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ANTIBACTERIAL ACTIVITY OF TiO_2 THIN FILMS AGAINST GRAM NEGATIVE OF BACTERIAL STRAINS

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Abstract: *Titanium dioxide (TiO_2) is one of the most important semiconductors. It has attracted growing attention in huge applications because of their unique properties. In this present survey we are interesting to investigate the resist growth of TiO_2 thin films with various dopants against gram negative of Escherichia coli bacteria. TiO_2 films were deposited on soda lime glass and tissue by two different methods (spray pyrolysis and sol-gel) in order to study their effect on antibacterial activity. We used two processes of bacteria and in both the results indicate that TiO_2 can resist the growth of E. coli. The zone of inhibition of this bacterium is found between 11 and 17 mm.*

Key words: *TiO_2, E. coli, antibacterial activity, dopants*

1. Introduction

Diseases caused by bacteria, viruses, fungi, and other parasites are major causes of deaths, disabilities, and social and economic disruption for millions of people. Infections acquired in hospital or in the course of medical treatment present a serious burden for patients and the health systems. Over 9.5 million people die each year due to infectious diseases in developing countries. There are many kinds of disease (e.g., listeriosis and strep throat) which can be caused by bacteria contamination of the environment. Escherichia coli (E. coli), gonococcus, salmonella, staphylococcus bacteria, streptococcus, and typhoid bacillus, are well known as common bacteria. For instance, enterohemorrhagic E. coli is very dangerous bacteria, which can cause serious diarrhea.

Therefore, the most urgent requirement for humans is to prevent these diseases by applying materials that are capable of killing or inactivating the causative bacteria. The development of new materials with high antibacterial properties has long been the goal of medical science.

Robust inorganic materials have received more recognition in the antibacterial market and photocatalytic products, because of their high heat resistance and long life expectancy. Especially, titanium dioxide (titania, TiO_2) has been recognized as the most familiar substance for widespread environmental applications [1], because of its biological and chemical inertness, strong oxidizing power, nontoxicity, and long-term stability. Nowadays, Titania nanomaterials with various morphologies have been used for wide applications such as an Instructions water treatment, air purification, hazardous waste remediation, environmental purification, and deactivation of bacteria [2].

The antibacterial activity of Titania strongly depends on its crystal structure, surface area, and surface morphology. Crystallized anatase nanoparticles (e.g., Degussa P-25) show excellent photocatalytic activity, which offers potentially a facile and cheap method to clean the environment from pollutant traces as well as from biological organisms such as bacteria and viruses. Mesoporous anatase with extremely high surface area can provide a higher amount of hydroxyl radical, which can increase the antibacterial activity compared to commercially available products [3].

2. Experimental

2.1. Preparation of films:

TiO_2 thin films on glass:

Doped and undoped TiO_2 were deposited by ultrasonic spray pyrolysis at 400 °C with 2.6 ml/l and 12 cycles (for 1 cycle: 20 s solution sprayed and 20 s paused). The solution of TiO_2 was prepared with 120 µl titanium isopropoxide dissolved in isopropanol then sprayed on soda lime glass.

TiO_2 films on tissue:

To deposit TiO_2 pure and doped by sol-gel method (dip-coating), we used 120 µl titanium isopropoxide dissolved in isopropanol and added acetic acid then took on various dopants such as: silver, Au Nanoparticles, Cobalt and Indium. The tissue was dipping in the solution for 15 s then dried for 10 min at 80 °C for 2 layers and 5 layers. The pieces of tissue cut at diameter of 1 mm.

2.2. Preparation of bacteria:

2.2.1 First method: (shaker)

We prepared liquid medium and agar medium for bacteria. For inoculums 1ml bacteria E. coli and 30 ml Nutrient Broth (NB) medium after 1 day or 24 hours, we took from this solution 100 µl and we sprayed in agar (plate). Then we put on film (agar) (100 µl on film) after this step, we put in the incubation then after 1 or 2 days we checked for which one has got inhibition zone .

2.2.2. Second method:

We took 1ml bacteria E. coli and added 9 ml N.B medium (Solution 1). After the first step, we took 100 µl from solution 1 and added 900 µl N.B medium (Solution 2). Then we took from solution 2:1 ml and put this quantity on our films. Finally, we put all samples with this method of preparation and we used the shaker for 2 hours at 37.5°C temperature. This was for incubation. After 2 hours, we took 100 µl from solution of samples. Then all plate waited for 1or 2days in the incubation at 37.5°C. After we shake the samples and reference then we counted the colonies.

For medium: Agar Nutrient Broth liquid, we put 8g in 1 litre and for Nutrient agar for plate (colonies) the weight employed is 20 g in 1 litre. To prepare medium, we use autoclave at 121 bar for 20 minutes.

3. Results and Discussion

The antibacterial activity of glass slide coated doped and undoped TiO_2 was tested against the Gram negative bacterium E. coli. The viable bacteria were monitored by counting the number of colonies. As shown in Fig.1, all the samples: TiO_2 3 % Co, TiO_2 3% In, TiO_2 5 % In, TiO_2 3 % Au NPs 2 layers, TiO_2 5 % Au NPs 5 layers, TiO_2 5 % Ag NPs 5 layers show the disappearance of colonies after 24 H of treatment. No bacterial growth was observed adjacent to the coating. As concluded the films can resist the growth of E. coli by the shaker Method.

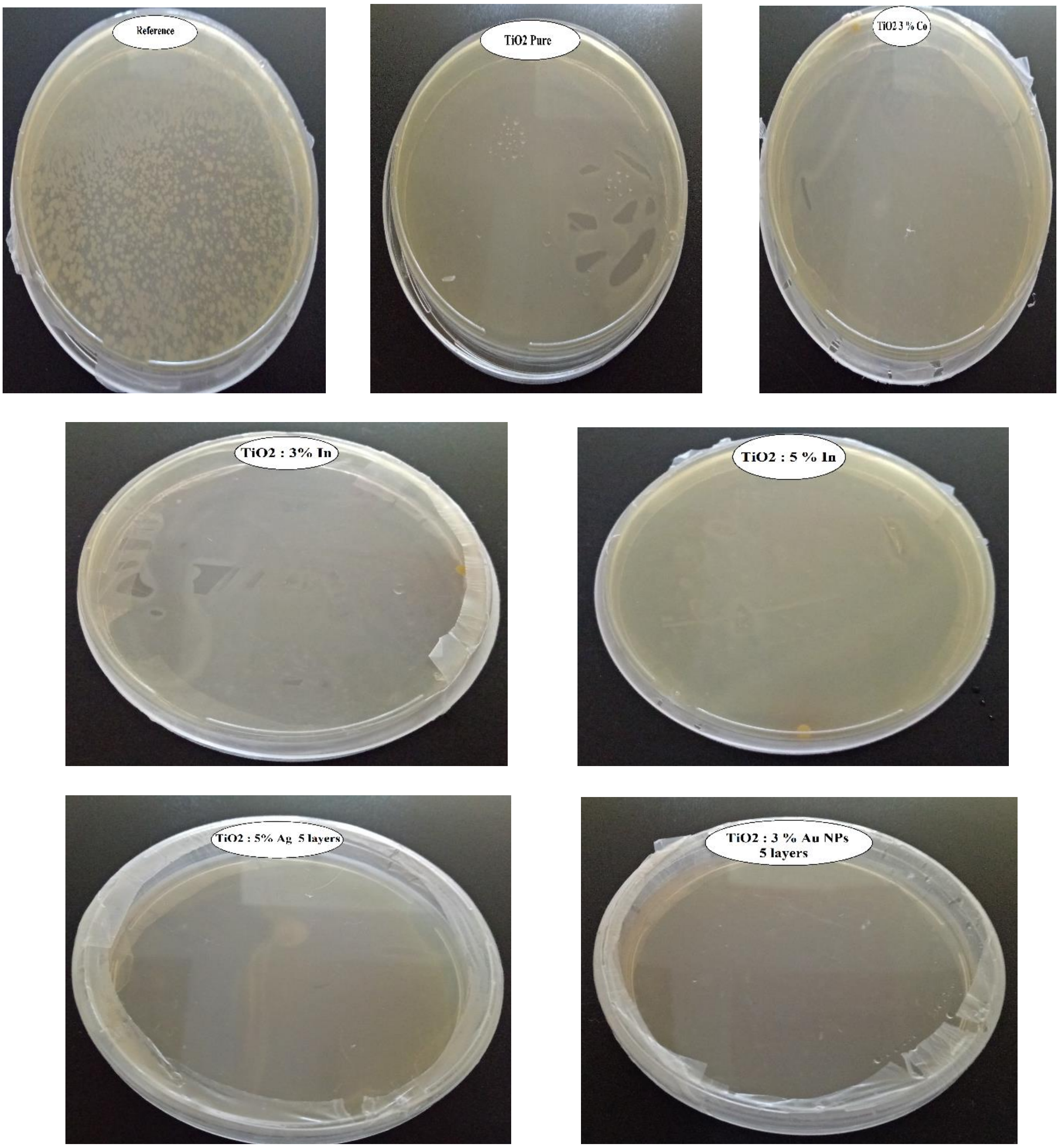

Fig.1: Doped and TiO_2 pure against gram negative bacterium E. coli

For the second method another set of bacterial tests was performed by applying the Kirby Bauer method, looking for zone of inhibition of the coated tissue. In these tests a tissue with and without TiO_2 coating partially covered the agar disk. Bacteria were then sprayed onto the surface of the coated disk. Bacterial growth was visualized after an overnight incubation in NB agar. No bacterial growth was observed adjacent to the coating, and for the higher percentage of TiO_2 coating the observed zone of inhibition was larger with 5 % Au NPs 5 layers. In comparison, many colonies were grown around the uncoated tissue as shown in Fig.2.

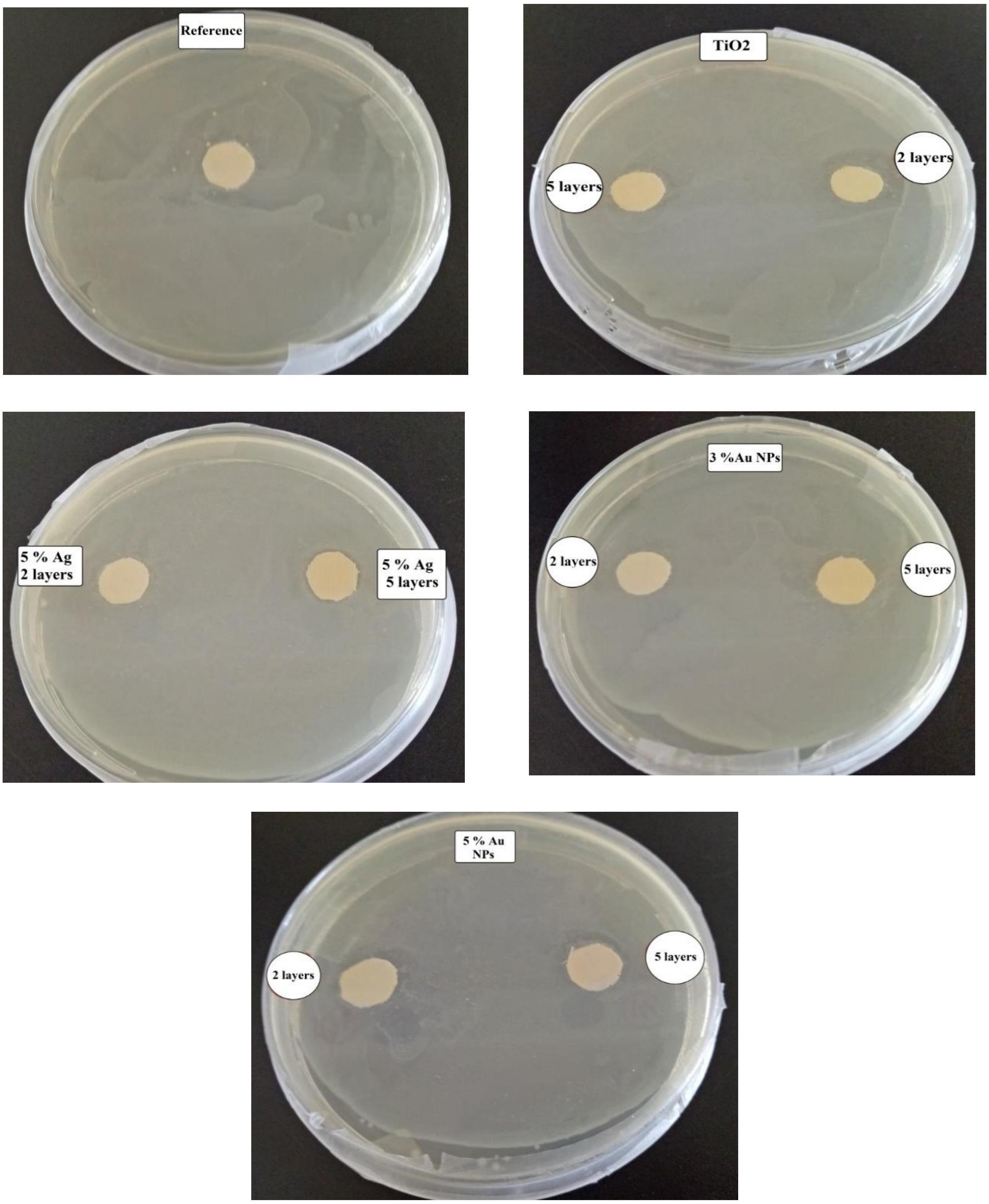

Fig.2: Doped and TiO_2 pure on tissue against gram negative bacterium E. coli

We have reported in Tab.1 the zone of inhibition for all samples prepared by sol-gel dip-coating on tissue. As observed the zone of inhibition is ranged between 11 and 18 mm. So the doped and undoped TiO_2 are harmful for this type of E. coli bacteria.

Tab.1 The zone of inhibition of TiO2 pure and doped against E. coli bacterium

TiO_2	Zone of inhibition (mm)
Reference	-
TiO_2 pure 2 layers	17
TiO_2 pure 5 layers	13
TiO_2 5% Ag 2 layers	11
TiO_2 5% Ag 5 layers	13
TiO_2 3 % Au NPs 2 layers	11
TiO_2 3 % Au NPs 5 layers	14
TiO_2 5 % Au NPs 2 layers	13
TiO_2 5 % Au NPs 5 layers	18

One of the factors influencing the antibacterial activity of the developed coating is the release of an active ingredient into the surrounding medium. The same observation was signed by G. Applerot et al. [4]. A detailed study of the mechanism of the activity of ZnO nanoparticles leads us to assume that the leaching of Zn ions governed by the Ksp of ZnO has a minor influence on the antibacterial activity. We have recently found [5] that the major components responsible for the bactericidal effect of ZnO nanoparticles were some species of oxyradicals in the solution, mainly hydroxyl radicals. These reactive-oxygen-species (ROS) were detected in electron spin resonance (ESR) studies. Moreover, the results of the antibacterial tests of the coated glass are in good agreement with the results obtained using pristine ZnO nanoparticles powders as an antibacterial agent [5]. Thus, it can be concluded that the major contributors of antibacterial activity of bacterium contains a large amount of cartenoid pigment, rendering it a higher resistance to oxidative stress [6].

4. Conclusion

The use of doped and undoped TiO_2 as a semiconductor for study the antibacterial activity was investigated. In this survey, two processes of bacteria were used with two deposition methods (Spray pyrolysis and dip-coating). The TiO_2 obtained were deposited with various dopants on glass slides and tissue with 1 mm as a diameter. The performance of glass slides coated with various percentages of TiO_2 as an antibacterial agent was investigated, and their excellent bactericidal effect was demonstrated by their zone of inhibition against gram negative bacterium E. coli.

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