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Research Article

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Characterization and antibacterial activity of Titanium Dioxide nanoparticles

Kaushita Banerjee and Padma Thiagarajan*

School of Biosciences and Technology, VIT University, Vellore, India

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ABSTRACT

Metal nano particles have an enormous potential with several applications in which they involve with great selectivity and high efficiency. In this context, titanium dioxide nanoparticles belong to a new generation of such particles, due to their brilliant and interesting optical, dielectric, and photo-catalytic characteristics. Hence they are now being widely used in electronic devices, cosmetics, pharmaceutics and other products. Extensive usage and applications has also lead to the indiscriminate dumping of their related products, after use, into the environment. Incremental amounts may also add up in the environment as "by products" and wastage during the synthesis of their commercial products. In this study, the characterization of commercially available titanium dioxide nanoparticles has been carried out by X-ray diffraction analysis and UV-VIS spectroscopy. Their toxicity with respect to certain bacteria, both MTCC cultures and also those isolated from environmental samples has been studied by suspending these particles in water and in acidic solution.

Keywords: Metal nanoparticles, Titanium di oxide nanoparticles, Analytical characterization, antibacterial activity

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INTRODUCTION

Nano particles are finding increasing applications in industries [1, 2] and during their life cycle process, may inevitably enter into natural ecosystems. This is true especially for entry into the soil which acts as a substantial sink to these particles [3]. Studies evaluating the impact of metal nano particles on microbes have been carried out. They have found these particles to be potentially toxic [4, 5]. Thus, the effect of engineered nanoparticles on ecosystems may affect either the population in general or a particular community. However, several reports involving the effect of metal nanoparticle toxicity on soil bacteria are inconclusive. The diversified results vary from their being non-toxic [6] and physiologically important [7] to considerably toxic [8], with several mechanism being proposed for their toxicity [9]. Due to accelerated developments in the field of nanotechnology, determining the potential toxicity of these nanoparticles on human health and on the environment is considered to be important. Titanium dioxide, less than a hundred nm in diameter, have become a new generation of advanced particles due to their novel and interesting optical, dielectric and photo-catalytic properties [10]. They are being used in many products such as additives, white pigments, food colorants, sunscreen, cosmetic creams [11] gas sensors [12] and solar cells [13].

In this study, the characterization studies of titanium dioxide nanoparticles using X-ray diffractometry and UV spectroscopy have been reported. The effect of these particles on several microorganisms, both standard cultures and those isolated from environmental samples have also been studied and recorded.

EXPERIMENTAL SECTION

Chemicals and media: Pure rutile Titanium dioxide nanoparticles and other fine chemicals were purchased from Sisco Research Laboratories Pvt. Ltd. Media chemicals were from Hi Media.

Microbial cultures: MTCC bacterial cultures were procured from Institute of Microbial Technology, Chandigarh, India. The test organisms used were 4300 **-** *Micrococcus luteus,* 3160 - *Staphylococcus aureus,* 441 - *Bacillus subtilis*, 8076 -*Pseudomonas aeroginosa*, 9493 - *Proteus mirabilis*, 7407 - *Klebsiella pneumoniae,* 443 - *Eshcherichia coli,* 3231 -*Salmonella typhimurium,* 111 - *Enterobacter aerogenes*

Environmental samples: Micro-organisms were isolated from three sources, *viz.*, soil, marine source and sewage by following standard procedures.

Characterizations of Titanium dioxide nanoparticles

a. UV analysis: The absorption spectra of the TiO_2 nanoparticles were measured by UV/Vis spectrophotometer equipped (Perkin Elmer Lambda 35) with an integrating sphere. The spectra were recorded at room temperature in the range of 250-450 nm.

b. XRD Analysis: X-ray powder diffraction (XRD) experiments on TiO_2 nanoparticles were conducted using Shimadzu 27000S spectrometer with at 40 kV, 40 mA using CuKα radiation. Scans were made in the 2θ range 10-80° with a scan rate of 1°/min in wide-angle diffraction.

Antibacterial activity of TiO_2 nanoparticles against MTCC cultures and environmental samples

The antibacterial activities of TiO_2 nanoparticles against nine MTCC organisms and the organisms isolated from environmental samples, were tested by the well diffusion method [14].The cultures were inoculated in nutrient broth at 37˚C for 24 hours, and swabbed onto Muller Hinton Agar (MHA) plates. A concentration of 1% TiO_2 in water was prepared and used to check for the zone of inhibition on standard bacterial cultures. Wells were punched onto the MHA plates using a sterile well borer and the sample was added to the wells. Water was used as the control. The plates were incubated at 37˚C for 24 hours and were checked for zones of inhibition. The activities against the standard cultures were also tested, following the same procedure, with 1 % TiO_2 prepared in dilute sulphuric acid based acidic water by checking for the zone of inhibition. Methicillin, rifampin, ciprofloxin, tetracycline and bacitracin discs were used as positive controls and the diluted acid were used as controls.

RESULTS AND DISCUSSION

Metal oxide nanoparticles are known to have antimicrobial activities and numerous other biomedical applications [1,2]. In this context, reports have shown TiO_2 nanoparticles to possess excellent photocatalytic activity [10]. It has also been reported that these particles check the multiplication and reproduction of certain bacteria by decomposing the cell membrane and its components. There has been increasing interest in utilizing photocatalytic properties of TiO_2 for disinfection of surfaces, air and water and controlling water pollution and in waste water treatment [15]. However, TiO_2 is an insoluble powder which is itself not dissolved during the degradation of complex organic compounds.

Characterisation of TiO_2 nanoparticles: Commercial rutile TiO_2 nanoparticles were analyzed by UV-VIS spectroscopy and X-ray diffraction technique. The UV spectrum showed maximum absorbance at 340nm as seen from Fig 1.

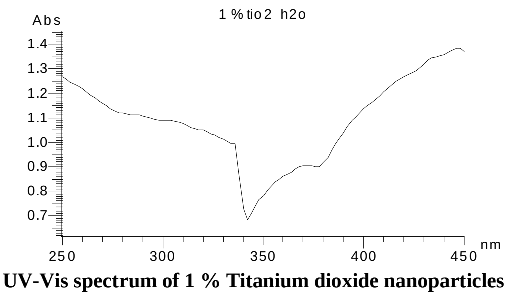

Fig 1: **UV-Vis spectrum of 1 % Titanium dioxide nanoparticles in water**

This is responsible for the photocatalytic activity of the nanoparticles to a major extent. Many reports of photocatalytic mechanism for antibacterial activities are based on UV/TiO_2 systems, which propose that there are different pathways for inhibiting bacterial growth, including the major ones which have detrimental effects on DNA molecules, and cell wall along with cell membrane damage and lysis that leads to leakage of cell contents [16, 17]. Decrease or loss of respiratory activities due to oxidation/loss of coenzyme A has also been observed [18].

Fig. 2 shows the XRD analysis results of Titanium dioxide nanoparticles in water shows narrow diffraction peaks of sharp intensity with these corresponding to the rutile phase (TiO2, JCPDS card #75-1753).

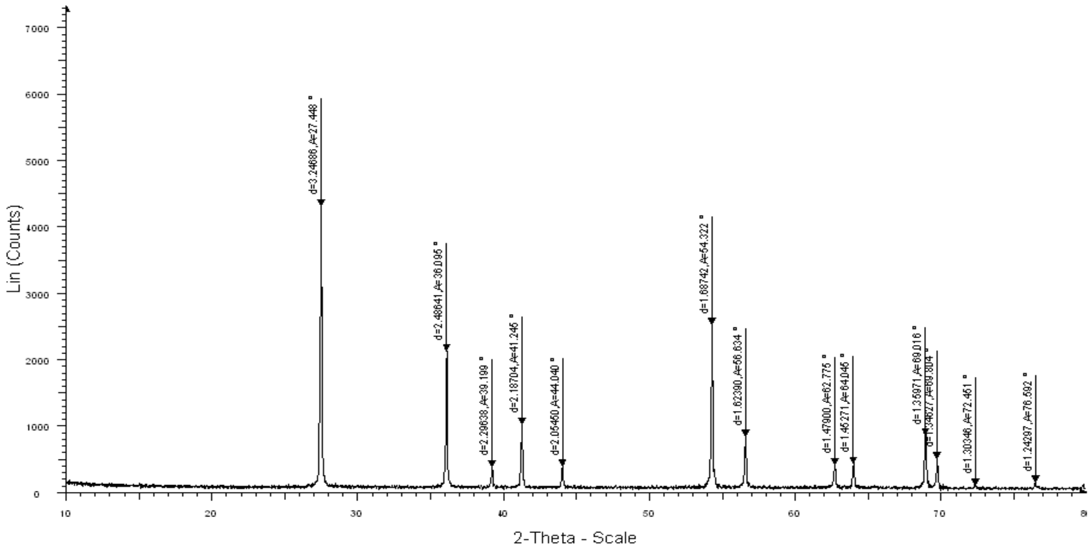

Fig 2: X ray diffraction of Titanium dioxide nanoparticles

Antibacterial activity of TiO_2 nanoparticles against MTCC cultures

No zone of inhibition was observed for 1% TiO_2 prepared in aqueous medium, against the nine MTCC bacterial cultures. However, with TiO_2 dispersed in acid, slight zones were seen, as compared to the plain diluted acid, in case of certain bacteria. Most of these zones were not very significant. The results are depicted in Table 1.

Table 1: Zones of inhibition (cm) with 1% TiO_2 in acid and diluted acid

MTCC cultures	1% TiO_2 in diluted acid	Diluted acid (cm)
441	1.4	1.00
9493	1.7	1.7
111	1.7	1.6
8076	1.5	1.4
3231	1.8	1.5
3160	1.5	1.4
443	1.3	1.5
4300	2.0	1.6
7407	1.7	1.6

Characterisation of bacteria isolated from environmental samples

Using colony morphology, the bacteria isolated from the various environmental sources were characterized and identified as *E.coli*, *Pseudomonas* sp., and *Bacillus* sp. The analysis is illustrated briefly in Table 2, 3 and 4.

Table 2: Colony characteristics of organisms isolated from sewage sample

Dilution	No. of colonies	cfu	Colour	Form	Margin	Elevation	Opacity	Consistency	Gram character
1/100	48 (4)	0.48	off-white	circular	entire	flat	opaque	butyrous	G +ve bacilli
			off-white	irregular	undulate	umbonate	opaque	butyrous	G +ve bacilli
			off-white	circular	entire	convex	opaque	mucoid	G +ve cocco bacilli
			Yellow	circular	entire	convex	opaque	butyrous	G +ve bacilli
1/1000	40 (7)	0.04	iridescent	irregular	undulate	flat	translucent	viscid	G -ve cocco bacilli
			off-white	irregular	undulate	flat	opaque	viscid	G +ve bacilli
			off-white	irregular	undulate	flat	opaque	butyrous	G -ve bacilli
			greenish yellow	irregular	undulate	flat	translucent	butyrous	G +ve bacilli
			off-white	circular	entire	flat	opaque	butyrous	G -ve bacilli
			off-white	punctiform	entire	flat	opaque	butyrous	G -ve cocco bacilli
			off-white	circular	entire	flat	opaque	brittle	G -ve bacilli
1/10000	12 (1)	0.0012	off-white	circular	entire	flat	opaque	viscid	G +ve bacilli

Table 3: Colony characteristic of organisms isolated from marine water samples

Dilution	No. of colonies	Cfu	Colour	Form	Margin	Elevation	Opacity	Consistency	Gram character
1/100	11 (1)	0.11	off-white	circular	entire	flat	opaque	butyrous	G -ve bacilli
1/10000	5 (4)	0.0005	off-white	circular	undulate	flat	opaque	butyrous	G +ve cocci
			off-white	irregular	undulate	flat	opaque	butyrous	G -ve cocco bacilli
			off-white	circular	entire	flat	opaque	butyrous	G +ve cocco bacilli
			off-white	irregular	undulate	flat	opaque	butyrous	G -ve cocco bacilli

Table 4: Colony characteristics of organisms isolated from soil sample

Dilution	No. of colonies	cfu	Colour	Form	Margin	Elevation	Opacity	Consistency	Gram character
1/100	26 (5)	0.26	off-white	irregular	lobate	flat	opaque	butyrous	G +ve bacilli
			off-white	circular	entire	flat	opaque	butyrous	G -ve bacilli
			off-white	circular	entire	flat	transluscent	butyrous	G +ve bacilli
			off-white	irregular	undulate	umbonate	opaque	viscid	G -ve bacilli
			off-white	irregular	undulate	flat	opaque	butyrous	G-ve bacilli
1/1000	5 (2)	0.005	off-white	circular	entire	flat	opaque	viscid	G -ve bacilli
			off-white	irregular	lobate	flat	opaque	butyrous	G +ve bacilli
1/10000	31 (2)	0.0031	off-white	circular	entire	umbonate	opaque	viscid	G -ve bacilli
			off-white	circular	entire	flat	opaque	viscid	G -ve bacilli

Water dispersed TiO_2 nanoparticles showed no inhibitory activity against organisms isolated from the environmental samples listed in Tables 2, 3 and 4, that largely included three ubiquitous organisms, *viz., E.coli*, *Pseudomonas* sp., and *Bacillus* sp. This is in contrast to previous reports wherein a concentration dependent inhibition has been observed [19]. In this study, there was an observable zone of inhibition with 1% TiO_2 prepared with diluted acid. This zone was also seen with the plain diluted acid and hence it can be inferred that it may be due to the acid which serves as a medium for the nanoparticle dispersion and not due to the particles themselves.

Though reports discussed previously show antibacterial activity for titanium dioxide particles, significant activity has not been observed in the present work. It has also been suggested that the resting stages, particularly bacterial endospores, fungal spores and protozoan cysts, are generally more resistant than the vegetative forms, possibly due to the increased cell wall thickness. The degradation of the microbes, whenever it happens is possibly due to cell wall damage and cytoplasmic membrane lysis. This may be due to the production of reactive oxygen species such as hydroxyl radicals and hydrogen peroxide that initially leads to leakage of cellular contents followed by cell lysis leading to complete mineralisation of the organism. It is inferred that the inhibition and degradation whenever observed is due to the medium of dispersion of the particles (in case of it not being water) and not because of the particles themselves. Hence it is recommended to use these particles as their dispersions in water and not in any other solvent as it may harm the environmental microbes in the long run if their disposal is not properly effected. Currently the dispersion these nanoparticles in natural oil based aqueous emulsions is being explored in our laboratory.

CONCLUSION

Commercial rutile TiO_2 nanoparticles were characterised using with UV-VIS and by XRD techniques. They showed an absorbance at 340nm and rutile crystal structure. Their one percent dispersions in water and dilute acid were tested for antibacterial activities against MTCC cultures as well as bacteria isolated from soil, marine and sewage sources. No activity was observed for water dispersions whereas an observable activity was seen with the sample prepared in acid which largely might have been due to the acid matrix. The microbial activity studies on the organisms isolated from environmental samples showed similar results. Thus, antimicrobial effects of the TiO_2 nanoparticles may essentially be due to their dispersion matrix and not per se because of the nanoparticles themselves. Hence they may be safely used for varied applied applications in different industries by dispersion in water or in other natural component based matrices.

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