

Synthesis and Characterization of TiO₂- Nanoparticles for Photodynamic

Cancer therapy

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Introduction

Titanium dioxide is one of the most promising semiconductors used in photolysis since it provides a good compromise between catalytic activity and stability in aqueous media [1]. Photocatalysis aided by TiO₂-nanoparticles is used in removing the organic chemicals which occur as pollutants in waste water effluents from industrial and domestic sources [2-4]. TiO₂ -NPs are proven to photo oxidise a variety of compounds such as phenols, chlorinated phenols, polychlorinated biphenyls, dioxins, DDT, surfactants, saturated aliphatic hydrocarbons, s-triazine, etc [5]. TiO₂ -assisted photocatalysis may also be useful in the removal of certain heavy metals including Hg, Ag, Pt, Pd, Rh and Au via their reduction followed by deposition at the catalyst surface [6]. For TiO₂-nanoparticles catalytic activity is dramatically increased at particle sizes below 20nm [7,8]. In recent years many researchers have considered the possibility of using non-toxic semiconducting nanoparticles in PDT [9,10]. Some nanomaterials, e.g., TiO₂, ZnO, and Fullerenes and their derivatives can generate singlet oxygen, [11] which is the main requirement for PDT.

Photoexcited TiO₂ has a strong ability to drive various chemical reactions due to both its strong oxidising and reducing ability [12]. To investigate the possible use of this effect for cancer treatment, the anti tumour activity of photoexcited TiO₂ particles was studied [13]. The first report of

TiO₂-photocatalysis in microbial effects was given by Matsunaga et.al., in 1985 [14]. Although TiO₂-NPs are well known for their photodecomposition of organic pollutants, few studies have been investigated for the application of TiO₂ in life sciences, especially in the field of cancer treatment [13,15]. TiO₂-NPs are easy to attach to the cell membranes and accumulate. They can also enter cytoplasm via phagocytosis [16].

Akira Fujishima et.al [13] reported the distinct cell killing effect of HeLa cells observed in vitro with photoexcited bulk TiO₂ particles and significant inhibition of tumour growth of HeLa cells observed in vitro by the treatment of TiO₂ particles with UV irradiation. Recently the photocatalytic killing effect of TiO₂ – NPs on LS-174-t human colon carcinoma cells has been reported by Ai-ping-zhang [17]. The cultured human colon carcinoma cells were effectively killed by photoexcited TiO₂-nanoparticles in vitro. Ti [18] and Ti alloy biomaterials for implants have been widely applied in the orthopaedic and dental fields. These materials possess excellent corrosion resistance, good mechanical properties and biocompatibility [19]. Only limited works have been reported on TiO₂ mediated PDT.

Since photoexcited TiO₂-nanoparticles are very good photocatalysts, and considering the fact that human erythrocytes serve as model cells to explore the cellular and molecular mechanisms of Photodynamic therapy (PDT)[20-26], the photohemolysis using TiO₂-NPs was studied. The photodynamic activity was studied as the function of light dose and drug dose. A further attempt was also made to understand the nature of the mechanism involved with the help of scavengers such as NaN₃ and Glutathione (reduced)[27-31].

Experimental section

Materials and methods

Titanium (IV) isopropoxide was purchased from Sigma Aldrich and all the other reagents used in this experiment were Analar grade, and double de-ionised water was used. The following flow-chart illustrates the details of the preparation, and characterization of TiO₂-NPs for photohemolysis[32-35].

Preparation of TiO₂-nanoparticles

TiO₂ nanoparticles were prepared by slight modification of the method described by Weijun Liu et al [20]. In brief, Titanium (IV) isopropoxide (5ml) was diluted with ethanol (5ml) and then hydrolysed by adding it to hydrochloric acid (30ml, 0.1M) dropwise under stirring at room temperature. The slurry thus obtained was refluxed at 70°C for 6 hrs with constant stirring. The colloidal solution obtained was sonicated for 4-5hrs and filtered on a glass frit to remove non-peptized agglomerate. The filtrate obtained was evaporated to get a gel which was then calcined to get a white powder of TiO₂-NPs[36-40].

Results and discussion

Characterization of TiO₂-nanoparticles

UV-Visible absorption spectrum of TiO₂-NPs

The UV-Visible absorption spectrum of TiO₂- NPs was scanned between 200 and 700nm (Fig 1).The absorption maximum occurs at 340nm and the band gap for it is 3.6 eV. There is a gradual increase of absorbance with the decrease of wavelength from 700-400nm, but at 400nm a sudden increase in absorbance is observed. Absorbance suddenly drops below 300nm[41-45].

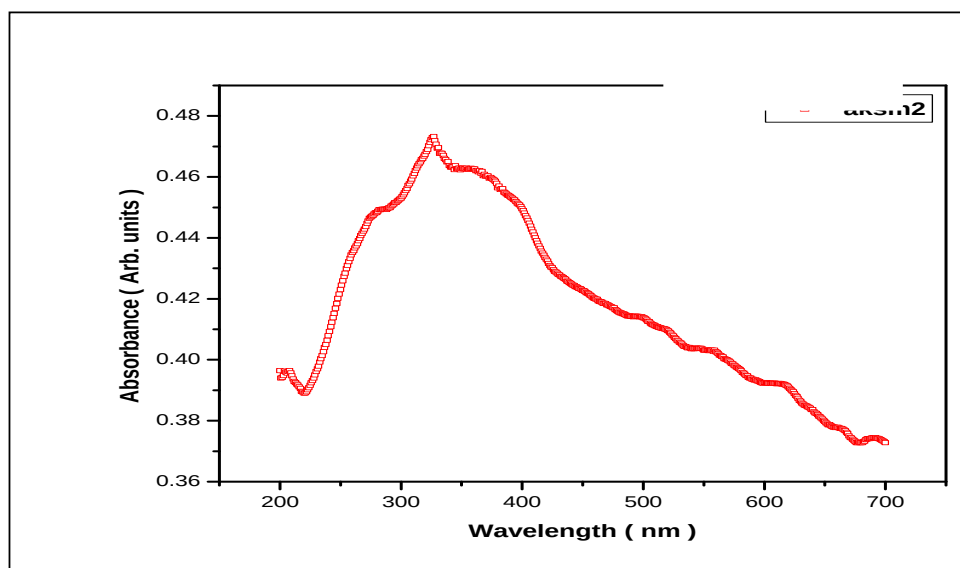


Fig.1 UV-Visible Absorption Spectrum of TiO₂-NPs

DRS-UV-Visible absorption spectrum of TiO₂-NPs

The DRS-UV-Visible spectral analysis of TiO₂-NPs was carried out between 200 and 700nm. The plot of absorbance versus wavelength is shown in Fig. 2. The spectrum illustrates increase in absorbance just below 400nm due to band gap excitation. By taking 350nm as the onset of absorbance the band gap value was calculated to be 3.5eV. The band gap value is consistent with that of UV-Visible absorption spectrum of TiO₂- NPs (Fig.1). Sharp absorbance lines below 250nm are due to TiO₂ particles of size much smaller than those absorbing close to 350nm.

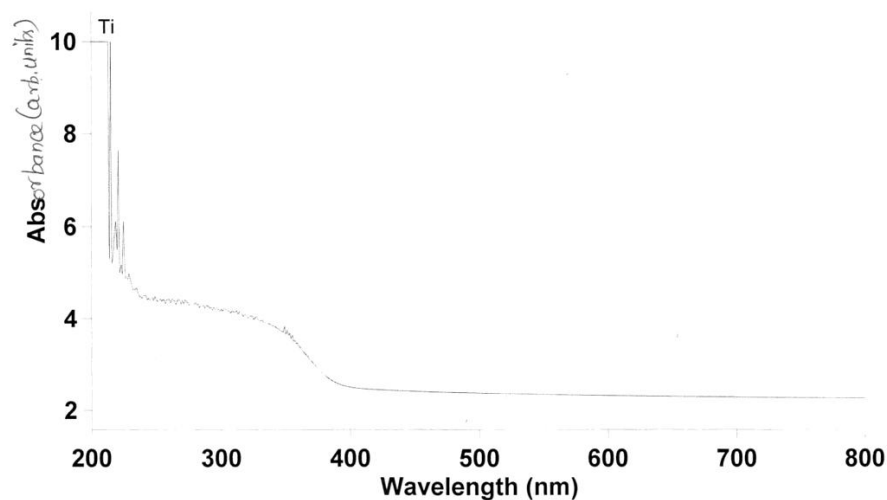


Fig.2 DRS-UV-Visible absorption spectrum of TiO_2 -NPs

Steadystate fluorescence spectroscopic characteristics of TiO_2 - nanoparticles

The fluorescence spectrum of TiO_2 -NPs, excited at 300nm, is shown in Fig.3 (a). The fluorescence gives a broad maximum close to 380nm. It is also close to band gap excitation shown in Fig.2. Hence TiO_2 -NPs after excitation gives maximum fluorescence only from the levels close to the lowest level of the conduction band. Again there is significant fluorescence at about 470 nm.

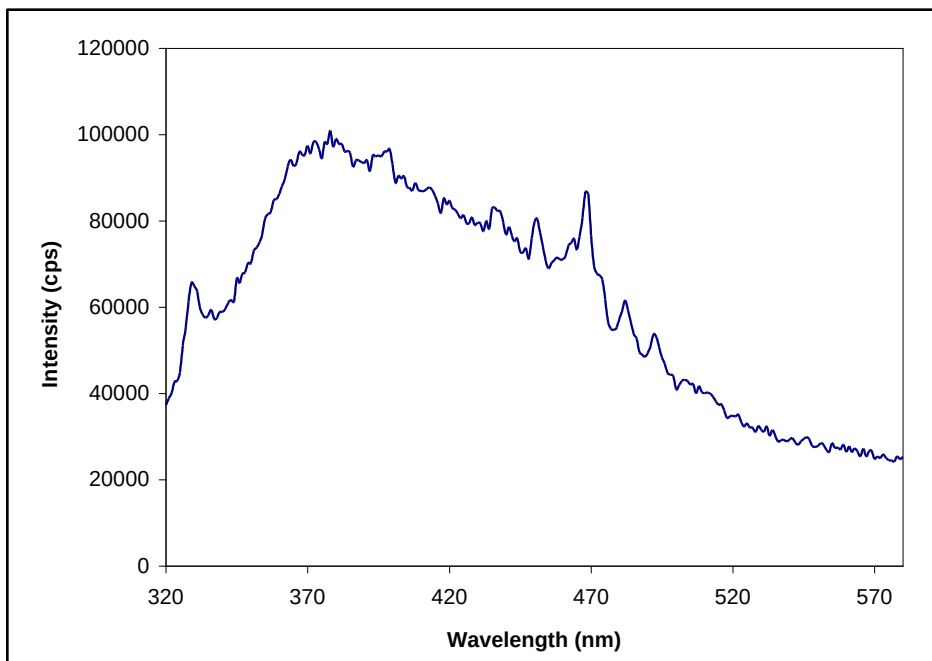


Fig.3(a) Fluorescence spectrum of TiO₂-NPs excited at 300nm

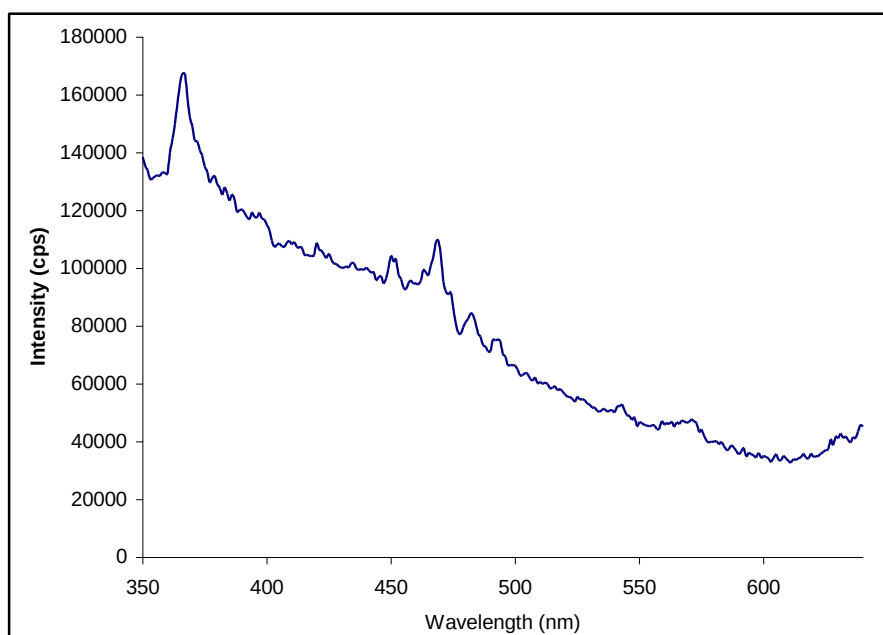


Fig.3(b) Fluorescence spectrum of TiO₂-NPs excited at 330nm

The fluorescence spectrum of TiO₂-NPs excited at 330nm is shown in Fig.3 (b). It depicts nearly similar features as that of Fig.3 (a). The fluorescence maximum lies close to 370nm.

Excited state kinetics of TiO₂ - nanoparticles

Photoluminescence lifetime measurement was carried out using time correlated single photon counting (TCSPC) technique and the samples were excited at 330nm. The measurements were made on solutions with a uniform concentration of 100µg /ml of TiO₂ - nanoparticles in solvents of different polarity. Totally seven solvents of different polarity ranging from double distilled water to n-octanol were used. Each observed decay curve is a combination of three exponential curves for the TiO₂ nanoparticles (dried at 120°C) in different solvent environments. The lifetimes and the respective relative amplitudes and average lifetime are shown in Table.1. The relative amplitude indicates the relative abundance of different sizes of grains. Since the relative amplitude of one species is dominating (above 80%) there is no much variation in the distribution of sizes. This is also supported by the XRD results and HR-TEM images (Fig 5& 9). Fig.4. shows the fluorescence spectra of TiO₂ -NPs in different solvents ranging from water to alcohols and there is shift in the emission maxima, hence the solvent polarity might play a significant role in the emission properties of TiO₂ -NPs. Fig.4a-g show the fluorescence decay curves of TiO₂-NPs in the above said solvents.

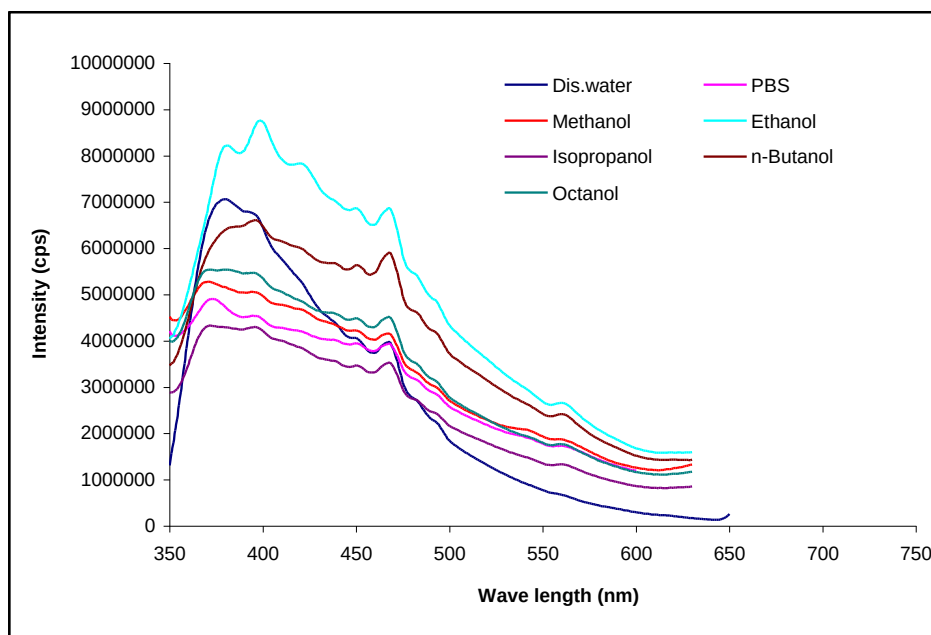
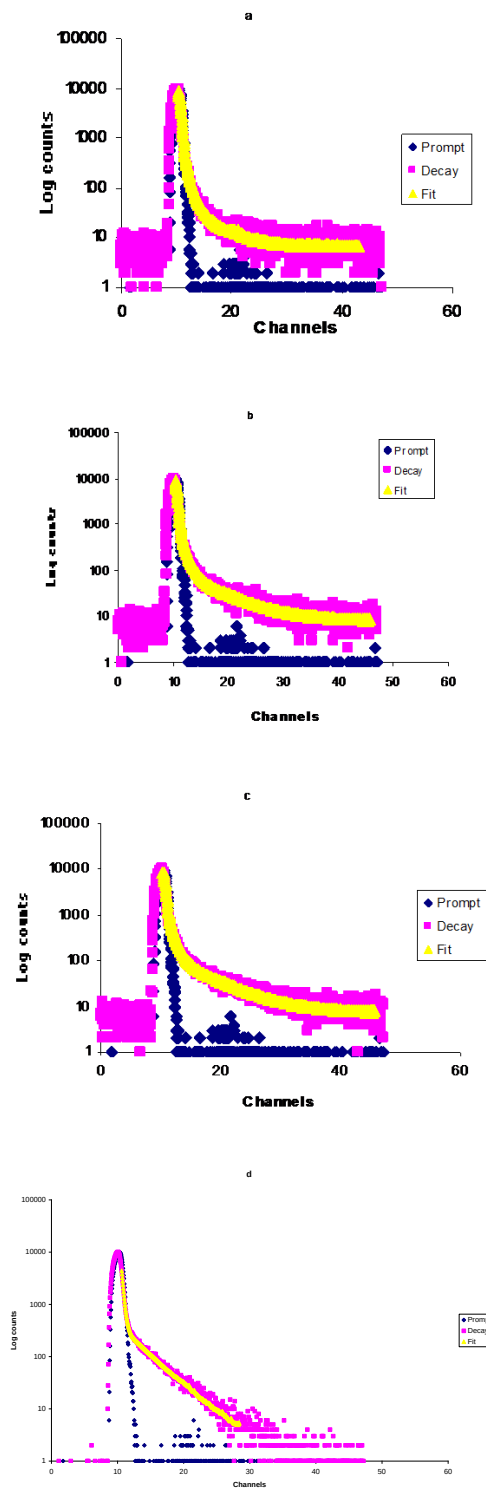


Fig. 4 Fluorescence spectra of TiO₂-NPs in different solvents excited at 330nm

Table 1 Fluorescence lifetimes of TiO₂-NPs in different solvents

Solvent	Viscosity	Dielectric constant	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_{avg} Fluorescence lifetime(ns)
Double distilled water	0.89	78.54	13.11	9.81	11.75	11.56
PBS	-	-	13.07	14.77	42.58	23.47
Methanol	0.544	32.63	11.45	16.24	53.42	27.04
Ethanol	1.094	24.30	12.46	16.89	71.74	33.70
iso-Propanol	2.038	18.30	14.24	17.29	21.52	17.68
n-Butanol	2.544	17.84	13.53	6.82	25.86	19.82
n-Octanol	7.288	10.30	14.44	16.48	28.55	15.41



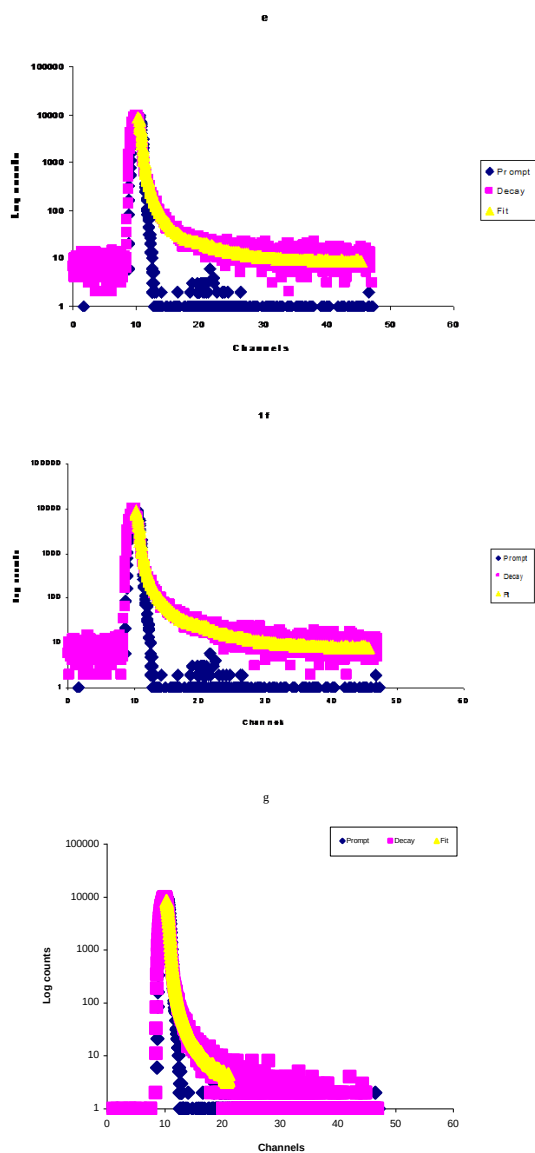


Fig.4 Fluorescence decay curves of TiO₂ -NPs in a) water b) PBS c) methanol d) ethanol e) isopropanol f) n-butanol g) n-octanol

A number of earlier reports have examined the influence of the solvent environment on xanthene dye photophysics [21] but almost no such study was reported using TiO_2 nanoparticles. Hence in the present study the influence of solvent environment was examined on the fluorescence lifetimes of TiO_2 nanoparticles which would provide important certification and data for future reference. In xanthene dyes shortest lifetime was observed in water and increased along the series of higher alcohols [22]. Unlike dyes, in the case of semiconductor nanoparticles whose optical properties are entirely different, a variation of fluorescence lifetime was observed with dielectric constant of the solvent. The lifetime increases from water to ethanol but it decreases in iso-propanol and then increases slightly even though the dielectric constant decreases from water to higher alcohols. This discrepancy may be due to the use of colloidal suspension of the semiconductor nanoparticles since they are not completely soluble in alcohols.

XRD analysis of TiO_2 -NPs

The XRD pattern of TiO_2 -NPs, calcined at 120°C , is shown in Fig.5. The patterns are very well comparable to anatase form of TiO_2 -NPs reported in the literature [20]. The phase is completely free of rutile form. The signals appear to be rather broad due to the nanosize. The five characteristic peaks at (2θ) about 25.63° , 38.23° , 48.08° , 54.4° and 62.9° correspond to anatase form of TiO_2 (JCPDS # 89-4921) identified to originate from (101), (112), (200), (105), (220) planes respectively. The XRD patterns could be indexed to Tetragonal structure with body centered cubic lattice with cell parameters $a = 3.777\text{\AA}$ & $c = 9.501\text{\AA}$. The space group was found to be $14_1/\text{amd}$ (141). The average crystal grain size calculated using the Scherrer equation was found to be 12.32nm which is well in accordance with HRTEM photographs of TiO_2 -NPs (Fig.9). The XRD patterns are very well comparable with that reported in literature [20].

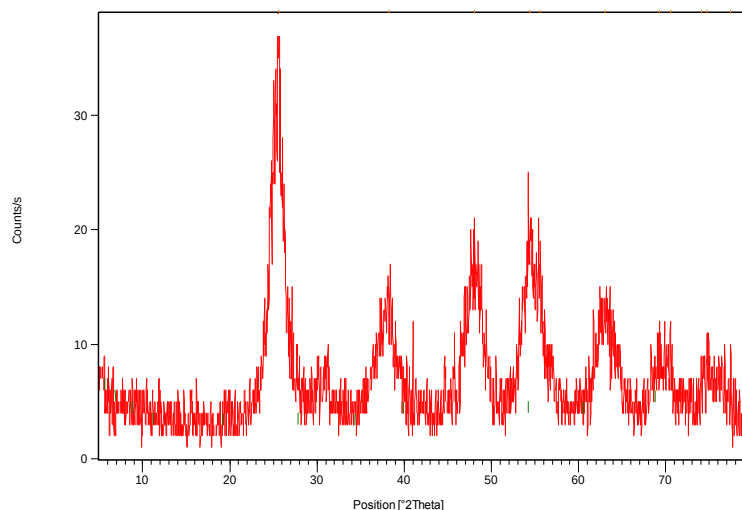


Fig.5 X-Ray diffraction patterns of TiO₂ –NPs dried at 120°C

FTIR Spectroscopy

The TiO₂-NPs were characterized by taking FTIR spectrum of the air dried sample. The spectrum was recorded in KBr matrix. The FTIR spectrum of TiO₂-NPs is shown in Fig.6. The bands centered at 3284 cm⁻¹ and 1618 cm⁻¹ are characteristic of ν-OH stretching and δ-H₂O bending modes respectively. The Ti-O vibration appears as a broad envelope between 1000-400 cm⁻¹. Since there is no C-H vibration band from 3000 to 2700 cm⁻¹, the NPs are free of adsorbed organics. We can thus consider that the hydrolysis reaction might lead to Ti (OH)₄ n.H₂O precursor. The spectrum is very well comparable with that given by Guillaume Sudant et. al, [23].

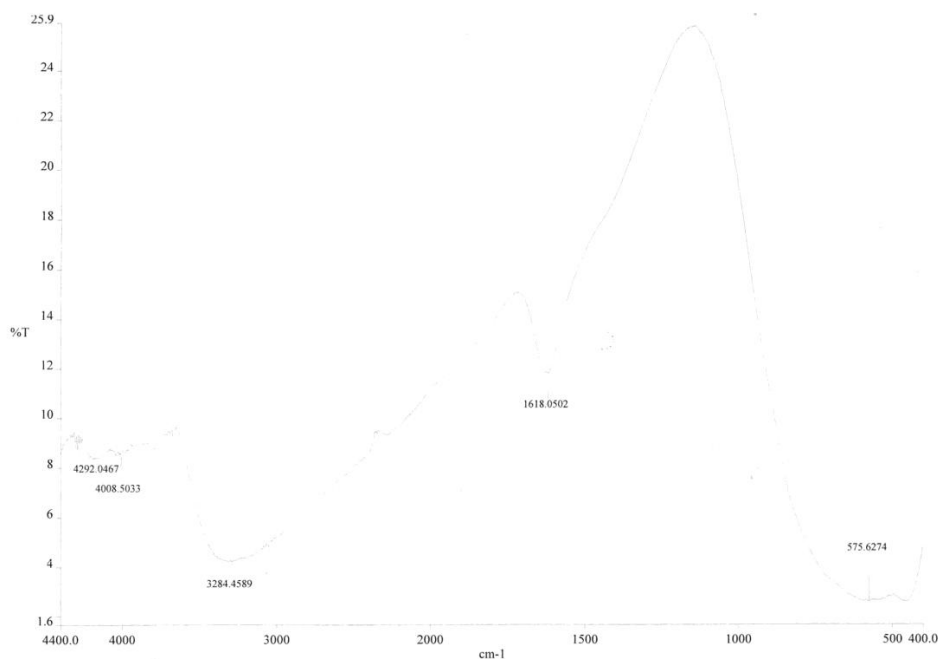


Fig.6 FTIR spectrum of air dried TiO₂ -NPs

Thermo gravimetric analysis (TGA)

The TGA of TiO₂ NPs was carried out between 50 and 900°C. The TGA trace of TiO₂-NPs is shown in Fig.7. There is a weight loss between 50-150°C due to desorption of adsorbed water. There is a gradual increase in weight loss from 300 to about 550°C. It is assigned to condensation of adjacent defective-OH groupings on the crystal surface. It also supports FTIR results described above. Again there is another weight loss from 610-750°C. It is ascribed to condensation of difficultly condensable defective-OH groupings. Thus the thermogravimetric study of TiO₂-NPs shows a monotonous mass decrease with temperature and is well comparable with that reported in the literature [23].

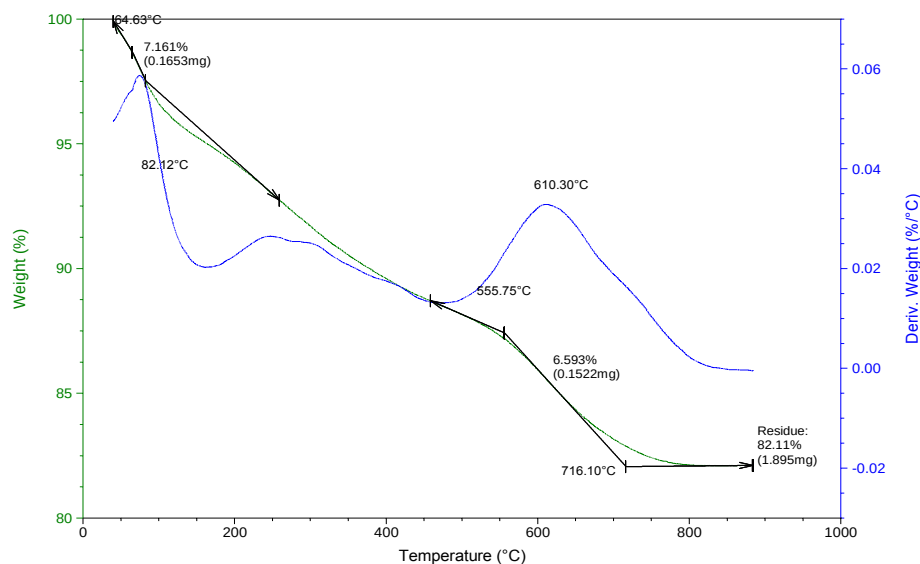


Fig.7 TGA thermogram of air dried TiO₂ -NPs

Differential scanning calorimetry (DSC)

The DSC analysis was carried out in the temperature range 50 to 400°C. There is an endothermic peak between 50 and 125°C (Fig.8). This peak coincides with the first major weight loss in the TGA trace shown in Fig.7. It is due to loss of adsorbed water. There is also a broad endotherm above 250°C. It is assigned to condensation of the adjacent defective Ti-OH groupings on the surface. It also supports the results of TGA as discussed above.

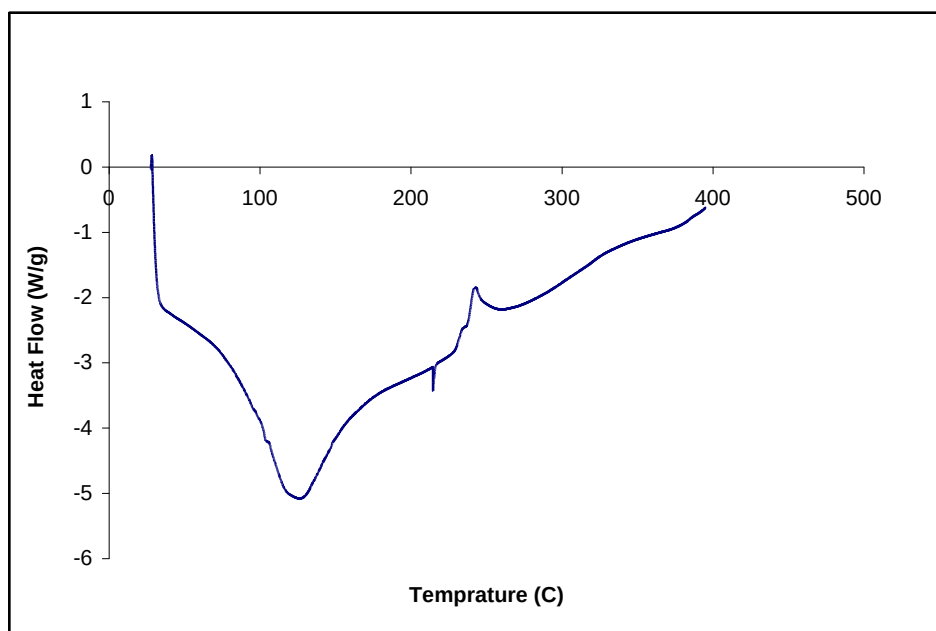


Fig.8 DSC Thermogram of air dried TiO₂ -NPs

HR-TEM pictures of TiO₂-NPs

The microstructure of TiO₂-NPs is illustrated by the HRTEM images. The HRTEM images of TiO₂-NPs are shown in Fig 9(a-c). All the images clearly illustrate particles of nearly uniform size. The particles are aggregated but the boundary between them is clearly seen. Fig.9(b&c) show the lattice planes of anatase crystallite of TiO₂-NPs. The particle size varies from 5-10nm. The size is also comparable to that derived by Scherrer equation in the XRD analysis.

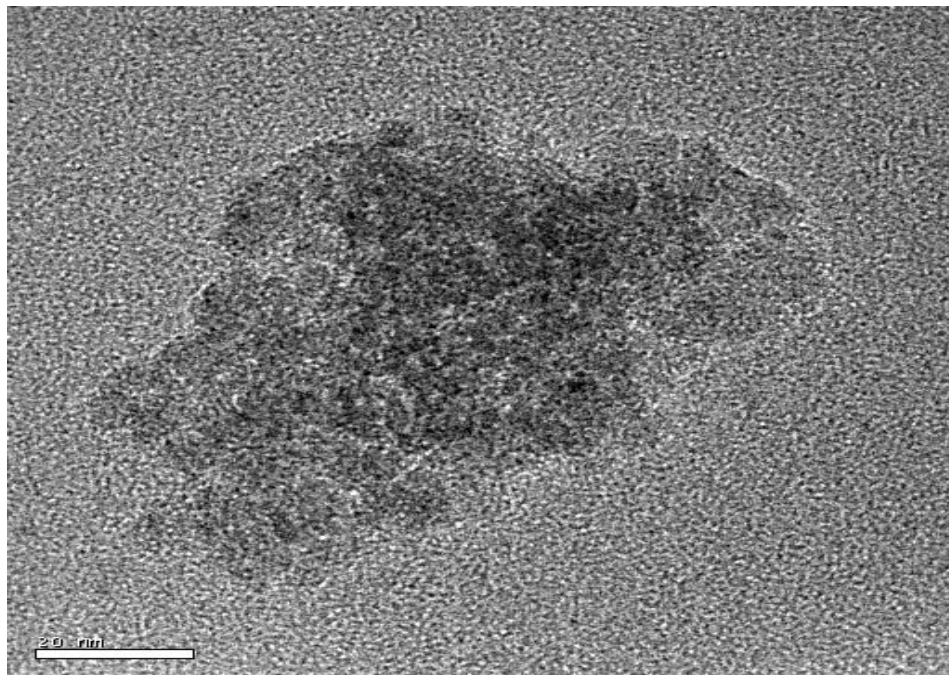


Fig.9(a) High resolution transmission electronic microscopy picture of TiO_2 -NPs.

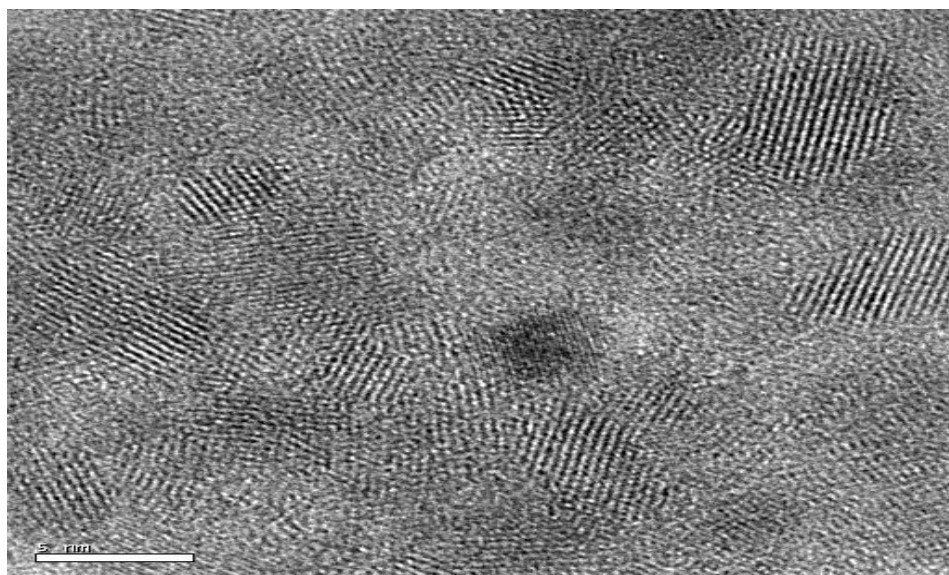


Fig.9(b) High resolution transmission electronic microscopy picture of TiO_2 -NPs

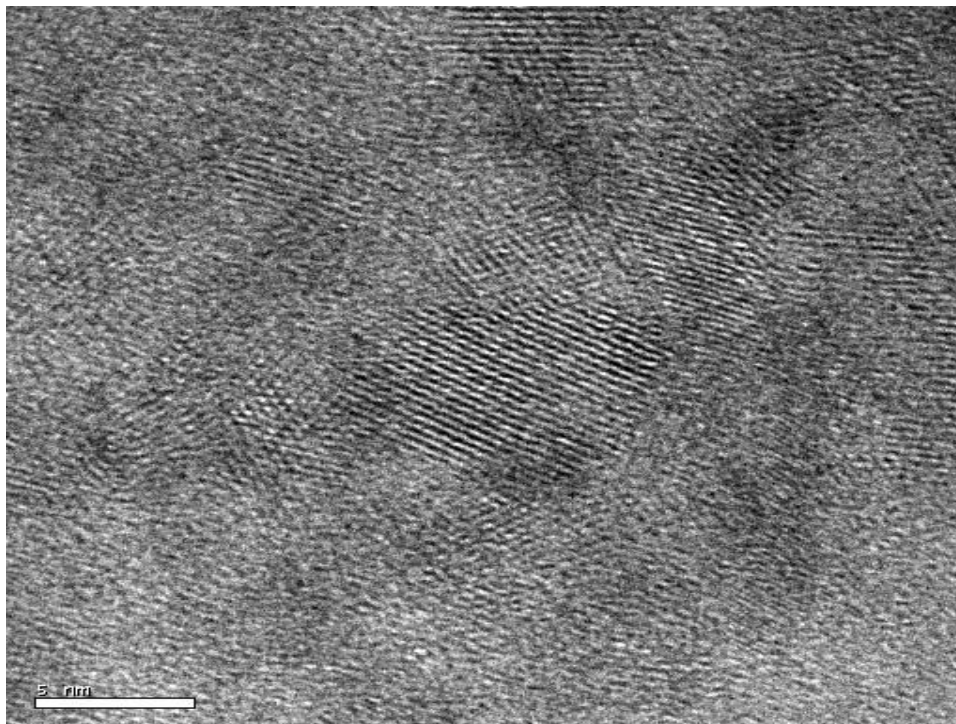


Fig.9(c) High resolution transmission electronic microscopy picture of TiO₂-NPs

Conclusion

Nanosize TiO₂ particles (below 10nm) were prepared, characterized and used as a nano-photosensitizer in the place of organic dyes in photodynamic therapy. In the present study human erythrocyte cells were effectively killed by photoexcited TiO₂-NPs. The photokilling of erythrocytes was considerably affected by the concentration of TiO₂-NPs and light dose. TiO₂-NPs. The study concludes that light irradiated TiO₂-NPs can be a convenient substitute for the conventional photosensitizers such as organic dyes.

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