusted to an optical density OD_{600nm} = 0.1 AU (10^6 CFU/ml). Bacterial suspension was inoculated on each plate by spread-plate method and then antibiotic sensitivity discs were placed on the surface of the medium. After that, TiO_2 NPs samples (1–0.5 mg/ml) were added from two stocks into each disc and incubated for 24 h at 37 °C and the antibacterial sensitivity was measured as the zone of inhibition in mm diameter, for the purpose of activation of TiO_2 UV light was applied within a time interval of 10 min reaching, adjusted.

Determination of the minimum inhibitory concentration (MIC)

The MIC of the bacterial strains was determined as concentration minimum of NPs which inhibits the growth of 90% bacterial population after incubation time of 18 to 24 h at 37 °C. Approximately 100 µl of distilled ACS water were added to each well of a micro titre plate containing 96 wells, and 200 µl of the stock aqueous suspension of NPs (10 mg/ml) were added to the second row wells of each from which series of geometric dilution common ratio of 2 were made. Approximately 100 µl of the bacterial suspension of 0.5 McFarland standard 100 folds dilute cell were added to each well, and the microtitre plate was incubated at 37 °C for 24 h in the dark, while shaking.

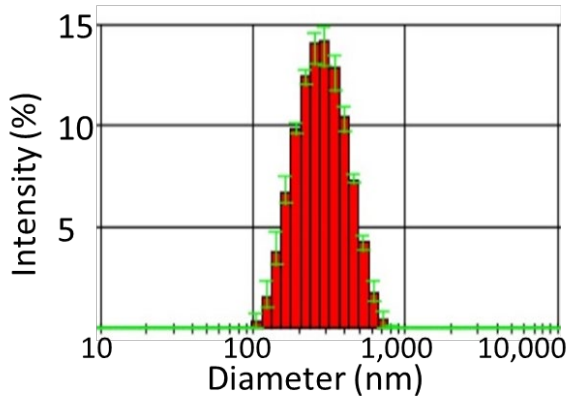
RESULTS AND DISCUSSION

TiO_2 NPs synthesis and characterization

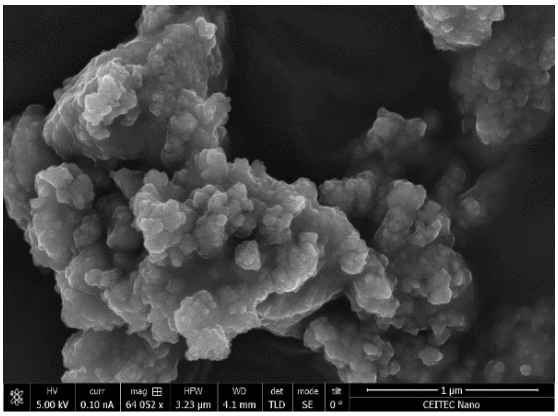
In this experiment, biphasic TiO_2 NPs hydrolysis condensation synthesis was obtained in liquid phase of TiO_2 dispersion and was determine the particle size distribution performed. The TiO_2 NPs particle size distribution in one particle fraction exhibited a mean size of about 250 nm (Figure 1A). X-ray scattering study showed that nearly 48.90% of mass of the particles was attributed to Ti atoms (Table 1). Oxygen and carbon atoms masses contributed to 28.95% and 22.15%, respectively. It is possible that the carbon was added during the synthesis procedure. Based on SEM imaging (Figure 1B), NPs are possibly agglomerates of smaller TiO_2 particles that are possibly electrostatically stabilized and may be mechanically disrupted.

Figure 1 Characterization of TiO_2 NPs

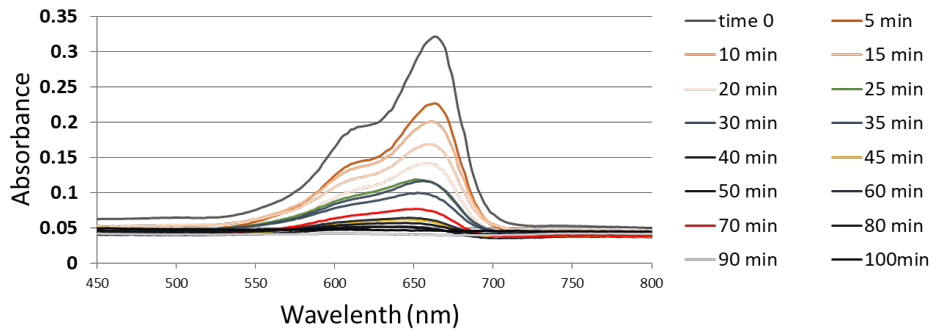
A) Dynamic light scattering



B) Scanning electron microscopy



C) Photocatalytic activity



The photocatalytic activity of synthesized TiO_2 NPs was determined by observing the changes in methylene blue concentration using UV-Visible spectroscopy after TiO_2 NP treatment and UV exposure (Figure 1C). TiO_2 NPs started to decolorize methylene blue dye within 10 min

and nearly all visible color was removed after 80 min. Fifty percent of the maximum peak absorbance (dye reduction) was observed within 20 min of exposure for both TiO_2 NP samples. This was confirmed by the reduction of the intensity of the characteristic peak position of untreated methylene blue with time (Figure 1C).

Table 1 Atomic composition via X-ray scattering

Atoms	Atomic conc. (%)	Error (%)	Mass conc. (%)	Error (%)
O 1s	38.71	0.83	28.95	0.69
Ti 2p	21.85	0.58	48.90	0.85
C 1s	39.44	0.92	22.15	0.67

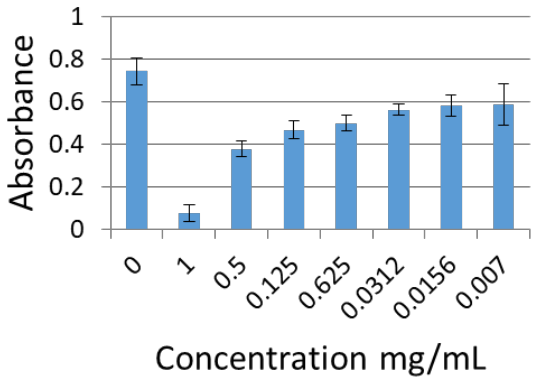
TiO_2 NPs antimicrobial activity

The MIC results for *S. aureus* were collected after 24 h of incubation (Figure 2A–B). The findings indicated that the TiO_2 of concentration over 1 mg/ml had antimicrobial activity when it was applied against *S. aureus* with higher selectivity than *E. coli* (data not shown), the results showed a stronger activity of higher concentration of TiO_2 with the absence of the UV light, on the other hand, the same method was carried out against the same strain of bacteria *S. aureus* but with the presence of the UV light as mentioned above in the material and methods, with less activity of the NPs (Figure 2A–B).

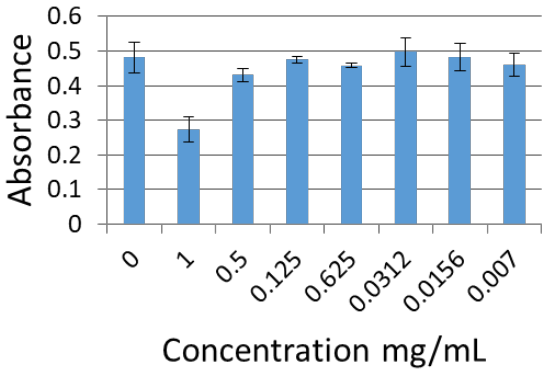
The disc diffusion procedure was conducted to assess the antimicrobial activity of NPs. Results showed no significant activity of TiO_2 NPs at two different concentrations. Inhibition zone diameter indicated that TiO_2 NPs had no antimicrobial effect against bacteria in the presence of UV light (Figure 2C).

Figure 2 Antimicrobial activity of TiO_2 NPs in Staphylococcus aureus

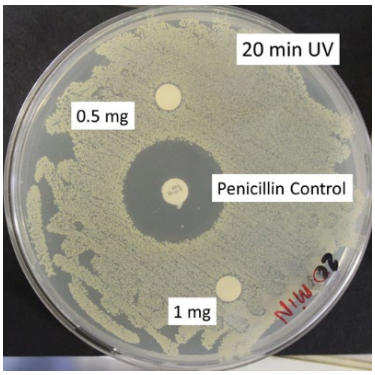
A) MIC test without UV

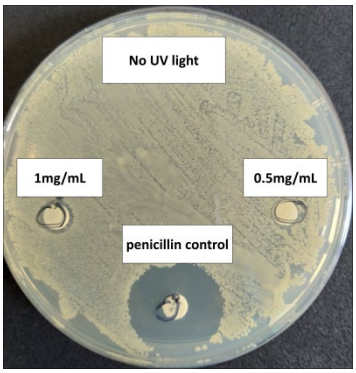


B) MIC test after 100 min UV exposure



C) Disc diffusion test





CONCLUSION

In this work, we have studied the potential of TiO_2 NPs activation by UV light to inhibit the growth of *S. aureus.* NPs were found to have a mean particle size of 250 nm, while SEM showed them to be formed in agglumerates. We have found that large amount of the atomic masses as estimated by XRS was attributed to Ti (~48.90%) while oxygen and carbon atomic masses were relativily similar. TiO_2 NPs started to decolorize methylene blue dye within 10 min and nearly all visible color was removed after 80 min. TiO_2 of concentration over 1 mg/ml had antimicrobial activity when it was applied against *S. aureus* as indicated by MIC, however disc diffusion method did not show any significant antimicrobial role.

Future work will involve modification of the particles to adjust the methodology of UV activation of TiO_2 NPs by using visible light. Such modification may include forming composites or coating or any other method that can expand the band gap.

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