Journal of Mines, Metals and Fuels, *71(8) : 1030-1036; 2023. DOI: 10.18311/jmmf/2023/35043*

Print ISSN : 0022-2755

Journal of Mines, Metals and Fuels

Contents available at: www.informaticsjournals.com/index.php/jmmf

Synthesis of ZnO-TiO_2 Nanocomposites for the Enhancement of Antibacterial Properties

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Abstract

The sol-gel process was used to create ZnO-TiO_2 nanocomposites with various weight percentages and studied for their antibacterial and optical properties. XRD and SEM analysis were used to analyze the structure and morphology of the obtained composite powders. Thus the resulting nanocomposites were examined for bacterial activities for the gram positive and gram negative bacteria's like (a) Staphylococcus, (b) Bacillus subtilis (c) Salmonella typhi (d) Pseudomonas aeruginosa. The synthesized ZnO-TiO_2 nanocomposites were found to be highly effective for 1% and 10% ZnO nano particles exhibit higher bactericidal activity with a high zone of inhibition of 8 mm, 11 mm against B. subtilis and 7 mm, 12 mm for S. aureus, 8mm, 10mm for P. aeruginosa and 6mm, 10mm for S. typhi bacterial strains. UV visible spectroscopy was used for optical studies and band gap measurement.

Keywords: *Bactericidal Property of Metal Oxides, Sol-Gel Synthesis, ZnO - TiO_2 Nanocomposites*

1.0 Introduction

The rise of bacterial disease poses a significant risk to human life on this planet. The biocompatibility of the synthetic antibiotics is also quite excellent, ensuring their persistent therapeutic potential. Since it frequently manifests in epidemics in biosensors, medical implants, public health events, hospital settings and food storage, bacterial contamination has become a serious issue[1]. People are becoming more aware of human health issues, diseases, and methods for preventing germs from endangering people's safety and health[2]. Traditional manual cleaning methods involving wiping are labor and time-intensive, cannot be standardized, and are not effective over the long run. The usage of harsh chemicals is also accompanied with a number of issues[3]. However, using these antibacterial wipes on all surfaces creates an environment where the resistant bacteria can survive[4]. Nanomedicine has gained popularity recently as an effective antibacterial agent. Metal oxide nanoparticles have been found to have exceptional antibacterial activity. In recent years metal oxide nano particles have evinced for their unique electronic and chemical properties. Metal oxide nanoparticles' (NPs) antibacterial effectiveness is influenced by a number of factors, i. Crystallanity, ii. Surface area, iii. Morphology, iv. Particle size, v. pH of the solution, vi. Capping/Stability, vii. Dosage including the type of microorganisms present[5]. In hospitals and other public spaces, antibacterial agents are frequently employed. A number of biocides, whether organic or metallic, have been tried to limit mould growth, although they can be hazardous and only work temporarily[6]. Recent research aims to create new types of safe and affordable biocidal materials because certain antimicrobial compounds are very unpleasant and hazardous[7]. Since food poisoning from *Staphylococcus aureus* (*S. aureus*),

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Legionella infections in full-time baths, and Drug-resistant bacteria like Methicillin-Resistant *S. aureus* (MRSA) are more prevalent in hospital infections, it is crucial that simple, inexpensive, and effective antibacterial methods need to be developed. TiO_2, one of the most investigated compounds, has shown to be a good anti bactericidal substance in the environmental and energy domains, including (i) self-cleaning surfaces (ii) air and water purification, (iii) water splitting and (iv) antimicrobial[8]. The most promising inorganic substance is Zinc oxide is a useful substance with a variety of applications. It has a variety of uses, in the fields of rubber industries, creams, face powders and also in the pharmaceutical sectors, as the ultra violet absorbers in the photo degradation process, in the electronic sectors and as sensors. Other notable applications of ZnO are packaging, crime investigation and fingerprint enhancement[9].

Zinc Oxide (ZnO) has received a lot of attention as a significant semiconductor photo catalyst because to its good qualities, which include affordability, high redox potential, nontoxicity, and environmental friendliness[10]. The formation of a nanocomposite system using ZnO added to TiO_2 is one of the useful coupling techniques. Nevertheless, this composite system demonstrates enhanced stability and considerable chemical activity with high ability to release trapped centre's on the TiO_2 surface which are responsible for the charge reduction and recombination active photo catalysis[11] and better anti-bacterial activity.

The eradication of the detrimental consequences triggered by microorganism attack has become a new problem. These antimicrobial therapies frequently make use of a variety of lipid-based nano particles, such as Nano Structured Lipid carriers (NLS) and Solid Lipid Nano Particles (SLP)[12]. However, in recent years, inorganic antimicrobial agents (such as ZnO and TiO_2) have become increasingly popular because of their increased stability and safety, which are lacking in organic antimicrobial agents[13]. Here, it is believed that the release of ions is the main driver of inorganic antibacterial agents' for antibacterial actions[12]. Typically, a bacterial medium is used as an indication to gauge how well these drugs disinfect[14]. Antibacterial metal and metal oxide nanoparticles have a major advantage including that their combined mechanism of action make it challenging for microbes to acquire resistance to them[15]. Silver nanoparticles are the inorganic antibacterial particles that have undergone the most thorough testing[16]. They can kill both Gram-positive and Gram-negative bacteria, being particularly effective against species that are resistant to conventional antibiotics. In this work we have synthesized the nanocomposites of ZnO–TiO_2 nanocomposites and tested these nanocomposites for the *S. aureus, B. subtilis*, and *S. typhi* bacterium cultures that are both gram-positive and gram-negative.

2.0 Experimental Procedure

2.1 Synthesis of ZnO and TiO_2 Nanocomposites

(i) Synthesis of TiO_2

Titanium dioxide and Zinc Oxide nanoparticles were synthesized by using sol-gel method. To prepare TiO_2, Titanium Tetra Iso-Propoxide (TTIP) solution was taken as precursor material. A 20 ml of TTIP solution was mixed to the solution of 10 ml of ethanol and 12ml of deionized water, after 1 hour of constant stirring at the temperature of 80^{0}C, concentrated nitric acid was added to maintain the pH of the solution, then the stirring was continued for 6 hours at 60^{0}C. White colored nano powder of TiO_2 was obtained after the calcination at 350^{0}C for 1.5 hours.

(ii) Synthesis of ZnO

Nanoparticles of ZnO were synthesized by considering Zinc nitrate and KOH as precursors. (0.4M) KOH solution was added to the mixture of (0.3M) Zinc nitrate and de-ionized water at room temperature with a continuous stirring of 20 minutes. The precipitate was then washed with ethanol and subjected to a 2-hour calcination at 6000°C to produce the ZnO nano powder.

(iii) Synthesis of ZnO and TiO_2 Nano Composites

The nano composite with the weight percentage 1, 10, 15 and 20 percentages of ZnO with respect to TiO_2 was fabricated by using the sol-gel process. In order to get the precipitation, ZnO and TiO_2 nanoparticles that have been synthesised are mixed with agitation for four hours at room temperature in 20 ml of distilled water in the appropriate weight ratio. It is filtered, after being aged for 24 hours, the item was cleaned with deionized water and dried for 12 hours at 1500°C. The produced precipitate was then used. The final step was calcining the resulting powder for 2.5 hours at 700^{0}C.

2.2 Antibacterial Procedure

(i) Bacterial Cultures

Microbial Type Culture Collection (MTCC) samples from Chandigarh's Institute of Microbial Technology were used for the test bacterial cultures. In order to test the antibacterial activity of the bacteria Gram-positive Gram-negative organisms, *S. aureus*, and *B. subtilis, P. aeruginosa* and *S. typhi* cultures were cultured on nutrient agar media.

(ii) Growth Inhibition Assay

Dual-culture agar diffusion experiment[17] was employed to evaluate the antibacterial effectiveness of chemical substances. Under aseptic conditions, Petri dishes received 20 ml of sterile Nutrient agar medium, which was then given time to set up *S. aureus, B. subtilis, P. aeruginosa* and *S. typhi* are examples of gram-positive bacteria were spread out evenly across 100 μl of the medium after it had solidified. To test for antibacterial agent diffusion, concentrations of 1 mg/ml of the sample were added to plates after it had been diluted with DMSO. Some transdermal drug delivery systems use DMSO because it increases the rate at which some substances are absorbed by organic tissues, including skin. In medicine, DMSO is primarily employed as a topical analgesic, a method of administering drugs topically, an anti-inflammatory, and an antioxidant. The plates were subsequently incubated at 37°C for the subsequent 24 hours. To see if there was a chance of antibacterial activity, the diameter of the inhibitory zone surrounding the well was measured in millimetres (mm). Agar discs came in three sets of three. Chloramphenicol was used as a comparison substance.

2.3 Material Characterization

Bruker powder diffractometer (Shimadzu-7000) with mono chromatized Cu-K (1.5406) radiation was used to characterize the synthesized $ZnO-TiO_2$ nanocomposites. The crystal size of the nanocomposites was determined using the Scherer formula. FTIR was used to investigate the stretching band frequencies. Scanning Electron Microscopy (SEM), a technique used to examine the morphology of nanocomposites, was carried out using a Zeiss electron microscope. The ability of the prepared nano composites was checked to enhance the antibacterial properties by Dual – Culture agar diffusion method.

3.0 Results and Discussion

3.1 Structural Studies

Figure 1 depicts room temperature XRD patterns of $ZnO-TiO_2$ nano composites in the 2θ range of 20 to 90 degrees. The diffraction peaks match the standard peaks very well. The hexagonal wurtzite structure of ZnO is represented by strong peaks at planes (100), (102) (110) (107) with 2θ values of 31.73, 56.55, and 62.83 degrees, respectively (JCPDS Card No - 3-888). Furthermore, the plane's peaks (213) (303) (402) (411) corresponded to TiO_2's

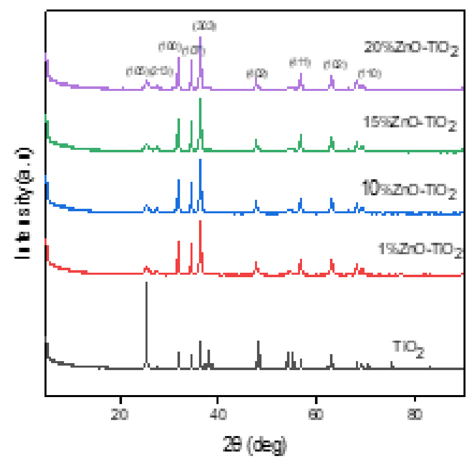

Figure 1. XRD spectra of 1, 10,15 and 20% $ZnO-TiO_2$ nanocomposites.

anatase phase (JCPDS Card No - 1-562). The crystalline hexagonal phase of $ZnTiO_3$ was identified by a single diffraction peak at the 2 value of 35.25 degrees.

The Scherer's equation was used to get the average particle size.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the particle's size and λ is the X-ray wavelength, θ is the glancing angle, β is the particle's full width at half maximum and K is a constant with a value of 0.89.

The Scherer's equation measured crystallite sizes ranging from 15nm to 17nm displayed in Table 1.

Table 1. Shows the evaluated crystallite size of ZnO-TiO_2 nanocomposites

Percentage of samples	Crystallite Size(nm)
1% ZnO-TiO_2	17
10% ZnO-TiO_2	16
15% ZnO-TiO_2	15
20% ZnO-TiO_2	15

3.2 Morphological Studies

SEM image analysis was used to examine the prepared ZnO-TiO_2's structural investigation. Surface morphology was found to be homogenous, and particle size was discovered to have a web-like structure. The images revealed the particle aggregation. Elemental composition is confirmed by EDAX results. Figure 2 displays the high-magnification SEM images of the ZnO-TiO_2 nanocomposite.

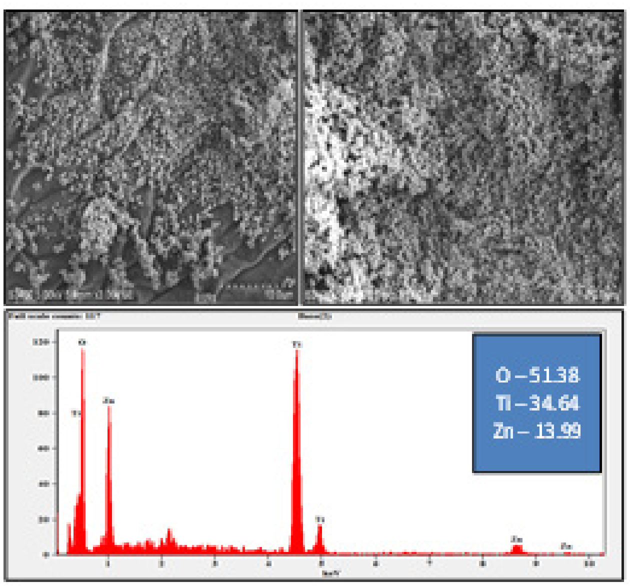

Figure 2. SEM and EDAX graph of 10% ZnO-TiO_2 nanocomposites.

3.3 FTIR Studies

The ZnO-TiO_2 nanocomposite was analyzed using FTIR in the 400 to 4000 cm^{-1} range shown in Figure 3. FTIR analysis revealed distinct TiO_2 signatures at 493.53, 699.11, 1350.11, 2185.93, and 3433.88 cm^{-1}. The IR peaks at 536.71, 692.65, 1590.01, and 3423.16 cm^{-1} represent the characteristic peaks of ZnO[18]. The presence of hydroxyl groups is associated with the band at 3500 cm^{-1}, and H-O-H bending vibration is associated with the band near 1590 cm^{-1} (-OH)

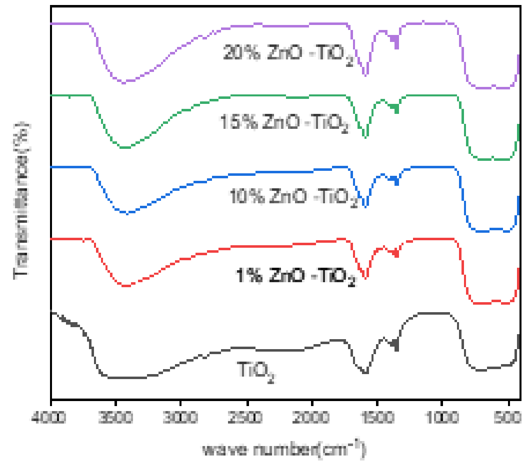

Figure 3. FTIR analysis of ZnO - TiO_2 nano composites.

3.4 Optical Properties

The optical absorption spectra of ZnO-TiO_2 nano-

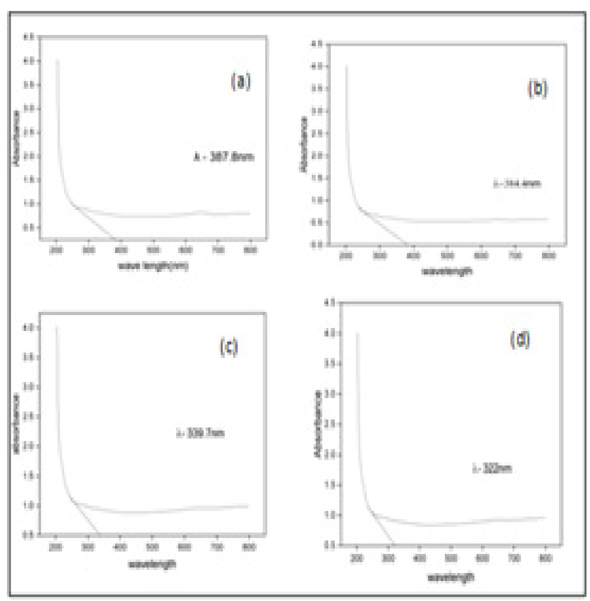

Figure 4. Optical absorbance spectra ZnO-TiO_2 nanocomposites.

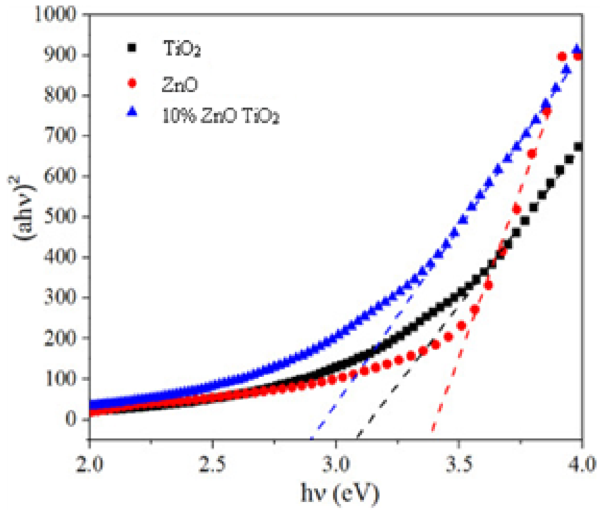

Figure 5. Tauc plot of Zno-TiO_2 nano composites

composites, as shown in Figure 4, demonstrated the relocation of the absorption band into the visible region. The sediment that forms during synthesis reduces absorbance, and discrepancies in absorbance can be explained by taking into account the production of agglomerates in the suspension. Most frequently, $ZnO-TiO_2$ samples exhibit this behavior. The electronic transitions occurring between the states located in the energy gap were attributed to the greatest absorption of wave length, which was discovered to be near 387 nm.

By using the tauc plot method, the optical band gap of the produced nanocomposites containing 10% ZnO and TiO_2 was examined in Figure 5. The 10% Zno-TiO_2 nanocomposites' findings were determined to be 2.9eV, which was lower than TiO_2's (3.2eV) and Zno's (3.9eV). Several oxygen-containing functional groups cause an electronic interaction between the ZnO and TiO_2 nanoparticles, shifting the edge of the valence band upward. This increases the efficiency of light absorption and lowers the bandgap energy of the nanocomposite.

4.0 Antibacterial Assay

The bacteria were exposed to 1%, 10%, 15%, and 20% $ZnO-TiO_2$ nanoparticles after 24 hours of incubation at 37^0 C, and the zone of clearance was manually examined. According to different ZnO weight percentages, the percentages of zone of inhibition for various bacterial strains are displayed in Figure 6 and the observations are outlined in Table 2. It is clear that the percentage of bacterial cell inhibition is quite low at higher concentrations of ZnO of 15% and 20%, respectively, with a

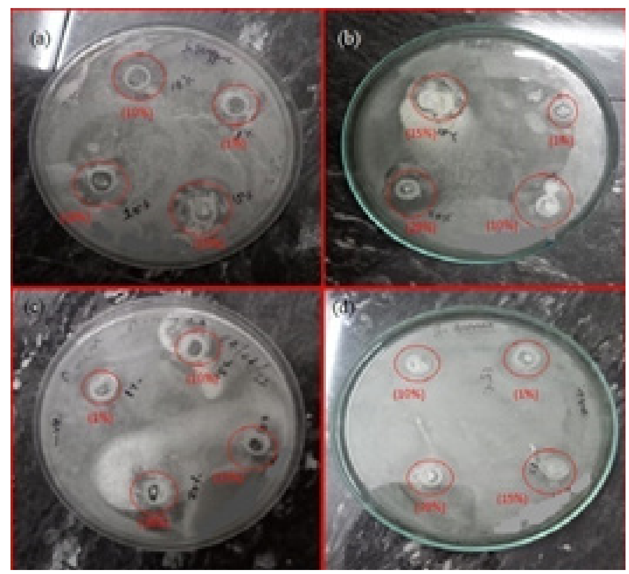

Figure 6. Antibacterial activity of 1%, 10%, 15%, and 20% $ZnO-TiO_2$ nanoparticles with zone of Inhibition of (a) *Staphylococcus aureus* (b) *S. typhi* (c) *Bacillus subtilis* (d) *P. aeruginosa.*

Table 2. Inhibition zone diameter of $ZnO-TiO_2$ nanoparticles against different bacterial pathogens

SI. No.	Name	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*S. typhi*
1	1% $ZnO-TiO_2$	8 mm	8 mm	7 mm	6 mm
2	10% $ZnO-TiO_2$	10 mm	11 mm	12 mm	10 mm
3	15% $ZnO-TiO_2$	6 mm	5 mm	5 mm	5 mm
4	20% $ZnO-TiO_2$	5 mm	5 mm	5 mm	5 mm

minimal inhibition of around 6 mm and 5 mm on all bacterial strains. Additionally, increased cell survival with greater decrease was the result of ZnO (10%) in NPs.

In comparison to 1% and 10% ZnO nano particles shown higher bactericidal activity with a high zone of inhibition of 8 mm, 11 mm against *B. subtilis* and 7 mm, 12 mm for *S. aureus,* 8mm, 10mm for *P. aeruginosa* and 6mm, 10mm for *S. typhi* bacterial strains. Therefore, it is clear after this assay that ZnO-TiO_2 at concentrations of 15% ZnO have improved antibacterial activity against all bacterial strains[19].

5.0 Conclusion

The sol-gel method was used for the synthesis of 1%, 10%, 15%, and 20% ZnO-TiO_2 nanocomposites. SEM images show a web-like structure, and EDAX analysis confirms the presence of Zn, Ti, and O. The FTIR spectrum depicts the typical vibrational modes of Ti-O as well as Zn-O. XRD peaks corresponded to the standard peaks. ZnO-TiO_2 nanocomposites significantly inhibit the growth of various microorganisms, significantly greater antibacterial action against the bacteria Gram-negative *P. aeruginosa* and *S. typhi* as well as Gram-positive *S. aureus* and *B. subtilis*.

6.0 Conflicts of Interest

It is here by the corresponding author declared on behalf of other authors that there is no conflict of interest among the authors

7.0 Acknowledgement

The authors are thankful to the material research center and management of Presidency University, Bangalore.

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