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ANTIBACTERIAL ACTIVITY OF SYNTHESIZED TIO_2 NANOPARTICLES

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ABSTRACT

Nanosized materials are attracting a great deal in biological and pharmaceutical applications. Recently, it has been demonstrated that metal oxide nanoparticles exhibit excellent biocidal and biostatic action against Gram positive and Gram negative bacteria. TiO_2 nanoparticles, have become a new generation of advanced materials due to their optical, dielectric, and photo-catalytic characteristics from size quantization. These properties have been applied in removing bacteria and harmful organic materials from water and air. In this work, antibacterial activity of synthesized TiO_2 nanoparticle by sol-gel process was investigated. The TiO_2 nanoparticle was characterized by X-ray diffraction, UV-Visible spectroscopy and scanning electron microscopy analyses. The antibacterial effect of the obtained nanoparticle was examined on *Esherichia coli* (MTCC 1687), *Pseudomonas Sp.* (MTCC 1688), *Salmonella Sp.* (MTCC 3858), *Shigella Sp.* (MTCC 11947) and *Vibrio Sp.* (MTCC 3906). The synthesized TiO_2 nanoparticle maybe was found to be effective against these bacteria. Thus the synthesized TiO_2 nanoparticles concluded as good inorganic antibacterial agents.

Keywords: TiO_2 nanoparticle, XRD, SEM, UV, Pathogenic bacteria

INTRODUCTION

Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructures materials, play an ever increasing role in science, as more and more products based on nanostructured materials are introduced to the market. Metal and metal oxide nanoparticles have high surface to volume ratio which is responsible for their fascinating properties such as antimicrobial, magnetic, electronic and catalytic activities. Generally properties of nanoparticles depend on size, shape, composition, morphology and crystalline phase. Among the various metal oxide nanoparticles, TiO_2 nanoparticles have wide range of applications in air and water purification, pigments, photocatalysis (Papp, 1993), solar energy conversion (Ding, 1998), sensor devices (Guidi, 1999), and antibacterial activities (Akbari 2017 and Sobhani Nasab 2017).

TiO_2 exists as three important polymorphs: rutile (tetragonal), anatase (tetragonal), and brookite (orthorhombic). Rutile is the only stable phase; anatase and brookite are metastable at all temperatures and can be converted to rutile after heat treatment at high temperatures. Dependent on structure and particle size, each crystalline phase exhibit different physical properties best suited to different applications. Titanium dioxide has a more helpful role in our environmental purification due to its nontoxicity, photo induced super hydrophobicity and antifogging effect. These properties have been applied in removing bacteria and harmful organic materials from water and air as well as in self-cleaning or self sterilizing surfaces for places such as medical centers. As TiO_2 is nontoxic, the American Food and Drug Administration (FDA) has approved TiO_2 for use in human food, drugs, cosmetics, and food contact materials.

Traditionally metal oxide nanoparticles were synthesized by various physical and chemical methods. Some commonly used synthetic methods are non-sputtering, solvothermal, reduction, sol-gel technique and electrochemical technique. Among various methods reported to date for titania synthesis sol-gel is one of the most important and promising method due to its low cost, simplicity, low temperature reaction kinetics and most importantly its application oriented nature. The control of crystallite size, shape, phase of titania, surface structure are influenced by choice of precursor, solvents, pH and temperature of sol, catalyst, sol concentration etc.,(Shalan 2013 and Loryuenyong, 2012).

In the present work, TiO_2 nanoparticles were synthesized through the sol-gel process and annealed at 400°C for 3 hours. The synthesized TiO_2 nanoparticles properties were studied through XRD, UV-Visible spectroscopy and SEM with EDAX analysis. The antibacterial effect of TiO_2 nanoparticles was investigated against pathogenic bacteria using Well diffusion method.

MATERIALS AND METHODS

Chemicals used

Titanium tetra isopropoxide [$Ti(OCH(CH_3)_2)_4$], Sigma-Aldrich, 97%] was used as precursor and Iso-propanol [$(CH_3)_2CHOH$], Sigma-Aldrich, 99.7%] was used as solvent for the preparation of TiO_2 nanoparticles. Deionized water was used throughout the experiment. All the glass wares were washed in dilute nitric acid and distilled water and dried in hot air oven.

Synthesis of TiO_2 nanoparticles

TiO_2 nanoparticles were prepared via sol-gel method using the precursor Titanium Tetra isopropoxide, deionised water and Isopropyl alcohol as the starting materials. First, the 4ml of TTIP was added to 50 ml of Isopropyl alcohol in a 100 ml beaker and stirred in a magnetic stirrer for 10 minutes. The solution becomes milky white in colour. For hydrolysis reaction 5ml of deionized water is added drop by drop to the mixed solution. Then the solutions was stirred continuously for 2 hours and left idle for 24 hours. The resulting solid product was filtered and washed several times by de-ionized water and dried at temperature of 100°C in oven for 30 minutes. In order to get finely divided nanopowder the sample was crushed in mortar and pestle and annealed at 400^0C for 3 hours in muffle furnace.

Characterization of TiO_2 nanoparticles

The crystallites of TiO_2 nanoparticles were characterized by X-Ray diffraction (XRD) using X'PERT-PRO X-ray diffractometer which was operated at 40 KV and 30 mA with $CuK\alpha_1$ radiation of wavelength 1.5406 Å. The surface morphological observations and elemental analysis were done by EV018 Carlzeiss SEM and S-Flash 6130 Bruker EDAX respectively. UV-Visible spectra were recorded in the range of 400 - 700 nm by using the (Schimadzu 1800) UV-VIS-NIR spectrophotometer.

Antibacterial activity of TiO_2 nanoparticle

Well diffusion method was employed to evaluate the antibacterial activity of TiO_2 nanoparticles. The antibacterial activity was done on five pathogenic bacteria namely *Esherichia coli*, *Pseudomonas Sp.*, *Salmonella Sp.*, *Shigella Sp.* and *Vibrio Sp.* Nutrient Agar medium was used to cultivate bacteria. Approximately, 25ml of molten and cooled nutrient agar media were poured in five sterilized petri plates. The plates were left over night at room temperature to check for any contamination to appear. The bacterial test organisms *E.coli*, *Pseudomonas Sp.*, *Salmonella Sp.*, *Shigella Sp.* and *Vibrio Sp.* were grown in nutrient broth for 24 h. After incubation, the above said broth culture 100µl of each bacterial strain was swabbed on petri plates. Then diffusion wells were prepared with the help of a sterilized stainless steel cork borer. TiO_2 nanoparticles were taken at the concentration of 50µl, 75µl, 100µl, 125µl, 150µl and 175µl for the antibacterial tests. All the petriplates were incubated at 37°C for 24 hours in an incubator. Then inhibitory action of nanoparticles on the growth of the bacteria was determined by the measurement of diameter of inhibition zone.

RESULTS AND DISCUSSION

Structural analysis

The X-ray diffraction pattern of the samples were recorded by a X'PERT-PRO X-ray diffractometer using the $CuK\alpha_1$ radiation (λ=1.54059 Å) in the range of 2θ between 15°- 80°. The powder X-ray diffraction patterns of the prepared TiO_2 nanoparticles (Figure 1) showed the presence of broad peaks. The broad peaks indicate either particles of very small crystalline size or particles are semi crystal in nature (Zhang, 2009).

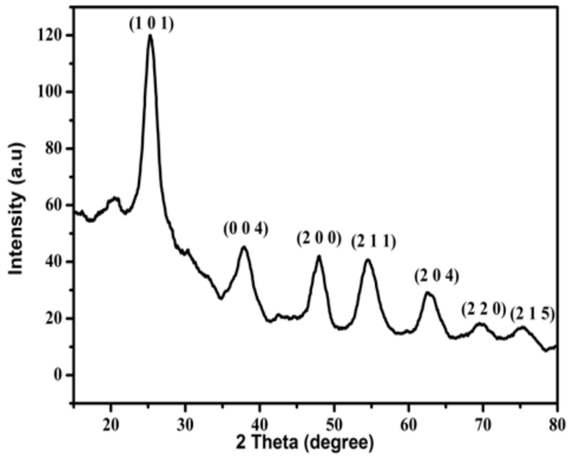


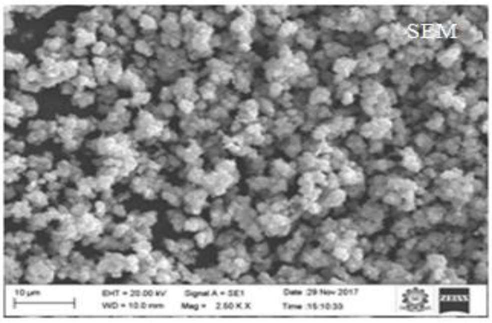
Fig.1: X-ray diffraction patterns of TiO_2 nanoparticles

From the XRD pattern it was observed that the TiO_2 nanoparticles possess preferential orientation along (1 0 1) plane which corresponds to tetragonal crystal structure of anatase phase with the crystallite size of 11nm. The XRD pattern exhibited diffraction peaks at 25.48°, 38.19°, 48.50°, 54.65°, 62.70°, 71.5° and 75.47° indicating TiO_2 in anatase phase with the corresponding planes of (1 0 1), (0 0 4),(2 0 0), (2 1 1), (2 0 4), (2 2 1) and (2 1 5) respectively (Ajay Sharma Karn 2014). All observed peaks are in good agreement with the standard spectrum (JCPDS no. 89-4203). The crystallite size was estimated by using Scherrer equation,

$$D = \frac{K\lambda}{\beta Cos\theta}(nm)$$

where D is the crystallite size, λ is the x-ray wavelength, β is the full width at half maximum (FWHM) value of the dominant peak and θ is the Bragg angle. The crystallite size was about 11 nm for the predominant peak.

Further structural study of the prepared TiO_2 nanoparticles were obtained using SEM image analysis. SEM is the scanning electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material. The Figure 2 shows the SEM and EDAX images of synthesized TiO_2 nanoparticles which is annealed at 400°C. The SEM images indicate the formation of irregularly shaped particles with some agglomeration (Manikandan, 2017). Yet some of the particles were found spherical in shape with clump distribution.



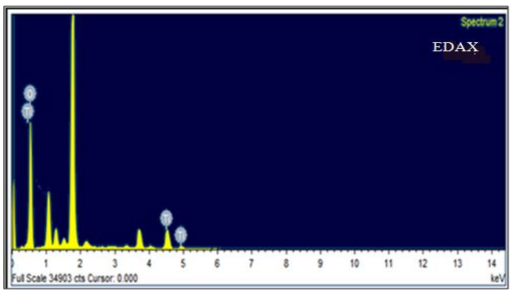


Fig. 2: SEM with EDAX spectra of TiO_2 nanoparticles

Optical analysis

UV-Vis spectroscopy was used to characterize the optical adsorptions of TiO_2 nanoparticles shown in Figure 3. From the figure it was observed that the absorption edges for TiO_2 nanoparticle is about 370nm which confirms the TiO_2 response under ultra violet region (Hwan, 2003). The fast decrease below 400 nm is due to absorption of light caused by the excitation of electrons from the valence band to the conduction band of TiO_2. The energy gap E_g of the TiO_2 nanoparticle was calculated by plotting $(\alpha h\nu)^2$ vs Energy shown in Figure 4, by extrapolating the straight line part of the plot to the photon energy axis.

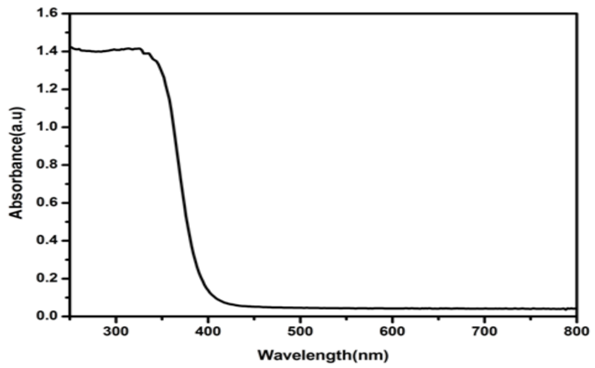


Fig. 3 : UV-Vis absorption spectrum of TiO_2 nanoparticles

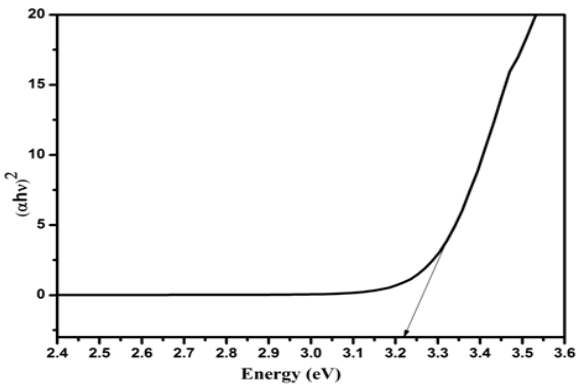


Fig. 4 Direct band gap of TiO_2 nanoparticles

The relationship between the absorption coefficient α and the incident photon energy hν is given by,

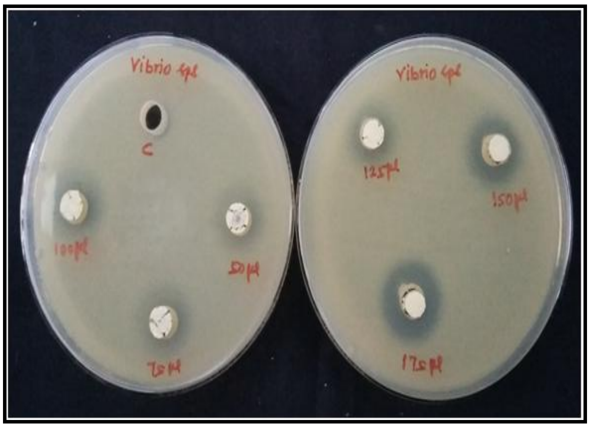
$$\alpha h\nu = A\ (h\nu - E_g)^n$$

where E_g is the separation gap between bottom of conduction band and top of valence band, hν is the photon energy and n is the constant, which depends on the probability of transitions, it takes values as 1/2, 3/2, 2 and 3 for direct allowed, direct forbidden, indirect allowed and indirect forbidden respectively. The band gap of TiO_2 nanoparticle was found to be 3.21eV and is attributed to be a promising material for a potential application (Edna 2017) such as in the fabrication of DSSC, photocatalytic activity, antibacterial activity etc.,

Antibacterial activity of TiO_2 nanoparticles

Antibacterial activity of TiO_2 nanoparticles was studied using five different gram negative microorganisms. Figure 5 depicts the antibacterial activity of TiO_2 nanoparticle. It is well known that the TiO_2 nanoparticle is an efficient antibacterial agent. The present study substantiates the antibacterial activity of TiO_2 nanoparticles.

According to several studies, it is believed that the metal oxides carry the positive charge while the microorganisms carry negative charges. This causes electrostatic attraction between microorganisms and the metal oxides which leads to oxidization and finally death of microorganisms (Zhang, 2009).



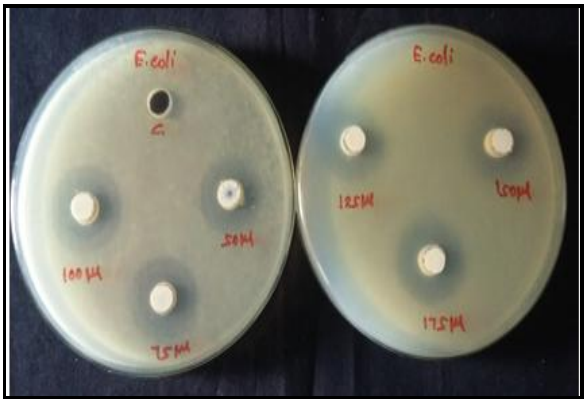


Fig. 5 : Well diffusion method for TiO_2 nanoparticles against pathogenic bacteria

They cause pits or holes on bacterial cell wall which could be associated with internalized particles, leading to increased permeability and to cell death (Holt 2005, Ravishankar Rai 2011). The TiO_2 nanoparticle showed minimum activity toward *Vibrio Sp.* (13mm) and the maximum toward *Salmonella Sp.* (25mm). The TiO_2 nanoparticles are effective in inhibiting the growth of gram negative bacteria.

Table 1: Zone of inhibition for TiO_2 nano-particles against Pathogenic bacteria

Pathogenic bacteria	Zone of inhibition (mm)					
	50µl	75µl	100µl	125µl	150µl	175µl
Vibrio Sp.	-	-	-	-	7	8
E.coli	10	12	14	15	16	17
Shigella Sp.	12	13	14	15	16	17
Pseudomonas Sp.	15	16	18	19	20	21
Salmonella Sp.	17	18	19	20	21	23

It is well known that if the diameter of antibacterial circle of one sample is larger than 7mm, it indicates better antibacterial activity.

However, if the diameter of antibacterial zone is equal to or less than 7mm, it means the sample has poorer antibacterial activity. The conditions may vary for different species of organisms. From the results in Table 1, it could be predicted that all samples have better antibacterial activity because of their zone diameter for TiO_2 nanoparticle. The control did not show any antimicrobial activity against the test bacterial strains. In addition, 175µl concentrations of TiO_2 nanoparticles showed better result as compared to 50µl for inhibiting growth of bacterium test organism. Therefore, the investigation on the antibacterial effect of nanoparticles against *E. coli*, *Pseudomonas Sp.*, *Salmonella Sp.*, *Shigella Sp.* and *Vibrio Sp.* reveals that the TiO_2 nanoparticle act as a strong antibacterial agent. This can be useful in various industrial and medical applications.

CONCLUSION

Titanium dioxide (TiO_2) nanoparticles were successfully synthesized by sol-gel method and annealed at 400°C for 3 hours to improve the crystallization. The annealed TiO_2 nanoparticles were characterized by powder XRD, SEM and UV-Vis spectroscopy. The X-ray diffraction analysis revealed that the prepared nanoparticle has tetragonal crystal structure of anatase phase with preferential orientation along (1 0 1) plane. The surface morphology study using SEM reveals that the particles are spherical in shape with agglomeration and the presence Ti and O was confirmed from EDAX measurements. The optical absorbance of the prepared nanoparticle TiO_2 was found to be 370nm which corresponds to the band gap of 3.21eV. The TiO_2 nanoparticles yield a well-pronounced antibacterial activity and it acts as a good antibacterial agent. It may be used in water purification as well as in other biomedical application for inhibiting the growth of pathogenic bacteria.

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