SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF TITANIUM DIOXIDE (TIO_2) NANOPARTICLES.

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Abstract

The high catalytic activeness of Titanium dioxide (TiO_2) nanoparticles makes it one of the most important and promising semiconductor oxide material, good gas-sensitive characteristics, di-electricity, high stability, low cost, non-toxic, and its potential use in any applications. These nano-sized particles exhibit beneficent wide band for absorption of UV, which has recently been used in sunscreen applications. In the present work Sol-gel method was used to prepare titanium dioxide (TiO_2) nanoparticles. The sample was characterized by powder X-Ray diffraction (PXRD), Energy Dispersive X-Ray analysis (EDX) and Scanning Electron Microscopy (SEM).The average particle size of the sample was calculated from PXRD peaks by Debye-Scherer's equation and size to be in nano range.The average crystallite size from sharp peak of Rutile was 22.41 nm, which was estimated by using the Scherer's formula.

Introduction

Titanium dioxide (TiO2) nanoparticles is such a material, mostly used in daily life applications due to high photo catalytic activity of titanium(Allen, 2008).Titanium nanoparticles has an excellent gas-sensitive properties(Chen and Mao, 2007).TiO_2 nanoparticles shows dielectric properties(Cao *et al.*, 1995), high stability and non-toxicity behavior (Sugimoto,2003). The optical property of Titanium dioxide makes it suited in the splitting of H_2O (Rao *et al.*, 1980). These materials exhibit broad band UV absorption, sunscreen applications (Sung *et al***.,** 2003). The sol-gel rout is a low cost method for the preparation of titanium dioxide (Zhou *et al*., 2006). As far as the treatment of infectious diseases is concerned, resistance has developed due to injudicious and insensible use of antimicrobial agents (Asai *et al*., 2005). From the time of immemorial, for the cure of infections ,the inorganic antimicrobials such as silver and copper have been in practice (Moghimi, 2005).Some of the new potential of nanoparticles are in the area of diagnostics and biomolecular detection of diseases as well as antimicrobials in therapeutics of infectious diseases (Jain *et al*., 2009).

The main purpose of our current work is to prepare high efficient nano scale particles with wide surface area and characterize the synthesized sample through these techniques like PXRD, SEM and EDX and performing the Antibacterial activity of titanium dioxide nanoparticles.

Materials and Methods

A solution was prepared by dissolving titanium dioxide (TiO_2) and tri sodium citrate ($Na_3C_6H_5O_7$) in 50 mL of deionized water. Tri sodium citrate solution then added to TiO_2 solution at a rate of 2mL/min to maintain 3:8 ratios. During the mixing procedure, the solution was constantly stirred for two hours and the temperature was maintained at 45^oC. Subsequently the obtained product was washed with distilled water for three times and centrifuged at 2000RPM for 15 minutes to separate out the precipitate. The resultant precipitate was dried using in an oven at 110 °C for 24 hours to get TiO_2 crystal. TiO_2 crystals were then grinded into a fine powder with mortar and pestle. Finally the fine powder was annealed at 550°C.

X-Ray Diffraction (XRD): Figure 1 shows the X-ray diffraction (XRD) patterns of synthesized titanium Dioxide sample. Powder XRD data was recorded on a Siemens D5000 diffractometer using Cu radiation with graphite monocrometer and divergence slit 1mm Scattered slit 1mm and Receiving slit 0.2mm and a detector used Scintillation counter in PCSIR Complex Karachi. Data was recoreded from 10 to 90˚ for 2θ using a step size of 0.05 degree/sec. First, we grinded the sample for powder form and this put in sample holder have dimension 5cm x 5cm and sample place in 2.5cm circle which is in center of holder. Sample was analyzed as random mounts. Phase and mineral identification of the sample evaluated and analyze by Diffrac^{plus} searching software version 7.0.108. According to PDF-2: release (2001), contains, 136,895 patterns. Software contains a computerized search-match function that co-relates the sample pattern by the International Center for Diffraction Data ICDD database.

Scanning Electron Microscopy (SEM): SEM was obtained from Karachi university centralized science laboratories -Pakistan
SEM of our sample was performed information of the particles size and characteristics of the synthesized sample. For SEM we use JSM 6380A, JEOL, Japan.

Energy Dispersive X-Ray (EDX): EDX was obtained from Karachi university centralized science laboratories –Pakistan. we use EX-54175 JMU for EDX.

Antibacterial Activity: Agar-well method is the method which was used to evaluate the antibacterial activity of compounds. 1 mL DMSO was used to prepare stock solution by dissolving 10 mg compounds in it. The swabbing is done to make the lawn of culture by using two hours old log phase culture turbidity of which is matching with 0.5 Mac Farland and then wells were made in Muller Hinton agar. 10 μL of stock solution was added into the wells (Perez *et al.*, 1990). The plates were incubated at 37$\pm$ 2°C for 24 -48 hours and results are noted by measuring the diameter of zone of inhibition in mm. Gentamicin was employed as a positive control and DMSO is taken as negative control (Vaghasiya *et al.*, 2009).

Minimum inhibitory concentration (MIC) against bacteria: Micro broth dilution method using 96-well microtitre plate was used to measure Minimum inhibitory Concentration (MIC) (Sherwani *et al*., 2011). Two fold serial dilutions of stock solution were made in 100 µL broth and subsequently 10 µL of 0.5 Mac Farland matched culture was loaded in all wells. One well was kept as antibiotic control while other was kept as culture control. The microtitre plate was incubated at 37 ºC for 24 hours. The last well showing no visible growth is taken as MIC.

Antifungal Activity: Antifungal activity of compounds was tested using agar-well method. Fungal suspension was made in the autoclaved normal saline and transferred aseptically into each Sabourd Dextrose agar (SDA) plates. All plates were incubated at 28$\pm$ 2°C for 1 week and results were measured in diameter of zone of inhibitions in mm.

Minimum inhibitory concentration (MIC) against fungi: Minimum inhibitory Concentration (MIC) of was determined by micro broth dilution method using 96-well microtitre plate (Sherwani *et al.,* 2011). Two fold serial dilutions of stock solution was made in 100 µL broth and subsequently each well was loaded with 10 µL culture matched with 0.5 Mac Farland index. One well is kept as only antifungal agent control while other is kept as culture control. Microtitre plate was incubated for 1 week hours at 37 ºC. The last well showing no visible growth is considered as MIC.

Results and Discussion

Powder X-Ray Diffraction (PXRD): Thepowder X-ray diffraction (PXRD) patterns of prepared titanium Dioxide sample has been shown in figure 1.
The x-ray diffraction analysis of our sample suggests that according to ICDD No. 01-073-2224 presence of Rutile (syn) "TiO_2" in major phase as shown in graph. The average crystallite size from sharp peak of Rutile is 22.41 nm, which is estimated by the Scherer's formula using the XRD spectra (Cullity and Stock,2001).In one of the earlier studies, carried out to assess the level of potential of silver and titanium against both opportunistic pathogens and found promising results with least toxicity (Martinez-Gutierrez *et al*., 2010). Many sunscreens contain titanium based nanoparticles not only to prevent the skin from rays as well as harmful microbes (Oberdorster *et al.,* 2005). Many earlier studies have also highlighted the relationship of antibacterial activity of agents with the size of developed nanoparticles (Stoimenov *et al.,* 2002). Moreover; such particles possess durability, less toxicity, heat resistance, greater selectivity (Brayner *et al*., 2006).

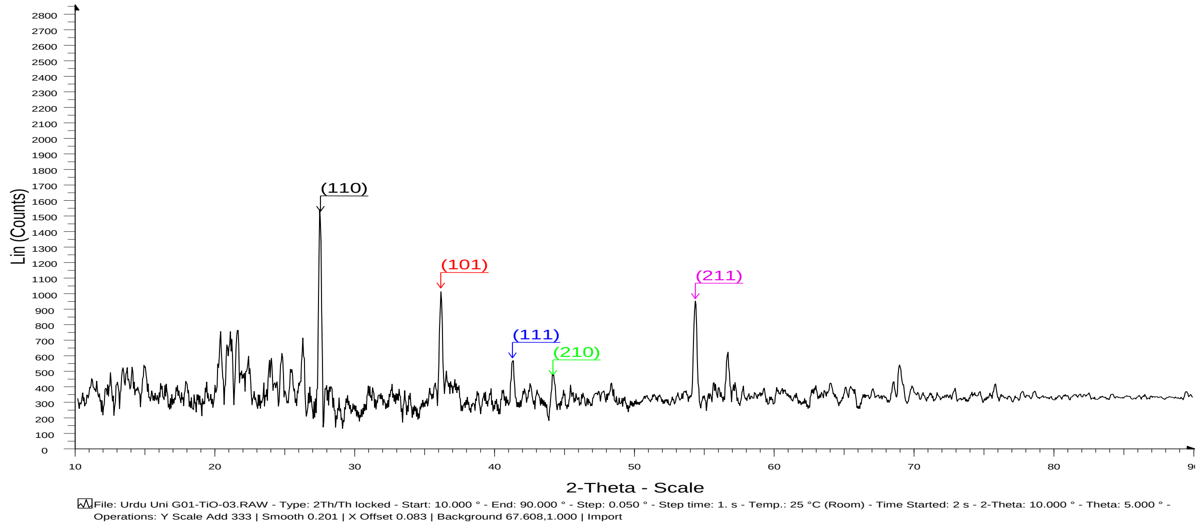

Fig.1.Powder XRD spectra of synthesized titanium.

Sherer formula

$D = K\lambda / \beta Cos\theta$

`Where D represents size of the particle, λ (1.54A^o) the wavelength of X-Rays, β denotes the full width at half maximum (FWHM) of the diffraction peak (radian), K is called coefficient (0.9) and θ is the angle of diffraction at the highest peak. In our current study, the size of the particle titanium dioxides ynthesized by Sol- gel rout was calculated to be 22.41 nm.

Scanning Electron Microscopy (SEM): The SEM images of the Titanium Dioxide nanoparticles synthesized by sol-gel rout is shown in Figure 2. The particles have almost uniform size and spherical in shape. Below images determine that there is no aggregation in titanium dioxide nanoparticles and they are evenly dispersed on the surfaces.

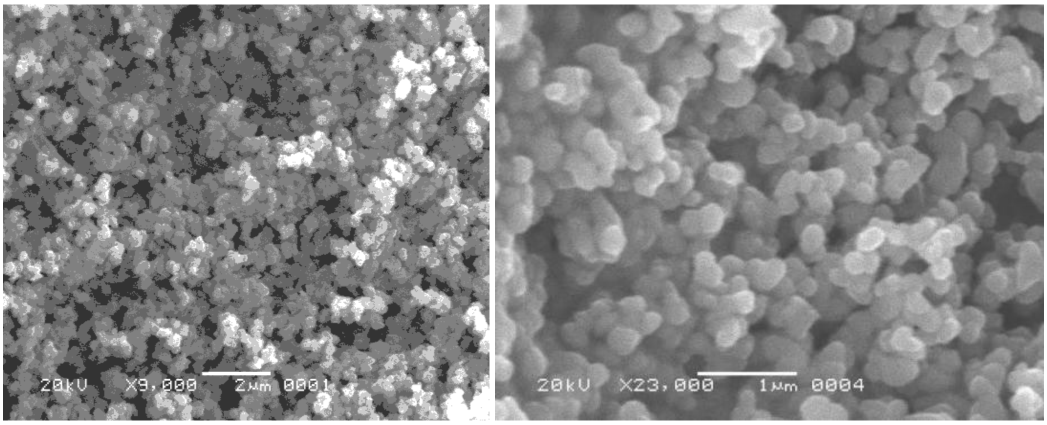

Fig.2. SEM images of Synthesized titania with different magnification.

Energy Dispersive X-Ray (EDX): It is an absorption technique as explained by Miroslav. Here we observe the absorption of x-rays in the material and then emission of other x rays which are resulted due to interaction of incident x rays with the electrons (Figure 3). The Table 1 shows the detailed analysis of EDS data of TiO_2 nanoparticles in which ZAF method is used for quantitative analysis.

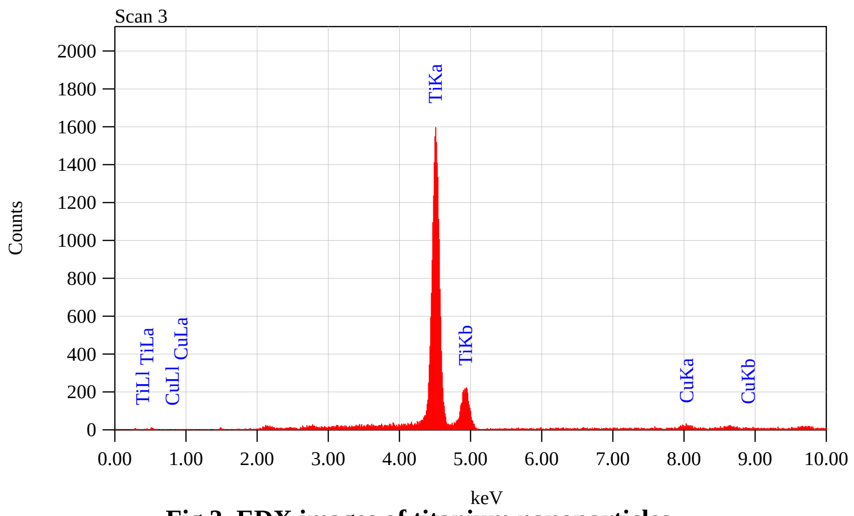

Fig.3. EDX images of titanium nanoparticles

Table 1.The detail analysis of EDS Spectrum in TiO_2 Nanoparticles (Fitting Coefficient: 0.2036).

element	Ke V	MASS %	ERROR%	AT%	MASS%
C K	0.277	7.20	0.04	13.19	4.5231
O K	0.525	47.67	0.24	65.60	24.4065
Al K	1.486	1.29	0.05	1.05	1.2807
Ti K	4.508	43.84	0.07	20.15	69.7896
TOTAL	100.00		100.00		

Antibacterial activity: Titatinum dioxide nanoparticles exhibited good activity against *Escherichia coli*, *E. coli multi drug resistant*, *Staphylococcus epidermidis*, *Streptococcus fecalis*, *Streptococcus pyogenes* zone of inhibitions ranged between 12 mm to 16mm (Table 2). However, the best activity was found against *Streptococcus fecalis* and *E. coli multi drug resistant*, MIC value 65 and 72 mg/ml (Table 3)

Table 2. Antibacterial potential of compounds in terms of zone of inhibition (mm).

Gram positive bacteria	**Zone of inhibition in mm (mean± S.D)**	**Gram negative bacteria**	**Zone of inhibition in mm (mean± S.D)**
Bacillus cereus	-	*Enterobacter aerogenes*	-
Bacillus subtilis	-	*Escherichia coli ATCC 8739*	-
Bacillus thruingiensis	-	*Escherichia coli*	12±1
Corynebacterium diptheriae	-	*E. coli multi drug resistance*	14±2
Corynebacterium hofmanii	-	*Klebsiella pneumoniae*	-
Corynebacterium xerosis	-	*Salmonella typhi*	-
Staphylococcus epidermidis	15±1	*Salmonella paratyphi A*	-
Streptococcus saprophyticus	10±2	*Salmonella paratyphi B*	-

M. smegmatis	-	*Shigella dysenteriae*	15±1
Streptococcus fecalis	16±1	*Serratia marcesens*	-
Streptococcus pyogenes	12±1	*Acinetobacter baumanii*	-
		Campylobacter jejuni	-
		Campylobacter coli	-
		Helicobacter pylori	-
		Hemophilus influenzae	-
		Vibrio cholerae	-
		Aeromonas hydrophila	12±0

Table 3: MIC of compound in mg/ mL.

Bacteria Extract	**MIC (mg/ml)**
Gram positive bacteria	
Streptococcus fecalis	65
Streptococcus pyogenes	200
Streptococcus saprophyticus	100
Staphylococcus epidermidis	144
Gram negative bacteria	
E.coli multi drug resistant	72
E.coli	100
Aeromonas hydrophila	100
Shigella dysenteriae	20

Antifungal activity: Antifungal activity was found only against *Microsporum canis*, *Penicillium sp and Rhizopus*. Results are presented in Tables 4 and 5.

Table 4. Antifungal potential of compounds (zone of inhibition in mm).

Yeasts	Zone of inhibition(mm)	**Dermatophytes**	Zone of inhibition(mm)	**Saprophytes**	Zone of inhibition mm
Candida albicans	-	*Microsporum canis*	12±1	*Aspergillus flavus*	-
Candida albicans ATCC 0383	-	*Microsporum gypseum*	-	*Aspergillus niger*	-
Saccharomyces cerevisiae	-	*Trichophyton rubrum*	-	*Fusarium specie*	-
Candida galbrata	-	*Trichophyton mentagrophytes*	-	*Penicilliumsp*	14±0
Candida tropicalis	-	*Trichophyton tonsurans*	-	*Rhizopus*	12±1
Candida kruzei	-			*Helminthosporum*	-

Table 5: MIC in mg/mL.

Yeasts	MIC mg/ml	**Dermatophytes**	MIC mg/ml	**Saprophytes**	MIC mg/ml
Candida albicans	-	*Microsporumcanis*	44	*Aspergillus flavus*	-
Candida albicans ATCC 0383	-	*Microsporumgypseum*	-	*Aspergillus niger*	-
Saccharomyces cerevisiae	-	*Trichophyton rubrum*	-	*Fusarium specie*	-
Candida galbrata	-	*Trichophyton mentagrophytes*	-	*Penicilliumsp*	80
Candida tropicalis	-	*Trichophyton tonsurans*	-	*Rhizopus*	100
Candida kruzei	-			*Helminthosporum*	-

Conclusions

By sol gel rout which is very economical, we have positively prepared Titanium dioxide (TiO_2) nanoparticles. XRD showed the formation of high transparent titanium dioxide nanoparticles. Our results demonstrated antifungal and antibacterial activities of titanium dioxide (TiO_2) nanoparticles.

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