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By

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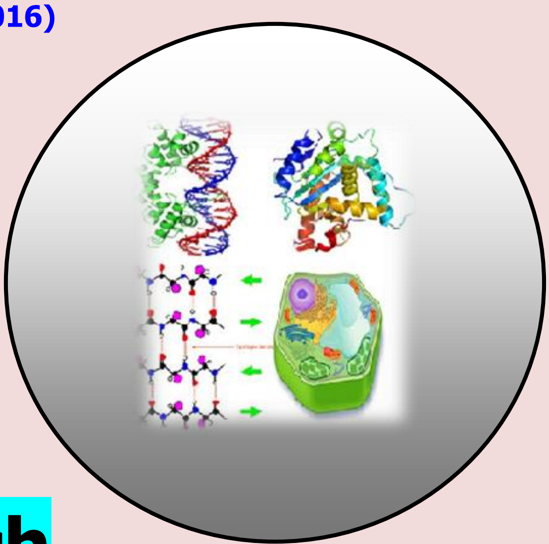

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Dr. B. Saravanan
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RESEARCH PAPER

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Antibacterial Efficiency of Titanium Doped Indium Oxide (In_2O_3: Ti) Thin Film

B. Saravanan and *V. Manivannan

Department of Physics, Marudupandiyar College, Thanjavur, Tamil Nadu, India
***Department of Physics, Presidency College, Chennai, Tamil Nadu, India**

ABSTRACT

Titanium–doped indium oxide (In_2O_3: Ti) thin films were prepared by thermal evaporation method on two different substrates. Nanocrystalline Titanium doped indium oxide (In_2O_3: Ti) thin films employing a much simplified spray pyrolysis technique in different substrate temperature environments (300, 350, 400 and 450 K) for the first time were fabricated. As deposited film was subjected to microbial resistance activity against Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginos and Bacillus subtilis which are common bacteria which spread easily to the host environment through the medium was studied. From the studies, it was found that at A4 (400 K deposition temperature) potential antibacterial efficiency of the thin film toward Escherchia coli and Pseudomonas aeruginos are stronger than that towards Staphylococcus aureus and Bacillus subtilis.

Key words: Indium Oxide, Titanium Dioxide, Thin Film, Antimicrobial Activity, Nanoparticles and Evaporation.

INTRODUCTION

Nano sized metal particles have great interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Metal nano particles have played vital role for solving environmental problems that affects humans. Besides semiconducting metal nano particles such as ZnO, TiO_2, CdS, Si, Ge, etc., Transparent conductive oxide (TCO) films such as tin oxide (SnO_2), zinc oxide (ZnO), titanium dioxide (TiO_2) and indium oxide (In_2O_3) have significant and potential role in the fabrication of next generation of optoelectronic devices in the UV region and display devices. Among these TCOs, In_2O_3 based thin films have recently gained much attention owing to its unique advantages over other oxide thin films.

In the recent decade, one of the most significant challenges faced by the world is the recurrence of infectious diseases and the bacterial contamination in all kinds of materials (Desselberger, 2000) Therefore, several antibacterial agents are widely used in day-to-day life for the prevention of public health issues caused by the ubiquity of micro-organisms and their ability to establish themselves (Kim *et al.*, 2005). When antibacterial agents are used in the new packaging materials for health care and food applications, the most crucial parameters to be taken care of are low toxicity to human beings and high efficiency in controlling bacteria. Currently, the antibacterial agents can be classified into two categories; organic and inorganic reagents. Inorganic antibacterial agents are more stable at high temperatures and pressures compared with the organic materials (Sawai, 2003).

Recently, investigations on preparation of In_2O_3 nanostructures with various forms such as nanotubes, nanobelts, nanofibers, wires and nanoparticles have been widely emphasized to extend their technological applications. Among these nanostructures, In_2O_3 with nanoparticulate form has been intensively studied to be used as a promising material for gas sensor applications. So far, nanoparticles of In_2O_3 have been synthesized by several techniques including sol–gel technique, pulse laser deposition, thermal decomposition, thermal hydrolysis, microemulsion, spray pyrolysis, mechanical chemical processing, hybrid induction and laser heating (HILH) method, nonaqueous synthesis, and hydrothermal synthesis (Maensiri, et al., 2008). Among other established synthesis methods, spray pyrolysis technique is simple and cost effective routes to synthesize nanocrystalline In_2O_3 by utilization of cheap, nontoxic and environmentally benign precursors are still the key issues. Recently, it is well established that the doping process improves the antibacterial efficacy of In_2O_3. Especially, the dopants like Sn, Mn, Mg, Sb, Ti, Co and N play a vital role in enhancing the antimicrobial activities of indium oxide etc. (Madahi *et al*., 2011) In this research work Titanium–doped indium oxide (In_2O_3: Ti) thin films were prepared by thermal evaporation method on two different substrates. As deposited film was annealed and subjected to microbial resistance activity against *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginos* and *Bacillus subtilis* which are common bacteria which spread easily to the host environment through the medium was studied. The reason for choosing spray pyrolysis technique is that, it is simple, inexpensive and has flexible process modifications for large area Transparent conductive oxide (TCO) coatings.The aim of the study to investigate the microbial resistance activity of Titanium–doped indium oxide (In_2O_3:Ti) thin film against *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginos* and *Bacillus subtilis*.

MATERIALS AND METHODS

Preparation of Thin film

Titanium doped Indium oxide (ITiO) films were deposited onto microscopic glass substrates using spray pyrolysis technique. The precursor of Indium (III) acetate (0.1nM) was dissolved in deionised water. A few drops of concentrated hydrochloric acid was added for complete dissolution and sprayed onto microscopic glass substrates with dimensions of 75x25 mmz at different substrate temperature (300, 350, 400 and 450 K). For doping of Ti, the TiCh (2 %) was used as the dopant source along with indium acetate at the optimum substrate temperature. The substrates were first cleaned with a water bath, followed by dipping in con. HCl, acetone and ethanol successively. Finally the substrates were rinsed in deionised water and allowed to dry in a hot air oven. After the deposition, the films were allowed to cool slowly to room temperature and washed with deionised water and then dried (Manoharan *et al*., 2013).

Microorganisms

The microbial strains employed in the biological assays were Gram - **Positive** bacteria: *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), Gram – **negative** bacteria: *Pseudomonas aeruginosa* (MTCC 2474), *Escherichia coli* (MTCC 119) Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), chandigarh, India.

Antibacterial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) using thin film. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginos* and *Bacillus subtilis* on Nutrient agar plates for bacteria. Using sterile forceps, thin films (A1) were laid down on the surface of inoculated agar plate. The plates were incubated at $37^{\circ}C$ for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Each sample was tested in triplicates. The antibacterial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in centimeters. The zones of inhibition of the tested microorganisms by thin films were measured using a millimeter scale.

RESULTS

The mean inhibition zone of thin film A1 was 5±0.35 cm for *Escherchia coli*, 1.34±0.079 cm for *Staphylococcus aureus,* 1.16±0.08 cm for *Pseudomonas aeruginos* and 1.32±0.09 cm for *Bacillus subtilis*. The highest microbial resistant were observed in the following order: *Escherchia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginos* represent in table 1 and Fig 1.

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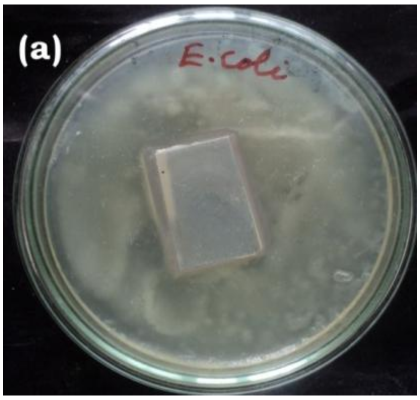
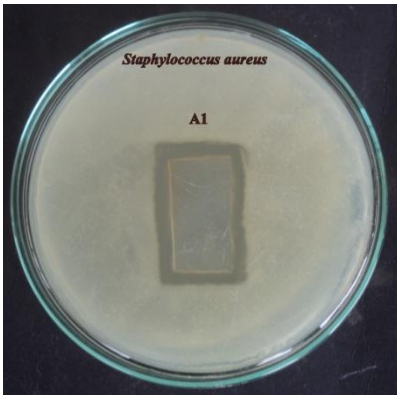
Table 1. Antibacterial activity of Titanium–doped indium oxide (In_2O_3:Ti) thin film.

Microorganisms	Result
Escherchia coli	5±0.35
Staphylococcus aureus (cm)	1.34±0.07
Pseudomonas aeruginos(cm)	1.16±0.08
Bacillus subtilis (cm)	1.32±0.09

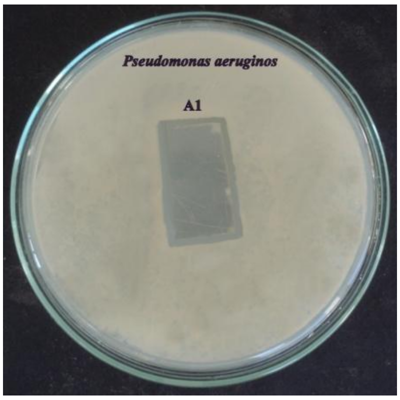
Values were expressed as Mean ± SD.



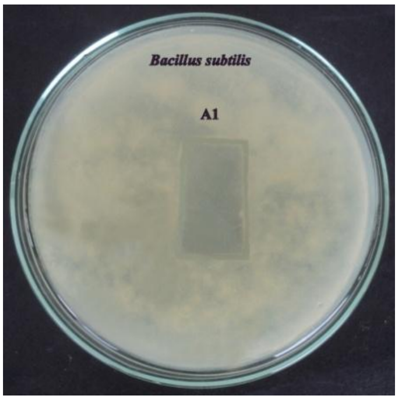
Escherichia coli



Staphylococcus aureu



Pseudomonas aeruginos



Bacillus subtilis

Figure 1. Antibacterial activity Titanium–doped indium oxide (In_2O_3: Ti) thin film.

DISCUSSION

Antibacterial agents are applied to many fields, such as food, care, packaging, synthetic textiles, environmental, and so on (de Moura et al., 2012; Pinto et al., 2009) . Chemical synthesis antibacterial agent is divided into two categories: organic and inorganic antibacterial agent. Organic antibacterial agent has many disadvantages, including the toxicity hazard to the human body and instability in high temperature and pressure (Selvam *et al.*, 2012). By comparison, inorganic antibacterial agent has the properties of heat resistance, long life, and chemical stability (Necula et al., 2012). In recent times, the usage of transparent conducting oxides (TCO) has become vital and wide due to their immediate applicability in various areas such as flat panel displays, photovoltaic cells, touch screens and in high speed operational electronic devices. More recently, titanium doped indium oxide thin films have attracted considerable attention due to their enhanced mobility and transparent conducting properties (Kaleemulla et al., 2007).

Nanocrystalline Titanium doped indium oxide (In_2O_3: Ti) films employing a much simplified spray pyrolysis technique in different substrate temperature environments (300, 350, 400 and 450 K) for evaluation of antibacterial activities. In our work, the antibacterial property results show that the titanium-doped In_2O_3 thin film against *Escherchia coli* and *Pseudomonas aeruginos* are better than *Staphylococcus aureus,* and *Bacillus subtilis.* The mechanism of antibactrial activity of Titanium doped indium oxide is discussed in this paper.

The bacterial cell wall can provide strength, rigidity, and shape for the cells and can protect the cells from osmotic rupture and mechanical damage. The bacterial cells can be divided into Gram-positive cells and Gram negative cells according to their cell wall structure. Besides, the wall of Gram-positive cells contains a thick layer of peptidoglycan (PG) of 20 to 80 nm, which is attached to teichoic acids. By contrast, Gram-negative cell walls are more complex, both structurally and chemically. The wall of Gram-negative cell contains a thin PG layer of 2 to 3 nm and an outer membrane of 8 to 10 nm, which covers the surface membrane (Hajipour et al., 2012).

Moreover, the antibacterial experiments were done in the dark, so there are no active oxide, hydrogen peroxide, and super-oxide. We can conclude that the In_2O_3 thin film are attached on the bacterial cell wall through electrostatic interaction, rupturing the cell walls, increasing the permeability, causing the leakage of cytoplasm, and leading to bacterial cell death. Figure 9 schematically illustrates the antibacterial mechanisms of titanium doped In_2O_3 thin film *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginos* and *Bacillus subtilis*. It may be that the cell walls of *Escherchia coli* and *Pseudomonas aeruginos* are broken easily due to the thin layer of PG, and the cell membranes burst; thus, the antibacterial properties of In_2O_3 thin film against *Escherchia coli* and *Pseudomonas aeruginos* are better than *Staphylococcus aureus* and *Bacillus subtilis*. Comprehensive analysis reveals that the antibacterial properties of titanium-doped In_2O_3 thin film are affected not only by the size but by the crystallinity. When the thin films are attached to the bacterial surface, titanium doped In_2O_3 crystals reacted with PG, teichoic acids, and lipoteichoic acids, and then the structure of bacterial cell wall is damaged.

The titanium-doped In_2O_3 thin film is crystalline nanorods synthesized from zinc acetate, and its antibacterial activities are lower than the others. Meanwhile, the bacterial cell wall is damaged slightly, and the electrical conductance of bacterial suspension is increased; it indicates that the destroy capacity of the thin film to bacterial cell wall and cell membrane is feeblish. This could be because of the weak doping level of titanium in In_2O_3 crystal, although the particle size is smaller than the others. When the titanium-doped In_2O_3 thin films are prepared from zinc nitrate, the particles are six prismatic crystals with big size. The bacterial cell wall is damaged seriously, and the electrical conductance of bacterial suspension is increased; it proves that the thin film' damage capability to the bacterial cell wall and cell membrane is great (Nair et al., 2011). It could be due to good doping level of titanium in In_2O_3 crystal and high dissolving ability of metal ion from the crystals.

The titanium-doped In_2O_3 thin films are square shape nanoparticles, which are synthesized from TiO_2. After treatment with them, the bacterial cell wall and cell membrane are damaged seriously, and the increase of electrical conductance of the bacterial suspension is greater than the others. It indicates that the capability of the thin film to the cell wall is high and makes the penetrability of cell membrane increased. This is due to high doping level of titanium and small size of particles. When the bacterial suspension is treated by the powders prepared from zinc sulfate, the antibacterial activity is weak and the damage degree of bacterial cell wall is slight. It demonstrates that the antibacterial activities of $In_2O_{3:}TiO_3$ crystal is better than other oxides. Furthermore, when the *E. coli* and *Pseudomonas aeruginos* cell walls are damaged by titanium-doped In_2O_3 thin film, the holes may be appeared on the cells; this may be because the thin cell wall and outer membrane are easy to break. When the *Staphylococcus aureus* and *Bacillus subtilis* cell walls are damaged by the thin film, the cell walls may become crinkly or honeycomb; this could be due to the thick layer of PG and the PG chemical network structure. On the basis of the above analysis, it is inferred that the antibacterial properties of the titanium-doped In_2O_3 thin film are relevant to the particle size and the crystallinity.

The Titanium doped indium oxide (In_2O_3: Ti) thin film with controlled sizes were synthesized from Ti salts. Antibacterial property results show that the Titanium doped indium oxide thin film have different antimicrobial activities. The antibacterial properties of the thin film prepared from TiO_2 are optimal. Moreover, the antibacterial action of the thin film toward *Escherchia coli* and *Pseudomonas aeruginos* are stronger than that towards *Staphylococcus aureus* and *Bacillus subtilis*.

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REFERENCES

Awoyinka, O. Balogun, I.O, Ogunnowo, A.A. (2007). Phytochemical screening and ivitro bioactivity Of Cnidoscolus aconitifolius (Euphorbiaceae). *J Med Plant Res*; 1(3): 63-65.

de Moura, M.R. Mattoso, L.H.C. Zucolotto, V. (2012). Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles and their use as active food packaging. *J Food Eng,* 109: 520–524.

Desselberger, U. Mrcrobes and infection. *J. Infect.* 40 (2000) 3–15.

Hajipour, M.J. Fromm, K.M. Ashkarran, A.A. Jimenez de Aberasturi, D. de Larramendi I.R. Rojo, T. (2012) Antibacterial properties of nanoparticles. *Trends Biotechnol*, 30:499–511.

Kaleemulla, S. Sivasankar Reddy, A. Uthanna, S. Sreedhara Reddy, P. (2007). Conducting properties of metals. *Mater. Lett.* 61 4309–4313.

Kim, J.S. E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. (2005). Impact of nanoparticle in microorganism. *Nanomedicine* 3 95–101.

Madahi, P. Shahtahmasebi, N. Kompany, A. Mashreghi, M. Bagheri-Mohagheghi, M.M. Hosseini, A. (2011) Antimicrobial activities of indium oxide. *Phys. Scr.* 84 035801–035805.

Maensiri, S. Laokul, P. Klinkaewnarong, J. Phokha, S. Promarak, V. Seraphin S. (2008). Indium oxide (In_2O_3) nanoparticles using *Aloe vera* plant extract: Synthesis and optical properties. *Journal Of Optoelectronics And Advanced Materials.* 10(3), p. 161 – 165.

Manoharan, C. Jothibas M. Dhanapandian S. Kiruthigal, G. Johnson Jeyakuma, R. S. (2013). Role of Titanium Doping on Indium Oxide Thin Films Using Spray Pyrolysis Techniques. Proceedings of the "International Conference on Advanced Nanomaterials and Emerging Engineering Technologies" (ICANMEET-20 /3)organized by Sathyabama University, Chennai, India in association with DRDO, New Delhi, India, 24-26.

Nair, M.G. Nirmala, M Rekha, K. Anukaliani, A. (2011). Structural, optical, photo catalytic and antibacterial activity of ZnO and Co doped ZnO nanoparticles. *Mater Lett,* 65:1797–1800.

NCCLS. (1993). National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. *PA: NCCLS Publications* 2-5.

Necula, B.S., van Leeuwen, J.P. Fratila-Apachitei, L.E. Zaat, S.A. Apachitei, I. Duszczyk, J. (2012). *In vitro* cytotoxicity evaluation of porous TiO2-Ag antibacterial coatings for human fetal osteoblasts. *Acta Biomater*, 8:4191–4197.

Pinto, R.J. Marques, P.A. Neto, C.P. Trindade, T. Daina, S. Sadocco, P. (2009). Antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers. *Acta Biomater,* 5:2279–2289.

Sawai. J. (2003). Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. *J Microbiol Methods.* 54 (2):177-82.

Selvam, S. Rajiv Gandhi, R. Suresh, J. Gowri, S. Ravikumar, S. Sundrarajan, M: (2012). Antibacterial effect of novel synthesized sulfated beta-cyclodextrin crosslinked cotton fabric and its improved antibacterial activities with ZnO, TiO_2 and Ag nanoparticles coating. *Int J Pharm*, 434:366–374.

Corresponding author: Dr. V. Manivannan, Department of Physics, Presidency College, Chennai, Tamil Nadu, India
Email: mayavelvan@gmail.com

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