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Antibacterial Activity of Zn Decorated TiO_2 Nanocomposites

[1]Kumarasinghe N.M.A, [2,*]Charitha Thambiliyagodage, [3]Madara Jayanetti, [4]Heshan Liyanaarachchi

[1,2,3,4]Faculty of Humanities and Sciences, Sri Lanka Institute of Information Technology, Malabe,10115, Sri Lanka

Email of the corresponding Author - *charitha.t@sliit.lk

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ABSTRACT

Bacterial infections have a significant public health impact. Infections are caused by bacteria in animals, plants as well as humans. Pathogenic bacteria can produce toxins, which are chemical poisons that interfere with cell function such as digestion of normal human enzymes, evasion of infection-fighting white blood cells, and immune clearance. Antibiotic prophylaxis is used to prevent bacterial infection. Antibiotic resistance is one of the most serious concerns in world health. Antibacterial nanoparticles are one possible answer to antimicrobial resistance. These nanomaterials not only kill antibiotic-resistant bacteria through various modes of action but, they can also be employed in conjunction with existing clinically relevant antibiotics to help overcome antimicrobial resistance mechanisms. In this study, anodized titanium dioxide (TiO_2) nanorods were treated hydrothermally with zinc oxide (ZnO) nanoparticles to give titanium (Ti) antibacterial properties. The antibacterial activity of synthesized samples was investigated by Agar Well Diffusion method at 40 mg/ml concentration, against gram negative *Klebsiella pneumoniae*. To determine the antibacterial activity, the diameter of the zone of inhibition was measured, and the resulting data were statistically analyzed. Zn/TiO_2 nano particles were characterized by using X-ray diffraction (XRD) Analysis.

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1. INTRODUCTION

Nanotechnology is defined as the understanding and control of matter at dimensions of roughly 1- 100 nm. Many structures with one or more nanometer dimensions can be found in nature, and many technologies have used them incidentally for many years, but it is only recently that it has been able to do so intentionally (Mukhopadhyay et al., 2009). Many technologies and industry sectors are benefiting from nanotechnology, including information technology, homeland security, medicine, transportation, energy, food safety, cosmetics, and environmental science (Mondal, 2020). Among them, the biological side is relevant to the biotechnology. Nanomaterials with different morphologies such as nanoparticles, nanotubes, nanorods have been used to inhibit the growth of bacteria.

The antibacterial activity of ZnO nanoparticles could be caused by seven different mechanisms by nanomaterials. First disruption of the cell wall and cytoplasmic membrane of the bacterial cell or pass through the cell wall and cytoplasmic membrane. Secondly, metal ions denature ribosomes and inhibit protein synthesis. Then ATP production is terminated because the metal ions deactivate respiratory enzymes on the cytoplasmic membrane. Reactive oxygen species produced by the nanomaterials disrupts the membrane. Then metal nanoparticles and reactive oxygen species bind to deoxyribonucleic acid and prevent its replication and cell multiplication. Further, ZnO nanoparticles accumulate in the pits of the cell wall and cause membrane denaturation. Finally, nanomaterials can perforate the membrane (Yin et al., 2020). Reactive oxygen species are produced by the nanomaterials in the presence of light source. Because of that while the other mechanisms are active in dark and light mechanism in which the reactive oxygen species is involved is active in the presence of light source (Dayem et al., 2017).

Bacteria are single-cell organisms that are microscopic in size. Bacteria can be found almost anywhere on the planet and are essential to the planet's ecosystems. Problem is sometimes bacteria cause adverse effects on all living beings (Brazier, 2019). Diseases are caused by bacteria in animals, plants as well as humans. The immune system of the body is weakened when bacteria invade the host, which opens the door for other bacteria to enter the body (Kim et al., 2020).

Objectives of this study are synthesis of Zn decorated TiO_2 nanorods and evaluation of the antibacterial activity of Zn decorated TiO_2 nanorods.

2. MATERIALS AND METHODS

2.1. SYNTHESIS OF TIO_2 NANOPARTICLES

First P25 TiO_2 (2 g) was dispersed into 80 ml of a 1 M NaOH solution. The suspension was sonicated for 1 h. Then the hydrothermal treatment method was applied to the suspension at 180 ℃ for 24 h. After that, the solid sample was washed with deionized water until the pH was neutral. Finally, the sample was dried at 180 ℃ in a dry oven.

2.2. SYNTHESIS OF ZNO NANOPARTICLES

$ZnSO_4.7H_2O$ (4 g) was measured and deionized water (20 ml) was added to dissolve $ZnSO_4.7H_2O$. Then the solution was titrated with 1 M NaOH under continuous stirring until a white gelatinous precipitate is formed. Then the hydrothermal treatment method was applied to the solution at 180 ℃ for 24 h. The resultant solution was filtered, then washed with deionized water until negative to and kept for drying at 180 ℃.

2.3. SYNTHESIS OF NANOCOMPOSITES

2.3.1. [TiO_2: ZnO = 1:1]

P25 TiO_2 (2 g) was dispersed in the minimum amount of DI water. Then $ZnSO_4.7H_2O$ (7.19 g) was measured and it was dissolved in the minimum amount of water. After that 1 M NaOH solution (50 ml) was added drop by drop into the mixed solution with continuous stirring. Then $ZnSO_4.7H_2O$ solution and P25 TiO_2 solution were mixed together. The solution was sonicated for 30 mins. Then the hydrothermal treatment method was applied to the solution at 180 ℃ for 24 h. The resultant solution was filtered, then washed with deionized water until negative to and pH to neutral. Finally, the precipitate was kept for drying at 180 ℃.

2.3.2. [TiO_2: ZnO = 2:1]

The synthesis procedure was the same as that reported in section 3.3.1, except for the amount of $ZnSO_4.7H_2O$. The amount that was used to synthesize this composite (TiO_2: ZnO = 2:1) is 3.6 g.

2.3.3. [TiO_2: ZnO = 1:2]

The synthesis procedure was the same as that documented in section 3.3.1, except for the amount of $ZnSO_4.7H_2O$. The amount that was used to synthesize this composite (TiO_2: ZnO = 1:2) is 14.38 g.

2.4. ANTI-BACTERIAL ACTIVITY

2.4.1. AGAR-WELL DIFFUSION METHOD

The antibacterial activity of synthesized composites was investigated using the agar-well diffusion method. The antibacterial effect of synthesized composites was tested against *Klebsiella pneumoniae* in a sterilized Luria Bertani broth medium. For the broth dilution assay, a 24 h aged bacterial culture was adjusted to obtain 5×10^5 CFU/mL with a 0.5 McFarland turbidity standard. The adjusted bacterial suspension was used within 30 min to avoid changes in the cell count. Nanoparticle suspensions were prepared by sonicating the synthesized nanoparticles in dimethyl sulfoxide for 1 h.

After that, bacterial strain was streaked over Mueller Hinton's agar by sterile cotton swabs, and after 15 min, wells were cut into the agar with the help of sterilized micropipette tips. Then the sonicated samples (40 mg/ml) were separately poured onto the wells using a micropipette and the plates were covered with parafilm. After that, the well plates were incubated at 37 °C for 24 h and experiments were conducted in triplicate. Finally, the inhibition zones were measured for each sample.

3. RESULTS AND DISCUSSION

3.1. ANTIBACTERIAL ACTIVITY

	M1 (mm)	M2 (mm)	M3 (mm)	M4 (mm)	M5 (mm)
K. pneumoniae	10.83±1.15	12.00±0.87	12.50±1.00	11.33±0.58	10.50±2.78

Table 01: Antibacterial activity of ZnO, TiO_2 and Zn/TiO_2 nanoparticles using the well diffusion method.

Depending on the microbe and the metal oxide concentration, metal oxide nanoparticles have different levels of microbial sensitivity. Figure 01 shows the antibacterial activity of ZnO, TiO_2 and Zn/TiO_2 nanoparticles against *Klebsiella pneumoniae*. Table 01 shows their corresponding inhibition zones. All these results are at a concentration of 40 mg/ml. There are 5 nanomaterials tested for antibacterial activity. They are M1 (Pure ZnO), M2 (TiO_2: ZnO = 1:1), M3 (TiO_2: ZnO = 1:2), M4 (TiO_2: ZnO =2:1) and M5 (Pure TiO_2).Figure 01 shows the antibacterial activity against *Klebsiella pneumoniae* of synthesized nanomaterials.

Inhibition zones for M1, M2, M3, M4 and M5 against *Klebsiella pneumoniae* are, 10.83±1.15 mm, 12.00±0.87 mm, 12.50±1.00 mm, 11.33±0.58 mm and 10.50±2.78 mm respectively (Table 01). The antibacterial activity of Zn-decorated TiO_2 is more potent when compared to pure

ZnO (M1) and pure TiO_2 (M5). Among them, M3 nanoparticles have the best antibacterial activity against *Klebsiella pneumoniae.*

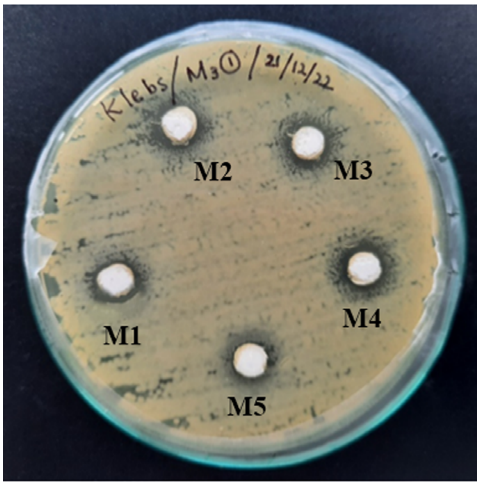

Figure 01: Antibacterial activity of synthesized nanoparticles against *Klebsiella pneumoniae*

3.2. X-RAY DIFFRACTION (XRD) ANALYSIS

To investigate the crystal nature of the produced composites, X-ray diffraction patterns were recorded. Figure 02 shows the XRD patterns for the synthesized samples. Figure 02e shows the XRD pattern of the pure ZnO consists of peaks at 32.07°, 34.73°, 36.67°, 47.84°, 56.86°, 63.11°, 68.15°, 69.36° and 77.18° which correspond to the (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (1 1 2), (2 0 1) and (2 0 2). XRD pattern of P25 TiO2 shows peaks at 27.57°, 36.18°, 41.37°and 56.73°which are attributed to (11 0), (1 0 1), (1 1 1) and (2 2 0) planes of the Rutile phase and the peaks at 25.46°, 37.95°, 38.72°, 48.17°, 54.07°, 55.18°, 62.83°, 68.94°, 70.34°and 75.21° assigned to (1 0 1), (1 0 3), (0 0 4), ((0 0 2), (1 0 5), (2 1 1), (2 0 4), (1 1 5), (2 2 0) and (2 1 5) the Anatase phase (Figure 02d). The obtained values are the same as the known values. (Khorsand Zak et al., 2011).The composites showed crystalline phase peaks of both TiO2 and ZnO which confirmed the presence of both phases in the composite. Figure 02a shows the XRD pattern of composite which is TiO_2: ZnO = 2:1. In this composite, the amount of P25 TiO_2 is higher than ZnO. With increase of P25 TiO_2 amount, diffraction peaks of TiO_2 started appearing with slight increase than ZnO.

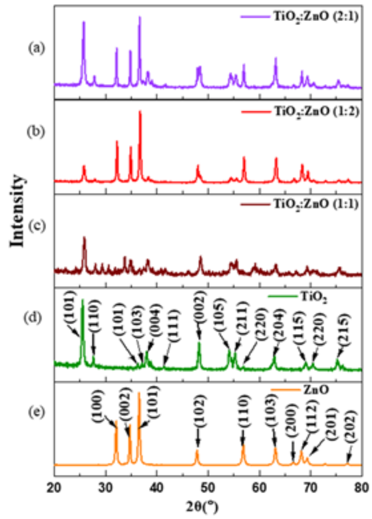

Figure 02: XRD patterns of synthesized samples

4. CONCLUSION

Highly pure TiO_2 nanoparticles and ZnO nanoparticles were successfully synthesized using P25 TiO_2 and $ZnSO_4.7H_2O$ respectively via hydrothermal synthesis pathways. ZnO nanoparticles were successfully coupled to TiO_2 in different weight ratios hydrothermally to synthesize Zn/TiO_2 nanocomposites. The antibacterial activity of ZnO decorated TiO_2 nanorods has been tested on inhibition of gram-negative *Klebsiella pneumoniae* bacteria in this study. The synthesized composites were effective in inhibiting the growth of bacteria strain. Compared to pure ZnO (M1) and pure TiO_2 (M5), the antibacterial activity of Zn-decorated TiO_2 shows greater antibacterial activity. Among them, TiO_2: ZnO = 1:2 (M3) nanocomposites exhibit superior antibacterial activity, as compared to other materials.

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