CHAPTER - VII

7. Biosynthesis of SnO$_2$ Nanoparticles using *Nyctanthes arbor-tristis* Extract and their Application

7.1. Introduction

Green synthesis is the pathway to design, develop and implement the chemical products and processes that reduce or eliminate the use and generation of hazardous substances to human health and environment [1]. Low cost production of nanoparticles in bulk quantities entails continuous flow processing [2]. Often, the chemicals used in nanoparticles manufacture, and the resulting byproducts, are toxic and/or flammable. In this context, rapid, room-temperature and green processes are desirable to minimize capital, design and environmental costs [3]. Several recent reports have made significant progress towards this goal by using amino acids, vitamins, polysaccharides and extracts of bioorganisms for the synthesis of Ag and Au nanoparticles [4]. The use of environmentally benign materials like plant extract [5], bacteria [6], fungi [7] and enzymes [8] for the synthesis of metal nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications, where toxic chemicals are not used for the synthesis. But this kind of synthesis has not been exploited for many versatile metal oxides.

During the past decade, SnO$_2$ nanostructures have been one of the most important oxide nanostructures due to their properties and potential applications [9]. Small particle size or large specific surface area is essential for the high performance SnO$_2$ [10]. Concerning the other facile metal oxides, the SnO$_2$ material is the most widely used sensitive layer for the chemical sensors
due to its wide band gap ($E_g$) of 3.6 eV at 300 K [11, 12]. The elements of SnO$_2$ such as particle size, shape, surface/volume ratio, and porosity which is well known for its gas sensing character to detect NO, NO$_2$, CO, H$_2$S and C$_2$H$_5$OH can be dramatically altered by the morphological and microstructural features of SnO$_2$ [13-15]. Various chemical synthesis methods have been reported for the preparation of SnO$_2$ nanoparticles with large surface area. Most of the studies have introduced the different precursors such as tin alkoxide and other tin salts for the fabrication of SnO$_2$ spherical-like nanoparticles [16-18]. To prepare powders with large specific surface areas, an alcoholic solvent is preferred to water because of its lower surface tension so that a “loose” powder can be obtained from collapsing of gel structure which will create environmental problems.

On the other hand, textiles, being the chiefly used product by mankind, efforts are being made to use them to safeguard our health. For this purpose, antimicrobial properties are being imparted to textile materials by chemically or physically incorporating various inorganic antibacterial agents on fibers or fabrics [19]. With the emergence of nanotechnology, material scientists have focused their attention on immobilizing metal nanoparticles like silver, copper etc on fibers because these metal based nanoparticles show excellent biocidal action but the high cost of these metals has limited their use as antibacterial agents on industrial basis [20-21]. Hence extremely promising agents for functionalisation are nanoparticles of metal oxides, in particular titanium dioxide, tin oxide and zinc oxide [22-24]. SnO$_2$ shows high absorption of UV radiation, antibacterial and antistatic properties, is a very interesting modifier used in the textile industry due to its prospective use for the production of safety garments and all kinds of fabrics for the construction [25]. SnO$_2$ has also attracted widely as antimicrobial agent in textile field
due to its higher photocatalytic efficiency, low cost, easy availability and unique physical and chemical properties [26].

Herein, the present work reports on the synthesis of tin oxide nanoparticles by the water extract of *Nyctanthes arbor-tristis* using tin tetrachloride as a metal salt precursor. The prepared SnO$_2$ nanoparticles were applied on cotton fabric for the antimicrobial activity evaluation which is shown in Fig. 7.1. *Nyctanthes arbor-tristis*, an Indian medicinal herbal plant which has diverse applications due to its functional moieties like diterpenoid nyctanthin, flavonoids, anthocyanins, essential oil, nyctanthic acid, tannic acid, ascorbic acid, stearic acid, methyl salicylate, palmitic acid, iridoid, phenylpropanoid etc [27-30]. This method aims to develop a very cheap, simple synthetic procedure so as to obtain the SnO$_2$ with low nanometer scale. The particles have been characterized by means of XRD, SEM, TEM, UV and FT-IR techniques. To the best of our knowledge, this is the first report for the rapid, room temperature and size-controlled SnO$_2$ nanoparticles protocol using a green reagent and their antimicrobial application on textiles.
Figure 7.1: Schematic representation of biosynthesis of SnO₂ nanoparticles using *Nyctanthes arbor-tristis* extract and their application
7.2. Results and Discussion

7.2.1. TG/DTA of the as-prepared sample

The TGA and DTA curve of as-prepared sample is shown in Fig. 7.2.1(a) & Fig. 7.2.1(b) respectively. TGA curve shows three different stages of weight loss in the temperature ranges of 40°C to 300°C, 300°C to 450°C and 450°C to 800°C. Weight loss of about 20% in the first step can be attributed to the removal of water molecules and decomposition of biomolecules of Nyctanthes extract adsorbed on the surface of the precursor. A steep weight loss in the second stage of about 25% can be attributed to the crystallization of the hydrated and amorphous as-prepared sample to crystalline rutile SnO$_2$ [31]. A minimum weight loss of about 5% occurred above 450°C can be due to the oxidation of residue carbon. The total weight loss of the as-prepared sample by the heat treatment between room temperature to 800°C is 50%. The remaining residual content percentage is 50% after the thermal analysis process.

To study the processes occurring during heat treatment, DTA study were carried out for the as-prepared sample. An endothermic peak appeared below 100°C may be due to the elimination of water molecules. A broad and expansive exothermic appeared with a maximum of 425°C may be due to the decomposition of organic biomatters with the progressive transformation of amorphous precursor i.e. tin hydroxide to crystalline SnO$_2$. Moreover, a big exothermic peak may be caused by the ordering of the irregularly distributed atoms and the integrity of the crystals [32, 33]. Hence the heat treatment of about 450°C is required for the conversion of amorphous tin hydroxide to crystalline SnO$_2$ nanoparticles.
7.2.2. XRD analysis of SnO$_2$ nanoparticles

The phase structure and crystallinity of as-prepared and calcinated SnO$_2$ nanoparticles can be characterized from its XRD pattern. As-prepared sample has no crystalline state which could be attributed from the amorphous behavior of its XRD pattern in Fig. 7.2.2(a). Crystalline nature of calcinated SnO$_2$ nanoparticles was confirmed from the sharp peaks of its XRD pattern in Fig. 7.2.2(b). All the diffraction lines are assigned to tetragonal crystalline phases of tin oxide which is consistent with the standard JCPDS data file 41-1445. The XRD pattern clearly indicates that no other peaks characterized for Sn or any other Sn based salts implying that the as-prepared sample have been completely decomposed during the calcination process. The diffraction peaks are markedly broadened due to the smaller sized crystalline particles [34].

All diffraction peaks observed in the 2θ range of 20°-80° can be indexed to the reflections of tetragonal rutile SnO$_2$. By using Debye-Scherer’s equation i.e. $D = \frac{0.89\lambda}{\beta\cos\theta}$, the crystallite size calculated from the maximum intense plane was 6.4 nm. Table 7.2.2 depicts that the crystallite size and other crystallinity parameters of SnO$_2$ nanoparticles for the crystallographic planes. The Bragg reflections appeared at 2θ value: 26.7°, 33.9°, 38.3°, 51.9°, 61.8° and 65.3° corresponds to the lattice planes of (110) (101) (200) (211) (220) and (301) respectively. The average crystallite size determined from all the diffraction peaks was 5.1 nm.

7.2.3. SEM analysis of SnO$_2$ nanoparticles

To investigate the surface morphology of individual SnO$_2$ nanoparticles, the sample was further subjected to the SEM analysis and their micrographs under different magnifications are shown in Fig. 7.2.3. SnO$_2$ nanoparticles appear in the form of spherical morphology with a
highly porous, foam-like structure under lower magnification, which may be due to long view of the particles, whereas the magnification transferring to higher range, the particles are in discrete definite shape with fine tiny agglomerated granules. The rate of hydrolysis was increased significantly by the combination of *Nyctanthes* extract with water, a commercial hydrolysing agent. A combination of nucleophilic attack and condensation hydrolysis cascade reactions produced low crystalline nanoparticles that display a tiny agglomeration in solution. Flavonoids and carotenoids compounds of natural extract saturated with tin hydroxide enabling rapid nucleation (faster induction time) that results in smaller particle size.

**7.2.4. EDX analysis of SnO$_2$ nanoparticles**

Structural identity of the formed biosynthetic product was further confirmed by EDX analysis. Appearance of tin and oxygen atom peaks alone in the EDX spectrum (Fig. 7.2.4) confirms the formation of tin oxide nanoparticles without any other impurities like tin salts. Moreover, complete decomposition of biological molecules of *Nyctanthes* extract of as-prepared sample also confirmed by the absence of any other peaks from the natural extract. Sn atoms are present in the EDX spectrum at 3.3 keV to 3.6 keV and O atom at 0.46 keV.

**7.2.5. AFM analysis of SnO$_2$ nanoparticles**

Exact size distribution of calcined SnO$_2$ nanoparticles was further confirmed by AFM analysis. AFM images were scanned in an area of 10 μm × 10 μm. Size of about 40-60 nm with agglomerated porous structured particles was observed in the topographic and 3D AFM image in Fig. 7.2.5(a) & Fig. 7.2.5(b) respectively. Maximum height distribution at 53 nm and width distribution at 38 nm is observed from the height and width distribution line profile spectrum
which is displayed in Fig. 7.2.5(c) & Fig. 7.2.5(d) respectively. Therefore *Nyctanthes arbor-tristis* extract produced the nanosized SnO$_2$ particles. AFM results are consistent with SEM reports thereby adding evidence for its structural information.

### 7.2.6. TEM analysis of SnO$_2$ nanoparticles

The panoramic morphology and size of the biosynthesized SnO$_2$ nanoparticles were further confirmed by adopting TEM analysis. TEM micrographs of SnO$_2$ nanoparticles and their corresponding SAED pattern are shown in Fig. 7.2.6. Lower and higher magnified TEM images of SnO$_2$ nanoparticles in Fig. 7.2.6(a) & 7.2.6(b) exhibit the structure of porous structure where it is difficult to predict the size of the particles individually. The observed TEM result coincides well with the morphological details arrived from its SEM analysis. SAED pattern in Fig. 7.2.6(c) reveals its monodispersive nature with lesser crystallinity compared to other biosynthetic metal oxides. Lesser crystallinity coincides well with the crystalline data arrived from XRD analysis. The lesser crystallinity of particles could be predicted by the appearance of diffraction rings without any dark spots. The diffraction rings could be indexed to the lattice planes of (110) (101) (200) (211) (220) and (301) corresponding to tetragonal rutile tin oxide which is consistent with the XRD result.

### 7.2.7. UV-Vis analysis of SnO$_2$ nanoparticles

Fig. 7.2.7(a) shows the optical absorption spectrum of plant mediated SnO$_2$ nanoparticles. Absorption peak at 314 nm is exhibited for SnO$_2$ nanoparticles which is almost consistent with bulk SnO$_2$ absorption value. Since the size of SnO$_2$ is nearly in nanosize, blue shift of the peak is observed in its UV-Vis spectrum which may arise due to quantum confinement effect. Hence the
energy is too small to bridge the band gap of these particles [35]. From the UV-Visible spectrum, the band gap plot in Fig. 7.2.7(b) was drawn between $(\alpha h\nu)^2$ vs photon energy $(h\nu)$ to estimate the band gap of prepared SnO$_2$ nanoparticles. The observed band gap value is 3.86 eV which is higher than that of 3.6 eV band gap of bulk rutile SnO$_2$. This quantum confinement can be attributed to the higher surface area of SnO$_2$ nanoparticles.

7.2.8. FT-IR analysis of SnO$_2$ nanoparticles

The interaction of surface functional biological groups with the metal can be studied by the valuable analytical tool FT-IR. FT-IR absorption spectra of aqueous extract of Nyctanthes arbor-tristis, as-prepared sample and calcinated SnO$_2$ are shown in Fig. 7.2.8. This technique was applied to determine the groups that were present in the Nyctanthes extract and to possibly predict their role for the synthesis of tin oxide nanostructures. It is observed that the broad band at 3445 cm$^{-1}$ can be related to hydrogen bonded -OH stretching vibrations possibly from phenolic groups of Nyctanthes extract [Fig. 7.2.8(a)]. The methylene group –CH stretching vibration is appeared as a small peak at 2921 cm$^{-1}$. Presence of amide groups of carbonyl compounds in Nyctanthes extract can be predicted by the absorption band at 1634 cm$^{-1}$ which is characteristic of –CO stretching vibration. Absorption peak at 1392 cm$^{-1}$ might correspond to –NH deformation of amino acids or –OH deformation frequency possibly from phenolic groups. The peaks at 1258 cm$^{-1}$ and 1058 cm$^{-1}$ could be attributed to $1^\circ$ or $2^\circ$ –OH in-plane bending vibration of alcoholic groups and –OH stretching vibrations of phenolic groups or antisymmetric stretching band of C–O–C groups of polysaccharides respectively. The peak appeared at 614cm$^{-1}$ is characteristic of –CH in-plane bending vibration.
After the reaction process of converting tin tetrachloride to tin hydroxide using Nyctanthes extract as hydrolytic agent, most of the peak corresponding to functional groups are slightly shifted with decreased intensity and a few disappeared are shown in Fig. 7.2.8(b). The peaks in the spectrum of as-prepared sample are shifted as follows: 3445 to 3442 cm\(^{-1}\), 1634 to 1640 cm\(^{-1}\), 1392 to 1396 cm\(^{-1}\), 1258 to 1240 cm\(^{-1}\) and 1058 to 1089 cm\(^{-1}\). The intensity of carbonyl groups are greatly decreased may be due to the coordination with metal ions to act as a nucleation site for nanoparticles formation. Appearance of new symmetric and asymmetric stretching vibrations of Sn-O-Sn bonds at 600-700 cm\(^{-1}\) in the FT-IR spectrum of the amorphous as-prepared sample confirms the role of bioorganic functional groups for the hydrolysis of tin tetrachloride salt [36]. In the FT-IR spectrum [(Fig. 7.2.8(c)] of SnO\(_2\) nanoparticles, there is no existence of characteristic peaks and bands for the functional groups such as hydroxyl, alkanes, C=C of benzene, aromatic amines and aliphatic amines (1040 cm\(^{-1}\) and 1053 cm\(^{-1}\)) corresponds to Nyctanthes extract [37]. Thereby confirms the nonexistence of Nyctanthes extract in the SnO\(_2\) nanoparticles after calcination. FT-IR spectrum verifies the complete removal of Nyctanthes extract after calcinating the as-prepared sample to get the pure SnO\(_2\) nanoparticles. A strong peak at wave number 648 cm\(^{-1}\) is observed is characteristic of the Sn–O–Sn stretching mode in SnO\(_2\) [38]. This band is assigned to the surface-bridging oxide formed by condensation of adjacent surface hydroxyl groups and in a way confirms presence of SnO\(_2\).

7.2.9. Probable reason for the formation of SnO\(_2\) nanoparticles

It is interesting to note that Nyctanthes can act well as a capping agent [39, 40], due to the presence of water soluble compounds such as flavonoids and carotenoids [41, 42] which is responsible to ensue the nanoparticles formation. Hence it may be inferred that these
biomolecules are mainly responsible for capping and efficient stabilization. Flavonoids that are firstly absorbed on the surface of SnO$_2$ nanoparticles then the exposed surface planes that are not attached by flavonoids tend to combine to reduce the surface energy to form a tetragonal structure. Therefore the *Nyctanthes* extract was found to be a promising one for the synthesis of lower size tin oxide nanostructures. The proteins could bind with nanoparticles either through free amine groups or crystalline residues in the proteins. The carboxylic groups are known to coordinate with metal ions, which may act as a nucleation site for nanoparticle formation which was confirmed from FT-IR analysis [43]. After the heating process, the organic functional groups of *Nyctanthes* extract are evaporated due to their low thermal stability with the simultaneous production of stable SnO$_2$ nanoparticles (Fig. 7.2.9).

**Figure 7.2.9: Formation of SnO$_2$ nanoparticles by *Nyctanthes arbor-tristis* extract**

7.2.10. SEM analysis of SnO$_2$ nanoparticles treated cotton

Fig. 7.2.10(a) and Fig. 7.2.10(b) are the SEM images of untreated and SnO$_2$ nanoparticles treated cotton. The fiber surface of the untreated cotton is smooth and sleek while the treated cotton appears in an inconsecutive way with some aggregation due to the minimal particle size led self-aggregating phenomenon of nano SnO$_2$. Consecutive SnO$_2$ nanoparticles range in a dispersed way leads to regular smooth surface. No notable difference in the particle size is
observed after treatment with cotton, presumably due to the subsequent collapse of the nanoparticles pushing them close together [44].

### 7.2.11. EDX analysis of SnO\textsubscript{2} nanoparticles treated cotton

The elemental analysis of the SnO\textsubscript{2} nanoparticles was performed using Energy dispersive X-ray spectra on the scanning Electron Microscope. Figure 7.2.11 shows the EDX spectrum of untreated cotton and SnO\textsubscript{2} nanoparticles treated cotton. Untreated cotton has presented only its carbon and oxygen atoms in its EDX spectrum [Fig. 7.2.11(a)]. The peaks around 3-4 keV, 0.5 keV and 0.2 keV corresponds to the binding energies of tin, oxygen and carbon atoms in the EDX spectrum of SnO\textsubscript{2} nanoparticles treated cotton [Fig. 7.2.11(b)]. Throughout the scanning range of binding energies, no peak belonging to impurity was detected on the spectra. The results indicated that the reaction product was composed of high purity SnO\textsubscript{2} nanoparticles.

### 7.2.12. Antibacterial evaluation of SnO\textsubscript{2} nanoparticles and treated cotton

Antibacterial activity of the SnO\textsubscript{2} nanoparticles and SnO\textsubscript{2} nanoparticles treated cotton were investigated against two strains, \textit{S. aureus} (gram positive bacteria) and \textit{E. coli} (gram negative bacteria) by agar diffusion method. The measured zone of inhibition values from Fig. 7.2.12 developed depending upon the size of the SnO\textsubscript{2} nanoparticles formed by the assistance of natural extract and SnO\textsubscript{2} nanoparticles treated cotton are registered in Table 7.2.12. Zone of inhibition is the area in which the bacterial growth is stopped due to bacteriostatic effect of the compound and it measures the inhibitory effect of compound towards a particular microorganism. From Table 7.2.12, it is obvious that the antibacterial activity of SnO\textsubscript{2} nanoparticles and SnO\textsubscript{2} nanoparticles treated cotton are significant.
The main reason for the inactivation of bacteria involves the direct interaction between SnO$_2$ nanoparticles and cell surfaces, which affects the permeability of membranes where nanoparticles enter and induce stress in bacterial cells, subsequently resulting in the inhibition of cell growth and eventually in cell death [45]. Besides, the more positive charge on the cell surface of gram positive bacteria interacts stronger with the SnO$_2$ nanoparticles than the gram negative bacteria. Hence the zone of inhibition values probably more against *S. aureus*, than *E. coli*, during the contact of nanoparticles.

7.2.13. Antifungal evaluation of SnO$_2$ nanoparticles and treated cotton

Studies on the antifungal activity of SnO$_2$ nanoparticles are not yet been widely explored and exploited. The antifungal activity produced by SnO$_2$ nanoparticles and SnO$_2$ nanoparticles treated cotton whose images are shown in Fig. 7.2.13 are about 10 mm and 11 mm against *C. albicans* and 6 mm and 7 mm against *A. niger* respectively. The zone of inhibition values are registered in Table 7.2.13. The different antifungal effects may result from different growth morphologies of these two fungi. By the use of SnO$_2$ nanoparticles, membrane damage and some pits that have been created cause intercellular components leakage. The damage to the cell membrane directly leads to the leakage of minerals, proteins and genetic materials, causing cell death [46]. In general, the intrinsic toxic properties of metal oxide nanoparticles, as well as the types of microbial cells are associated with the species sensitivity of metal oxide nanoparticles [47]. The study clearly represents that the antifungal activity of green synthesized SnO$_2$ nanoparticles and SnO$_2$ nanoparticles treated cotton are due to the obvious protein leakage of fungal species.
7.3. Conclusion

In summary, for the first time, synthesis of SnO$_2$ nanoparticles using Nyctanthes arbor-tristis is demonstrated in this report. Specifically this work describes an environmentally friendly method to synthesize tin oxide nanoparticles by the novel application of Nyctanthes. It fortifies the screening of a new plant as a potential source of capping agent for the synthesis of well shaped smaller size SnO$_2$ nanoparticles. The synthesized SnO$_2$ nanoparticles are biocompatible which is very important from the aspects of biomedical application. This study shows that nanosize SnO$_2$ particles can be synthesized under lower pressure and in cheaper ways. This green approach may find various medicinal as well as technical applications. This method of synthesis using the plant extracts may be extended to other metal oxides. Furthermore, considering the potential antimicrobial implication of these metal oxide nanoparticles by the treatment on cotton, treatment techniques can be varied using suitable chemistry for desired applications.
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Table 7.2.13: Zone of inhibition of SnO\textsubscript{2} nanoparticles and SnO\textsubscript{2} nanoparticles treated cotton against fungal strains

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<td>2</td>
<td>SnO$_2$ nanoparticles treated cotton</td>
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