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## Synthesis, Characterization and Antimicrobial Studies of SnO<sub>2</sub> Nanoparticles

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**Abstract :** This study was involved to synthesize and investigate the antimicrobial properties of highly pure nanocrystalline  $\text{SnO}_2$  by simple chemical method. In the nanorods,  $\text{SnO}_2$  nanoparticles, with a size of about 74 nm, the  $\text{SnO}_2$  nanoparticles were maximum antibacterial activity against both bacterial and fungal with the zone of inhibition for Klebsiella pneumoniae, Staphylococcus aures, Salmonella typhi and Ascerpergillus Flavus, Ascerpergillus Niger from  $25\mu$ g/ml and  $100 \mu$ g/ml respectively.  $\text{SnO}_2$  nanoparticles showed good activity against both Gram-negative and Gram-positive bacteria confirming these as future broad spectrum antibacterial a cost effective way and to study its antimicrobial properties. We observed an effective antibacterial and antifungal activity of the  $\text{SnO}_2$  nanoparticle against bacteria and fungi. The results showed that  $\text{SnO}_2$  nanoparticles enhanced the good antibacterial activity.

**Keywords :** SnO<sub>2</sub> Nanoparticles, Klebsiella pneumoniae, Staphylococcus aures, Salmonella typhi.

## 1. Introduction

Nanochemistry is the science of tools, technologies and methodologies for chemical synthesis, analysis and biochemical diagnostics performed in nanolitre to femtolitre domains. It is the use of synthetic chemistry to make nanoscale building blocks of desired shape, size, composition, surface structure, charge and functionality. Nanotechnology is leading to the production of many types of nanoparticles such as metal, metal oxide, doped and un-doped metal and metal oxide etc. The antibacterial studies of the doped and un-doped SnO<sub>2</sub> nanoparticles are also being studied [1]. The conductivity and optical properties of SnO<sub>2</sub> are largely dependent on the particle size and shape of the nanocrystallites [2-5]. Because of its excellent optical, electrochemical, and catalytic properties it is being used in many practical applications such as solid-state sensors, solar cells, Libatteries and optoelectronic devices. Large band gap semiconductors, such as TiO2, SnO<sub>2</sub>, SiO<sub>2</sub> and ZnO are suitable photocatalytic materials [6-7]. Tin(IV) oxide plays a very important role in the field of gas sensing and

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catalysis and as a transparent conducting oxide [8]. The activities of nanoparticles are directly dependent on the bacterial strain i.e., Grampositive and Gram-negative because they have differences in their cell wall. More recently, researchers have produced nano-sized wires and tubes. Nanowires have remarkable optical, electronic and magnetic properties, so they will prove useful in storing computer data. Carbon nanotubes may lead to new building materials, being much stronger and higher than steel. Metal oxide nanoparticles shows a great demand in the chemical, electronic and pharmaceutical industries [9-11]. Different methods have been adopted to synthesize nanoparticles like sol gel method [12-13] hydrothermal method, deposition-precipitation method. Antimicrobial properties have been demonstrated for metallic nanoparticles, metal oxide powders and nanoparticles [14]. The antibacterial property makes it desirable in hospital, Pharmaceutical and food industries where there is need for hygienic situation [15].

The present investigation synthesized nano-sized  $SnO_2$  by novel simple chemical method and study their antibacterial and antifungal activities against Klebsiella pneumonia (Gram negative bacteria), Staphylococcus aures, Salmonella typhi(Gram positive bacteria) and Ascerpergillus Flavus, Ascerpergillus Niger(Fungai) as a model by two methods.

#### 2. Experiments

#### 2.1 Antimicrobial studies

The in-built  $SnO_2$  systems have increased biological potency, more so with the system having smaller rings. On this basis, the synthesized  $SnO_2$  powder was evaluated for their in-vitro antibacterial and antifungal activities. The evaluations were carried out using "Agar plate technique" for both antibacterial and antifungal activity. Glasswares used in the present investigation were thoroughly washed with deionised water and dried.

For culturing bacteria, Nutrient agar medium and for fungi, Muller Hinton agar agar medium were used. Both the bacterial and fungal cultures were inoculated in nutrient booth and Muller Hinton agar booth respectively and incubated for overnight. Stock solution was prepared next day. 1 mg of each different sample was dissolved in 1.0 ml of DMSO.

#### 2.2 Preparation of nutrient agar medium

Table-1 Nutrient agar was mainly used for the isolation of bacteria in pure culture.

## 2.3 Sterilization

The above media were sterilized in an autoclave at 151 bs for 15 min. After sterilization, the medias were poured into sterile petri dish each of 35 ml. The sterile cotton swab was dipped into the nutrient and Muller Hinton agar media over night for bacteria and fungi culture respectively. The excess inoculation was removed by pressing the swab against the inner wall of the culture tube. Petri dishes were sterilized in a hot air oven at  $160^{\circ}$ C for 3 hrs.

#### 2.4 Preparation of media

The compositions of media were weighed separately and dissolve in approximate amount of water. After the sterilization, the media was allowed to cool for sometimes and at bearable heat, the media was poured into the Petri dishes aseptically. The depth of the medium should be approximately kept as 4mm. After solidification, the dishes were dried for 30 min in an incubator to remove excess moisture from the surface.

#### 2.5 Inoculation

#### 2.5.1 Preparation of Inoculum

Only clinical isolates were used for the sensitivity test. The bacteria culture was maintained in nutrient agar slants. 48 hrs old culture was used as source of inoculum. The fungal isolates were maintained in Muller Hinton agar agar slants. Spores were collected from 5 days old culture and used as source of inoculum.

#### 2.5.2 Method of Inoculation

A loop full of bacterial culture was suspended in 10ml of sterile distilled water. 0.5ml of this was pipetted out in to sterile Petri dishes over which 20ml of nutrient agar medium was poured and mixed thoroughly. The entire agar plates were swabbed herizontally, vertically and outer edge of the plate to ensure heavy growth over the entire surface. All the culture plates were allowed to dry for about five min in the prepared agar media plates. The well was prepared with equal distance in the size of 4 mm. The prepared well was filled with different concentration of various samples using sterile pipettes. All the plates were incubated for 24 hours at 37°C. Then the presence of zone of inhibition could be measured on the plates.

20ml of Muller Hinton agar agar medium was poured into sterile Petri dishes and allowed to solidify. A loop full of fungal spores were suspended in 10ml of sterile distilled water. A loop of this suspension was placed in the centre of Petri dishes. The agar surface of the plate was looped in 3 directions by turning the plate 60° angle between each looping. The lid of the petri dishes was closed and kept at room temperature for 5-10 minutes to dry. The inoculum confluent growth was desirable for accurate results.

#### 2.5.3 Incubation

The inoculated petri dishes were incubated at  $27^{\circ}$ C for a period of 2 days in the case of bacteria and 5 days in the case of fungi.

#### 2.5.4 Micro organisms used

The following clinical pathogens were used to check the antibacterial activity of synthesized SnO<sub>2</sub>.

- **\*** Gram negative bacteria
  - Klebsiella pneumoniae
- Gram positive bacteria
  - Staphylococcus aureus
  - Salmonell Typhi
- Fungi
  - Ascerpergillus Niger
  - Ascerpergillus Flavus

## 2.5.5 Preparation of synthesized SnO<sub>2</sub> sample disc

The disc preparation technique followed for both antibacterial and antifungal activity of a  $SnO_2$  sample was same. The  $SnO_2$  is insoluble in water but soluble in con HCl. Exactly 0.1mg of dried powder sample was individually weighed. Then the stock was prepared by dissolving it in con HCl and used to study antibacterial and antifungal sensitivity.

#### 2.5.6 Application of Antibiotic discs

The antibiotic disc was removed from their respective vials with the help of a sterilized forceps and carefully placed in the petri dishes, at least 22-24mm away from the edge. The antibiotic disc served as positive control, sterile distilled water disc served as negative control and prepared  $\text{SnO}_2$  discs were placed at a considerable amount of distance to place on the media overlapping at the zone. Then the disc was pressed gently on the surface of the medium. The petri dishes were allowed to stand at room temperature for 30 minutes or refrigerated at 15 min for prediffusion.

#### 2.5.7 Incubation

The plates were incubated at 37°C for about 20 hrs for antibacterial activity and at 27° for about 20hrs for antifungal activity.

#### 2.5.8 Reading the results

The zone of inhibition of each antibiotic was measured at the end of incubation period. The zones were measured from the discs showing complete inhibition and diameters of the zones were recorded to the nearest millimeter.

Ingredients	Gm/1000ml		
Peptone	5.0 gm		
Beef extract	3.0 gm		
NaCl	5.0 gm		
Yeast extract	2.0 gm		
Agar	15.0 gm		
Distilled water	1000 ml		
рН	$6.8 \pm 0.2$		

Table-1	Nutrient	agar was	mainly us	sed for t	he isolation	of bacteria in	pure culture.

PH (at  $25^{\circ}$ C) =7.3 ± 0.2 Table-2 Composition of Muller Hinton agar

Ingredients	Concentration gms/litre		
Beej injusion	300.00		
Casein acid hydrolysate	17.50		
Starch	1.50		
Agar	17.00		

## 3. Results and Discussion

#### 3.1 Antimicrobial study

Table 3 presents the antimicrobial activity of SnO<sub>2</sub>. From the table, it can be seen that, the nanocrystalline SnO<sub>2</sub> inhibits effectively the growth of staphylococcus aures bacteria than Klebsiella pneumoniae and it poorly inhibits the growth of Salmonella typhi bacteria in almost all concentrations of SnO<sub>2</sub>. As the concentration of micro-organism increases, the growth of bacteria also increases. From the Fig 1, it can be concluded that, the synthesized SnO<sub>2</sub> powder has strong antibacterial activity towards staphylococcus aures. The order of antibacterial activity of SnO<sub>2</sub> on growth of the three bacteria are staphylococcus aures > Klebsiella pneumoniae > Salmonella typhi. Table 4 gives the antifungal activity of SnO<sub>2</sub>. From the table it can be seen that, the synthesized SnO<sub>2</sub> has very good antifungal activity towards Ascerpergillus Flavus and Ascerpergillus Niger in all concentrations of SnO<sub>2</sub> from 25 µg/ml and 100 µg/ml. It has comparable antifungal activity on growth of both fungi shown Fig 2.

	Growth in				
Micro organism	25µl	50µl	75 μl	100 µl	
	SnO <sub>2</sub>	SnO <sub>2</sub>	SnO <sub>2</sub>	SnO <sub>2</sub>	
Staphylococcus aures	15 mm	16 mm	18 mm	20 mm	
Klebsiella pneumoniae	16 mm	18 mm	17 mm	21 mm	
Salmonella typhi	15 mm	17 mm	19 mm	20 mm	

Table-3: Antibacterial activity of SnO<sub>2</sub>



Fig.1 Antibacterial activity of SnO<sub>2</sub>

	Growth in				
Micro Organism	25µl SnO <sub>2</sub>	50µl SnO <sub>2</sub>	75 μl SnO2	100 μl SnO2	
Ascerpergillus Flavus	8 mm	10 mm	12 mm	15 mm	
Ascerpergillus Niger	8 mm	9 mm	12 mm	14 mm	

Table-4: Antifungal activity of SnO<sub>2</sub>

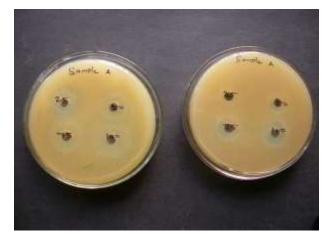


Fig.2 Antifungal activity of SnO<sub>2</sub>

The relative antibacterial activity of  $SnO_2$  suspensions of particles with size 74 nm toward Klebsiella pneumoniae, Staphylococcus aures, Salmonella typhi was studied qualitatively in aqueous Nutrient broth by disk diffusion. A standard testing protocol was employed that is applicable to inorganic metal oxides. The antifungal activity of  $SnO_2$  suspensions of particles with size 74 nm towards Ascerpergillus Flavus, Ascerpergillus Niger was studied qualitatively in aqueous Muller Hinton agar broth.

The ability of the antimicrobial agent to rupture bacterial cells is tested by the disk diffusion method and the results are given in table 4. The presence of an inhibition zone clearly indicate that the mechanism of the biocidal action of  $SnO_2$  involves disrupting the membrane. The high rate of generation of surface oxygen species from  $SnO_2$  leads to the death of the bacteria.  $SnO_2$  suspensions was incubated with Klebsiella pneumoniae, Staphylococcus aures, Salmonella typhi in aqueous Nutrient broth. Bacterial growth was studied by visually inspecting the nutrient broth for turbidity. If the material being tested does not kill but instead inhibits the growth of bacteria (Bacteriostatic agent), the bacteria will grow when it is removed from the solution containing the material, and colonies will be observed upon plating an aliquot. If the material being tested is bactericidal, the absence of bacterial colonies will be observed upon plating. To establish whether the suspensions were bacteriostatic or bactericidal,  $25\mu$  lit,  $50\mu$  lit,  $75\mu$  lit,  $100\mu$  lit aliquots were taken from the incubated Nutrient broth, each containing SnO<sub>2</sub> Klebsiella pneumoniae, Staphylococcus aures, Salmonella typhi and were plated on Nutrient agar plates and incubated for 18-20 h.

SnO<sub>2</sub> suspension with a concentration in the range of 25-100µl effectively inhibits the bacterial growth. No significant antibacterial activity was observed at concentrations less than 10µl. The SnO<sub>2</sub> suspension with 74nm particles is more effective. This can be explained on the basis of the oxygen species released on the surface of SnO<sub>2</sub>, which cause fatal damage to microorganisms. Highly reactive species such as OH,  $H_2O_2$  and  $O_2^{2^2}$  were formed. The generated  $H_2O_2$  can penetrate the cell membrane and kill the bacteria. Since, the hydroxyl radicals and superoxides are negatively charged particles, they cannot penetrate into the cell membrane and must remain in direct contact with the outer surface of the bacteria; however,  $H_2O_2$  can penetrate into the cell.

The detailed mechanism for the activity of  $SnO_2$  is still under debate. One possible explanation of the antibacterial effect of  $SnO_2$  is based on the abrasive surface texture of  $SnO_2$ .  $SnO_2$  nanoparticles have been found to be abrasive due to surface defects. Although metals and metal oxides are known to be toxic at relatively high concentrations, they are not expected to be toxic at low concentrations. No colonies were observed at this PH. This indicates that a pH in the range of 6-8 does not affect the growth of the bacteria, irrespective of the metal ions present.

#### Conclusion

Antimicrobial activity of the  $SnO_2$  nanoparticle has been stuided against three bacteria namely Staphylococcus aures, Klebsiella pneumonia and Salmonella typhi, and two fungi Ascerpergillus flavus, Ascerpergillus niger.  $SnO_2$  suspension with a concentration in the range of 25-100µl effectively inhibits the bacterial and fungi growth. From the antimicrobial studies it can be concluded that,  $SnO_2$  nanoparticle have strong antibacterial activity and antifungal activity.

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