IOP Conf. Series: Materials Science and Engineering **360** (2018) 012007 doi:10.1088/1757-899X/360/1/012007

Synthesis, Characterization and Anti – Bacterial Activities of SnO_2 Nanoparticles Using Biological Molecule.

Nancy John*, Malu Somaraj[1] and Nisha J Tharayil[2]

[,1] Department of Physics, S.N College, Kollam, Kerala, India*

[2] Department of Physics, S.N College for Women, Kollam, Kerala, India

E-mail: nancypninan@gmail.com

Abstract

Nano – crystalline SnO_2 particles have been synthesized by co–precipitation method using DNA, a biomaterial, as capping agent. The nanoparticles obtained were characterized using XRD, SEM and TEM. The XRD confirms the crystalline nature and formation of SnO_2 nanoparticle. The optical properties were studied using FTIR spectroscopy. The effect of SnO_2 nanoparticles on the growth of some common food and water poisoning pathogens were studied. To understand the mechanism of antibacterial activity and to confirm the role of reactive oxygen species, the samples were annealed at different temperature and the bacterial activity is probed. Antibacterial studies were done against three bacterial strains namely, Staphylococcus aureus (ATCC 9144), Shigella flexineri (ATCC 2908) and Escherichia Coli (ATCC 25922).

1 Introduction

Emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high [16]. Therefore, there is a pressing demand to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections. Organic compounds used for disinfection have some disadvantages, including toxicity to the human body, therefore, the interest in inorganic disinfectants such as metal oxide nanoparticles is increasing.

As an n – type wide band gap semiconductor (E_g = 3.6 eV), SnO_2 is one of the most intensively studied materials owing to its technological applications. The anomalous variation in the size and shape dependent properties of tin oxide nanoparticles makes it a promising candidate in optoelectronics, sensing, laser, solar cell, photocatalytic and biological applications.

Metal oxide nanoparticles are found good inhibitor to bacterial strains. The antimicrobial efficiency of metal oxide nanoparticles depends on the particle size, presence of light, composition of aqueous medium used in assay etc. Electrostatic interactions are responsible for the attachment of nanoparticles to the bacteria. These interactions changes the integrity of cell membrane of bacteria and toxic free radicals are released which induce oxidative stress on bacteria [2, 15]. Using biomolecules as a templating agent to synthesis inorganic nanoparticles is an effective method to fabricate functional materials with well-defined structure and controllable dimensions. Among biological molecules DNA have been extensively used as a bio template because of their physicochemical stability and unique structure [6 – 8].

In this paper, samples were prepared by co – precipitation technique using DNA as capping agent and their activities against various bacterial strains were studied. Antimicrobial studies were done against three bacterial strains namely, Staphylococcus aureus (ATCC 9144), Shigella flexineri (ATCC 2908) and Escherichia Coli (ATCC 25922).

2 Experimental

2.1 Materials

The chemicals used for the synthesis of nanoparticles, namely Stannic Chloride Pentahydrate, and Sodium Carbonate were obtained from MERCK. DNA is obtained from calf thymus. The bacterial cultures were obtained from Institute of microbial technology, IMTECH, Chandigarh.

2.2 Synthesis of SnO_2 nanoparticles

SnO_2 nanoparticles were prepared from Stannic Chloride Pentahydrate and Sodium carbonate using DNA as the capping agent with the help of a magnetic stirrer. The white precipitate obtained was separated from the reaction mixture and washed several times with distilled water to remove impurities and traces of chemicals used. The wet precipitate was allowed to dry naturally and then thoroughly grounded to obtain metal carbonate precursor in the form of fine powder. Upon heating the obtained metal carbonate precursor to 400°C, it decomposes to form metal oxide. The calcinations temperature of the sample were fixed upon the TGA measurements. The white coloured precipitate changed into pale yellow which showed the formation of tin oxide nanoparticles. The nanoparticles annealed at 500˚C and 700 ˚C are denoted by S1 and S2.

2.3 Characterization

The XRD studies were carried out using Bruker AXS D8 Advance model and TEM was recorded using Jeol/ JEM 2100 model from STIC, Cochin. SEM of the samples were taken by Hitachi S- 3400 N and the chemical composition was examined using EDAX attachment Nortan system six attached to the SEM unit. The FTIR studies of the samples were carried out in a Perkin – Elmer FTIR Spectrophotometer between 300 cm^{-1} and 4000 cm^{-1}.

3 Results and Discussions

3.1 EDAX analysis

The chemical composition of SnO_2 nanoparticles prepared at the optimized conditions was extracted from the energy dispersive X – ray spectrum (EDAX) which is shown in Figure 1. The EDAX analysis exhibits clear peaks of only Sn and O elements, whereas no additional peaks were detected, which means the prepared samples are exempted from the starting precursors [4, 8]. The percentage of Sn and O in the sample is shown in table 1.

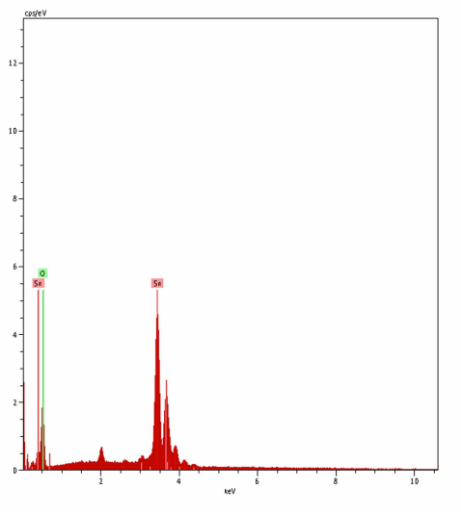


Figure 1. EDAX of S1

Table 1: Result of EDAX analysis showing the percentage of Sn and O in the sample.

Element	Mass %	Atom %
O K	74.12	27.85
Sn L	25.88	72.15
Total	100	100

3.2 XRD analysis

The XRD pattern of the synthesized samples (S1 annealed at 500˚C and S2 annealed at 700 ˚C) are shown in figure 2. The observed and standard (h k l) planes confirmed that the product is of SnO_2 having a tetragonal structure, which is in good agreement with the literature, values (JCPDS No 41 – 1445).

The average crystallite sizes of the samples were calculated using Debye-Scherer equation

$$\mathbf{D} = \frac{K\lambda}{\beta cos\theta} \quad \text{.....} \quad (1)$$

Here D is the crystallite size and β full width half maximum of the peak with diffracting angle θ.

The microstrain analysis shows that the residual stress and strain of the material is an important mechanical property of the material. The strain present in the lattice also contributes to

broadening of the XRD peaks. The microstrain, e and dislocation density, *D* can be calculated using the following equations,

$$e = \left(\frac{\lambda}{d\cos\theta} - \beta\right)\left(\frac{1}{\tan\theta}\right) \quad \text{.....} \quad (2)$$

$$D = \frac{1}{d^2} \quad \text{.....} \quad (3)$$

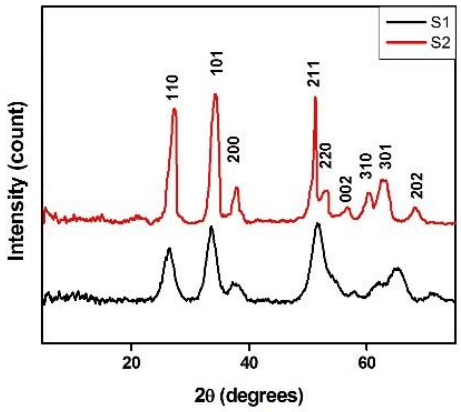


Figure 2. XRD pattern of samples S1 and S2.

Table 2: Parameters obtained from XRD of the three samples with particle size.

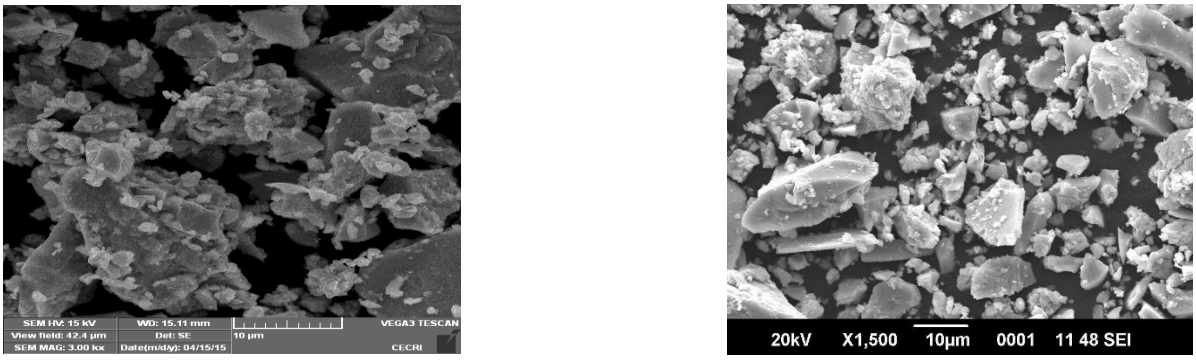
Sample	h k l	d observed	d JCPDS	Particle Size (nm)	Microstrain	Dislocation density
S1	110 101 211	63.39314 2.66438 1.7721	3.3470 2.6427 1.7641	5 ± 1	0.2092	4.73
S2	110 101 211	3.39343 2.67045 1.77184	3.3470 2.6427 1.7641	7 ± 1	0.0079	2.12

It is clear that from XRD patterns of the samples, the intensity enhanced with annealing temperature. The estimated average particle size, micro strain and dislocation density of the samples are given in table 2. As the annealing temperature increases the diffraction peaks gets sharper. The two possible mechanisms involved in the narrowing of peak, one is particle size effect and the other is lattice strain. These nanocrystals have lesser lattice planes compared to the bulk, which contributes the broadening of peaks in the diffractogram. The non- existence of any other overlapping peak in the standard peak position indicates that the prepared samples have only a single phase.

The microstrain and dislocation density decreases with annealing temperature. This may be due to the fact that when crystallite size become too small, the internal pressure exerted by the surface tension on the nanostructured material will create a stress field which induces lattice strain. When the crystal size is increased, stress reduces and thus lattice strain is reduced. In short, as temperature increases crystalline defects decreases thereby reducing dislocation density and lattice strain.

3.3 Scanning Electron Microscopy (SEM)

Scanning electron microscopy of samples are shown in figure 3. It reveals that the particles are agglomerated. The particles are more dispersed on annealing. The homogeneity in shape and size of the nanoparticles in the sample S2 appears to be better than that of S1 under the resolutions taken.

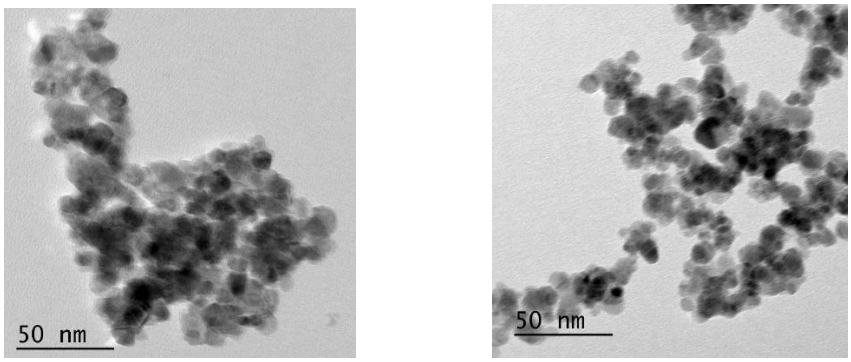


S1 **S2**

Figure 3. SEM images of samples S1 and S2

3.4 Transmission Electron Microscopy (TEM)

The TEM spectrum of SnO_2 nanoparticles are shown in (Figure 4). The particles are irregular in shape with the size ranges between 5 and 13 nm, which is in reasonable agreement with the results obtained from the XRD patterns.



S1 **S2**

Figure 4. TEM images of samples S1 and S2

3.5 Fourier Transform Infrared Spectroscopy (FTIR)

In order to evident the presence of functional groups, FTIR spectroscopic analysis is carried out for synthesized metal oxide nanoparticles. Figure 5 gives the FTIR spectra of samples S1 and S2. The spectrum has been recorded in the range 4000cm^{-1}- 300cm^{-1}

The presence of water molecules adsorbed on the presence of tin oxide during handling or bending and stretching vibrations of OH groups can be seen in 3431 cm^{-1} and 3446 cm^{-1}. The bands at 1612 cm^{-1} and 1636 cm^{-1} have been assigned to lattice vibrations due to decreasing the intensity which leads to overtones and combinations. The bands around 512 and 613 cm^{-1} have been attributed to Sn – 0 stretching modes of Sn – O – Sn, respectively, revealing of the presence of SnO_2. Table 3 shows the bands and assigned vibrations of S1 and S2.

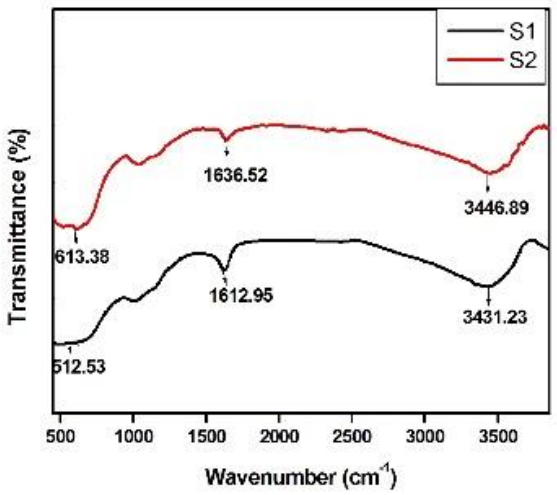


Figure 5. FTIR spectra of samples S1 and S2

Table 3: The major bands obtained in the FTIR spectrum of S1 and S2

Bands (cm^{-1})	Assignments
512-613	Sn-O stretching vibrations
1612 - 1636	Presence of oxygen radicals
3431-3446	Stretching vibrations of OH attached with surface

3.6 Antibacterial Studies

The antibacterial studies were done in CEPC laboratory and technical division, Kollam, Kerala, India. Test organisms were collected from Institute of Microbial Technology, IMTECH, Chandigarh. The technique used here is filter paper diffusion technique.

The bacterial strains were maintained on their respective medium in slants at 2 - 8°C. Muller Hinton Agar was used for bacterial culture. MHA was prepared and sterilized at 121°C for 15 minutes. After sterilization required volume of the medium (20 ml) was poured in the sterile petri dishes and allowed to solidify. Pure culture was selected as innoculam. 3 – 4 colonies were selected and transferred them into about 5ml of suitable broath such as Tryptone Soya Broath (TSB). It is incubated at 37°C for 2 – 8 hours till light to moderate turbidity develops. A sterile non – toxic swab is dipped on a wooden applicator into the standardized inoculum and the soaked swab is rotated firmly against the upper side wall of the tube to express the excess fluid. The entire agar surface of the plate is streaked with the swab three times, turning the plates at 60 angles between each streaking. The inoculums were allowed to dry for 5 – 15 minutes with lid in place. The disc is allowed to impregnate with the sample, approximately 30 µl, using aseptic technique. This is incubated immediately at 37°C and examined after 16 – 18 hours. The zone showing complete inhibition is measured and the diameters of the zones to the nearest mm is studied.

The antibacterial activity against three bacterial strains namely Staphylococcus aureus, Shigella flexineri and Escherichia Coli is analyzed for the samples. For Staphylococcus aureus and Shigella flexineri, S2 had a higher inhibition zone than S1. It indicates that inhibition zone increases on annealing temperature for the two bacterial strains whereas for Escherichia Coli, S2 has lower inhibition zone than S1. The zone of inhibition of S1 and S2 against bacterial strains are given in table 4. Nano particles tend to absorb on the bacterial surface and dehydrogenation due to respiration process occurs at the cell membrane in bacteria. The high surface to volume ratio of these nanoparticles is the factor for their increased chemical and biological activity. The active oxygen species generated by the presence of SnO_2 nanoparticle interact with the cell membrane of the bacteria and are easily penetrated through it.

Table 4: Results obtained for the anti - bacterial activities of the prepared samples.

Parameters	**Diameter of clear inhibition zone in mm**	
	S1 (mm)	**S2 (mm)**
Shigella flexneri	**17**	**19**
Escherichia Coli	**10**	**6**
Staphylococcus aureus	**7**	**9**

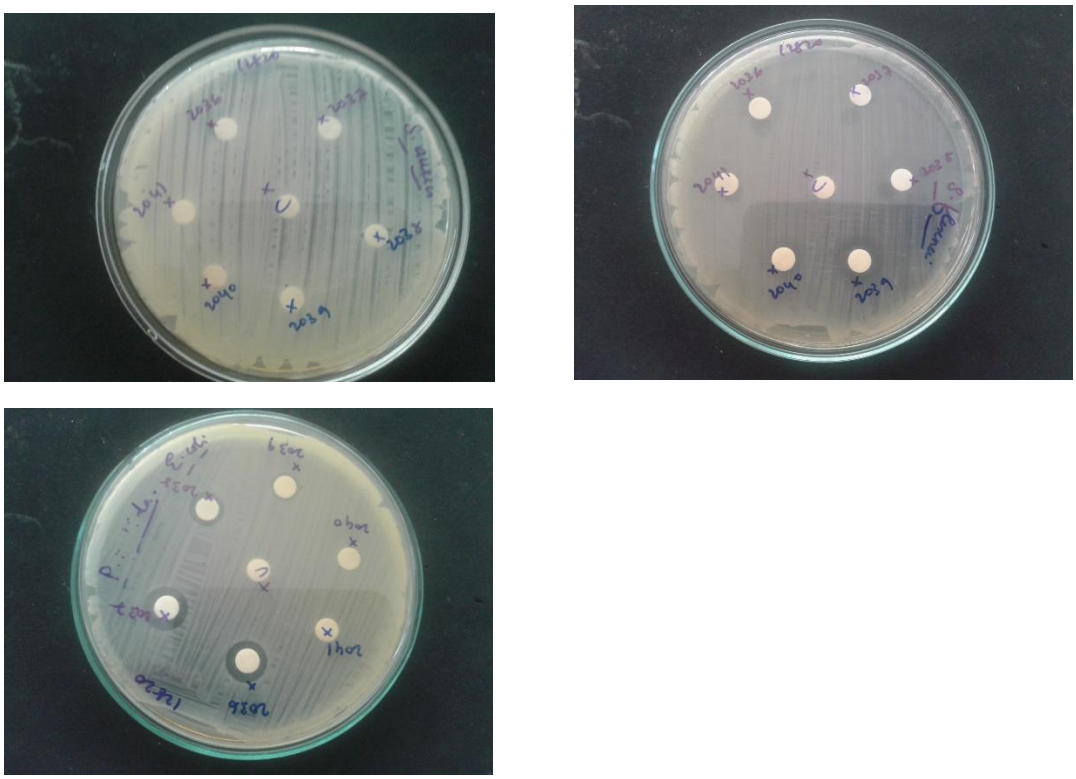


Figure 6. Photograph showing the inhibition zones obtained for different bacterial strains by S1 and S2.

4 Conclusion

An environment friendly method of synthesizing SnO_2 nanoparticles using DNA were successfully done. EDAX shows the presence of Sn and O and their presence in stochiometric proportion. It is clear that from XRD patterns of the samples, the intensity enhanced with

annealing temperature. The non- existence of any other overlapping peak in the standard peak position indicates that the prepared samples have only a single phase. The TEM shows particles are of size in between 5 and 13 nm which is in good agreement with XRD results. The morphology and shape of structure improves on annealing. Antibacterial activity increases on annealing temperature. SnO_2 nanoparticles using DNA as capping agents exhibit significant antibacterial activity.

Acknowledgements

The first author is grateful to STIC Cochin, CECRI, Karaikudi, Department of Optoelectronics, Kariavattom and CEPCI, Kollam for providing facilities for analysis.

References

[1] R. Jalal, E.K. Ghorshadi, M. Abareshia, Mmoosavi, A.Yousefi, P. Nancarrow; Materials Chemistry and Physics, 121(2010), 198-201.

[2] P.P Sharmila, S, Sagar, Nisha. J.Tharayil, Nanoscience and Nanotechnology, 8 (2014), 312-314.

[3] Nasrin Talebian,Hoda Sadeghi Haddad Zavvare, Journal of Photochemistry and Photobiology B: Biology, 130 (2014), 132 – 139.

[4] A. Ayeshamarium, R. Perumal Samy, Journal on Photonics and Spinctronics, 2 (2013), (4 – 8).

[5] Ravindra P. Sngh, Vineet K. Shukla, Raghavendra S. Yadav, Prashant K. Sharma, Prashant K. Singh, Avinash C. Pandey. Adv. Matt. Lett., 2 (2011), 313 - 317 .

[6] S. Matin Amininezhad, Alir eza Rezvani, Mehdi Amouheidari, S. Mohamad Amininejad, Sajjad Rakshani, Zahdan J Res Med Sci,, 16 (2015), 29 – 33.

[7] Sedumadavan Sudhaparimala, Arumugham Gnanamani A. Asit Baran Mandal, American Journal For Nanoscience and Nanotechnology,Bohn P W, Elimelech M, et al, Nature, 452 (2008), 301 – 310.

[8] P. P. Sharmila, Nisha j. Tharayil, International Journal of Material Science and Engineering, 2 (2014), 147 – 151.

[9] Simin Tazikeh, Amir Akbari, Amin Talebi, Emad Talebi, Material Science – Poland, 32 2014, (98 – 101).

[10] J. H. Choi, H. Tabata, T. Kawai; J.Cryst. Growth, 226 (2001), 493.

[11] N. Padmavathy, R. Vijayaraghavan, Sci. Tech. Adv.Matter, 9 (2008).

[12] Vennila Raj, Kamaraj Palanisamy, M. Arthanareeshwari, Chemical Science Review and Letters, 2 (2013), 293 – 299.

[13] H. R. Pouretedal, A. Shafeie, and M. H. Keshavarz, Journal of the Korean Chemical Society, 56 (2012), 484 – 490.

[14] Saba Ahmed, Muhammed Akhyar Farrukh, Madiha Khan, Muhammed Khaleeq–ur-Rahman and Muhammed Ashraf Tahir, Canadian Chemical Transactions, 2 (2014), 122 – 133.

[15] Archita Bhattacharjee, M. Ahmaruzzaman, Journal of Colloid and Interface Science, 448 (2015), 130 – 139.

[16] M. Kolar, K. Urbanek, T. Latal, Antibiotic selective pressure and development of bacterial resistance. Int J Antimicrob Ag, 17 (2001), 357–363.