Electronic Supplementary Material (ESI) for Toxicology Research

A comparative cytotoxicity study of TiO_2 Nanoparticles under Light and Dark Conditions at Low Exposure Concentrations

SUPPLEMENTARY INFORMATION

Swayamprava Dalai, Sunandan Pakrashi, Suresh Kumar R. S., N. Chandrasekaran, Amitava Mukherjee*

Centre for Nanobiotechnology

VIT- University

Vellore-632014

*** Corresponding author.**

Dr. Amitava Mukherjee

Professor & Assisstant Director

Centre for Nanobiotechnology

VIT University

Vellore-632014

Email: amit.mookerjea@gmail.com

UV-Vis Absorbance Spectroscopy of TiO_2 NPs

Another characterization process of TiO_2 NPs includes UV-Vis absorbance measurement. TiO2 NP dispersed in lake water gave a near-UV absorbance peak at 336nm. Hasan et al. measured the maximum absorption wavelength of synthesized TiO_2 NPs at 337 nm.[1] The dynamic aggregation process of TiO_2 NPs was monitored using a UV-Vis spectrophotometer at 378 nm, analyzing the sedimentation of the NPs in different water matrices.[2]

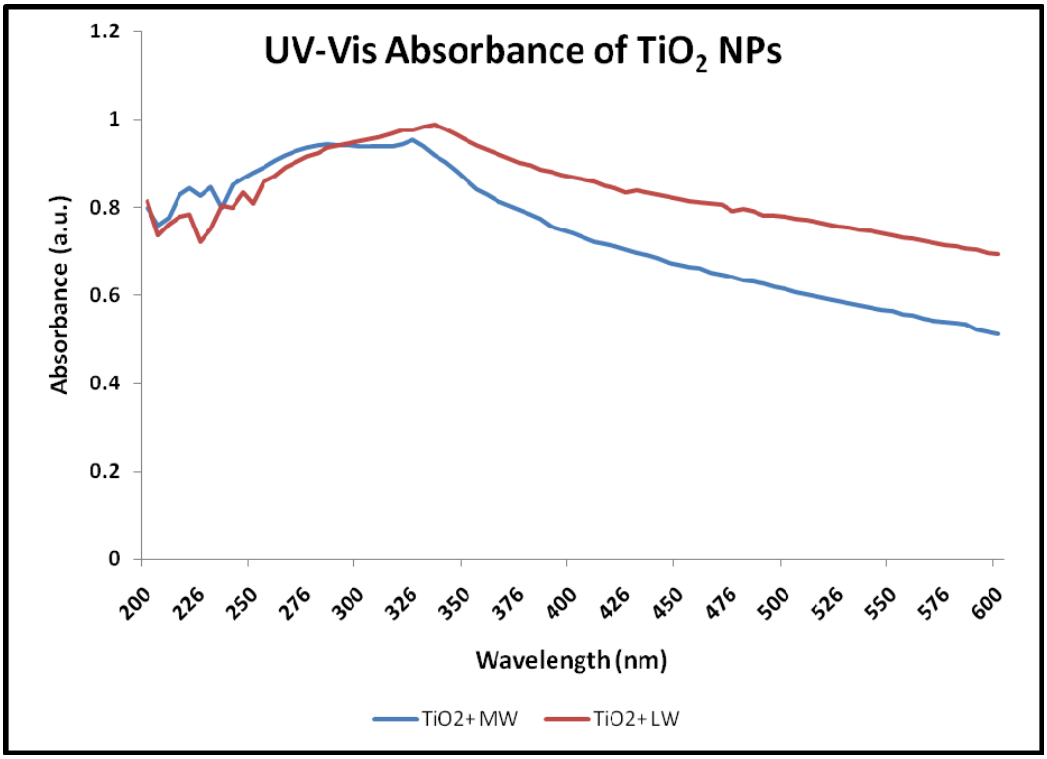


Figure S1: The UV-Vis Spectroscopy of TiO_2 NPs dispersed in Millipore water (MW) and Lake water (LW)

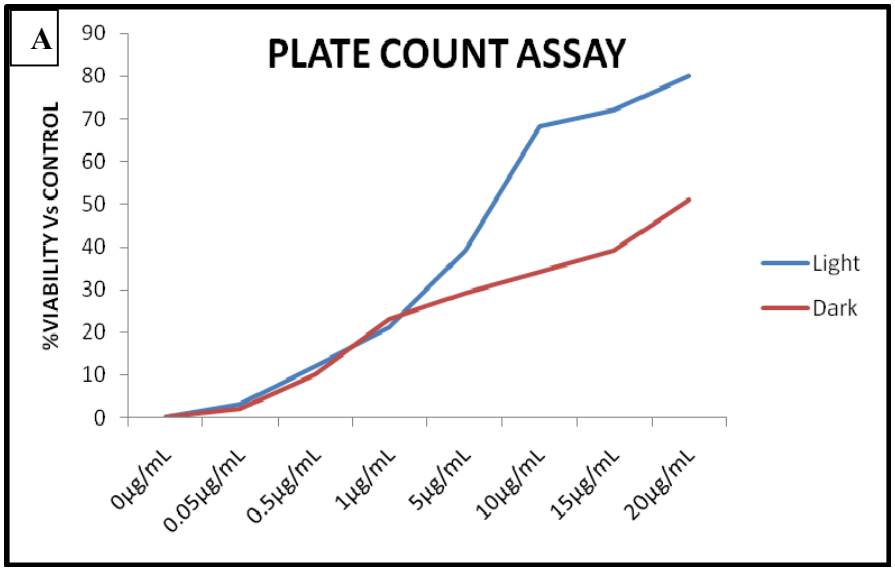
MTT Assay

MTT assay was performed following the standard protocol.[3] After the interaction period, 20 µl of MTT dye (5mg/mL) was added to 500 µl of sample in reaction vials. The vials were incubated

with proper mixing in dark (30ºC, 4 h). Two hundred microliters of DMSO was added to each vial to solubilize the formazan crystals. Samples were then loaded on a 96-well microtiter plate and absorbance was recorded at 570nm with a reference wavelength of 690nm (ELISA plate reader, Biotek, Powerwave XS2). Interference shown by TiO2 NPs with MTT dye was subtracted from the results. The decrease in bacterial viability in test samples was calculated with respect to control.

EC_{50} Calculation

Bacterial cells were interacted with different concentrations of NPs (0.05, 0.5, 1.0, 5.0, 10.0, 15.0 and 20.0 µg/mL) under light and dark conditions. EC_{50} value was calculated using plate count assay and MTT assay (Figure: S2). The EPA Probit Analysis Program, Version 1.5 was used for calculations. Though lower concentrations of NPs showed comparative toxicity towards bacteria, with increase in concentration the photocatalytic activity of TiO_2 NPs became more prominent.



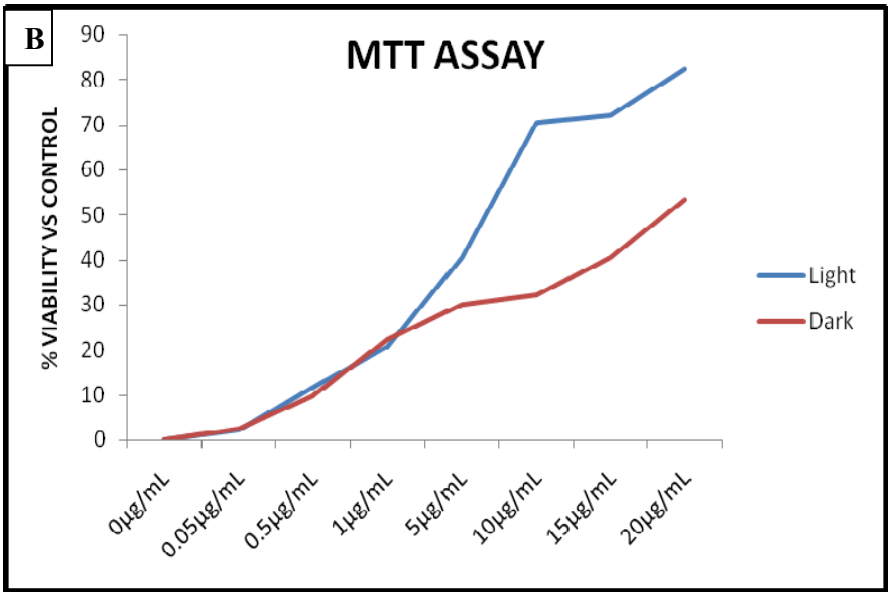


Figure S2: Percentage reduction in cell viability compared to control (A): Plate Count; (B): MTT assay

XRD

X-ray diffraction analysis of titanium dioxide nanopowder showed seven diffraction peaks at 25.166º, 37.69º, 47.85º, 54.89º, 62.4º, 70.03º and 75.22º respectively. Peaks at 25.166º and 47.85º were found to be distinctive. The XRD pattern confirmed that TiO_2 NPs had anatase phase predominantly (Figure: S3). Researchers have shown that the nano-TiO2 NPs in the anatase phase were capable of producing greater ROS than that in the rutile phase using X-ray absorption fine structure spectrometry (XAFS).[4]

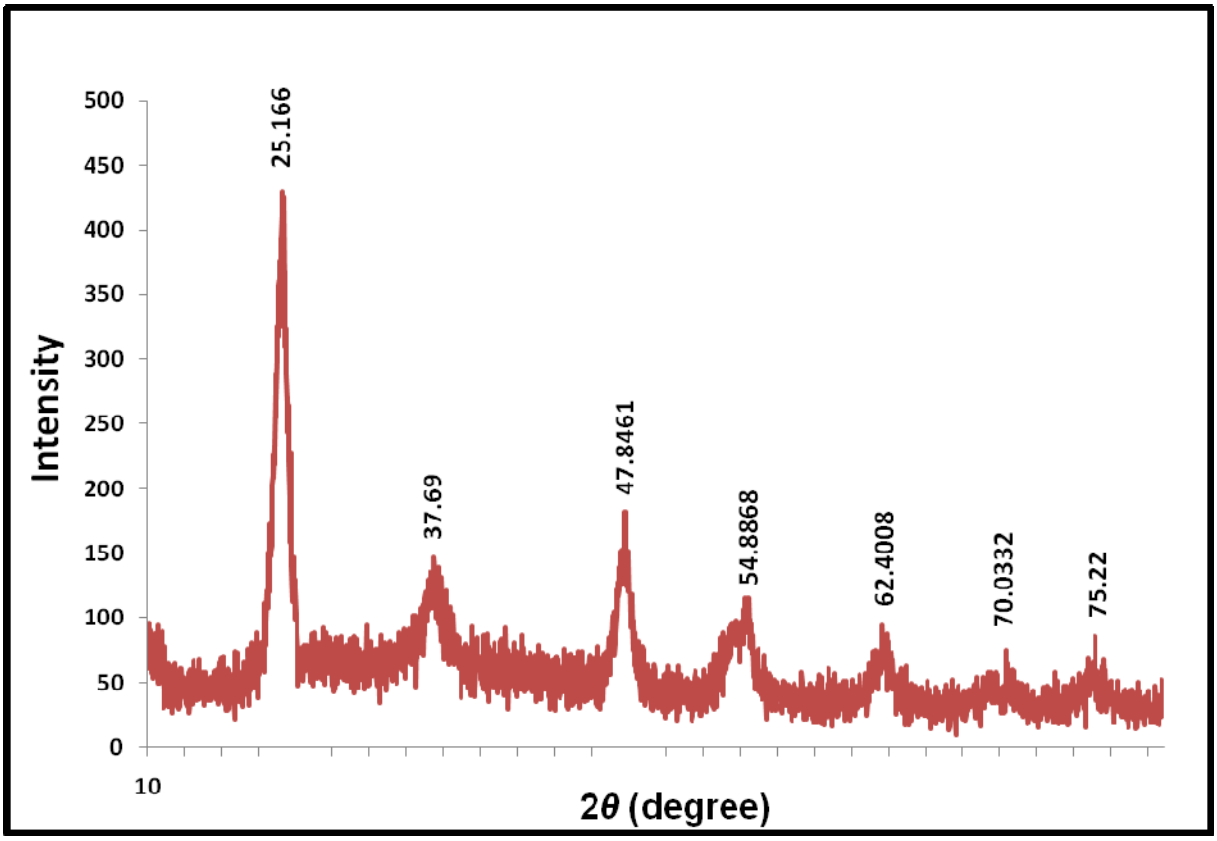


Figure S3: XRD spectra of as-received TiO_2 NPs

References:

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2. A. A. Keller, H. Wang, D. Zhou, H. S. Lenihan, G. Cherr, B. J. Cardinal, R. Miller and Z. Ji, *Environ. Sci. Technol.*, 2010, **44**, 1962–1967.
3. T. Mossman, *J. Immunol. Methods*, 1983, **65**, 55-63.
4. C. Jin, Y. Tang, F. G. Yang, X. L. Li, S. Xu, X. Y. Fan, Y. Huang and Y. J. Yang, Biol. *Trace Elem. Res.*, 2011, **141**, 3–15.