CHAPTER VI
SUMMARY AND SCOPE OF FUTURE WORK

6.1 Summary
6.2 Future outlook
SUMMARY

6.1 Titanium dioxide (TiO₂) has been the most intensively investigated binary transition metal oxide in the past four decades. The apparent merits of TiO₂ including elemental abundance, good chemical and thermal stability, nontoxicity, easy synthesis, flexibility to get tuned in diverse morphologies etc. have attracted much research interest. In this work, we have synthesised five nanostructured titanium dioxide, namely multishelled titania hollow nanospheroids, mesoporous assembled titania nanocuboids, titania nanotubes, titania nanobelts and titania nanoparticle adopting various synthesis procedures.

The first chapter begins with advancements in nanotechnology applied to metal oxides, with special emphasis on titanium dioxide. This includes a brief history of photocatalysis and also explains the importance of titania as photocatalyst. Details on its structure, different methods of preparation as well as some relevant applications are addressed here. The necessity for modification of titanium dioxide, various strategies adopted in this direction to enhance the application potential and merits of these modification procedures are also highlighted in this section citing appropriate literature. An outline of present research work was also incorporated.

Chapter 2 contains a detailed description of chemicals/reagents used, the experimental conditions for the preparation of various titania
nanostructures and its modifications techniques alongwith catalyst notations. Here we also discussed the theory and experimental basis behind various characterisation techniques adopted here. The experimental set up for the measure of photocatalytic activity studies and biological studies is well discussed in this part.

The third chapter summarised the results of various characterisation techniques of these synthesised titania nanostructures. Various microscopic analysis confirmed the size and morphology of the synthesised nanostructures. Crystal phases were analysed by XRD and Raman spectra. Mesoporous nature was further confirmed with low angle XRD and nitrogen adsorption desorption isotherms. Nitrogen adsorption desorption studies light on the porosity aspects, which are important in tuning the activity. UV- Visible diffuse reflectance spectrum showed that absorption of the synthesised nanostructures are in the UV-range. Various crystal planes are observed from the high resolution transmission electron microscopic images. Crystalline/amorphous nature was revealed from the SAED images. The dynamic light scattering analysis provided an idea about particle size distributions, zetapotential and polydispersity index of the synthesised systems. This chapter also included the formation mechanism of various nanostructures prepared, with appropriate literature status.

The fourth chapter deals with the photocatalytic applications of titania nanostructures. This chapter is divided into two sections, Section A and Section B. First part of Section A described the UV light assisted photocatalytic degradation of organic pollutant taking methylene blue
Conclusions and Scope of Future Work

Dye using the synthesised TiO$_2$ photocatalyst nanostructures. All the prepared systems showed good photocatalytic activity towards dye degradation while multishelled titania hollow nanospheroids was observed to be the superior one, completely degrading within 35 minutes. The highlight of this study is that the photocatalytic activity is achieved with a catalyst dosage which is commendable low compared to many previous reports. Much superior dye degradation efficiency was noticed for multishelled system where the catalyst amounts only to $\frac{1}{4}$th of the value reported under identical experimental conditions. Mineralisation studies by COD analysis were also conducted and it followed the same order. Commercially available TiO$_2$ was tested for dye degradation and is observed to be having the lowest value. The higher activity of multilayered structure is due to the multiple reflections of UV light when falls on the structure due to its sphere in sphere nature which effectively utilises the light to boost up the photocatalytic process. Mesoporous assembled titania nanocuboids is exhibiting good photocatalytic activities due to its high surface area and exposed active facets. The biphasic structure and reasonable surface area value make titania nanotubes a better photocatalyst than Titania nanobelt. Among the synthesised systems, titania nanoparticle shows the lower activity owing to the presence of rutile phase and lower surface area of the systems, towards dye degradation. Second part of section A comprises the photocatalytic antibacterial and antifungal studies of the prepared nanostructures. E.Coli, gram negative bacteria and the fungus Candida albicans were chosen as the microbes for the experiment. All the Titanium dioxide systems exhibited antimicrobial activity due to its strong oxidizing property when exposed to light. With a fixed time course of light radiation, multiwalled titania hollow nanospheroids exhibited maximum inhibition of gram negative bacteria E.Coli with
minimal catalyst concentration. Further the antifungal activity was evaluated by Optical Density method and the multiwalled titania hollow nanospheroids itself show the minimum number of viable cells with a predetermined concentration and fixed time course of light irradiation. The commendable activity to remove organic pollutant and pathogenic organisms highlights the applicability of multishelled system as a self cleaning material in walls and tiles of house, kitchen, hospitals, etc. Hence contact angle measurement was also performed to examine the hydrophilicity under UV radiation and it was observed that the contact angle was decreased to a considerable extent showing the hydrophilic nature of titania based systems upon UV irradiation.

Section B of the fourth chapter describes the characterisation and photocatalytic water splitting ability of cocatalysts loaded mesoporous assembled titania nanocuboids and titania nanotubes. The cocatalysts, copper and silver were loaded using photodeposition method. Excellent candidature of these systems was proved by visible light assisted photocatalytic removal of methylene blue dye. Hydrogen production by water splitting reactions in visible light emphasised their visible light response. The cocatalyst modified systems were found to be effective in reducing the charge carrier recombination as evident from the photoluminiscant spectra. Among the synthesised systems, the activity of copper decorated mesoporous assembled structures was found to be much higher which produced 16673.2μmoles/g of Hydrogen. Based on the results we can conclude that copper loaded titania nanostructures are cost-effective and efficient catalysts in the realm of photocatalytic water splitting.

The fifth chapter deals with the invitro cytotoxic studies of the synthesised systems in human breast cancer cell lines [MCF-7]. The
order of IC$_{50}$ value being TNT $<$ MHS $<$ TNP $<$ TNB $<$ MT as per MTT Assay. Morphology analysis and DAPI images confirmed visible evidence for the unhealthy cells in the treated ones compared to the control cells. In order to utilise the porosities of the synthesised nanostructures, Encapsulation experiments were done using the drug, 5-Fluorouracil. All the drug loaded samples showed lower IC$_{50}$ values related to their parent systems due to the presence of cytotoxic drug. Drug uptake per milligram of drug the loaded sample were analysed by HPLC. Highest uptake was shown by mesoporous assembled structures and correspondingly its IC$_{50}$ value (30 µg/mL) was decreased to a considerable extent than its parent system, MT (50.01 µg/mL). The over expression of folate receptors on cancer cells are utilised to specifically kill the tumour tissues by modifying the systems with folic acid. IC$_{50}$ values were found to decrease to a considerable extent when compared to the mere drug loaded systems. Targeting effect of folic acid and the synergetic effect of two cytotoxic agents namely Titanium dioxide and 5-Fluorouracil. Thus this type of biomolecule-based nanostructures would be beneficial for smart multifunctional nanomedicine systems. As folate modified 5-FU incorporated mesoporous assembled titania nanocuboids showed lowest IC$_{50}$ value, further investigation on the cancer intracellular mechanism were done using the system. Finally we concluded that system triggered caspase-dependent and ROS-dependent apoptosis in MCF-7 cancer cells through mitochondria-mediated pathway. Such a system can be developed as a novel apoptosis inducer against cancer cells.
6.2 Future Scope

- Achievement of single crystalline multishelled titania hollow nanostructures of uniform size through the synthesized requires indepth study of the various reaction conditions and reagent concentrations. Investigation on the effect of reagent concentrations and nature of alkali on the size and morphology of this complex hollow structure can be pursued.

- The photocatalytic activity of multishelled titania hollow nanospheroids can be extended to the visible region by doping with appropriate metals, nonmetals etc.

- Hydrogen production by photocatalytic water splitting using copper cocatalyst loaded multishelled titania hollow nanospheroids and titania nanobelt can be monitored.

- Scope of nano titania for biomedical applications can be further increased if the oxide is made more biocompatible. This will also enable to perform in vivo studies using the system.

- 5Fluorouracil is reported to be stable against skin enzymes which make it a good candidate for transdermal delivery. Certainly nanocarriers could improve skin targeting, enhancing the drug's ability to reach and penetrate into tumor tissues. Besides nanocarriers reduce the skin irritation by avoiding the intimate contact of the drug with skin surface and also increase the drug stability. It has been concluded from the study that the titanium dioxide nanostructures especially mesoporous
assembled titania entrapped with anticancer drug has significant cytotoxic effects. We have developed a folic acid based gel which can entrap sufficient amount of drug and these 3 Dimensional network of course result in slow release of the drug. Hydrogels in general have got more attention in the drug delivery industry because of their tunable chemical and three-dimensional (3D) physical structure most attractive features are their high water content, biocompatibility and environment responsiveness. When nanoparticle entrapped anticancer drug was dispersed in this gel, it can effectively release drug in a sustained manner. It has been proven that Folic acid is a good candidate for topical micronutrient delivery as its bioactive forms is useful in the maintenance of genomic integrity via enhancement of DNA synthesis, DNA repair, and maintenance of epigenetic regulation. Folic acid has been reported to form supramolecular gel varying its composition with the ratio of DMSO to water solvent mixture. And this drug can entrap molecules which can be released in the medium. The prepared folic acid gel and its ability to encapsulate molecules are evident from figure shown below (Figure 1).
Figure 1. Folic acid based supramolecular gel [Left] and the gel encapsulated Rhodamine dye [Right]

The gel comprises the solvents water, dimethyl sulfoxide and DMSO. When applied to skin, it penetrates and will pass into the tissue. The release of 5-FU from titanium dioxide to the gel network imbibes the drug and thus enter into the skin. Actually, 5-FU cream and solution have been used to treat skin cancers in clinical settings. Therefore, the development of the Folate targeted 5-Fu loaded titania nanostructures as a topical therapeutic agent could be an achievable design for treatment of melanoma. However, pharmaceutics studies are needed to realize this Design.