Influence of Substrate Temperature on Antibacterial Activity of Silver Doped Titanium Dioxide Thin Films

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Abstract - The present study emphasizes how the substrate temperature plays a crucial role in influencing the antibacterial activity of Silver doped Titanium dioxide (Ag: TiO_2) thin films; prepared by a spray pyrolysis technique using perfume atomizer method. The prepared samples were of polycrystalline nature and possessed with expected optical properties. In addition to the conventional characterizing studies, an effort has been made to observe the critical role of substrate temperature upon antibacterial resisting behaviour of the chosen sample against a few common and air/media borne bacteria like Escherchia coli and pseudomonas aeruginos.

I INTRODUCTION

In recent times metal oxides play a crucial role in various technological applications due to their distinguishing physiochemical characteristics just as optical, electronic, magnetic, catalytic and antibacterial properties. The world-wide problem in human and animals, that is, usage of antibiotics and dosage of the same. Hence, antibiotic resistant bacteria with different mechanism are probed continually and used in newly invented drugs. Here, the search for an antibacterial resisting samples have been investigated. The antibacterial activity of the TiO_2 thin film has attracted many researchers due to its existence in high potential environment, energy fields including air and water purification systems, sterilization and hydrogen evolution and photo electro chemical conversion among others [1-3]. Moreover, the TiO_2 in powder form has frequently used in many fields such as in cosmetics, pharmaceutical, paint and paper industry. On the contrary, when the size of the sample particles reduces to smaller grain sized particles (i.e nano particles), it expected that an entirely different chemical, magnetic, optical and structural features may serve the requirements of many researchers.

TiO_2 is a photo-catalytic agent with good chemical stability and optical competency; has been used extensively for killing different groups of microorganisms including bacteria, fungi and viruses [4-7]. Hence, the photo-catalytic activity of TiO_2 nanoparticles with silver dopants [8-9] is expected to excel in this study. Moreover, it worth to dope silver nanoparticles as they have certain prospective applications such as biosensing, biodiagnostics, optical fibers, antimicrobial and photocatalytic uses. Silver ions also have simplest photo-catalytic oxidation reactions between oxygen molecules in the cell and covalently bonded hydrogen atoms of thiol groups through disulfide bonds (R-S-S-R), lead to blocking of respiration and cell death of the bacteria [10]. Moreover, another remarkable mechanism of Ag nanoparticles is related to the formation of free radicals and consequent free-radicals induced oxidative damage for the cell membranes of bacteria [11-13]. Considering the foresaid aspects, it has been aimed to investigate the antibacterial activity of silver doped titanium oxide thin film, with the incorporation of deposition temperature, here.

II. EXPERIMENTAL DETAILS

Silver doped titanium dioxide thin films were synthesized by a simplified spray pyrolysis technique with fixed concentration ($TiCl_4$ = 4 at. % and $AgNO_3$ = 2 at. %) of various substrate temperature (300° C – 450°C (insteps of 50° C)). For the preparation of spray solution, at first, required amount of Titanium (IV) chloride (99.9 % from Alfa Aser) was dissolved in a mixture of doubly distilled water and 10 ml of ethanol. Separately, silver nitrate ($AgNO_3$ 99 % from Alfa Aser) as a dopant material was dissolved in double distilled water; finally both the solutions were mixed together using magnetic stirrer.

Then, the final transparent solution was manually sprayed onto preheated glass substrate, during spray process the rate of spray was maintained at 2ml/min, substrate was kept 30 cm distance from the spray gun (Perfume bottle), angle between nozzle and substrate was fixed at 45° and the time duration of spray deposition was 20 to 30 minutes [14]. Hence, the overall reaction process can be described as thermal decomposition of starting materials in the presence of water and air. The prepared samples were labled as A1 for 300° C, A2 for 350 ° C, A3 for 400° C and A4 for 450° C.

III CULTURE OF MICRO ORGANISM

Nutrient Agar (NA-Himedia) Media for Bacteria

A. Composition of Media

Animals tissue : 5.00 g
Sodium chloride : 5.00g
Beef extract : 1.50g
Yeast extract : 1.50g
Agar : 15.0g

B. Preparation of medium

Suspend 28.0 grams in 1000 ml distilled water. Heat to boiling and dissolve the medium completely.

Sterilize by autoclaving at 15 Ibs pressure (121° C) for 15 minutes. Mix well and pour into sterile Petri plates.

C. Microorganisms

The microbial strains employed in the biological assays were Gram negative bacteria: Pseudomonas aeruginos (MTCC 2474) and Escherichia coli (MTCC 119) obtained from microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

D. Preparation of pure culture (24 hours)

A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37° C for 24 hours except for microorganisms which was incubated at 25°C for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 2° C until use.

E. Measurement of zone of inhibition

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 centimeter scale.

IV EVALUATION OF ANTIBACTERIAL ACTIVITY

Antibiogram was done by disc diffusion method [15-16] using Ag doped TiO_2 thin films. 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, spread and allowed to dry for 10 minutes. 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 (Seen in Fig.3-Fig.4). Briefly, inoculums containing Escherchia coli and Pseudomonas aeruginos on Nutrient agar plates for bacteria. Using sterile forceps, thin films (A1, A2, A3, and A4) were laid down on the surface of inoculated agar plate. The plates were incubated at 37^0 C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Each sample was tested in triplicate.

V RESULTS AND DISCUSSION

A. Structural analysis

The structural analysis was carried out in order to examine that the crystalline behavior of Ag- doped TiO_2 thin films. XRD patterns of the prepared samples at various temperatures ranging from 300^0 C - 450^0 C depicted in Fig. 1. It can be seen that the amorphous nature was observed for the sample prepared at 300^0 C, the samples were prepared with another temperature such as 350^0 C, 400^0 C and 450^0 C exhibit well defined diffraction peaks which is appear at 27.8^0, 32.2^0, 46.2^0 and 25^0 exhibited two different type of TiO_2 structure such as rutile and anatase phase. However, the peak intensity is found to be high when the sample prepared at 450^0 C, which indicating that a better crystallinity. Therefore, 450^0 C is the optimum temperature to obtained well adherent and uniform Ag doped TiO_2 films. The sample prepared at 350°C, the low intensity peaks were found which may due to the low crystalline growth of the film on substrate. The grain size of the samples were calculated from Scherrer formula, it is found to be 48 nm, 19 nm, 29 nm and 10 nm for the substrate temperature being 300^0 C, 350^0 C, 400^0 C and 450^0 C respectively.

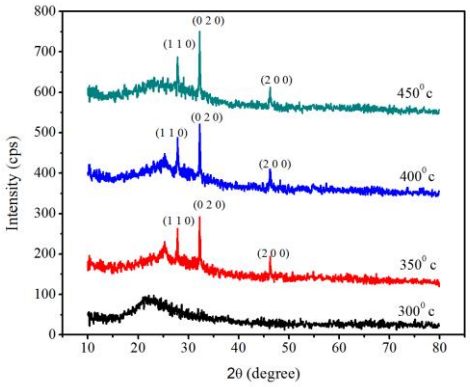

Fig.1. XRD patterns of Ag- doped TiO_2 Thin films

B. Optical analysis

The transmission spectra of Ag- doped TiO_2 films were characterized with the wavelength range of 300 - 1100 nm. The transmittance behavior of Ag- doped TiO_2 samples were recorded with respect to substrate temperature (300° C, 350° C, 400° C and 450° C) as shown in Fig. 2, a maximum transmittance of above 75% was found in visible to NIR region, when the film prepared at 450° C. The samples prepared at 350° C-450° C has above 60 % of visible transmittance was due to the well crystalline nature of the samples. For n=1/2 the transition data provide the best linear curve in the band edge region, which shows that the transition is direct in nature. The band gap of the prepared films were calculated using Tauc's plot by plotting $(\alpha hv)^2$ *Vs hv*, deduced to 2.7, 2.8, 3.1 and 3.3 *eV* with respect of substrate temperature (300° C - 450° C). The increment of band was due to Burstein-Moss (BM) effect, according to BM effect raising the Fermi level into the conduction band of degenerate semiconductor leads to energy band broadening.

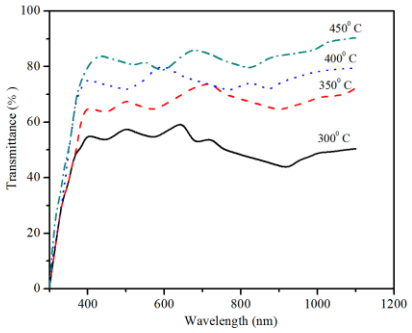

Fig. 2. Optical spectra of Ag- doped TiO_2 Thin films

C. Antimicrobial activity

The mean inhibition zone of thin film A1 was 5±0.35 cm for Escherchia coli and 1.16±0.08 cm for

Pseudomonas aeruginos. The highest microbial resistant were observed in Escherchia coli compared with Pseudomonas aeruginos. The mean inhibition zone of t A2 was ± 0.42 cm for Escherchia coli and 1.45±0.10cm for Pseudomonas aeruginos, high value was found for E.coli. Similarly, the mean inhibition zone of thin film A3 was 5±0.35 cm for Escherchia coli and 2.11±0.14 cm for Pseudomonas aeruginos and for A4, 7±0.49 cm for Escherchia coli and 2.28±0.15 cm for Pseudomonas aeruginos. The highest microbial resistant were observed in Escherchia coli than Pseudomonas aeruginos. Among the different thin film, A4 has highest antimicrobial activity obtained against Escherchia coli compared with other thin films.

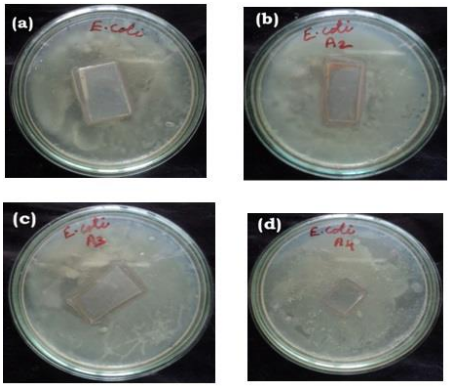

Fig. 3. Antimicrobial activity of Ag- doped TiO_2 Thin films (Escherichia coli)

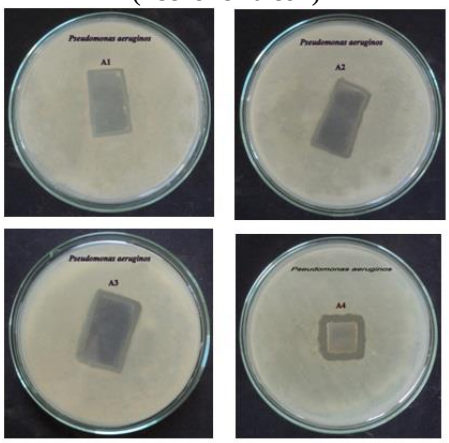

Fig.4. Antimicrobial activity of Ag- doped TiO_2 (Pseudomonas aeruginos)

Nanocrystalline Silver doped Titanium oxide (TiO_2: Ag) films employing a much simplified spray pyrolysis technique irrespective of the deposition temperatures (300° C, 350° C, 400° C and 450° C) for evaluation of antibacterial activities. In this work, the antibacterial property results show that the Ag doped TiO_2 thin films against Escherchia coli better than Pseudomonas aeruginos. The mechanism of antibacterial activity of these samples that 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 two categories such as 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 [17]. Moreover, the antibacterial experiments were done in the dark, so there are no active oxide, hydrogen peroxide, and super-oxide. Hence, the Ag-doped TiO_2 thin film are attached on the bacterial cell wall through electrostatic interaction, rupturing the cell walls, increasing the permeability, causing the leakage of cytoplasm which leads to bacterial cell death. Fig. 3-4 schematically illustrates the antibacterial mechanisms of TiO_2: Ag thin film Escherchia coli and Pseudomonas aeruginos. 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 the samples against Escherchia coli and Pseudomonas aeruginos are better than other microbials (Staphylococcus aureus, Bacillus subtilis etc.,). Comprehensive analysis revealed that the antibacterial activities (Fig. 5) of those samples were affected by both crystalline size and crystallinity. When the thin films are attached to the bacterial surface, Ag- doped TiO_2 crystals reacted with PG, teichoic acids, and lipoteichoic acids finally the structure of bacterial cell wall is damaged. 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 feeble. This could be because of the optimum temperature (450° C), although the particle size is smaller than the other samples. The bacterial cell wall is damaged and the electrical conductance of bacterial suspension is increased; it proves that the enhancement of antibacterial activity of Ag- doped TiO_2 thin film damage capability to the bacterial cell wall and cell membrane [18]. It concluded that the deposition temperature could be one of significant parameter for the preparation of thin films for antibacterial activity.

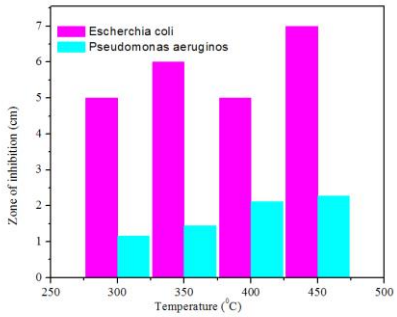

Fig.5. Comparative analysis two different antimicrobial activity of Ag- doped TiO_2 thin films with respect to deposition temperature

VI CONCLUSION

Silver doped titanium dioxide thin films (Ag doped TiO_2) with controlled sizes were synthesized from different deposition temperatures. The results of antibacterial properties show that the Ag doped TiO_2 thin

film have different antimicrobial activities. The antibacterial properties of the thin film prepared from TiO_2 are optimal and for the sample prepared by 450^o C has enhanced antibacterial activity compared with other samples (300^o C, 350^o C and 400 ^{o} C). Also, this samples have highest value of microbial resistant was found in E. coli than P. aeruginos.

VI ACKNOWLEDGEMENT

The authors wish to thank the University Grants Commission, New Delhi, for the financial support in the form of Rajiv Gandhi National Fellowship (F1-17.1/2011-2012/RGNF-SC- TAM-438/dt.06.06.2012).

VI REFERENCES

[1] R. Kanomi, J. Clin. Orthod. **31** (1997) 763.
[2] U. Fritz, A. Ehmer, P. Diedrich, J. Orofac. Orthop. **65** (2004) 410.
[3] L. Huang, J.L. Shotwell, H. Wang, Am. J. Orthod. Dentofac. Orthop. **127** (2005) 713.
[4] L. Carlsson, T. Rostlund, B. Albrektsson, T. Albrektsson, P.I. Branemark, Acta Orthop. Scand. **57** (1986) 285.
[5] L. Montanaro, D. Campoccia, C.R. Arciola, Biomaterials **28** (2007) 5155.
[6] A. Mo, J. Liao, W. Xu, S. Xian, Y. Li, S. Bai, Appl. Surf. Sci. **255** (2008) 435.
[7] B. Xin, L. Jing, Z. Ren, B. Wang, H. Fu, J. Phys. Chem. B **109** (2005) 2805.
[8] S.K. Lee, A. Mill, Platinum Met. Rev. **47** (2003) 61.
[9] A. Isse, S. Gottardello, C. Maccato, A. Gennaro, Electrochem. Commun. **8** (2006) 1707.
[10] B. Ohtani, K. Iwai, S.I. NishiMoto, S. Sato, J. Phys. Chem. B **101** (1997) 3349.
[11] C. He, Y. Xiong, X. Zhu, Thin Solid Films **422** (2002) 235.
[12] G. Zhao, H. Kozuka, T. Yoko, Thin Solid Films **277** (1996) 147.
[13] J.M. Herrmann, H. Tahiri, Y. Ait-Ichou, G. Lassaletta, A.R. Gonzalez- Elipe, A. Fernandez, Appl. Catal. B **13** (1997) 219.
[14] V. Sivaranjani and P. Philominathan, Int. J. Thin. Fil. Sci. Tec. **4** (2015) 9.
[15] G.S. Vicente, A. Morales, M.T. Gutierrez, Thin Solid Films **403** (2003) 338.
[16] T. Sekiya, S. Kamei, S. Kurita, Lumin. **87** (2000) 1142.
[17] M. Paulose,K.M. Gopal, O.K. Varghese, K. Shankar, C.A. Grimes, Photo chem. and Photo bio. A: Chem. **178** (2006) 15.
[18] Z. Huang, P.C. Maness, D.M. Blake, Edward J W, Sharon L S, William A J, Photo chem. and Photo bio. A: Chem. **130** (2000) 170.