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SYNTHESIS AND CHARACTERIZATION OF TIN OXIDE NANOPARTICLES WITH ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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ABSTRACT

Nanomaterials have unique properties compared to their bulk counterparts. For this reason, nanotechnology has attracted a great deal of attention from the scientific community. In this work ultra-fine tin oxide has been synthesized by chemical precipitation method. X-diffraction method confirm the formation of SnO_2 nanoparticles with average size of the range of 4 to 173 nm. Antibacterial activity of the SnO_2 nanorods has been studied against three bacteria namely *Staphylococcus aures, Klebsiella pneumonia* and *Salmonella typhi*, and two fungi *Aspergillus flavus, Aspergillus niger* exhibit is their antimicrobial behavior against pathogenic bacteria.

Keywords: Nanomaterials, Antibacterial and Antifungal Activity

INTRODUCTION

Development of the antibiotic conflict pathogens has become a severe health issue and thus, several studies have been reported to improve the current antimicrobial therapies. It is known that over 70% of bacterial toxicities are unaffected to one or more of the antibiotics that are generally used to eliminate the infection **[1]**. Enlargement of new and effective antimicrobial agents seems to be of paramount importance.

Recently, nanotechnology has presented the great potentials in various fields of science and technology. Pharmaceutical nanotechnology with numerous advantages has growingly attracted the attention of many researchers **[2]**. There has been a massive allowance of nanomaterial applications and uses as a consequence of basic and applied research from scientists all over the world. One such class of nanomaterials is metal oxide nanoparticles (NPs), ranging in size from 1 to 100 nm and available in different shapes and sizes. Metal nanoparticles possess unique physical and chemical characteristics linked to their nanometre size, thus offering versatility. Metal nanoparticles are key constituents in catalysis, diagnosis, drug delivery, semiconductors, sensing and solid oxide fuel cells **[3, 4]**. Beyond these uses, metal nanoparticles are utilised as antimicrobial agents **[5-7]** and hence have received much attention recently. Metal and oxygen elements combine to form metal nanoparticles where both high and low melting-point metals can be found. Based on their different electronic structures, metal nanoparticles exhibit metallic, semiconductor and insulator characteristics. Metallic and semiconductor metal nanoparticles are formed by the combination of oxides with metals from groups 3–12 of the periodic table, whereas insulator MeOs are formed from metals in groups 1, 2 and 13–18 **[8-10]**. The semiconductors are further classified into i-, n- and p-types **[11]**. Intrinsic semiconductors are i-type semiconductors having properties both of insulators and conductors. The n-types are electron-excess semiconductor oxides with free electrons as charge carriers and will have either excess cations or deficient anions. The p-types are electron-deficit semiconductors with a cation-deficient oxide and this cation vacancy provides the additional electrons for reactivity. These features confer unique chemical and physical properties to metal nanoparticles through which they interact with biological systems **[12]**. The different properties of metal nanoparticles are the major contributors to antimicrobial activity. The alkalinity of the calcium oxide (CaO) and magnesium oxide (MgO) NP surface is the significant component in conferring antimicrobial activity **[13]**. These alkali metal nanoparticles are more soluble owing to their contribution to alkalinity in the medium, which cannot be found with metal nanoparticles semiconductors such as neutral zinc oxide (ZnO) NPs **[14 - 16]**. SnO_2 is an important material due to its properties such as high degree of transparency in the visible spectrum, strong physical and chemical interfaces with adsorbed species, low operating temperature and strong thermal stability in air (up to 500^{o}C). It is an n-type semiconductor with a band gap of 3.6 to 3.8 eV **[17]**.

In this study, Ultra fine SnO_2 nanoparticles is successfully synthesized by simple chemical precipitation method and have been employed to evaluate the antimicrobial activities.

2. EXPERIMENTAL PROCEDURE

Materials

Tin chloride ($SnCl_2$, Merck 99 %) as starting material, trichloroethylene as surfactant, absolute HCl as a solvent, ortho phosphoric acid as precipitator were used to synthesize tin oxide nanoparticles. All the solutions were prepared using double distilled water.

Synthesis of SnO_2 powder

To 0.5 M $SnCl_2$, 10 ml of HCl was added. The mixture was then heated at 40°C and to the clear solution, 20 ml of H_3PO_4 was added drop by drop. The solution was continuously stirred for 3 hrs at room temperature. After 3 hrs the product was formed completely and the solution was decanted and the product was washed with water and ethanol. Then it was filtered and dried in hot air oven (or) in muffle furnace and the yield was noted.

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.

Preparation of nutrient agar medium

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

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
pH	6.8 ± 0.2

Composition of Muller Hinton agar

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

pH (at 25°C) =7.3 ± 0.2

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 160ºC for 3 hrs.

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.

Inoculation

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.

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 horizontally, 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 37ºC. 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 60º 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.

Incubation and micro organisms

The inoculated petri dishes were incubated at 27ºC for a period of 2 days in the case of bacteria and 5 days in the case of fungi. The following clinical pathogens were used to check the antibacterial activity of synthesized SnO_2. For Gram negative bacteria, *Klebsiella pneumonia* was used, Gram positive bacteria, *staphylococcus aureus* and *Salmonella typhi* was used, finally Fungi, *Aspergillus niger* and *Aspergillus flavus* was used

Preparation of synthesized SnO_2 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.

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 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.

Incubation

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

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.

RESULTS AND DISCUSSION

XRD analysis

The surface nanostructure and texture of the synthesized SnO_2 nanorods were analysed by XRD. **Figure 1** shows the XRD pattern of synthesized SnO_2 nanorods. Sharp peaks are observed which indicate the powder is made up of nanocrystals. The XRD inferred that the SnO_2 nanorods are having only one phase with different crystal sizes and planes.

The results indicate that, the product comprises of pure phase and there is no impurity peaks. The sharp peaks indicate that the products were well crystallised. The diffraction peaks were in good agreement with those given in the standard data (PCPDF, 79-0207) for SnO_2 and showed a good crystallinity. This means that, as the prepared materials were crystallized in a hexagonal rutile structure of SnO_2 with the mean crystallite size, d is in inverse proportion to the full width at half maximum β($d=0.89\ \lambda\ /(\ \beta\cos\theta)$, indicating that, the initial grain size to decrease.

The mean crystallite size d, was measured from the XRD (D.MAX-YB, RIGAKU) peaks at a scanning rate of 5°/min based on Scherrer's equation

$$d = \frac{0.9\lambda}{\beta\cos\theta'}$$

where λ is the wavelength of the X-ray, θ is the diffraction angle, and β is the full width at half maximum. The size of the SnO_2 crystallites ranged from 4 to 173 nm and the average crystallite size is 74 nm.

Surface morphology

Figure 2 shows the surface morphologies of the synthesized SnO_2 powder. From the SEM images, it can be observed that, the synthesized SnO_2 particles are having rod shape, with varying diameters ranging from 400-800 nm. It can be seen that, some of the rod shaped particles are agglomerated and appear as platelets or sheets. Form the size, shape and diameters of the particles, one can infer that, the synthesized SnO_2 powder is the agglomeration of nanorods. The nanostructure and size can be further confirmed by XRD studies.

Deformation area near the crystal grain boundary is seen. May be it was caused by squeezing between crystals. From **Figure 2**, it can be seen that, the crystal lattice was distorted by the grain squeeze which reveals that, deformation always occurs around crystalline exterior.

Antimicrobial study

Table 1 presents the antimicrobial activity of SnO_2. From the table, it can be seen that, the nanocrystalline SnO_2 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_2. As the concentration of microorganism increases, the growth of bacteria also increases. From the studies it can be concluded that, the synthesized SnO_2 powder has strong antibacterial activity towards *staphylococcus aureus*. The order of antibacterial activity of SnO_2 on growth of the three bacteria are *staphylococcus aureus* > *Klebsiella pneumoniae* > *Salmonella typhi*.

Table 2 gives the antifungal activity of SnO_2. From the table it can be seen that, the synthesized SnO_2 has very good antifungal activity towards *Aspergillus flavus* and *Aspergillus niger* in all concentrations of SnO_2 from 25µg/ml and 100µg/ml. It has comparable antifungal activity on growth of both fungi.

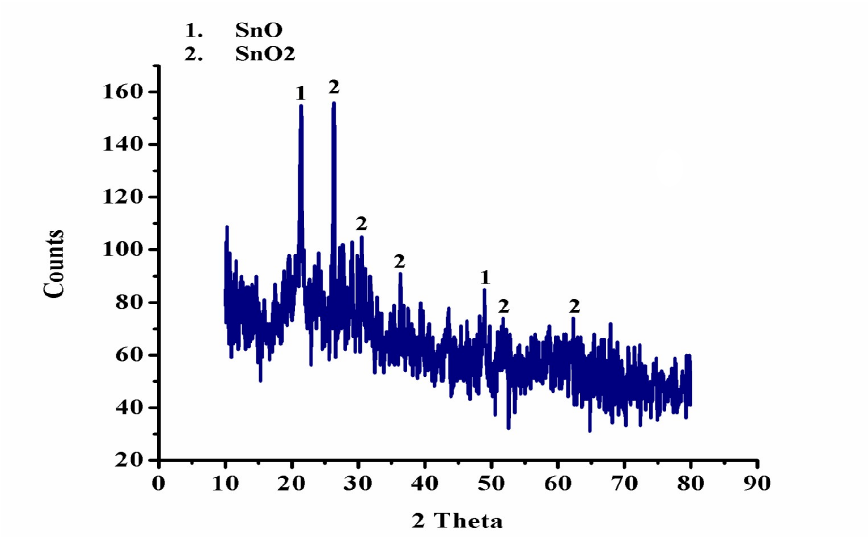

Figure 1: X-ray diffraction pattern of the SnO_2

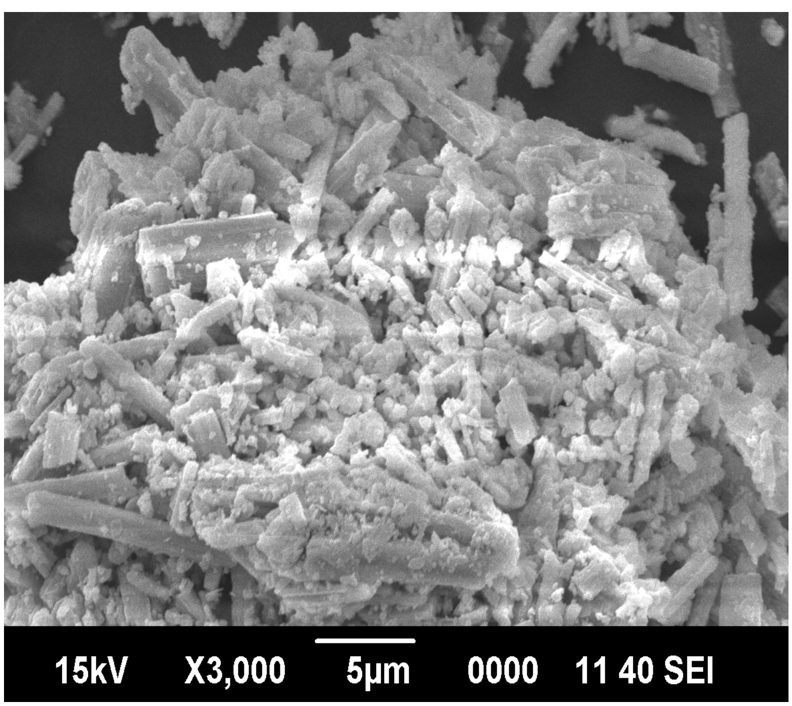

Figure 2: SEM image of as synthesized SnO_2

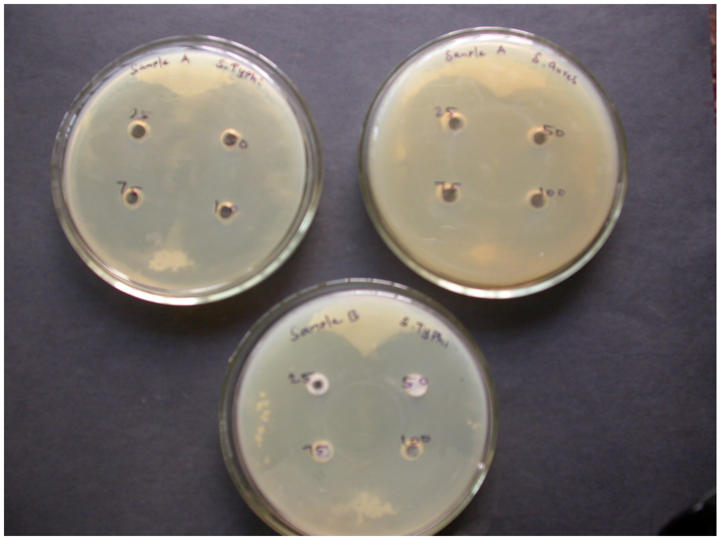

Figure 3: Antibacterial activity of SnO_2

Figure 4: Antifungal activity of SnO_2

Table 1: Antibacterial activity of SnO_2

Micro organism	**Growth in**			
	25µl SnO_2	**50µl SnO_2**	**75 µl SnO_2**	**100 µl SnO_2**
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-2: Antifungal activity of SnO_2

Micro Organism	**Growth in**			
	25µl SnO_2	**50µl SnO_2**	**75 µl SnO_2**	**100 µl SnO_2**
Aspergillus flavus	**8 mm**	**10 mm**	**12 mm**	**15 mm**
Aspergillus niger	**8 mm**	**9 mm**	**12 mm**	**14 mm**

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 *Aspergillus flavus*, *Aspergillus 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 2**. 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 bacteris (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, 25µ lit, 50 µ lit, 75 µ lit, 100 µ lit aliquots were taken from the incubated Nutrient broth, each containing SnO_2 *Klebsiella pneumoniae, Staphylococcus aures, Salmonella typhi* and were plated on Nutrient agar plates and incubated for 18-20 h.

SnO_2 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_2 suspension with 74nm particles is more effective. This can be explained on the basis of the oxygen species released on the surface of SnO_2, which cause fatal damage to microorganisms. Highly reactive species such as OH, H_2O_2 and O_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.

CONCLUSIONS

Ultra fine stannous oxide nanorods were synthesized by novel simple chemical method. The process parameters such as concentration of $SnCl_2$ phosphoric acid and HCl, stirring time, process temperature and other conditions were optimized to get ultra-fine nanorods. The nanostructure, surface morphology and size of the nanorods were studied by SEM and XRD. Antibacterial activity of the SnO_2 nanorods has also been studied against three bacteria namely *Staphylococcus aures*, *Klebsiella pneumonia* and *Salmonella typhi*, and two fungi *Aspergillus flavus*, *Aspergillus niger*. From the SEM, EDS and XRD analyses it can be concluded that, the synthesized SnO_2 is rod shaped with average crystallite size ranging from 4 to 173 nm. From the antimicrobial studies it can be concluded that, SnO_2 nanorods have strong antibacterial activity and antifungal activity.

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