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Antimicrobial Activity of TiO_2 NPs against Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923

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Abstract*—Titanium dioxide (TiO_2) nanoparticles (NPs) colloidal that have been synthesized via Nd-YAG laser technique in pure distilled water. The produced NPs were characterized by UV-Vis absorption spectrophotometer and Transmission Electron Microscope (TEM). The surface Plasmon resonance of the colloidal determined by UV-Vis absorption spectra and the absorption peak located at 200nm. TEM exhibits that the size of NPs ranged between 10-80 nm. The antimicrobial activity was tested against Escherichia coli ATCC (gram negative bacteria) and Staphylococcus aureus ATCC 25923 (gram positive bacteria). Prepared TiO_2 NPs exhibited inhibitory activity in both bacteria strains with best selectivity against gram-negative bacteria.

Index Terms*— Antimicrobial activity, TiO_2 NPs, pulsed laser technique.

I. Introduction

Antimicrobial agents like antibacterial, antifungals have been used to inhibit or kill a wide range of microbes such as bacteria and fungi [1, 3] S. aureus is a pathogenic microbe responsible for a wide series of diseases which includes skin infections, surgical site infections, bacteremia and pneumonia [4-7] E. coli is on the preponderant facultative anaerobes in the human gastrointestinal tract. E. coli strains provide health benefit but may be harmful to the host. A pathogenic E. coli can cause diarrheal disease, serious sequelae, urinary tract infection, meningitis, and septicemia [8]. Bacteria cells are surrounded by the plasma membrane, a lipid bilayer which contains opposing monolayers, or leaflets, of phospholipids with the hydrophilic head groups facing the extracellular and intracellular solutions, and the hydrophobic tails facing each other [9-12]. NPs have distinct characteristics that are not available in bulk materials. Uniqueness characteristics of nanoparticles attributed to their structures, size (usually ranging from 1 to 100nm), higher surface-to-volume ratios (increased the located atoms at the grain boundaries) thus they exhibit significantly novel physical, chemical, and biological properties [13-16]. The possibility of NPs permeation inside the bacterial cell membrane due to their potential toxicity mechanism such as diffusion, endocytosis and channel implication so NPs have been used to be an antimicrobial agent against microbes such as bacteria [17-23]. Moreover NPs provided wide range of application in varied fields including solar energy conversion, catalysis, medicine, biotechnology, environmental electronics and optics [24-28]. Semiconductor materials such as TiO_2 NPs (3.2 eV) are produced at a large scale for remediation and destruction of organic pollutants due to their unique physicochemical properties [29-44]. The bactericidal activity of TiO_2 NPs attributed to oxidative stress that generated through their interaction with the cell membrane followed by cell membrane oxidation by Reactive Oxygen Species (ROS) like H_2O_2, which can damage elicit lipids, proteins and DNA [45-73].

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II. Experimental Work

A. Preparation of Colloidal NPs:

Titanium target in the form of square-shaped was fixed at the bottom of glass vessel containing 5 ml of double distilled de-ionized water as shown in Figure 1. Titanium target (97% purity) was focused with Q-switched Nd: YAG laser (pulse duration=10 ns, 1064nm, repetition rate 6 Hz and focal length =10 cm) operating at 280 mJ. Digital weighed was used to weigh the target sample before and after the ablation to determine the concentration of NPs.

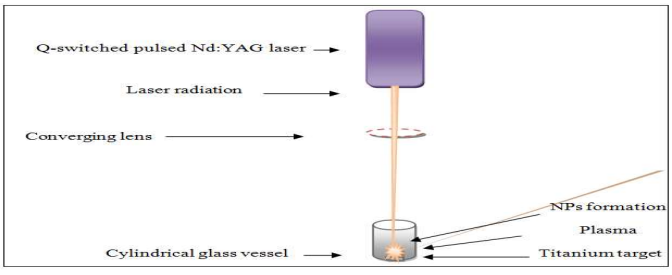

Fig. 1: experimental set up

B. Influence of NPs on E. coli and S. aureus:

TiO_2 NPs was tested for antimicrobial activity by broth dilution method [11] against E. coli and S. aureus. E. coli was cultured in MacConkey agar while S. aureus was cultured and maintenance in mannitol salt agar. Both bacterial strains with 100 µl of 1× 106 cell/ml were inculcated in 5 ml of Muller Hinton broth (MHB) then TiO_2 NPs with concentrations of prepared TiO_2 NPs (19, 37, and 75)µg/ml and standard TiO_2 NPs (100,200,300) were added. All tubes were incubated at 37º C for 24 hrs, bacterial growth was tested by measuring the absorbency at 450nm using UV-1100 spectrophotometers.

The inhibition rate of tested bacteria (%) was expressed as follows:

$$\text{Inhibition rate (\%)} = \left(\frac{\text{Control}-\text{Test}}{\text{Control}}\right) \times 100$$

Control = Bacterial absorbency at 450 nm wavelength.

C. Statistical Analysis:

The data were statistically evaluated using ANOVAI for significance testing $p \leq 0.05$.

III. Results and Discussion.

A. Optical properties:

UV-vis absorption spectrophotometer (Shimatzu SP8001) was used to determine the surface Plasmon resonance of the colloidal. When the laser beam incident on the immersed target, visible cloud was observed at the immersed target surface. When the surface of the metal absorbed the laser radiation, high density plasma was formed followed by generation of shock waves propagated through the liquid and generation cavities bubble with high pressure, subsequently the cavities bubble exploded and NPs diffused in the liquid [12,16]. When laser pulses were enhanced the created particles increased and the solution changed to gray color. UV-vis spectrophotometer showed one peak of absorption spectrum in the ultraviolet region (around 200 nm) as shown in Fig. 2.

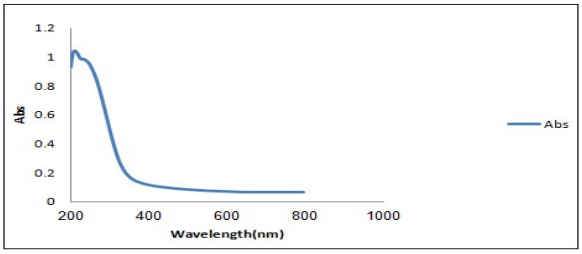

Fig. 2: absorption spectrum of TiO_2 NPs colloidal

B. Transmission Electron Microscopy (TEM):

Particle size distribution and morphology of TiO_2 NPs were characterized by Transmission Electron Microscopy (TEM) type CM10 pw6020, Philips-Germany. Figure 3 (a and b) shows particles shape (spherical shape) and distribution of particle size that ranged between 10 and 80 nm.

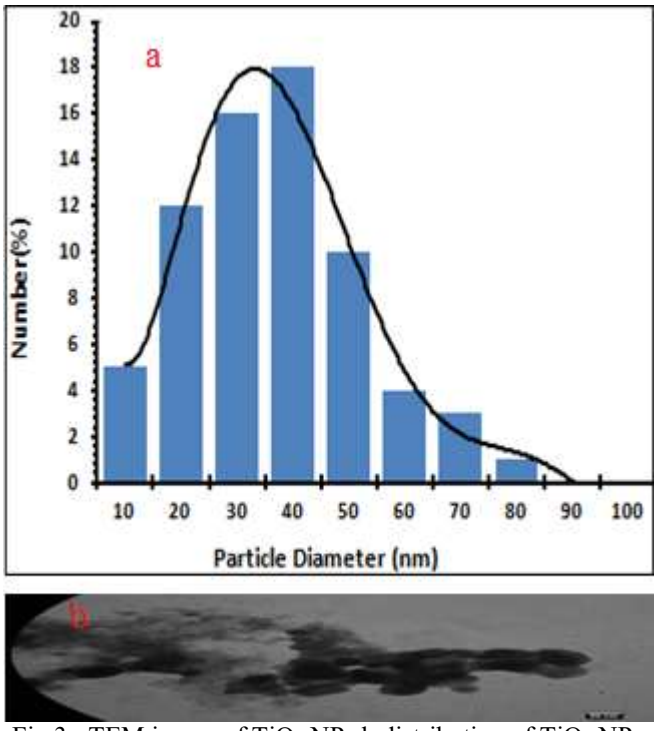

Fig.3: TEM image of TiO_2 NPs b distribution of TiO_2 NPs

C. Antimicrobial activity

The bacterial optical density of bacterial species was measured using UV-Vis spectrophotometer. As shown in Tables 1 and 2, the best suppressing bacterial strains growth was at high concentration of TiO_2 NPs. It is recorded that TiO_2 NPs are effective bactericidal agent for both gram positive and gram negative strains of bacteria but the activity against gram negative bacteria was more than that in the gram positive bacteria as shown in Fig. 4.

Table.1 Absorbency of Escherichia coli treated with different concentrations of standard and prepared TiO_2 NPs at 450 nm

Conc µg/ml	TiO_2 Prepared	Conc µg/ml	TiO_2 Standard
0.0	0.69±0.011	0.0	0.69±0.011
19	0. 46±0.1*	100	0.61±0.02
37	0.31±0.02*	200	0.45±0.02*
75	0.26±0.03*	300	0.41±0.011*

- Each number refers M±SD for three replicates.
- * Significant at ($P \leq 0.05$).

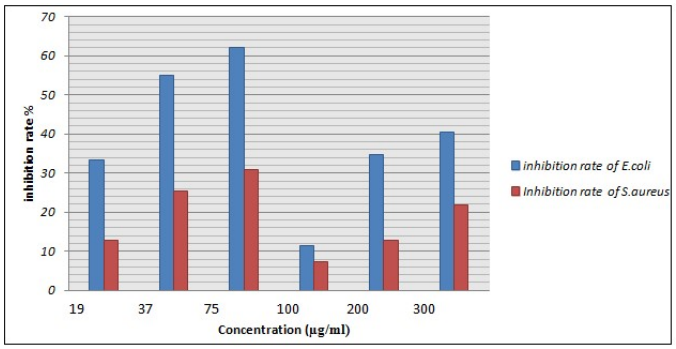

Fig. 4: inhibition rate of tested bacteria after treated with prepared and standard TiO_2NPs

When NPs entering the cell, NPs can produce intracellular H_2O_2. This metabolite naturally produced via Cells. This is the reason why a specific mechanism occurs to counteract the presence of hydrogen peroxide for cell's detoxifying. Catalase is a tetrameric heme-containing enzyme, and is one of the key antioxidant enzymes shows in almost every aerobic organism, catalyzing the breakdown of hydrogen peroxide to water and molecular oxygen to protect cells against the toxic effects of hydrogen peroxide [5].

IV. Conclusion

This report demonstrated that the titanium dioxide NPs can be easily generated by laser ablation technique of immersed metal in liquid. UV-Vis absorption spectrum referred that the surface Plasmon resonance of TiO_2 NPs colloidal located at 200 nm. Antimicrobial activity was performed on various species of bacteria. The findings suggest that TiO_2 NPs have significant antimicrobial activity against E. coli and S. aurerus and more significant effect on the viability of E. coli than S. aurerus therefore TiO_2 NPs is a promising and suitable for biomedical applications.

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