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# ANTIMICROBIOLOGICAL ACTIVITY STUDIES ON THE SYNTHESIZED TiO<sub>2</sub> AND TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-RGO NANOCOMPOSITES BY HYDROTHERMAL METHOD

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**Abstract** – Nano sized materials are plays vital role in biological and pharmaceutical applications. Especially, metal oxide nanoparticles are known to possess antibacterial properties. TiO<sub>2</sub>, a well-known p-type semiconductor and it has high photo reactivity, broad-spectrum antibiosis and chemical stability. These properties have been applied in removing bacteria and harmful organic materials from water and air. In order to enhance the antimicrobial activity of TiO<sub>2</sub>, prepared a new ternary nano composite, TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO by hydrothermal method. These nanoparticles and nanocomposites were characterized by X-ray diffraction (XRD), Scanning electron microscope (SEM) and Fourier Transform infrared spectroscopy (FTIR). The antibacterial effect of these obtained nanoparticles and nano composites was examined on *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 118), and *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 2405). Based on this study, we may conclude that the synthesized nanocomposites were found to be effective against these bacteria.

**Keywords** - TiO<sub>2</sub>, TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO, hydrothermal method, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*

## I. INTRODUCTION

Nanoparticles, because of their small size have distinct properties compared to the bulk, thus offering many new developments in the fields of biosensors,

biomedicine, and bio nanotechnology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders [1].

The rapid adaptation of microorganisms and resistance to a wide range of antibiotics has led to emergence of different infectious diseases. A curative treatment towards these diseases is becoming a difficult and herculean task. Researchers have also been focusing on development of new materials as well as new coating processes on materials suitable for food packaging to protect the food stuff from microbial attack. The development of nanoscience and nanotechnology within the last decades provides opportunities to deal with this problem [2]. Hence developing the new agents to inhibit microbial growth is necessary. The metal oxide nanoparticles exhibit excellent biocidal and biostatic action against gram positive and gram negative bacterial [3]. Recently, metal oxide nanoparticles have been considered as antibacterial and good inhibitor of different bacterial strains due to their selective toxicity to biological systems [4]. Metal oxide nanoparticles are used for a large variety of applications including catalysis, sensors, optoelectronic materials and environmental remediation [5]. Controlled syntheses of metal oxide nanoparticles are essential for several applications and solution phase methods provide a large degree of control over the synthesis products [6,8].

The activities of nanoparticles are directly dependent on the bacterial strain i.e., Gram-positive and Gram-



negative because they have differences in their cell wall. Electrostatic interactions are directly responsible for the attachment of nanoparticles to bacteria. These interactions changes the integrity of cell membranes of bacteria and toxic free radicals is released, which induce oxidative stress on bacteria [7]. Various nanoparticles of metal oxides have been synthesized and are found to be a good inhibitor of different bacterial strains[4-6] such as silver[9], oxides of copper, zinc[10]and gold[11]. Till date, the research in the field of biosynthesis mainly focused Ag Nps [7,699 papers, 59 %], Au Nps followed by ZnO [4,640 papers, 36 %] CuONps [690 papers, 5 %][12]. Although nanoparticles (NPs) of silver, zinc oxide and copper oxide are already used in several antimicrobial applications, those nanomaterials may also pose hazard to other organisms when released to the environment [13]. Therefore, current research work focuses on metal oxides like  $\text{TiO}_2$  and the composites of metals as effective antibacterial agents. Titanium dioxide ( $\text{TiO}_2$ ) is a self-cleaning and self-disinfecting material for surface coating and as photo catalyst in many applications, titanium dioxide has a major role in our environmental purification due to its nontoxicity, photo induced super-hydrophobicity and antifogging effect. And also it used extensively for killing different groups of microorganisms including bacteria, fungi and viruses, because it has high photo reactivity, broad-spectrum antibiosis and chemical stability[14].  $\text{TiO}_2$  has been chosen for the nanoparticle coating on HDPE films since the antimicrobial activity of  $\text{TiO}_2$  nanoparticles is well established [15-16]. These properties have been applied in removing bacteria and harmful organic materials from water and air, as well as in self-cleaning or self-sterilizing surfaces for places such as medical centres[17-18]. The  $\text{TiO}_2$  nanoparticles have been reported to decrease the toxicity of bacteria [19-20]. An earlier report on  $\text{TiO}_2$  suggests that want to improve the antimicrobial activity. In this research work we synthesized a ternary nano composite  $\text{TiO}_2$ - $\text{Fe}_2\text{O}_3$ -rGO by suitable method with the help of haematite ( $\text{Fe}_2\text{O}_3$ ) and reduced Graphene oxide (rGO) for enhancing the antimicrobial activity.

## II. EXPERIMENTAL SECTION

### 2.1 Materials & Methods

All the chemicals were Analytical grade Reagents and were used without any further purification. All reactions were carried out using double distilled water. Graphite powder, high grade hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (30%), hydrochloric acid (HCl), titanium tetra butoxide (TTBO), ferric nitrate (Fe

( $\text{NO}_3$ )<sub>3</sub>.9 $\text{H}_2\text{O}$ ) were procured from Merck chemical Ltd, India.

Nutrient Agar, Peptone, Beef extract, Agar-agar, Nutrient broth, Ethanol, Dimethyl sulphoxide, Neomycin (Broad spectrum antibiotic) were purchased from Himedia, Mumbai, India

**2.2 Test Organisms:** The microbial strains, *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 118) *Staphylococcus aureus* (MTCC 96), *Klebsiellapneumoniae* (MTCC 2405) were collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh.

### 2.3 Synthesis of $\text{TiO}_2$ - $\text{Fe}_2\text{O}_3$ -rGO Ternary Composite

#### 2.3.1 Synthesis of $\text{TiO}_2$ nanorods:

The compound was prepared by taking 1.36 mL of Titanium tetrabutoxide (TTBO) and dissolved in 30 mL of distilled water. Then 30 mL of 30%  $\text{H}_2\text{O}_2$  solution and 3 mL of 37% HCl were added in sequence. The resulting solution was then transferred to a 100 mL Teflon-lined autoclave. The autoclave was sealed and maintained at 150°C for 12h in a program controlled oven. After 12h reaction, the autoclave was allowed to cool till room temperature was attained. The white precipitate was washed with double distilled water and ethanol. Finally, the product was dried in a vacuum oven at 70°C for overnight.

#### 2.3.2 Synthesis of $\text{TiO}_2$ - $\text{Fe}_2\text{O}_3$ -rGO Ternary composite

Initially GO was synthesized by a modified Hummers method [21] 0.01M  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  ethanol solution (50 ml) was slowly added to 0.5 g of  $\text{TiO}_2$  powder containing appropriate amount of GO. The suspension was vigorously stirred for 12 h and the solvent was evaporated at 70°C using a hot plate. The obtained products were washed with ethanol and then the solid powder was dried at 70°C subsequently the sample was calcinated at 300±5°C for 6 h.

#### 2.3.4 Determination of antimicrobial activity

Active cultures were generated by inoculating a loopful of culture in separate 100 ml nutrient/potato dextrose broths and incubating on a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain  $5 \times 10^5$  cfu/ml.

#### 2.3.5 Instrumentation

The prepared samples were characterized using Powder X-ray diffraction (XRD) patterns were

obtained (D8Focus, Bruker instrument, Germany make) with Cu Ka radiation ( $\lambda=1.5406 \text{ \AA}$ ) in the  $2\theta$  range from 10 to 90 with a step size of  $0.02^\circ\text{S}^{-1}$ . The accelerating voltage and applied current was 40 kV

### III. RESULTS & DISCUSSIONS

#### 3.1 XRD

The XRD patterns of  $\text{TiO}_2$  and the ternary composite ( $\text{TiO}_2\text{-Fe}_2\text{O}_3\text{-rGO}$ ) are shown in Fig. 3.1. Fig.3.1(a) shows Pure  $\text{TiO}_2$  diffraction peaks at  $2\theta = 27.4^\circ, 36.1^\circ, 39.2^\circ, 41.2^\circ, 44.0^\circ, 54.3^\circ, 56.6^\circ, 62.8^\circ, 64.1^\circ, 69.1^\circ, 69.9^\circ$  and  $72.5^\circ$  were assigned to the (110), (101), (200), (111), (210), (211), (220), (002), (310), (301), (112), and (311) diffraction planes, respectively (JCPDS, no. 73-1765)[22]. The obtained rutile  $\text{TiO}_2$  crystal phase did not change after coupling with  $\text{Fe}_2\text{O}_3$  and rGO. XRD analysis revealed that, the synthesised composite consist of anatase phase also, it shows peak at  $2\theta$  value of  $25^\circ$  the separate peak for rGO at  $2\theta = 25^\circ$  was not observed, which is presumably due to the presence of the Anatase (101) peak at  $25.3^\circ$ [23,24]. However, the mixed phase titania having the better antimicrobial activity than pure  $\text{TiO}_2$ [25]. Therefore, the ternary composite synthesized here can exhibit well anti-microbial activity.

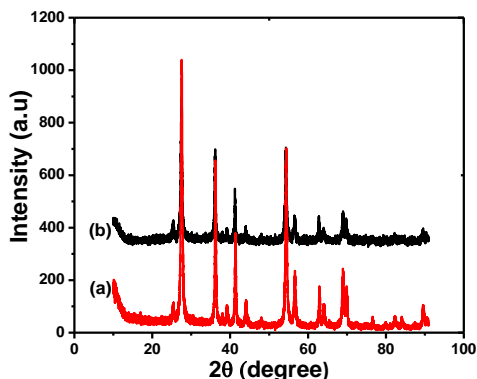


Figure 3.1: XRD images of a)  $\text{TiO}_2$  and b)  $\text{TiO}_2\text{-Fe}_2\text{O}_3\text{-rGO}$

#### 3.2 FTIR

Figure 3.2. (a) and (b) shows the FTIR spectrum of  $\text{TiO}_2$  and  $\text{TiO}_2\text{-Fe}_2\text{O}_3\text{-rGO}$  composite. The hydroxyl groups exhibits stretching frequency at 1250 and broad band at  $3420 \text{ cm}^{-1}$ [26, 27]. The peaks at  $680 \text{ cm}^{-1}$  and  $480 \text{ cm}^{-1}$  assigned to Ti-O-Ti and Fe-O-Fe stretching bands. The FT-IR spectrum of rGO reveals that the C=O and OH group intensity decreases, which indicates the removal of oxygen containing groups through synthesis method. However the rGO still contains oxygen containing

functional groups, which can induce interaction between metal oxide and rGO. A few of functional groups have been disappeared in ternary nano composite due to calcination of precursor material.

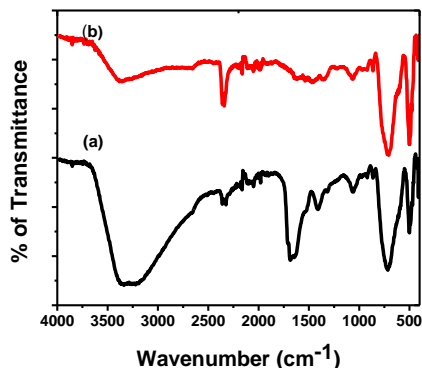


Figure 3.2: FTIR images of a)  $\text{TiO}_2$  and b)  $\text{TiO}_2\text{-Fe}_2\text{O}_3\text{-rGO}$

#### 3.3 SEM

The surface morphologies of pure material  $\text{TiO}_2$  and the ternary composite ( $\text{TiO}_2\text{-Fe}_2\text{O}_3\text{-rGO}$ ) were examined using SEM. Fig. 3.3(a) shows the formation of aggregated  $\text{TiO}_2$  nano rods. Fig. 3.3 (b) shows the surface morphology of the ternary composite. In the case of ternary composite,  $\text{Fe}_2\text{O}_3\text{-TiO}_2$  nano rods were completely embedded with the rGO.

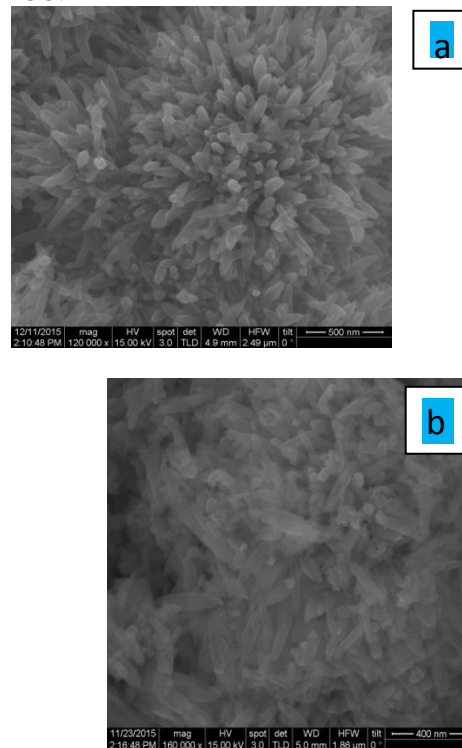


Figure 3.3: SEM images of a)  $\text{TiO}_2$  b)  $\text{TiO}_2\text{-Fe}_2\text{O}_3\text{-rGO}$

### 3.4 Antibacterial Activity

Synthesized nano particles were subjected to antibacterial assay using the agar well diffusion method[28, 29].

Nutrient agar (20 ml) was dispensed into sterile universal bottles, which were then inoculated with 0.2 ml of cultures, mixed gently and poured into sterile Petri dishes. After setting, a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make four uniform wells in each Petri dish. The wells were filled with DMSO containing nanoparticles (1mg/ml) and allowed for diffusion for 45 min. The plates were incubated at 37°C for 24 h for bacteria. Neomycin was included in the positive control and DMSO as negative control. The inhibition zones were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

#### 3.4.1 Antibacterial activity of TiO<sub>2</sub>

The antibacterial property of the TiO<sub>2</sub> was evaluated against two Gram positive and two Gram negative bacterial strains using agar well diffusion method (Figure-3.4.1).

Table-1 shows the effect of TiO<sub>2</sub> on the growth of both Gram positive and Gram negative bacteria. TiO<sub>2</sub> showed significant antibacterial activity on Gram positive bacterial strains.

Of the bacterial strains tested, TiO<sub>2</sub> strongly inhibited the growth of Gram positive bacteria - *Bacillus subtilis* (25 mm) and *Staphylococcus aureus* (23mm) at a concentration of 200 µg. On the other hand, TiO<sub>2</sub> moderately inhibited the growth of Gram negative bacteria- *Escherichia coli* (10mm) at a concentration of 200 µg (Table-1). These nanoparticles did not show any inhibitory effect on the growth of *Klebsiella pneumoniae* (Figure-3.4.1).

Haghi et al., (2012)[30] reported that the synthesized TiO<sub>2</sub> nanoparticles has efficient antibacterial effect against E.coli and can be used as an antibacterial agent for different purposes and nanomaterials are known to inactivate cellular enzymes and DNA by binding to electron-donating groups such as Carboxylates, Amides, Indoles, Hydroxyls, Thiols, and etc. causing cause little pores in bacterial cell walls, leading to increased permeability and finally cell death.

Joost et al. (2015)[31] reported that visualization of bacterial cells under scanning electron microscopy (SEM) showed enlargement of the E.coli cells, distortion of cellular membrane and possible leakage of cytoplasm after 10 min of exposure to photo activated TiO<sub>2</sub>. Antibacterial effects of TiO<sub>2</sub> were also reported against pathogenic bacteria by Ali et al., 2016[32]

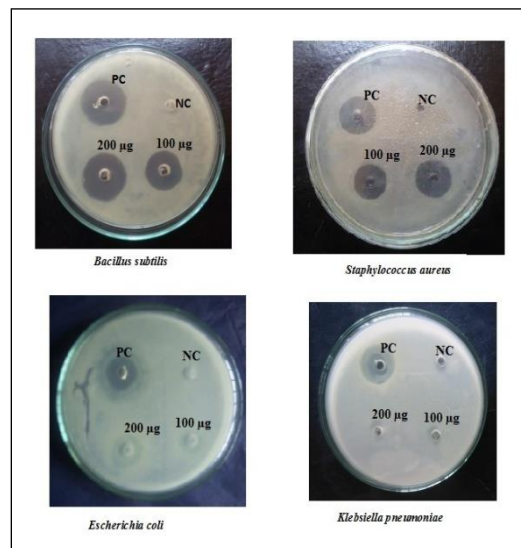


Fig. 3.4.1 Antibacterial activity of TiO<sub>2</sub> nanoparticles PC – Positive control – Neomycin, NC- Negative control – DMSO, 100, 200 µg of TiO<sub>2</sub>

Table-1. Effect of TiO<sub>2</sub> on the growth of bacteria

Name of the bacterial strain	Diameter of zone of inhibition (mm)		
	Compound TiO <sub>2</sub>		Neomycin (20 µg)
	100 µg	200 µg	
<b>Gram positive</b>			
<i>Bacillus subtilis</i>	11	25	27
<i>Staphylococcus aureus</i>	12	23	26
<b>Gram negative</b>			
<i>Escherichia coli</i>	5	10	26
<i>Klebsiella pneumoniae</i>	-	-	26

Bacterial strains were spread on agar plates. Different amounts of TiO<sub>2</sub> (100 µg & 200 µg) were placed in the wells. Controls contained Neomycin (20µg) in place of TiO<sub>2</sub>. The incubation period was 24 h at

37°C. Zone of inhibition was measured as described in standard methods.

Table-2. Effect of TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite on the growth of bacteria

### 3.4.2 Antibacterial activity of TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite

The antibacterial properties of the TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite were evaluated against two Gram positive and two Gram negative bacterial strains using agar well diffusion method (Figure-3.4.2). Table-2 shows the effect of TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO on the growth of both Gram positive and Gram negative bacteria. TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO showed significant antibacterial activity on Gram positive bacterial strains. Of the bacterial strains tested, TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite strongly inhibited the growth of both Gram positive bacteria - *Bacillus subtilis* (25 mm) and *Staphylococcus aureus* (24 mm) at a concentration of 100 µg and Gram negative bacteria - *Escherichia coli* (25mm) and *Klebsiella pneumoniae* (26 mm) at a concentration of 100 µg (Figure-3.4.2).

This is the first report informed by us that the composite TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO synthesized by hydrothermal method exhibited potent antibacterial activity against both Gram positive and Gram negative bacteria. The composite exhibited strong antibacterial activity and is more advantageous than the TiO<sub>2</sub> nanoparticles against bacterial strains tested. Gupta et al. 2013 reported the structural and optical properties and comparative photocatalytic activity of TiO<sub>2</sub> and Ag-doped TiO<sub>2</sub> nanoparticles against both gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria under visible-light irradiation.

Name of the bacterial strain	Diameter of zone of inhibition (mm)		
	TiO <sub>2</sub> -Fe <sub>2</sub> O <sub>3</sub> -rGO composite		Neomycin (20 µg)
	50µg	100 µg	
<b>Gram positive</b>			
<i>Bacillus subtilis</i>	12	25	26
<i>Staphylococcus aureus</i>	11	24	25
<b>Gram negative</b>			
<i>Escherichia coli</i>	12	2	27
<i>Klebsiella pneumoniae</i>		5	
	13	26	26

Bacterial strains were spread on agar plates. Different amounts of TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite (50µg & 100µg) were placed in the wells. Controls contained Neomycin (20µg) in place of TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite. The incubation period was 24 h at 37°C. Zone of inhibition was measured as described in standard methods.

#### IV. CONCLUSION

In the present study, TiO<sub>2</sub> and TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO nanocomposites have been successfully synthesized through hydrothermal method. The synthesized nanoparticles and nanocomposite and their characterizations were investigated. The nanoparticles and nano composite were found to exhibit elevated levels of bactericidal activity when tested for their antibacterial activity. TiO<sub>2</sub> showed less antibacterial activity when compared to its nano composite. This nano composite can be more effective when combined with antibiotics. In the coming days, this nano composite will play a significant role in the area of medical research for the production of effective antibiotic against different antibiotic resistant bacteria.

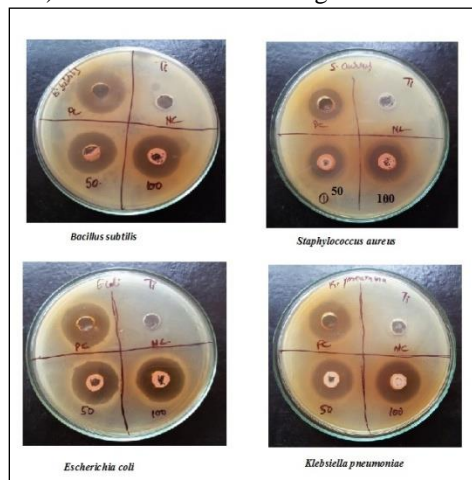


Fig. 3.4.2 Antibacterial activity of TiO<sub>2</sub>-Fe<sub>2</sub>O<sub>3</sub>-rGO composite PC – Positive control – Neomycin, NC- Negative control – DMSO 100, 200 µg of composite



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