

The Antibacterial Activity of SnO₂ Nanoparticles against *Escherichia coli* and *Staphylococcus aureus*

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Received: February 19, 2014; Accepted: May 5, 2014

Background: An increasing amount of populations all over the world are reported to have no access to clean and fresh water. Some traditional chemical disinfectants (free chlorine, chloramines, and ozone) are already widely used in the water industry; alarmingly, many of these are carcinogens. Furthermore, the resistance of microorganisms to these common chemical disinfectants is increasing; superior alternatives are therefore necessary. The development of nanoscience and nanotechnology within the last decades provides opportunities to deal with this problem.

Objectives: In this study, the solvothermal method was employed to synthesize SnO₂ nanoparticles with spherical morphology in the absence of templates or structure-directing agents under mild conditions. The nanoparticles used to in the inactivation efficiencies for two microorganisms under UV irradiation and dark conditions.

Materials and Methods: In this experimental study, tin dioxide (SnO₂) nanoparticles have been synthesized via a simple solvothermal method in the absence of templates or structure-directing agents under mild conditions. The prepared SnO₂ sample was employed for the inactivation of Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 29213) in MilliQ water under dark and UV illumination. The antibacterial activity of the synthesized SnO₂ nanoparticles was evaluated using bacterium as per colony count method.

Results: SnO₂ nanoparticles were successfully synthesized via solvothermal method under mild condition. SEM image showed SnO₂ nanoparticles were spherical when a mixture of water and ethanol used as solvent. The prepared SnO₂ nanoparticles were employed for the inactivation of gram-negative *Escherichia coli* (ATCC 25922) and gram-positive *S.aureus* (ATCC 29213) in MilliQ water. It was found that SnO₂ nanoparticles show much higher activity against *E. coli* than *S.aureus*.

Conclusions: According to the result of this study, SnO₂ nanoparticles are an excellent antibacterial agent for bactericidal applications.

Keywords: SnO₂ nanoparticles; Bacterial inactivation

1. Background

The removal of bacteria from water is an extremely important process for drinking and sanitation systems especially against concerns on growing outbreaks of water borne diseases [1]. In the United States, only between 2003 and 2005 there were four reported waterborne disease outbreaks attributed to pathogens in drinking water affecting 282 people [2]. Conventional methods for disinfection of water are dependent on chemical agents, that are ineffective against cyst-forming protozoa such as *Giardia* and *Cryptosporidium* and also these methods often produce harmful by-products.

Nanotechnology is considered as a new generation of technology that can have a great impact on economies through new consumer products, manufacturing methods and materials usage [3]. This technology can lead to cost effective and high performance water treatment systems [4]. By the

use of nanotechnology, implementation of oligodynamic nanoparticles for water disinfection is being explored. Oligodynamic nanoparticles based disinfection includes the use of metals such as silver, gold, zinc, tin and copper due to their antimicrobial properties. Besides their oligodynamic nature, they also possess catalytic properties [5].

In 1985, Matsunaga et al. [6] reported the antibacterial properties of TiO₂ particles for the first time, which attributed to the high redox potential of the surface species, affording non-selective oxidation of bacteria. Hashimoto et al. [7] have reviewed the antibacterial effects and detoxifying actions of TiO₂ photocatalyst on ceramic tiles. Since then, many photocatalytic inactivation studies have been conducted on a wide spectrum of organisms including cancer cells, phages, viruses, bacteria, fungi, algae and protozoa. Large band gap semiconductors, such as TiO₂, SnO₂,

SiO₂ and ZnO are suitable photocatalytic materials [8-10]. Among these Tin oxide (SnO₂) is an important n-type metallic oxide semiconductor with wide band gap (3.6 eV). Because of its unique electronic, optical, electrochemical and catalytic properties, SnO₂ were extensively used in solar cells, transparent conducting electrodes, solid-state sensors, rechargeable Li batteries and optical electronic devices [11, 12]. The conductivity and optical properties of SnO₂ are largely dependent on the particle size and shape of the nanocrystallites [13-15]. To obtain quantum size SnO₂ nanocrystallites, the sol-gel method [16] and hydrolysis of SnCl₂·2H₂O were carried out [17]. Recently, SnO₂ Quantum dots were also fabricated using hydrazine hydrate as the mineralizer instead of NaOH by a hydrothermal route [18].

However, it is still a great challenge to fabricate the nanostructure SnO₂ with controlled-size and tunable shapes by wet chemical methods [19].

2. Objectives

In this study, the solvothermal method was employed to synthesize SnO₂ nanoparticles with spherical morphology in the absence of templates or structure-directing agents under mild conditions. The nanoparticles used to in the inactivation efficiencies for two microorganisms under UV irradiation and dark conditions.

3. Materials and Methods

3.1. SnO₂ Powder Preparation

In this experimental study, the solvothermal method was employed to synthesize SnO₂ nanoparticles.

In a typical process, desired amount of SnCl₄·5H₂O and 1.2 g NaOH were mixed with 20 mL de-ionized water under magnetic stirring for 10 minutes. Twenty milliliter absolute ethanol was dropped slowly into the solution to make the white precipitation. The mixture was stirred for 24 hours and then the whole mixture was transferred into 50 mL Teflon-lined autoclave. After the reaction completed, the resulting solid product was filtered and washed several times by de-ionized water, absolute ethanol and finally dried at 120°C in air for 24 hours.

3.2. Antibacterial Performance

The antibacterial activity of the synthesized SnO₂ nanoparticles was evaluated using bacterium as per colony count method. To examine the bacterial growth rate, cultures (bacterial strain) were grown in the nutrient broth medium (NB) supplemented with SnO₂ nanoparticle colloidal suspension at concentration of 20 ppm. The SnO₂ nanoparticles were added into 10 mL NB medium which was added with 200 µL bacteria at a concentration of 1.5 × 10⁶ CFU (colony forming unit). The culture of nanoparticle free medium as blank was grown under the same reaction conditions and was used as a control. The samples and blank were illuminated with four 8 W lamps (Philips UV-A, k

= 365 nm), at room temperature for 0.5, 1, 2, 3, 4 and 5 hours, respectively. The temperature of the culture medium was controlled using the water circulation through the reactor. During each time period, the sample containers were placed horizontally onto a shaker platform and agitated at a speed of 225 rpm. After catalyst centrifugation and separation, 100 µL of each bacteria suspension was dispersed on the Nutrient Agar medium. The number of colonies forming units (CFUs) were counted taking into account the dilution factor after 24 hours of incubation at 37°C. The respective data are the average of values obtained from triplicate runs. The catalysts under consideration have not shown a decrease in their photo induced micro biocide efficiency when the antimicrobial experiments were repeated three times using the same samples. No significant decrease in CFU in nanoparticle free medium observed after UV light exposure. This indicates that the photochemical inactivation was negligible under UV-A irradiation with low energy.

3.3. Sample Characterization

To determine the crystal phase composition of the prepared photocatalysts, X-ray diffraction (XRD) measurement was carried on a Bruker, D8 ADVANCE XRD diffraction spectrometer with a Cu K α line at 1.5406 Å and a Ni filter for an angle range of 2 θ = 20 - 70°. To determine the band gap energy of the photocatalysts, UV-vis spectroscopy measurement was carried out using a double-beam spectrophotometer Cary 500 scan. UV-vis spectrophotometer operated over the range of 190 - 800 nm at a resolution of 2.0 nm. Philips XL30 scanning electron microscope (SEM) measurements were also used to investigate the morphology of the samples with an accelerating voltage of 17 kV.

4. Results

The XRD pattern of SnO₂ powder is shown in Figure 1. The patterns of SnO₂ powder display the (110), (101) and (211) diffraction peaks of the tetragonal rutile structure for SnO₂ crystalline phase (space group: P4₂/mnm, a = 0.4756 nm, c = 0.3175 nm) at 2 θ about 26.5°, 34° and 52.1°, respectively. No remarkable shift and no characteristic peaks for impurity, such as SnO, ZnSnO₃, and Zn₂SnO₄ in diffraction peak were detected. The surface energies of SnO₂ follow the trend (110) < (100) < (101) < (001), indicate the c-axis to be the preferential growth direction [20].

By conducting experiments in solutions, the morphology is therefore dictated by the crystal symmetry as well as by the surface energy in the aqueous environment and thus the most stable crystal habit is directly generated without template or surfactant. Further structural characterization of the SnO₂ powders was performed by SEM. It was found that the hydrothermal treatment led to synthesized spherical nanoparticles with 66 nm diameters (Figure 2). The morphology of the final products may be explained by both crystal growth and nucleation theory in which the synthesis is separated into two steps: nucleation stage and crystal growth. In the nucleation stage,

Sn(OH)₆²⁻ nucleus are created which decompose to SnO₂. At lower hydrothermal temperature instead of crystal growth stage, the greater the number of nuclei was created in the solution where nuclei attracted species and other nuclei around them to form spherical shapes.

UV/vis spectroscopy was used to characterize the optical adsorptions of SnO₂ sample as shown in Figure 3. The fundamental absorption edge of the sample corresponds to electron transitions from valence band to conduction band and this edge can be used to calculate the optical band gap of the sample. The optical transition of SnO₂ crystals is known to be a direct type [21]. In this case, the absorption coefficient is expressed [22]. The band gap energies (E_g) were calculated on the basis of the corresponding absorption edges and by extrapolating the horizontal and sharply rising portions of the curve and defining the edge as the energy of the intersection (not shown here).

E. coli and *S. aureus* cells were exposed to different samples for up to 5 hours to assess any bactericidal effect of the catalyst under UV light and dark conditions. Figures 4 and 5 show the relative number of bacteria survived after the antibacterial experiments.

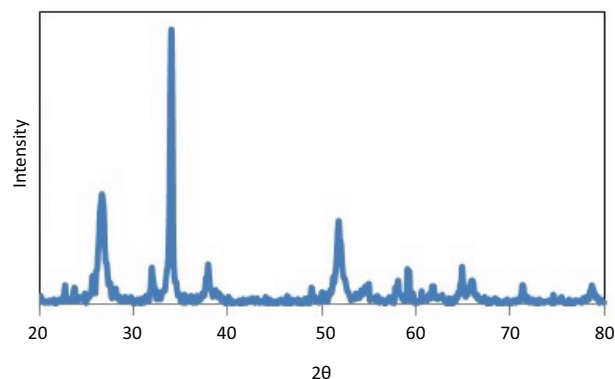


Figure 1. The X-ray Diffraction Pattern of SnO₂ Nanoparticles

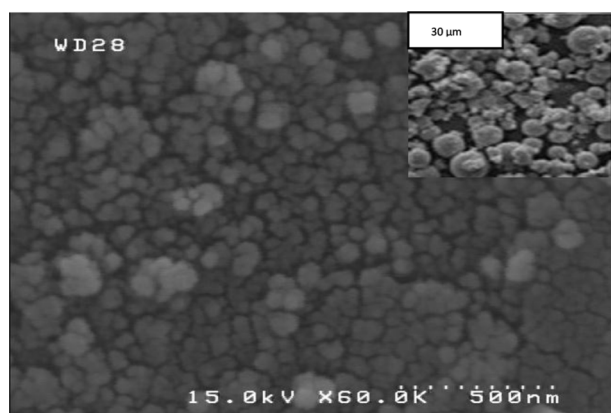


Figure 2. The SEM Image of SnO₂ Nanoparticles

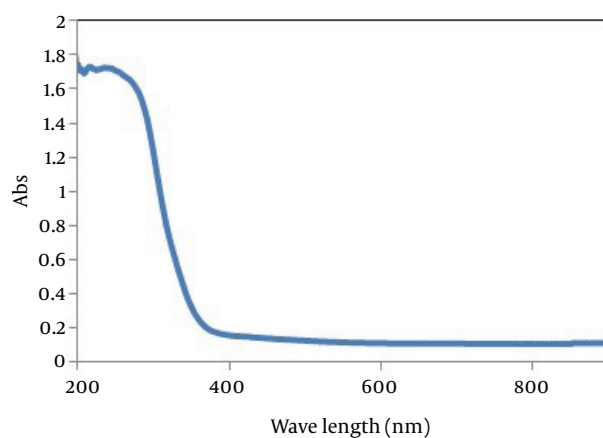


Figure 3. The Absorption Spectra of SnO₂ Nanoparticles

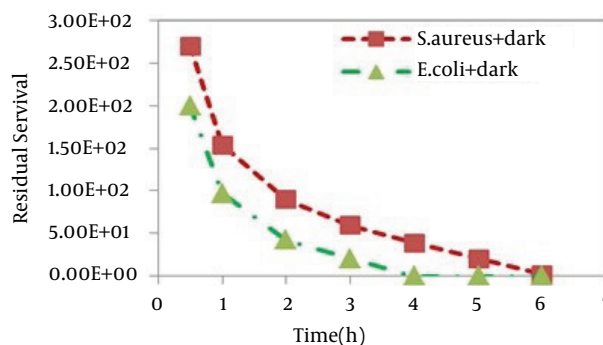


Figure 4. Plot of the Survival Number of *Escherichia coli* and *Staphylococcus aureus* Under Dark Condition

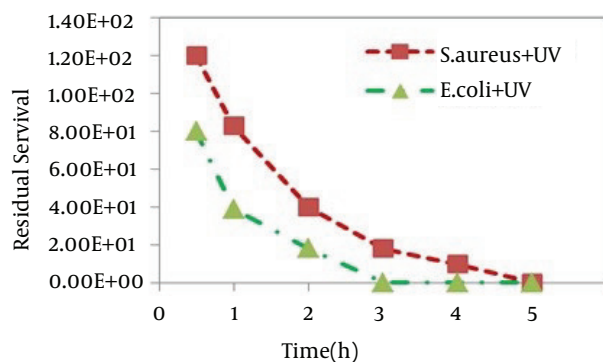


Figure 5. Plot of the Survival Number of *Escherichia coli* and *Staphylococcus aureus* Under UV Condition

5. Discussion

In this study it was found that SnO₂ nanoparticles had a good antibacterial activity against Gram-positive and Gram-negative bacteria. The antibacterial effect of the synthesized photocatalyst towards *E. coli* and *S.aureus* was investigated by initially performing controlled experiments. Two individual runs were performed as control ex-

periment. Blank control experiments (bacterial solutions without any catalyst) under dark and UV light irradiation were performed and no significant decrease in CFU of nanoparticle free medium were observed. In other controlled experiments, bacterial cultures were incubated with nanoparticles in dark. Similar experiments as mentioned above were performed by incubating bacterial cultures with nanoparticles under UV illumination. The larger band gap of SnO₂ sample enhances its UV-light absorption ability resulting in the formation of highly reactive oxygen species (ROS) which are responsible for bacterial cell damage. Therefore, UV illumination improved SnO₂ sample activity compared to dark condition. It is worthy to note that in the absence of UV light, all samples showed bactericidal activity. In contrast, nano-TiO₂, a common kind of bactericidal material, is photoactive and requires light irradiation in order to exhibit effective bactericidal activity.

The nanoparticle morphology, surface area, surface charge, its material solubility and degree of aggregation in a particular system being studied will strongly influence the availability of nanoparticles for uptake into cells and resulting toxicity in aquatic systems [23, 24]. The following factors may affect the antibacterial activity of SnO₂ powders: 1- Highly reactive oxygen species (ROS) generated including hydroxyl groups (OH), superoxide anions (O₂⁻) by single-electron reduction which does not require UV irradiation [25, 26]. However, photo catalytically induced oxygen bacterial cells. 2-pH: in our work, pH of the powder samples dispersed in physiological saline was 7.5, irrespective of the sample. 3- Membrane dysfunction [27]. 4- Nanoparticle internalization [28]. Species improve the bactericide effect of UV-illuminated samples. It has been generally accepted that likely mechanism of antibacterial photoactivity of metal oxides is due to interaction with highly reactive oxygen species (ROS) which would result in oxidative damage of the cell membrane or inside the cells [29, 30]. These ROS can include hydroxyl groups, superoxide anions, and hydrogen peroxide [31]. However, it is still a subject of investigation as to which of these reactive oxygen species are directly involved in the damage to bacterial cells.

Catalyst deactivation is one of the drawbacks on the application photocatalytic degradation processes; deposition of photo insensitive species on the photocatalyst surface blocking its active sites. The economy of the photocatalytic process depends upon how many times a catalyst can be reused without losing its efficiency and the type of regeneration it requires. The SnO₂ used catalyst was regenerated by treating with boiling distilled water and then by drying it in a hot air oven at a temperature of 100°C. The catalysts under consideration have not shown a significant decrease in their photoinduced and dark microbiocide efficiency when the antimicrobial experiments were repeated three times using the same sample.

SnO₂ nanoparticles show much higher activity against *E. coli* than *S.aureus*. The higher deactivation efficiency in the case of Gram-negative bacteria as compared to Gram-positive ones was reported earlier [32, 33]. This difference can be attributed to the differences in the cell wall structure inherent in Gram-negative and Gram-positive bacteria. Gram positive and Gram negative bacteria have similar internal, but very different external structures. A Gram positive bacterium has a thick peptidoglycan layer that contains teichoic and lipoteichoic acids. A Gram negative bacterium has a thin peptidoglycan layer and an outer membrane that contains lipopolysaccharide, phospholipids, and proteins. This can lead to the conclusion that the bacteria photoinactivation rate is governed not only by cell wall thickness but also by the morphology of cell envelop and resistance of outer membrane to the reactive oxygen species produced at the photocatalyst surface. Figure 6 photographically shows two obtained results including the higher inactivation efficiency under UV illumination for both bacteria and much higher activity against *E. coli* than against *S. aureus*. In this study, the solvothermal method was employed to synthesize SnO₂ nanoparticles with spherical morphology in the absence of templates or structure-directing agents under mild conditions. The nanoparticles used to in the inactivation efficiencies for two microorganisms under UV irradiation and dark conditions. It was found that SnO₂ nanoparticles show much higher activity against *E. coli* than *S.aureus*.

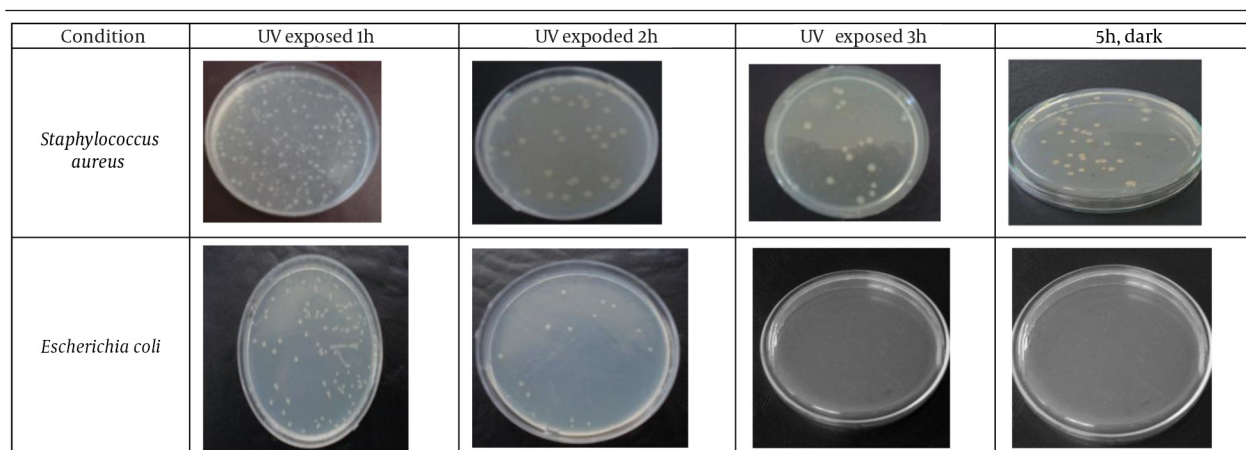


Figure 6. Photographs Demonstrating the Effect of Sample on the Number of CFU Following Exposure to UV Light for 1, 2, 3 Hours and without UV After 5 Hours

Acknowledgements

The authors wish to thank the Islamic Azad university of Shahreza and university of Sistan and Baluchestan for technical support.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Financial Disclosure

The authors declare no conflict of interest.

Funding/Support

Islamic Azad University of Shahreza.

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