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Chemical synthesis, characterization and applications of TiO2/GOx Bioconjugates system.

Usama Ahmed[1] Muhammad Hamza[2] Syed Faizan Ul Hassan[3] Muhammad Hamza Tarteel[4] Sadaf Sultana[5] Areeba Bibi[6]

Department of Medicine, School of Biomedical Engineering, Shenzhen University Medical School, Shenzhen 518060, PR China
Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Pakistan
Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Pakistan
Institute of Animal and Dairy Sciences, Faculty of Animal Husbandry, University of Agriculture, Pakistan
Institute of Biochemistry and Biotechnology, PMAS arid agriculture University, Pakistan
Department of Microbiology, Faculty of Biomedical and Applied Sciences, The University of Haripur
Correspondence: Muhammad Hamza[2] mhamza6297@gmail.com

ABSTRACT

Nanoparticles are particles with properties that differ from their bulk counterparts and have a size between 1 and 100 nm (NPs). Nanoparticles have recently been used in a variety of scientific sectors. NPs are typically created unchecked in huge quantities and in bigger sizes, posing a threat to the environment when released as aggregates. Capping or stabilizing substances are required in the creation of nanoparticles to improve biological functionality, avoid nanoparticle clustering or agglomeration, improve colloidal stability, and inhibit uncontrolled nanoparticle development. The functionality, particle size, shape, magnetic, and optical properties of nanoparticles are all affected by the capping agent used. The bio-conjugate of nanoparticles is critical for biomedical applications such as drug delivery in cancer therapy, hyperthermia, contrast agent magnetic resonance imaging, wound healing, tissue engineering, and antibacterial activity. As a potential capping agent, biocompatible, benign, and biodegradable surfactants are used. By employing a simple chemical process, we were able to successfully synthesis TiO2 nanoparticles and Glucose oxide (GOx) immobilised on TiO2 nanoparticles, which we then analyzed using UV visible spectrophotometer, scanning electron microscopy (SEM), and FTIR techniques. After doping with GOx, TEM revealed a drop in the crystalline size of TiO2 nanoparticles (from 25–30 nm to 15–20 nm). To examine the therapeutic potential of both types of TiO2 nanoparticles and GOx immobilised on TiO2 nanoparticles, antibacterial and antifungal activities were assessed

in vitro. The addition of GOx as a doping agent to TiO2 nanoparticles significantly improves both biological activities. Out of the several Gram-positive and Gram-negative bacteria isolates employed in this study, Klebsiella pneumoniae was shown to be the most vulnerable bacterial strain. Aspergillus flavus has been found to have good fungicidal action. This study reveals that GOx immobilised TiO2 nanoparticles are more effective than undoped TiO2 nanoparticles, as evidenced by several biological experiments. As a result, GOx-doped TiO2 nanoparticles have a greater chance of being used in biomedicine to treat a variety of ailments.

1. INTRODUCTION:

Nanotechnology is the branch of science that uses materials at the nanometer scale. Nanobiotechnology is the rapidly emerging field involving interface between nanotechnology and biology. This is interdisciplinary field having applications in almost all areas of biology. Titanium dioxide (TiO_2) nanoparticles, often called titania, are the most extraordinary nanoparticles among all transition metal oxide nanostructures due to their distinctive behavior. TiO_2 nanoparticles are strong oxidizing agents capable of very high photocatalysis. The three polymorphic forms in which the crystalline structure of TiO_2 nanoparticles exists are rutile, anatase, and brookite (Ali et al., 2018; Ovais et al., 2018)

TiO2 nanoparticles are very cost-efficient and easy to synthesize on laboratory and industrial scale. These nanoparticles have been accepted by the "Food and Drug Administration (FDA)" as the non- toxic, biocompatible, and chemically stable substances. Nanostructures of TiO2 have profound applications in the agriculture and food industry, energy, ceramics, wastewater treatment, cosmetics, medical devices, pharmacy, and theranostics of various diseases. These are excellent sanitizers (Kurade, Waghmode, Xiong, Govindwar, & Jeon, 2019). The significance of TiO_2 nanoparticles lies in the fact that they can solve problems and challenges related to the agricultural, medical, and environmental fields (Rehman et al., 2020).

Nowadays, scientists and engineers have developed different novel ways to develop nanoscale materials to get the advantages from the enhanced qualities such as lighter weight, higher strength, increased and controlled light spectra, greater chemical reactivity, and efficiency as compared to their large-scale counterparts. It is considered that nanotechnology could be the next Industrial Revolution (Kumar, Aadil, Ranjan, & Kumar, 2020). At nanoscale, surface area to volume fraction increases. So, the optical,

mechanical, chemical and catalytic properties of nanoparticles are changed as compared to bulk material which is not on nanoscale. Nanostructures also have broad UV absorbance ranges (Zhang, Yan, Tyagi, & Surampalli, 2011). **Pushpamalini, Keerthana et al. 2021** reported that Titanium dioxide nanoparticles were synthesized by using different leaf extracts such as piper betel, moringa oleifera and *Ocimum tenuiflorum*. X-ray diffraction, scanning electron microscope and Fourier transform-infrared is used to characterized the titanium dioxide nanoparticles. **Anandgaonker, Kulkarni et al. 2019** explained that Biosensor dependent on glucose oxidase is popularized and advertised for high selectivity of GOx. Affectability execution of biosensors can be expanded by joining of nanomaterials with GOx. The nanotubes were stacked by glucose oxidase GOx by cross connecting shaping an anode of amperometry glucose by sensor.

Straight reaction for 1.5 seconds to 1-10mM glucose was seen by created GOx/HTNTs/Ti terminals. The affectability and detection cutoff of biosensors was 1.541 microampere mM-1 cm-2 and 59micromolar individually. It was obvious from results that affectability can be upgraded by interesting 3D construction of GOx /HTNTs-Titanium terminal in-situ creation**.**

Gromada & Fiedurek (1996) investigated that Aspergillus niger was named as the greatest GOxproducer. Various medium components and metabolic inhibitors have an effect on GOx. The enzyme activity was enhanced when (NH4)2HPO4 was used instead of NaNO3 in the medium. The enzyme activity was boosted by 269.6% when magnesium ions were added. In the presence of hematin (1 mM), choline (40 mM), and Tween 80, A. niger yield was enhanced to 31.4-53.9 percent (0.1 percent). The synthesis of enzymes and the time course of growth were also investigated, and intracellular activity of glucose oxidase was raised by 68.3 percent in the presence of sodium orthovanadate (1 mM).

TiO_2 nanoparticles were used in combination with antibiotics including ceftazidime and cefotaxime against multidrug resistant *Pseudomonas aeruginosa*. The samples were isolated from sputum, pus, broncho-alveolar lavage, and endotracheal tract. On exposure to UV light for 1-hour, bactericidal effect was observed at >350 μg/ml. Even the MIC values of nanoparticles were 6 folds higher than the antibiotics. Hence, the combination of antibiotics and TiO_2 nanoparticles improved the antimicrobial activity

(Jesline, John, Narayanan, Vani, & Murugan, 2015).

Cancer is still one of the leading causes of death around the world. The photodynamic therapy (PDT) utilizes nanoparticles, which uses reactive oxygen species (ROS) generation take place via stimulation of light absorbing photosensitizers causing cell damage. PDT is expended in the treatment of various malignancies and abnormal vasculatures. But it is limited due to toxic singlet oxygen production and high photosensitivity of treated patients. In PTT, nanoparticles convert the energy of photon into heat owing to their explicit physicochemical characteristics and generating hyperthermia in tumor tissues. A promising candidate with definite features for the application in PTT of tumors is TiO_2 nanoparticles (Kong et al., 2018).

Glucose oxidase is an oxidoreductase enzyme. It has molecular weight ranges from 130-174 kDa (Kalisz, Hendle, & Schmid, 1997). As enzymes are very specific in nature, β-anomericform of glucose instead of α-anomeric is more suitable substrate for glucose oxidase (Kusaiet al., 1960).

These waste materials in water contain high amounts dye colorants and are also somewhat carcinogenic. Enzyme including are peroxidases, laccase and reductases can also be used for the disintegration of effluents. The horse radish peroxidases are prepared by entrapping it in calcium alginate beads, this process is still under study (Qiu, Zhang, Wang, & Liang, 2007). An antitumor potential of TiO_2 nanoparticles loaded with paclitaxel has been enhanced with the incorporation of chitosan due to its anti-inflammatory and anti-oxidant properties against osteosarcoma (Venkatasubbu, Ramasamy, Ramakrishnan, & Kumar, 2013). Jesline and colleagues demonstrated the antimicrobial activity of TiO_2 nanoparticles against MRSA to evaluate the effect of TiO_2 nanoparticles against the biofilm formation by MRSA using tissue culture plate method. Total 30 isolates were taken and out of them 22 were involved in strong biofilm formation and 2 were weak in the formation of biofilm. The TiO_2 (Jesline, John, Narayanan, Vani, & Murugan, 2015).

3 . MATERIAL AND METHOD

This research work was done from November 2020 to July 2021 in Department of Chemistry, Government College University Lahore, Pakistan. All the chemicals used in this research were purchased from Sigma (USA). Chemicals such as glucose ($C_6H_{12}O_6$), glucose oxidase enzyme, potassium mono hydrogen phosphate(K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), dinitro salicylic acid (DNSA), titania

powder, phosphate buffer, benzoquinone, isopropyl alcohol, glutaraldehyde, tetra iso-prop-oxide, sodium hydroxide (NaOH) used in this research work.

3.1. Glass apparatus

Glassware such as beakers, conical flasks (250 ml), stirrers, funnels, pipettes, measuring cylinders, test tubes and petri dishes was used. Water bath and condenser was also employed.

3.2. Green synthesis of TiO_2 nanoparticles:

Green synthesis of TiO_2 nanoparticles was done by using the dry leaves of *Moringa oleifera*. The aqueous solution of leaf extract was prepared by using the 10 grams of powder of moringa leavesin 100 ml of deionized water and this ALE was boiled at 60 Degree centigrade for 10 minutes to make it free from pathogens.

Figure 3.1: Aqueous Leaf extract formation of Moringa leaves

After that, the aqueous leaf extract was filtered by using Whatman filter paper 1. Titanium dioxidenanoparticles were synthesized by mixing 10 ml of filtered aqueous leaf extract solution in 90 mlof 5milli molar of titanium dioxide powder solution in acidic media in measuring flask at 50 Degree centigrade. The dark brown coloration of the solution confirms the synthesis of Titanium dioxide nanoparticles (Sivaranjani & Philominathan, 2016). The powder of titanium nanoparticles was obtained and characterization was done by SEM, FTIR,particle size analyzer, and UV-Visible spectrophotometer.

3.1. Chemical synthesis of titanium dioxide nanoparticles:

20 ml of tetra iso prop-oxide TTIP solution was put drop by drop into a solution of 10 ml iso propylalcohol and 12 ml of deionized water with constant stirring at about 80 ^{o} C in a

beaker.

0.8 ml of concentrated HNO_3 is added after one hour into the above solution for almost 6 hours with constant stirring at 60 ^{o} C until a highly viscous solution is obtained. This viscous sol gel washeated at 300 ^{o} C for two hours openly (Sharma, *et al*,.2014). After annealing, 2 grams of titanium nanoparticles were taken, grided to powder and the characterization of nanoparticles was done (Sivaranjani & Philominathan, 2016).

Figure 3.4: Open atmosphere annealing of TiO2 at 300 0 C and Prepared TiO_2nanoparticles

3.3. Preparation of potassium phosphate buffer, immobilization of GOx.

100 mml (0.1 M) Potassium phosphate buffer was prepared for the immobilization of glucoseoxidase enzyme. The pH of buffer was maintained at 5

The composition of buffer is given in Table.

Table 1.4: Composition of buffer

Chemicals	Molecular weight	Molarity	Molecular weight in stock g/mol 1000ml.
KH_2PO_4	136.086 g/mol	1.1 M	12.814 gm
K_2HPO_4	174.2 g/mol	0.1 M	1.017 gm

To prepare 0.1M of potassium phosphate buffer of (pH 5), 1.017g of K_2HPO_4 was weighed and 12.814 g of KH_2PO_4 was weighed and these weighed quantities were dissolved in water and then volume was raised up to 1000 ml by maintaining the pH of the buffer.

3.4. Immobilization of glucose oxidase enzyme with TiO_2 nanoparticles.

Solution of 200 micro liters of nanoparticles (30mg/1ml) was prepared. This solution of nanoparticles was dispersed in 100 mml, (0.1M) phosphate buffer of pH 5, containing 0.2volume/volume glutaraldehyde in ultrasonic for 20-25 minutes at room temperature. 1 ml of GOx solution was added to the above solution and it was incubated at 150 RPM for 30 minutes. TiO2 nanoparticles with GOx were collected and washed with phosphate buffer for three times.

Figure 3.5: Washing of Immobilized GOx, and Sonication

3.5. Biological Potential of TiO_2 Nanoparticles and TiO_2/GOx Bioconjugate:

3.5.1. Antibacterial Activity

The antibacterial activity of TiO_2/GOx bioconjugate nanoparticles was tested against a variety of gram-positive and gram-negative bacteria strains. This was accomplished using the agar disc diffusion method, which was modified somewhat from Haq et al. process. Bacillus subtilis, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), and resistant Streptococcus haemoliticus were the Gram-positive bacteria used. Gram-negative bacteria such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and resistant Pneumococcal aeruginosa were employed to determine antibacterial potential. All strains were grown at 25°C on 2% nutritional agar (NA) and then stored in the refrigerator. Then, 100 µl of tryptic soy broth (TSB) was put on agar medium, which was used to cultivate bacterial colonies. In dimethyl sulfoxide (DMSO), a stock solution of 1 mg/mL of nanoparticles was prepared, from which a final concentration of 50 µg/mL was obtained by extracting 50 µl of solution and loading it onto discs on petri plates. The plates were incubated for 24 hours at 37°C. The negative control was DMSO, while the positive controls were Roxithromycin and Cefixime. The inhibitory zones were measured with a vernier caliper.

3.5.2. Antifungal Activity

To investigate antifungal activity of nanoparticles, the agar disc diffusion method was utilized, with minor modifications, as described by Akhtar et al. Fusarium solani, Aspergillus flavus, Aspergillus fumigatus, and Aspergillus niger were the fungal strains used. On sabouraud dextrose agar, all strains were kept in the refrigerator at 4°C (SDA). After cultivating fungal strains on SDA, a 50 µg/mL solution of nanoparticles was put onto discs discovered on petri plates containing medium. The prepared petri plates were incubated for 72 hours at 28 degrees Celsius. The negative and positive controls were DMSO and Clotrimazole, respectively. The inhibitory zones were measured using a Vernier caliper.

4. RESULTS & DISCUSSION:

4.1. Characterization of TiO_2 Nanoparticles and Immobilized GOx.

4.1.1. UV-Visible Analysis:

The color change in the reaction mixture proves the formation of titanium dioxide nanoparticles. The optical activity of nanoparticles is measured by UV-Visible spectrophotometer. The maximum absorption range of nanoparticles lies in

UV-Visible range. TiO_2 nanoparticles synthesized by green and chemical synthesis After 5 hours, the UV-Vis spectrum was taken for the reduction of titanium ions that showed the maximum absorption of light at 360 nm range (Sivaranjani, *et al*,.2016). The analysis of TiO2 nanoparticles was done by UV-Visible spectrophotometer (within the range of 200-800 nm. TiO_2 nanoparticles have the saliant excitation absorption characteristic. The following is the UV-Visible spectra of titanium nanoparticles.

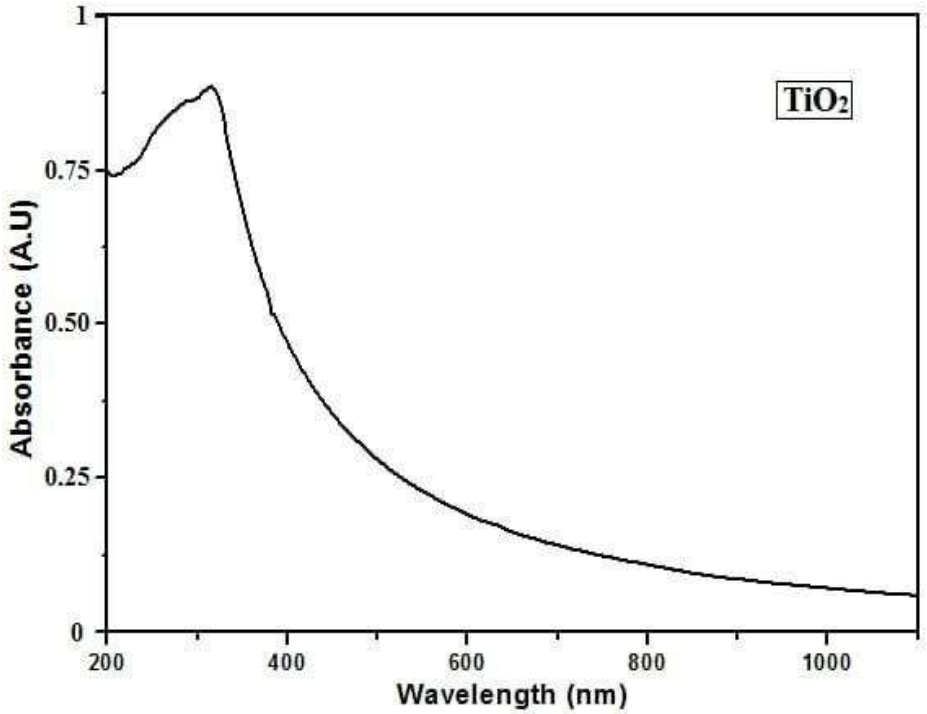
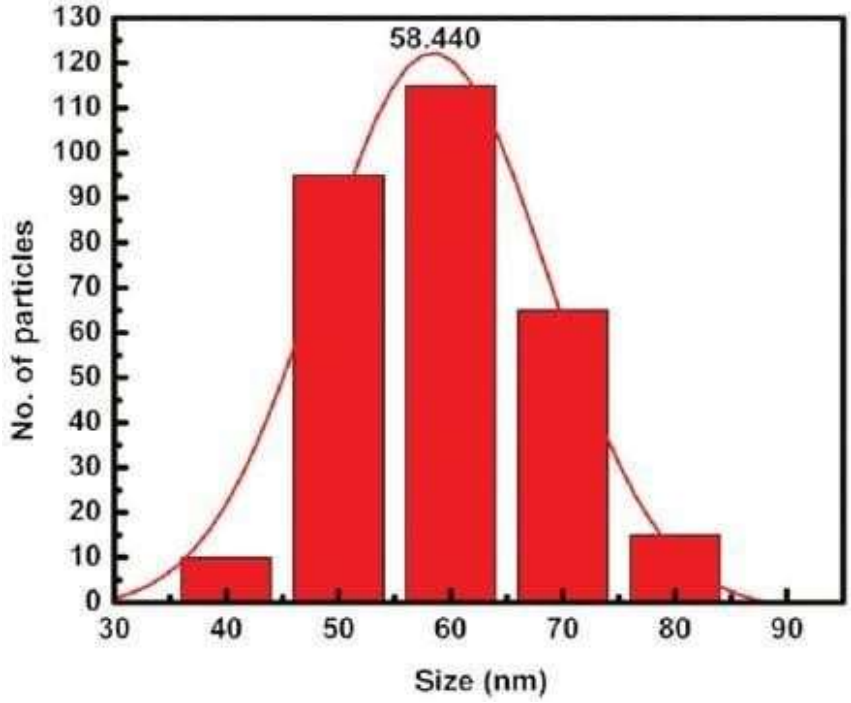


Figure 4.1: UV-Visible spectra of titanium nanoparticles

4.1.2. Particle size analyzer:

The size of TiO_2 nanoparticles is measured by the particle size analyzer after the ultrasonic dispersion of nanoparticles in solvent. The significant time of dispersion for the TiO_2 nanoparticles is 30 minutes. Ethanol and deionized water can be used as a dispersion solvent for TiO_2 nanoparticles.


58.440
No. of particles
Size (nm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
30
40
50
60
70
80
90

Histogram

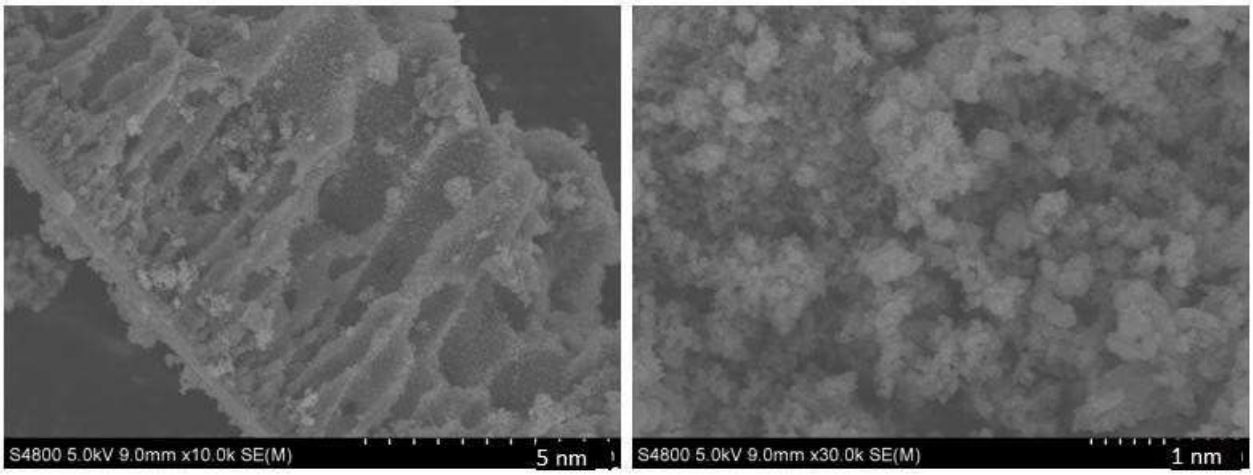


Figure 4.2: SEM 1(A) low resolution image and 1(B) high resolution images of TiO_2nanoparticles

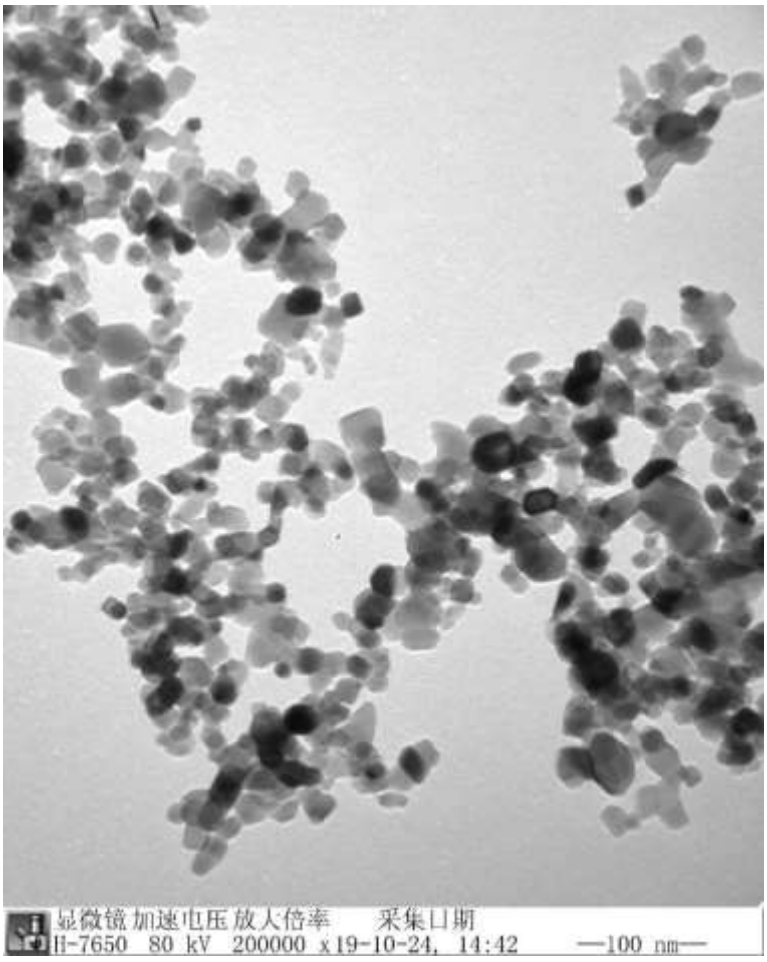


Figure 4.3: TEM image of TiO_2 nanoparticles

4.2. Immobilization of glucose oxidase enzyme with TiO_2-Nps (UV-Visible analysis)

UV-Visible spectrophotometric analysis depicts that the enzyme activity of enzymes increases after the immobilization. The resulting absorbance of free enzyme, the supernatant and the glucose oxidase immobilized on TiO_2 nanoparticles was measured at 540 nm (Kona, Qureshi, & Pai, 2001). The following results depict the enhanced activity of enzymes after the immobilizationof glucose oxidase enzymes.

Table 1.5: Immobilization results

NO	Sample	Enzyme Units (U/ml)
1	Enzyme before immobilization	43.6
2	Enzyme after immobilization	54
3	Supernatant	3.5

4.3. Immobilization of GOx on TiO_2

It was observed from the table, when enzyme was immobilized on nanoparticles. Its activity increases because more active site is available for catalysis as compared to free enzyme. It was observed in (Batool *et al*., 2021) enzyme activity increased after immobilization. When material approaches to nanoscale; the size decreases and surface to volume ratio increases. Due to these remarkable property nanoparticles was chosen as an immobilizing matrix. % Age of immobilization was calculated by using following formula;

$$\%\text{Age of immobilization} = \frac{\text{Activity of GOx initially added- Activity of GOx in supernatant}}{\text{Activity of GOx Initially added}} \times 100$$

90.4%

4.3. FTIR Analysis of GOx/TiO_2 bioconjugate:

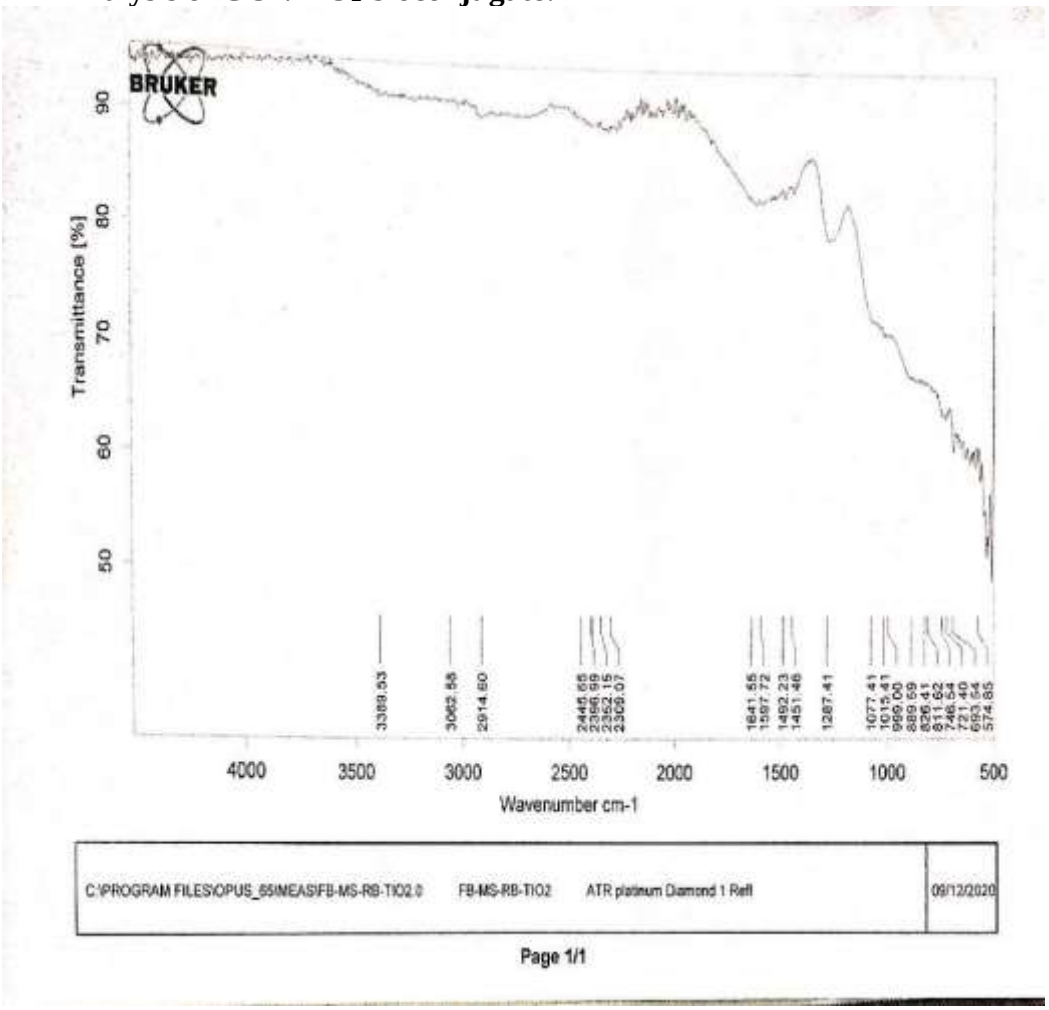


Figure 4.4: FTIR spectra of immobilized GOx.

FTIR analysis was used to assess for glucose oxidase immobilization on nanoparticles. Enzyme onthe surface of nanoparticles showed separate peaks at 1641 cm 1 and 1597 cm 1 (peptidic bond stretching vibrations of -C—O group) and amide-II (N – H in-plane bending and C – N stretchingmodes of the polypeptide chains), respectively.

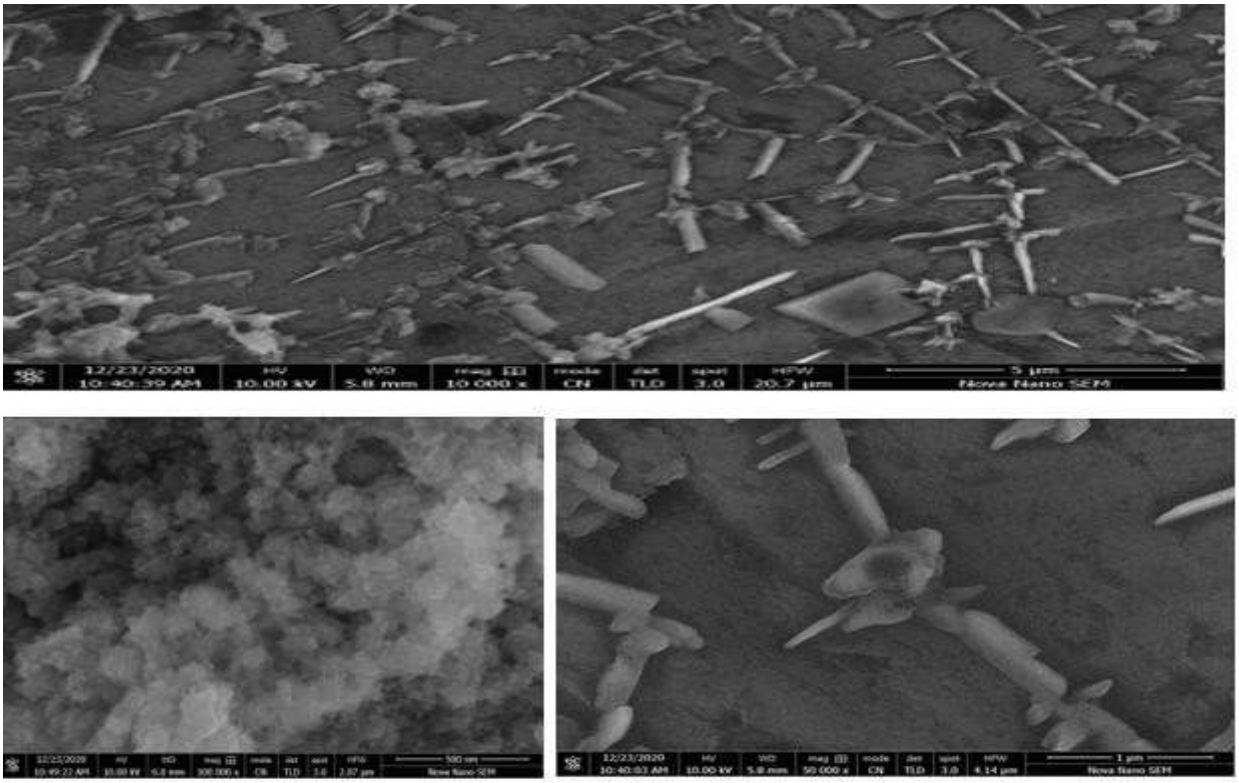


Figure 4.5: SEM images of immobilized GOx with TiO_2.

The morphological changes in nanoparticles after the immobilization of glucose oxidase were seen using scanning electron microscopy images of GOx/ TiO_2 Biconjugate. Other researchers have looked into structural changes in nanoparticles after they've been loaded onto their surfaces.(Kazmi et al., 2019).

4.4. Antibacterial Activity

Antibacterial activity of both bare TiO_2 nanoparticles and immobilized GOx-TiO_2 nanoparticles was investigated and shown to be powerful inhibitors of Gram-positive and Gram-negative bacteria, as shown. TiO_2 nanoparticles have also been shown to be potent antibacterial agents in the literature (Alavi & Karimi, 2018). Because of the varied functional groups present on their surfaces, different strains have different levels of bacterial inhibition (Javed, Ahmed, ul Haq, Nisa, & Zia, 2017). The electrostatic interaction between positively charged nanoparticles and negatively charged bacterial surfaces induces bactericidal activity through the generation of reactive oxygen species (ROS), which causes cell death once bacterial synthesis machinery malfunctions (Caratto et al., 2017). Figure 4.14 depicts the mechanism of TiO_2 nanoparticles' bactericidal action.

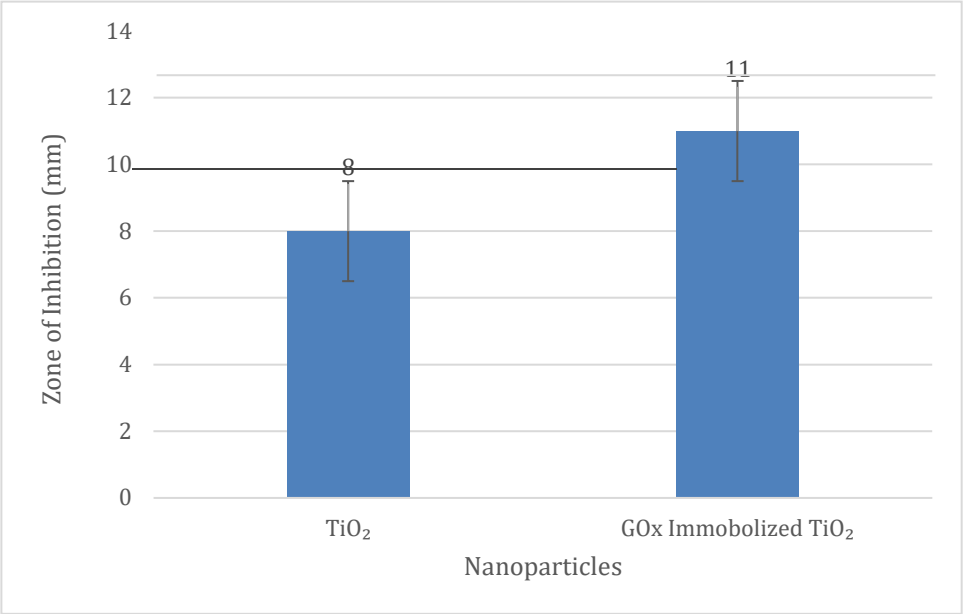


Figure 4.6: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx Immobilized TiO_2 nanoparticles against Gram-positive bacterial strain; Bacillus subtilis

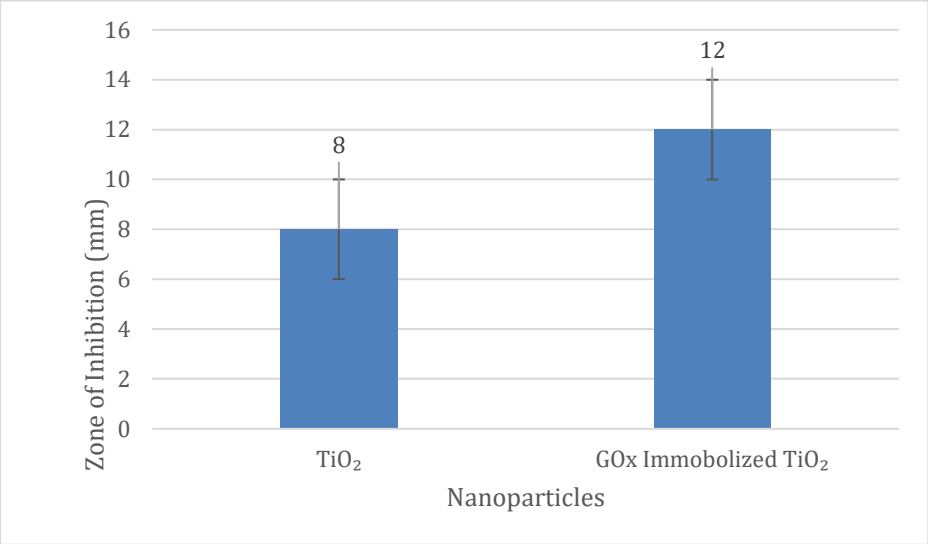


Figure 4.7: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO2 nanoparticles against Gram-positive bacterial strain; Staphylococcus aureus

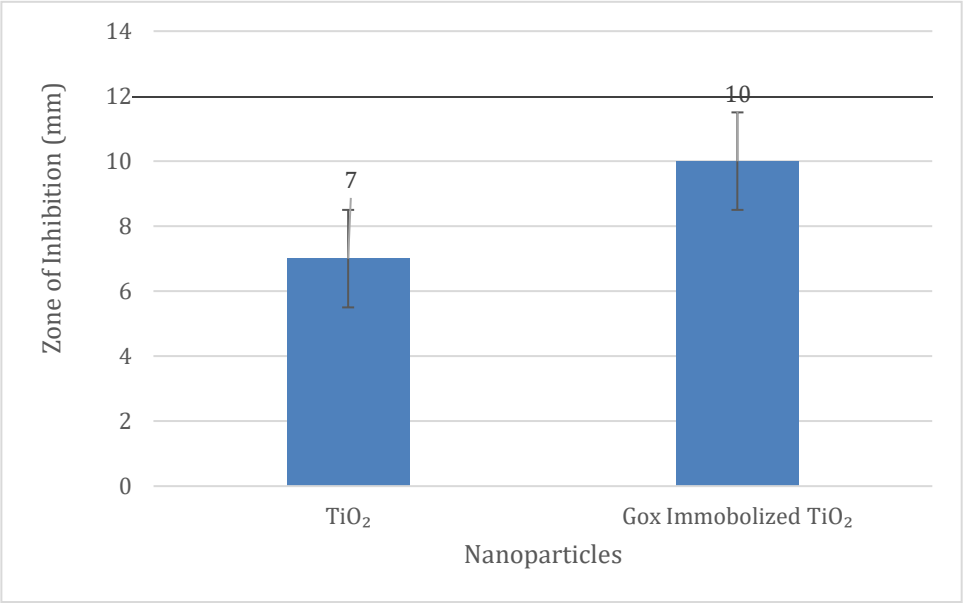


Figure 4.8: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO_2 nanoparticles against Gram-positive bacterial strain; Methicillin resistant Staphylococcus aureus

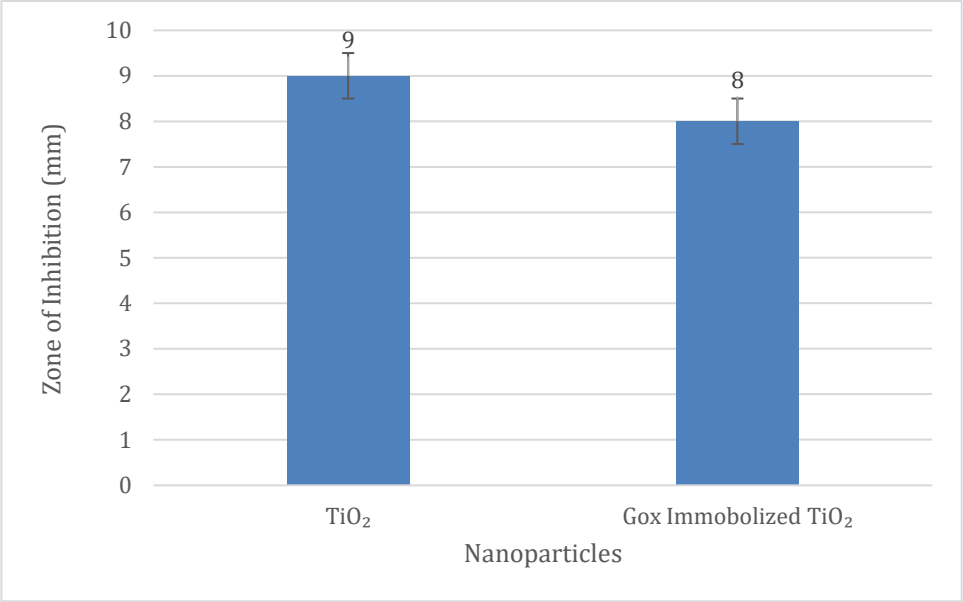


Figure 4.9: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO_2 nanoparticles against Gram-positive bacterial strain; Methicillin resistant Streptococcus Haemoliticus

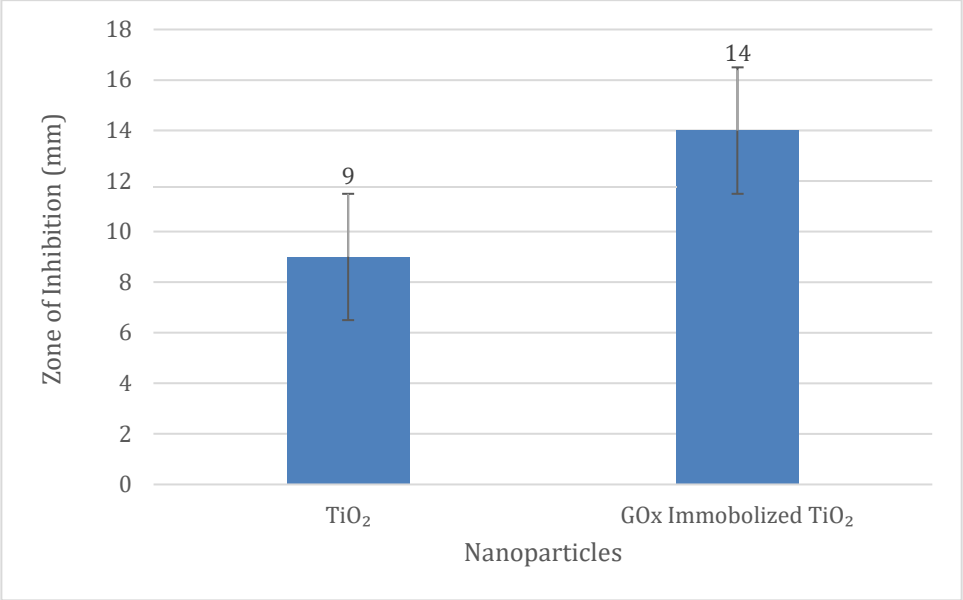


Figure 4.10: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO2 nanoparticles against Gram-negative bacterial strain; Pseudomonas aeruginosa

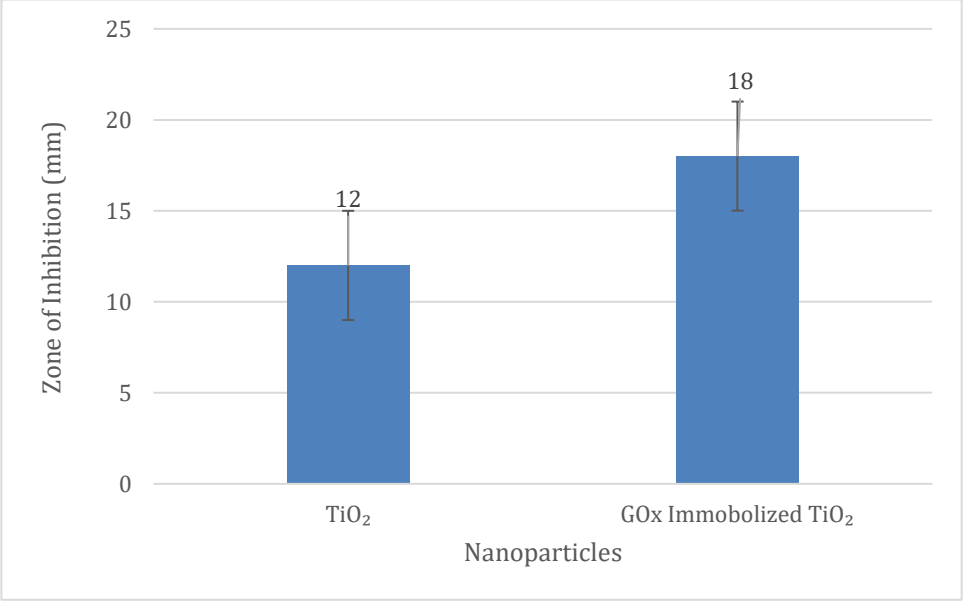


Figure 4.11: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO2 nanoparticles against Gram-negative bacterial strain; Klebsiella Pneumonias

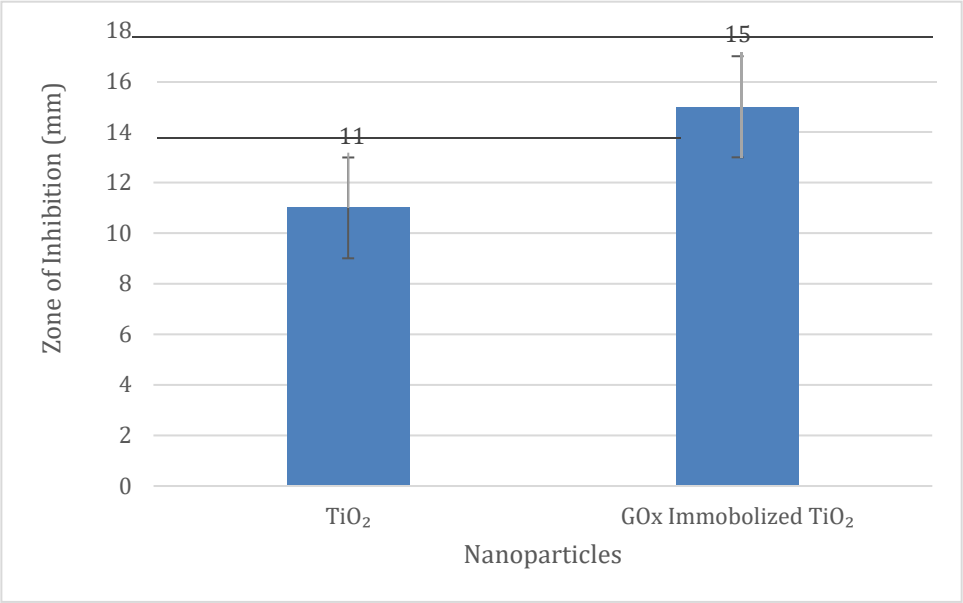


Figure 4.12: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO_2 nanoparticles against Gram-negative bacterial strain; Escherichia Coli

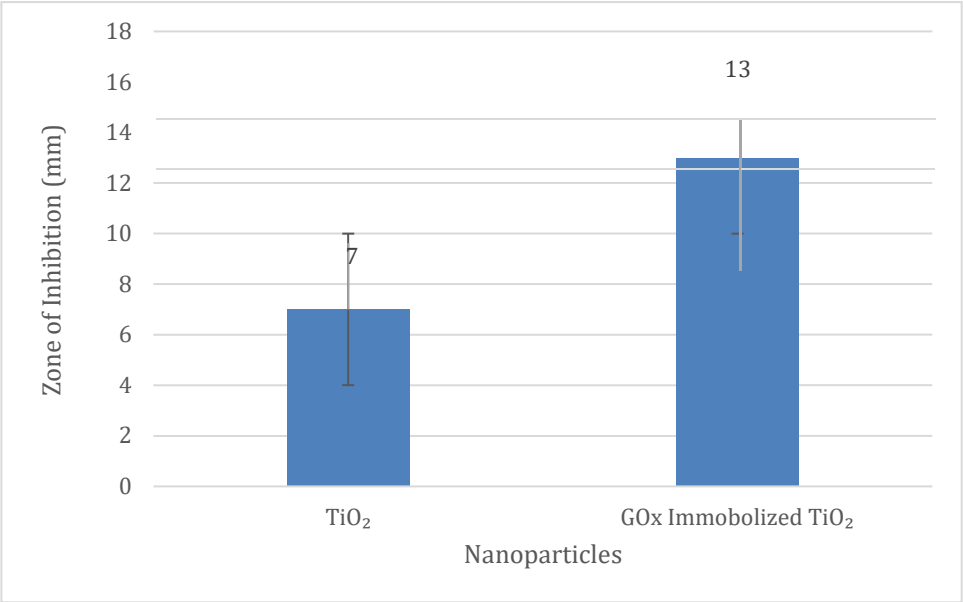


Figure 4.13: Antibacterial activity exhibited by TiO_2 nanoparticles and GOx Immobilized TiO_2 nanoparticles against Gram-negative bacterial strain; Pneumococcal aeruginosa

When compared to Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus, methicillin resistant Staphylococcus aureus (MRSA), and resistant Streptococcus haemoliticus), the antibacterial activity against Gram-negative bacteria (Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and resistant Pneumococcal aeruginos The difference in biological potential between Gram-positive and Gram-negative bacteria may be due to differences in cell wall composition and thickness (Javed, Usman, Tabassum, & Zia, 2016). Furthermore, in the current circumstance, TiO_2 nanoparticles are thought to more efficiently permeate Gram-negative bacterial cell walls via chemical bonding, resulting in oxidative damage from excessive ROS generation and eventual mortality following translocation into the cytoplasm and nucleus. Gram-negative bacteria are therefore more susceptible to TiO_2 nanoparticles than their Gram-positive counterparts. (Ripolles-Avila, Martinez-Garcia, Hascot, & Rodrguez-Jerez, 2019) recently conducted antibacterial investigations on TiO_2 nanoparticles and found no significant differences in their behavior against Gram-positive and Gram-negative bacteria.

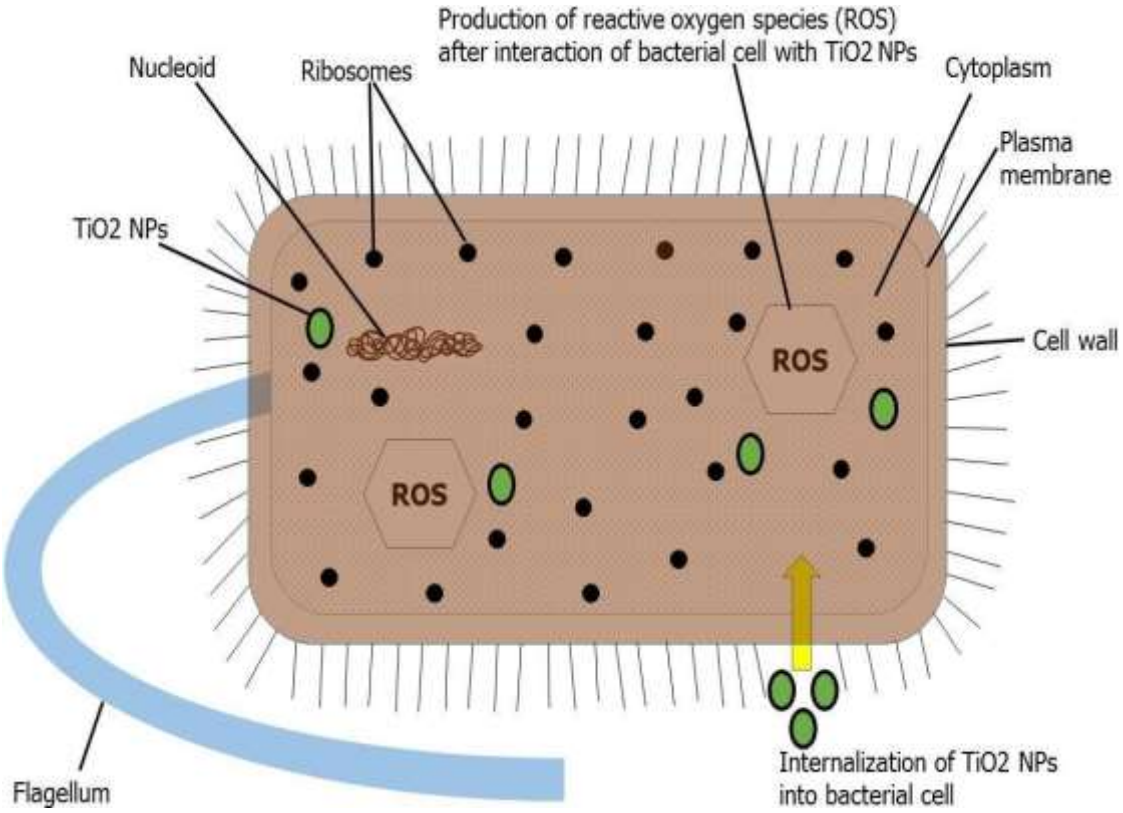


Figure 4.14: Diagrammatic illustration of mechanism of bactericidal accttivity of TiO_2 nanoparticles

In compared to bare TiO_2 nanoparticles, the GOx immobilised on TiO_2 nanoparticles demonstrated a clearer bactericidal action against all bacterial strains, indicating the importance of the doping agent. In this study, GOx immobilized on TiO_2 nanoparticles had the best antibacterial efficacy against Klebsiella pneumoniae (zone of inhibition 17 mm). Doping is thought to generate a reduction in nanoparticle size, a higher surface area, and, as a result, more antibacterial activity (Maheswari, Ponnusamy, Harish, Ganesh, & Hayakawa, 2020). Furthermore, it has been suggested that the synergistic impact of GOx and TiO_2 nanoparticles is responsible for the improved antibacterial activity of GOx immobilized on TiO_2 nanoparticles.

4.5. Antifungal Activity

As shown in Figure 4.15, the TiO_2 nanoparticles tested for antifungal potential against Fusarium solani, Aspergillus flavus, Aspergillus fumigatus, and Aspergillus niger were only mildly effective against Aspergillus flavus (zone of inhibition 8 mm and 12 mm for TiO_2 nanoparticles and GOx Immobilized on TiO_2 nanoparticles, respectively). Our findings back up a prior study that found TiO_2 nanoparticles to be antifungal against Candida albicans (F. Haghighi, Roudbar Mohammadi, Mohammadi, Hosseinkhani, & Shipour, 2013). The mechanism of fungicidal action is unknown. However, it is assumed to involve the production of reactive oxygen species (ROS) and oxidative degradation, which results in cellular death.

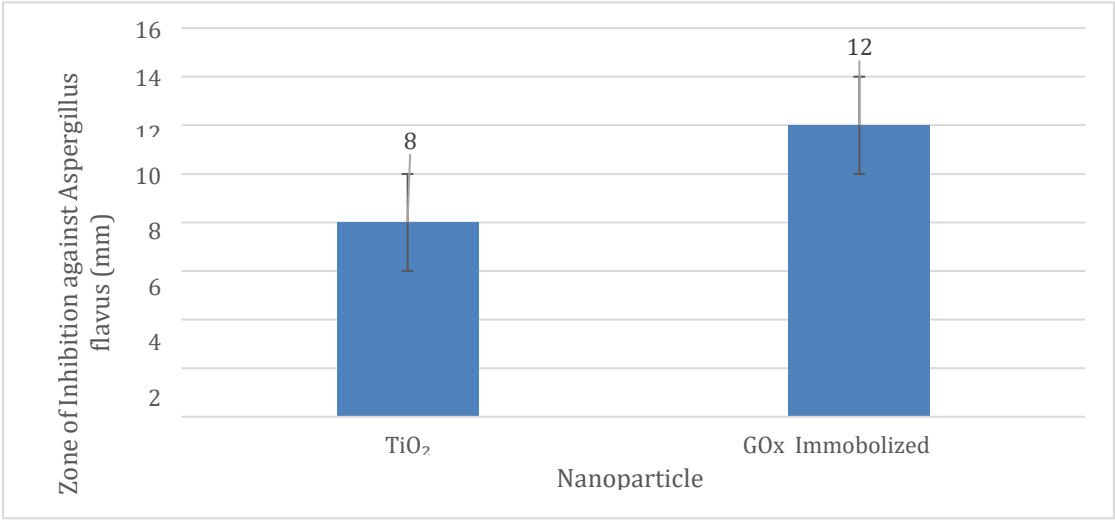


Figure 4.15: Antifungal activity exhibited by TiO_2 nanoparticles and GOx ImmobilizedTiO_2 nanoparticles against Aspergillus flavus

4.6. CONCLUSION

Several studies on the biological application of TiO_2 nanoparticles have been published. However, investigations on the comparative impacts of doping agent/surfactant on the biological nature of these nanoparticles have yet to be published, as far as we are aware. For optimal optimization and customizing of the materials for theranostic biomedical applications, nanoparticles-based theranostic biomedical applications required material at the nanoscale level. The size of nanoparticles used in in vitro applications is determined by the delivery methods. As a result, the goal of this experiment was to look into the broad therapeutic potential of produced undoped and GOx-doped TiO_2 nanoparticles using a variety of in vitro biological activities. The efficiency of GOx-doped TiO_2 nanoparticles was demonstrated in all bioassays, and modest biological activities were achieved in the case of GOx doping. Finally, these nanoparticles are highlighted as potential instruments for treating a variety of human and animal illnesses. However, more rigorous testing of all of these nanoparticles' biological actions on normal human cells is definitely recommended. The mechanisms underlying GOx doped TiO_2 NP's improved therapeutic potential, as well as their metabolic routes, should be investigated further. These researches will aid in identifying the precise material that can be further altered to achieve the desired outcomes. This will pave the path for further molecular level studies, such as gene expression and microRNA, to be conducted in metabolic engineering.

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