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Synthesis, Characterization and Antimicrobial Activity of Ce Doped TiO_2 Nanoparticles

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A B S T R A C T

Titanium dioxide (TiO_2) and cerium (Ce) doped TiO_2 nanoparticles were prepared by the sol-gel method using titanium tetraisopropoxide and cerium (III) nitrate hexahydrate as the precursor materials of TiO_2 and Ce, respectively. The bond configuration and anatase phase of the TiO_2 nanoparticles were analyzed using Fourier transform infrared (FTIR) spectrum in the wavenumber range from 400 to 3800 cm^{-1}. The antimicrobial activity and zone of inhibition of pure and Ce doped TiO2 nanoparticles on *E. coli* strain were investigated. Doped TiO2 nanoparticles showed elevated levels of antimicrobial activity than pure TiO2 nanoparticles. From these comparative studies, we have found that cerium doped TiO2 nanoparticles possess high bactericidal activity which was confirmed by Colony Forming Unit (CFU) with and without TiO2 nanoparticles inoculated on LB agar. This inhibition was further confirmed by Kirby-Bauer method and growth curve studies.

Introduction

The rapid adaptation of bacteria 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. Hence the development of new effective antimicrobials to combat these diseases is essential. Nanotechnology is the engineering of functional systems at the molecular scale. The concepts that seeded nanotechnology were first discussed in 1959 by Richard Feynman. Due to the electronic confinement of nano objects, their optical, chemical, magnetic and antimicrobial properties are different from those of larger objects. The key molecules in biology such as DNA, enzymes, receptors, antigens, antibodies, and oxygen carriers can be included in the dimension of nanometres. Molecular self organization around nanoparticle utilizing the tools of surface science can be of use in the fundamental life processes. In the present era, nanoparticles are found to be a boon for the biomedical research as they possess elevated levels of

bactericidal activity in treatment of infectious diseases. Hence developing the new agents to inhibit microbial growth is necessary. Metal and metal oxides show potential antimicrobial activity. Metal oxide nanomaterials can be easily prepared by the cost effective sol-gel method (Vishwas *et al*., 2014; Vishwas *et al*., 2011; Vishwas *et al*., 2010; Vishwas *et al*., 2012) at room temperature. The TiO2 nanoparticles can be effectively used to decrease the toxicity of bacteria (Kiran Gupta *et al*., 2013; Razi Ahmad and Meryam Sardar, 2013; Thomas Verdier *et al*., 2014). Combination therapy with metal nanoparticles has proven to increase the activity of the antibiotics. Bacterial resistance to antibiotics is a major risk factor in the present society. It has lead to the development of such effective antimicrobials which are human and also animal friendly. This has indeed lead to the dissemination of resistant strains of bacteria. Particle size was also an essential parameter which determined the antimicrobial effectiveness of the metal nanoparticles. TiO2 has three forms: Anatase, rutile and brookite. Among these, anatase is highly unstable, small, isolated and sharply developed crystals and also more commonly occurring modification of TiO2 as reported by Vishwas *et al*., 2009. We have preferred the anatase form due to its excellent optical, photo catalytic and antimicrobial properties from size quantization.

Materials and Methods

Titanium tetraisopropoxide and ethanol were taken in the volume ratio of 1:7 and continuously stirred in a 100 ml beaker for 1h using a magnetic stirrer maintaining temperature of 70^{o}C. Then 10 ml of deionized water was added drop wise for complete hydrolysis. The solution changed from colorless to white precipitate. The precipitate was filtered using Whatman filter paper and cleaned and dried as reported previously by Vishwas *et al*., 2014. Ce doped TiO_2 nanoparticles were prepared by dissolving 3 wt. % cerium (III) nitrate hexahydrate [$Ce(NO_3)_3.6H_2O$] (Spectrochem Pvt. Ltd., Mumbai, 99.50%) in TiO_2 sol with continuous stirring and repeated the same procedure of preparation of TiO_2 nanoparticles. Ce doped and un-doped TiO_2 nanoparticles were annealed at 500^{o}C for 3 h in air and subjected to infrared spectral characterization and antibacterial activity.

Eosin methylene blue agar, Luria agar and Luria broth, Pure TiO2, Cerium doped TiO2, and all other reagents used are of analytical grade.

Preparation of Stock Solution

Stock solution of TiO2 nanoparticles (both pure and doped) with concentration of 1mg/ml was prepared and suspended in distilled water. The above solution was sonicated for 5 minutes to get a homogeneous suspension and then kept under UV rays for 30 minutes for the activation of nanoparticles. In each experiment, fresh stock solution (sonicated and UV activated) was prepared.

Kirby-Bauer Test

The antibacterial effect of TiO2 nanoparticles was performed for comparing the inhibition on *E.coli* by cerium doped and pure TiO2 nanoparticles. A loopful of *E.coli* culture was added to 5 ml of Luria broth and incubated for 3 h. About 25 ml of sterile Luria agar was poured into 3 sterile petriplates and allowed to solidify. By spread plate technique, 100 µl of inoculum was added on the agar media. Two 10 mm diameter wells were cut in the agar media. In one well, 50 µl of TiO2 nanoparticles solution was added and in another well, streptomycin was added as control. The

plate was incubated at 37ºC for 16 h and observed for zone of inhibition.

Colony Forming Unit (CFU)

Six petriplates with different concentrations of 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1.0 mg/ml and one petriplate without TiO2 nanoparticles was taken as control. 100 µl of 10^{-5} serially diluted culture was spread on the agar media and incubated for 18-24 hours. Growth was observed and colonies were counted.

Effect of TiO2 in Liquid Media

Seven 100ml conical flasks were taken and 60ml of Luria broth was added and autoclaved. To the conical flasks, 0.03mg/ml, 0.06mg/ml, 0.13mg/ml, 0.25mg/ml, 0.50mg/ml, 1.0mg/ml concentrations of TiO2 was added. One flask without TiO2 was taken as control. 100µl of inoculum was added and incubated at 37ºC for 18 hours. Optical Density reading was taken at 600nm.

Effect of TiO_2 in Solid Media

Luria agar was poured into petriplates in duplicates and allowed to solidify. 100 µl of test culture was spread on the surface of agar media. Using cork borer, six wells of 10 mm diameter were cut and 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml concentrations of TiO2 was added. One well without TiO2 was taken as control and then incubated at 37ºC for 18 h. Zone of inhibition was measured and tabulated.

Growth Curve Studies

Six 250 ml conical flasks were taken. 100 ml Luria broth was added to the flasks and autoclaved. 100 µl of freshly grown *E.coli* culture was added. 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml, 1000 µg/ml concentrations of TiO2 were added. One flask without TiO2 was taken as control. Flasks were incubated at 37ºC and Optical Density at 600 nm was read every hour till 8 h.

Results and Discussion

The structural characterization was performed using XRD and found to be anatase phase of TiO_2 after annealing at 500^{o}C in air and the crystallite sizes were approximately equal to 15 nm [9]. Nanomaterials exhibit strong inhibiting effect towards a broadened spectrum of bacterial strains. The inhibitory activity of TiO_2 is due to the photocatalytic generation of strong oxidizing power when illuminated with UV light at wavelength of less than 385 nm for 30 minutes. TiO_2 particles catalyze the killing of bacteria on illumination in UV light. Generation of active free hydroxyl radicals by photo excited TiO_2 particles is responsible for the antibacterial activity. Doped TiO_2 nanoparticles are more inhibitory when compared with the pure TiO_2 nanoparticles. Doping increases the activity, since the empty sites are filled with cerium.

Fig.1 shows the FT-IR spectra of TiO_2 and Ce-doped TiO_2 nanoparticles annealed at 500^{o}C in air. A broad absorption band was observed between 3800 to 3000 cm^{-1} which is related to stretching hydroxyl (O-H) group, representing the water as moisture. A broad absorption band from 2960 to 2800 cm^{-1} is due to C-H stretching vibrations.

The peak at 1626 cm^{-1} were indicated to stretching vibrations of C=O which formed from TTIP and ethanol. The peak between 830 and 420 cm^{-1} is associated with the Ti-O stretching bands and is attributed to anatase phase of TiO_2. It is clear that the sharp peak

at 480 cm^{-1} has been shifted to 413 cm^{-1} with the doping of Ce.

Kirby-Bauer test is depicted in Fig.2, where the zone of inhibition in TiO2 doped with cerium was found to be 15-18 mm with a concentration of 1 mg/ml. For comparison, TiO2 was not added in another well which was taken as control.

Fig. 3 shows the Colony Forming Unit, as the concentration of metal oxides was increased; the colony growth was considerably decreased. In 1 mg/ml concentration of cerium doped TiO2 nanoparticles, just 38 colonies were observed but in 0.2 mg/ml concentration 214 colonies were observed whereas for pure TiO2 nanoparticles, 120 colonies were observed at concentration of 1.0 mg/ml and 290 colonies observed for 0.2 mg/ml concentration.

Fig.4 depicts the effect of TiO2 in liquid media that is the TiO2 nanoparticles decreased the turbidity of bacteria with increase in concentration of TiO2 nanoparticles. More the concentration of TiO2 in the liquid medium, less the turbidity.

From Fig.5, it is evident that both cerium doped TiO2 and undoped TiO2 have bactericidal activity. Comparatively pure TiO2 has shown more bactericidal activity.

Fig.6 a shows the growth curve studies of cerium doped TiO_2 nanoparticles. Concentration of 1 mg/ml showed the highest inhibitory activity which is depicted through the above graph. The elevated levels of bactericidal activity can be observed through the graph.

Fig.6 **b** shows the bactericidal activity of pure TiO2 nanoparticles which is described at different time intervals. Here too increase in bactericidal activity can be observed clearly.

Fig.7 shows the effect of TiO2 in solid media, as the concentration of TiO_2 nanoparticles was increased, the zone of inhibition was also found to increase but not as much as the antibiotic streptomycin which was used as the control.

Table.1 Colony Forming Unit with Different Concentrations of Doped and Un-doped TiO_2 Nanoparticles

Number of colonies counted in petriplates with different concentrations		
Concentration of Titanium dioxide in mg/ml	**Cerium doped Titanium dioxide**	**Pure TiO_2**
Control	253	312
0.2	214	290
0.4	163	254
0.6	115	203
0.8	82	176
1.0	38	120

Table.2 Growth Curve Studies with Different Concentrations of Doped and Un-doped TiO_2 Nanoparticles

O.D at 600nm						
Concentration in µg/ml	1st hour		2nd hour		3rd hour	
	Pure TiO_2	Doped TiO_2	Pure TiO_2	Doped TiO_2	Pure TiO_2	Doped TiO_2
200µg	0.19	0.03	0.20	0.07	0.22	0.13
400 µg	0.16	0.02	0.17	0.06	0.21	0.10
600 µg	0.12	0.03	0.16	0.05	0.18	0.09
800 µg	0.11	0.02	0.16	0.04	0.17	0.07
1000 µg	0.07	0.01	0.10	0.02	0.15	0.07
O.D at 600nm						
Concentration in µg/ml	4th hour		5th hour		6th hour	
	Pure TiO_2	Doped TiO_2	Pure TiO_2	Doped TiO_2	Pure TiO_2	Doped TiO_2
200µg	0.25	0.15	0.28	0.18	0.29	0.20
400 µg	0.24	0.13	0.27	0.14	0.28	0.15
600 µg	0.21	0.10	0.23	0.11	0.23	0.13
800 µg	0.19	0.09	0.20	0.10	0.21	0.12
1000 µg	0.16	0.06	0.17	0.07	0.17	0.08

Fig.1 FTIR Spectra of (a) Un-doped and (b) Ce-doped TiO_2 Nanoparticles

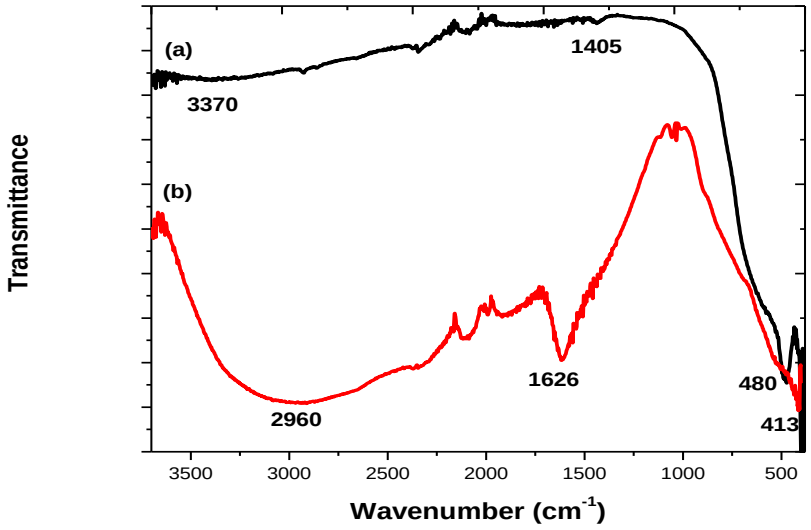


Fig.2 Kirby-Bauer Test of Ce-doped (A) and Un-doped (B)TiO_2 Nanoparticles

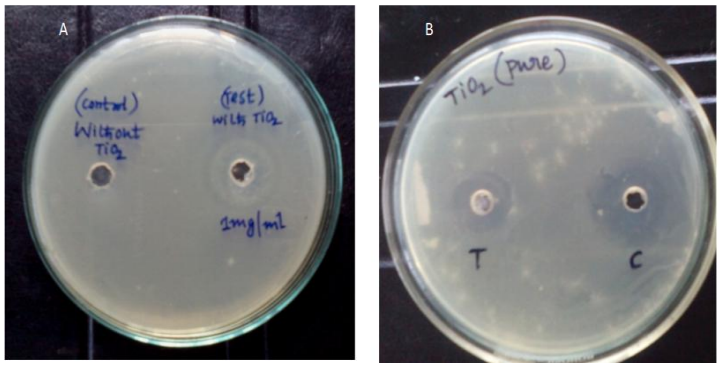


Fig.3 Colony Forming Unit of Ce-doped (A) and Un-doped (B)TiO_2 Nanoparticles

Fig.4 Effect of TiO_2 in Liquid Media of Ce-doped (A) and Un-doped (B)TiO_2 Nanoparticles

Fig.5 Comparative Antibacterial Activity of Doped and Undoped TiO_2 Nanoparticles in Liquid Media

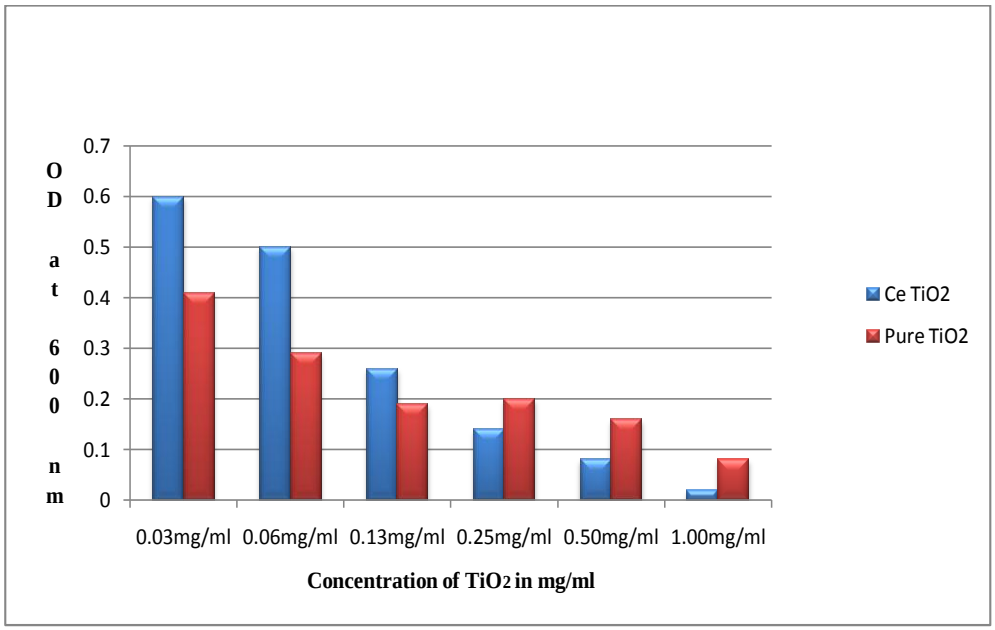
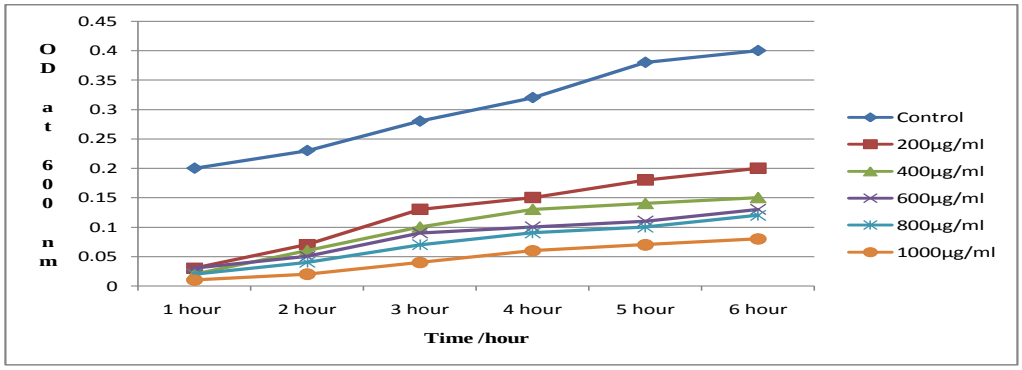


Fig.6 Growth Curve Study of *E.coli* in the Presence of (a) Ce-doped TiO_2 Nanoparticles (b) Un-doped TiO_2 Nanoparticles



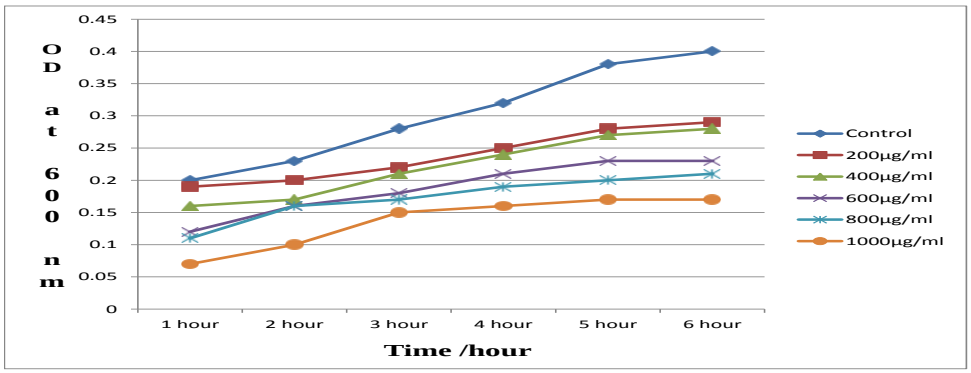
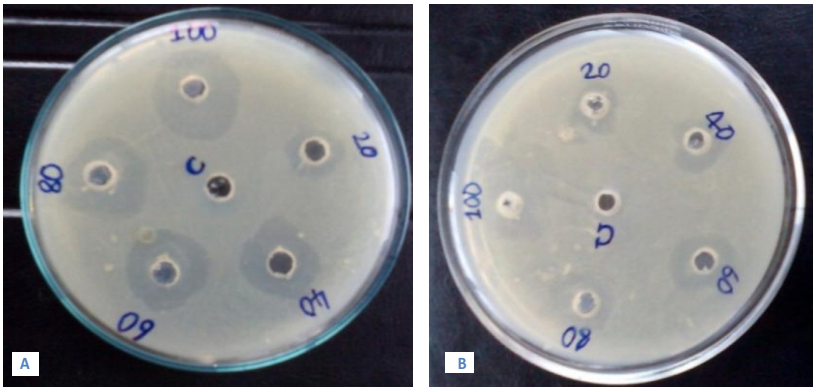


Fig.7 Effect of TiO_2 in Solid Media of Ce-doped (A) and Un-doped (B)TiO_2 Nanoparticles



In conclusion, From the above comparative studies, Ce-TiO2 nanoparticles found to show elevated levels of bactericidal activity when performed on different relevant methods of testing antibacterial activity. The nanoparticles can be more effective when combined with antibiotics. In the coming days, TiO_2 nanoparticles will play a significant role in the area of medical research for the production of effective antibiotics against different antibiotic resistant bacteria and it is the need of the hour.

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