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Synthesis Characterization of TiO_2doped Ferric Oxide Nanoparticles and their Antibacterial Activity

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Abstract:
The Present work outlines the antibacterial activity of TiO_2doped Ferric oxide nanoparticles synthesized through chemical combustion method where ferric nitrate is used as precursor material and urea as fuel with the assistant of Tween 80, a non-ionic surfactant. The obtained Fe_3O_4nanoparticles were characterized by X-ray diffraction, SEM with EDAX. From XRD and it was found to be 43–50 nm. The study aims to determine the antibacterial activity of TiO_2 doped Ferric oxide nanoparticles. The synthesized metal oxide nanoparticles were analyzed for antibacterial activity by disc diffusion method for zone of inhibition and by micro dilution plate method for MIC and showed strong antibacterial activity against bacterial species.

Keywords: TiO_2doped Ferric oxide, antibacterial activity, Escherichia coli, Staphylococcus aureus.

I. INTRODUCTION

'Nanotechnology- there is plenty of space at the bottom', this sentence was never thought to be as powerful as much it could revolutionize the worldwide industries. The world is now turning towards enormous applications of nanotechnology especially with the inorganic materials [1]. Due to its high aspect ratio, nanoparticles are used not just for electrical or optical applications but also for biological utilities [2].Free radicals are generated in human body due to pollutants, radiation and pesticides. These radicals will attack biomolecules such as proteins and lipid and hence these radicals need to be scavenged in order to prevent the deleterious effect in body [3].

1. Materials and methods

1.1 Synthesis of TiO_2 doped Ferric oxidenanoparticles

The synthesis of magnetite (Fe_3O_4) Nanoparticles was done by chemical combustion. The required amount of ferric nitrate (0.1 M) was dissolved in 20 ml of deionized water under the magnetic stirrer for 10 min. The fuel urea and ammonia (0.1 M) were dissolved separately in 30 ml of distilled water respectively. The surfactant TWEEN80 (0.07 M) was dissolved in 20 ml of distilled water and was kept under stirring for 10 min separately. Fuel solution was mixed with oxidizer solution which was under stirring followed by mixing of surfactant solution. The whole solution was kept under stirring for 15 min for stirring. The solution was placed on a hot plate to initiate the reaction.When the temperature had started to increase, the solution boiled and fumes gushed forth from the solution; as the temperature increased above 100 ^{0}C, the solution started to evaporated leading to an increase in the viscosity of the liquid and smouldering started eventually self-ignition took place forming the final product (Fe_3O_4). The powder was collected from the beaker and calcinated for 1 h at 400 ^{0}C [4].

1.2 Synthesis of TiO_2 doped Ferric oxidenanoparticles

Stoichiometric ratio of synthesized magnetite (Fe_3O_4) Nanoparticles was dissolved in 20ml of ethanol sonicated for 20 minutes to form solution A. Then Solution B was formed by dissolving pre calculated amount of Titanium iso Propoxide in 10 ml of deionized water, 10 ml of ethanol and 1ml of acetone.
The Solution B is transferred to Solution A under vigorous stirring, sonicated for 10 minutes then stirred at 70 ^{0}Cfor 4 hours. Precipitate was separated by Magnet, washed with deionized water and ethanol. Dried at 80 ^{0}C for 6 hours and calcined at 400 ^{0}C for 2 hours. The nanoparticles were collected for characterization and antibacterial application.

1.3 Characterization of prepared samples

TiO_2 doped Fe_3O_4nanoparticles were characterized by XRD to find out the phase formation. X-ray diffraction patterns were obtained from Rigaku Ultima IV X-ray Diffract meter with CuKα radiation and diffraction angle range 2θ= 10º to 70º operating at45 kV and 30 mA. To study the size and morphology of the product, SEMwas performed on a Vega 3 Tescan scanning electron microscope.

1.4 Antibacterial studies

Antibacterial activity assessment of TiO_2 doped Fe_3O_4 nano particles were done using agar well diffusion method as per CLSI guidelines[5] for Zone of Inhibition (ZOI) and MIC. The lawn of bacterial inoculum was spread uniformly on a sterile Muller Hinton Agar plates and wells were made using sterile borer. In each well, 100 µl of TiO_2 doped Fe_3O_4nanoparticles with 1000,500 and 250 µg/mlwas added separately and the plates were incubated at 37 ºC for 24h, after which ZOI was measured and readings were taken in triplicate against the standard antibiotic *i.e.,* ciprofloxacin. For determination of antibacterial activity by Minium Inhibitory Concentration, a100µl of sterile CAMHB broth was added to all 96 wells except first column three wells of the microtitre plate $A_1B_1C_1$ to which only 200µl TiO_2 doped Fe_3O_4nanoparticles were added. From first three wells (A_1B1C_1) of plate, 100µl of the TiO_2 doped

Fe_3O_4nanoparticles were double diluted till $A_{12}B_{12}C_{12}$. Then 10µl bacterial suspension of approximately 10^{-6}CFU/ml was added to each dilutions. A growth control (bacterial cell suspension + 100µl broth medium) from G_1 to G_{12} and broth control (only broth medium 100µl) from H_1 to H_{12} were also done on plate. The positive control consists of the 0.1%ciprofloxacin was also placed in the plate $D_1E_1F_1$ to $D_{12}E_{12}F_{12}$. The plates of gram –ve *Escherichia coli* and gram +ve *Staphylococcus aureus* were then incubated at 37° C for 24hours. After incubation, 10µl of working solution of resazurin was added to all wells. The plates were wrapped with aluminum film and incubated at 37°C for 1hour.The change in color was then assessed visually i.e., from purple to pink or colorless was recorded as positive (growth). The lowest concentration at which there is no colour change occurred was taken as the MIC value.

2. Result and discussions

2.1 Characterization

TiO_2 doped Fe_3O_4nanoparticles from x-ray diffraction(XRD) confirms crystalline structure with particle size 43-50nm.The XRD pattern of TiO_2 doped Fe_3O_4nanoparticles is shown in Figure

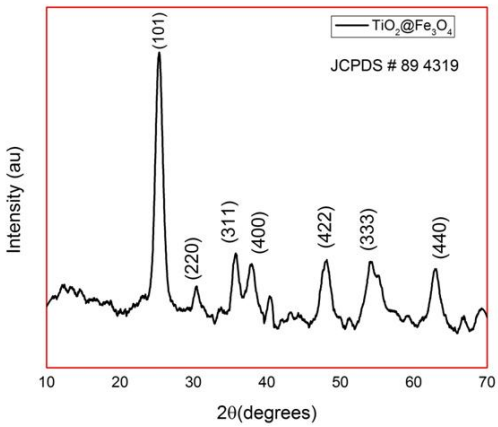

Figure.1. the X-ray Diffraction pattern of TiO_2 doped Fe_3O_4nanoparticles

The structural characterization was performed using SEM. The nanoparticles were readily identifiable, the particle dimension increased, size distribution was wide and nanoparticles were homogenously distributed. The SEM pattern of TiO_2 doped Fe_3O_4nanoparticlesare shown in Figure 2. The EDAX confirms the presence of Iron and Titanium in the synthesized TiO_2 doped Fe_3O_4nanoparticles shown in Figure 3.

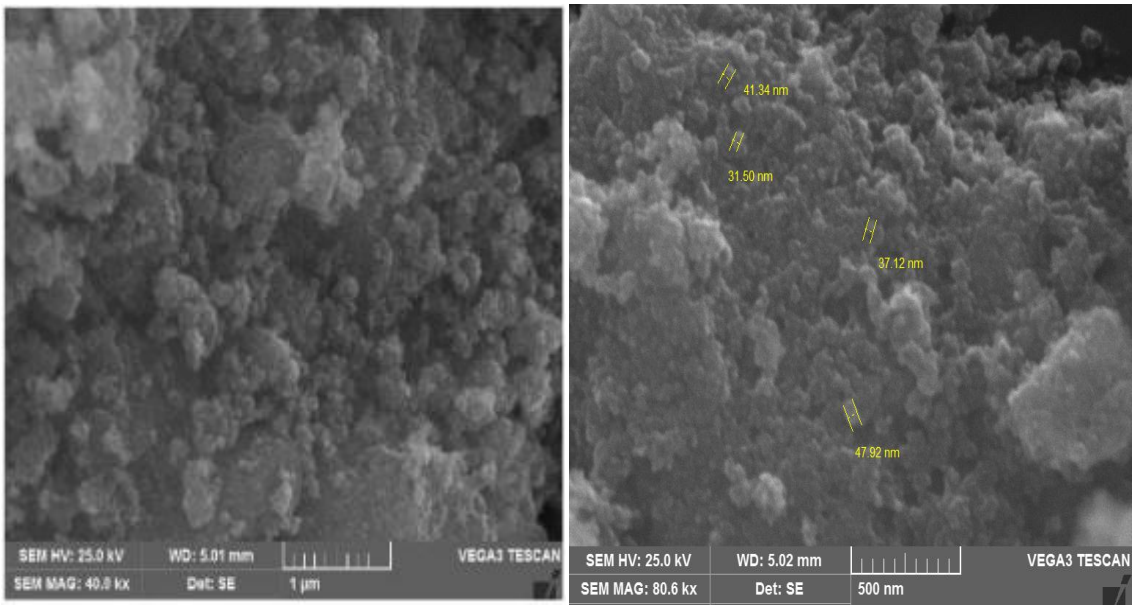

Figure.2. The SEM image of TiO_2 doped Fe_3O_4nanoparticles.

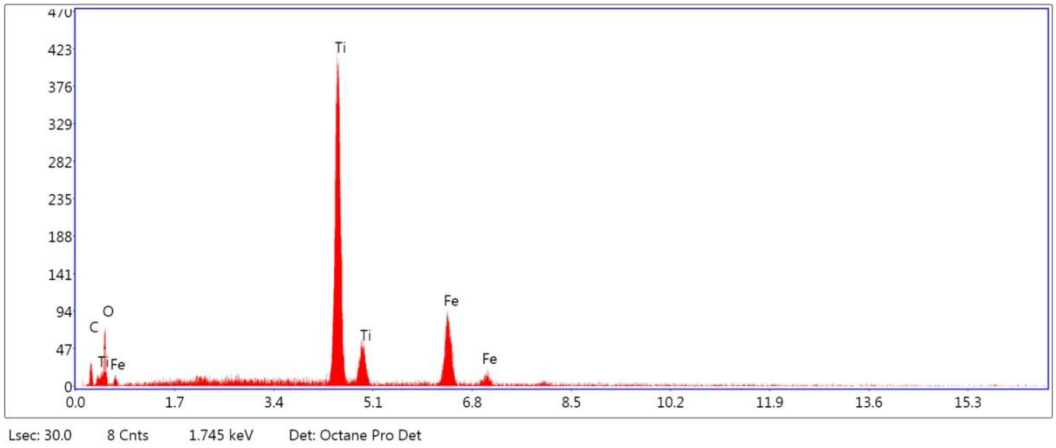

Figure.3. EDAX image of TiO_2 doped Fe_3O_4nanoparticles

2.2 Antibacterial studies

The *E. coli and S. aureus*cells were exposed to TiO_2 doped Fe_3O_4nanoparticlesfor antibacterial activity by ZOI method [6-9]and the results are as shown in Table 1 with inhibition from TiO_2 doped Fe_3O_4nanoparticlesagainst *S. aureus*as shown in Figure 4.

Table.1. ZOI antibacterial activity of TiO_2 doped Fe_3O_4nanoparticles

Sample Name	Conc. (µg)	ZOI# (mm)	
		E. coli	***S. aureus***
TiO_2 doped Fe_3O_4nanoparticles	1000	10	13
	500	5	9
	250	-	3
Ciprofloxacin	1000	36	38

#The zone of inhibition is exclusive of zone of well diameter

Figure.4. ZOI for standard and TiO_2 doped Fe_3O_4 nanoparticles against*S. aureus*bacterium

The Minimum Inhibitory Concentration [10-12] readings were taken from concentration ranging from 1000µg to 0.488 µg for TiO_2 doped Fe_3O_4nanoparticles. MIC of TiO_2 doped Fe_3O_4nanoparticleswere found to be 250 µg against *E.coli* whereas 125 µg for *S. aureus.*

Table.2. MIC antibacterial activity of TiO_2 doped Fe_3O_4nanoparticles

Sample Name	Conc. (µg/ml)	MIC (µg or µl/0.1ml)*	
		E. coli	***S. aureus***
TiO_2 doped Fe_3O_4nanoparticles	1000-0.488	250	125
Ciprofloxacin	01	<0.488	<0.488

* MIC value is expressed as mean of triplicate, $n = 3$

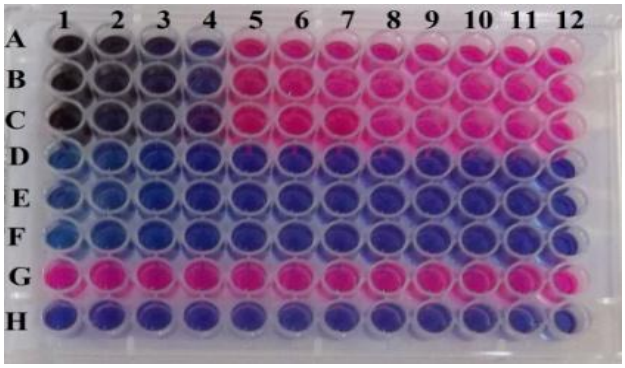

Figure.5. MIC for standard and TiO_2 doped Fe_3O_4 nanoparticles against*S. aureus*bacterium

3. CONCLUSIONS

We have demonstrated that TiO_2 doped Fe_3O_4 nano particles showed a significant antibacterial activity against gram negative *E. coli* and gram positive *S. aureus*organisms as the concentrations increased (i.e., 250,500 and 1000 µg) and its prevalent that TiO_2 doped Fe_3O_4 nanoparticleshad better antibacterial activity from both ZOI and MIC test methods.

4. ACKNOWLEDGEMENT:T

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