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Determining the antibacterial effect of ZnO nanoparticle against the pathogenic bacterium, *Shigella dysenteriae* (type 1)

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ABSTRACT

The nanomaterials have important application in different field of science such as biology and pharmacology, which draws the attention of biologists towards this field of study more than before. As the worldwide mortality rate is high due to the pathogens and especially because of the bacteria associated dysenteriae and the antibacterial effect of metal nanoparticles is well known from centuries- these materials can be used to annihilate *Shigella dysenteriae*. Here, we study the antibacterial characteristics of metal nanoparticle; ZnO against *Shigella dysenteriae* (type 1). Firstly, solid state pyrolytic reaction process has been used to synthesize ZnO nanoparticles. The characteristics were investigated by XRD, SEM and UV-Visible spectrometer. The optical density (OD) of *S.dysenteriae* was observed in the presence of 0.05% ZnO. This study showed that the presence concentration of nanoparticle was insufficient to the demonstrated antibacterial activity. But a considerable decrease in the bacterial number was observed in the presence of 0.5% and 1% ZnO nanoparticles. A 1.7 times decrease in the OD of *S.dysenteriae* was recorded of control group ($p<0.001$). The recorded in OD was 2.2 and 3.1 times in the presence of 0.5 and 1% ZnO nanoparticles respectively ($p<0.05$). Thus, ZnO nanoparticle can be candidate of antibacterial agent.

Keywords:*Nanoparticle, Bactericidal, Shigella dysenteries, ZnO*

INTRODUCTION

Scientists believe that the nanotechnology is multidisciplinary. Characteristics of nanomaterials differ enormously from those of the bulk materials [1]. These differences in characteristics have made nanotechnology applicable in different fields of science. Nanomaterials are being used in many branches of science such as harmful microorganisms, recognition and treatment of various diseases [2].

Nanotechnology has also invaded engineering, biology, chemistry, medicine physics etc. Metallic nanoparticles have different functions such as antibacterial characteristics [3]. Metallic nanoparticles are continuously being used in the manufacture of manufacture of bactericides, but unfortunately the application in these processes reduces the antibacterial characteristics of nanoparticles. However, in the meantime, inorganic nanoparticles seem to have been good bactericides because of their tolerance to high temperatures [4].

Nanomaterials have highly attractive structures as they have high potential for specific functions, especially in the biological and pharmacological studies and because of this fact; these materials are significantly important in modern pharmacology. Inorganic nanoparticles can annihilate 650 infected cells within 4 hours [5]. Effect of nanomaterials on microorganisms is very important as the latter constitutes the lowest level of food chain and ecosystems [6 -7].

Bactericidal characteristics of Zn nanoparticle and their oxidized forms have been numerously reported. Human beings suffer from mycosis, bacterial, viral and parasitic diseases. Thus the annihilation of these microorganisms is an important problem for human survival. For example, *Shigella dysenteriae* (type 1) has been reported as a principal cause, while the mortality ratio in E1 Salvador was 1.2%, while 0.6% of which was epidemic [8].

It is sad to say that 75000 children died in Bangladesh because of its epidemic state and 35000 deaths were observed in the non-epidemic state. During past 30 years, *S.dysenteriae* (type 1) was reported to have caused pandemic state in Central America, Bangladesh, South Asia, Central Asia and South Africa [9]. Endemic state of this disease is seen in about 10% of the children fewer than 5 years in the developing countries, while, over 75% cases are accompanied by death. Therefore annihilation of this case is an urgent issue [10].

Thus, the present study demonstrates the antibacterial characteristics of ZnO nanoparticles against *S.dysenteriae*. It is hoped that our research group can produce the nanoparticle-containing antibacterial fabric against *S.dysenteriae* [11] in near future studies.

MATERIALS AND METHODS

Bactericidal strains and culture conditions

S.dysenteriae type 1 (ATCC 1020) was used during the present experiment. Nutrient Agar (V264547; E. Merck Co; Darmstadt, Germany) and Nutrient Broth (VK493267; E. Merck Co; Darmstadt, Germany) were used in growing and maintaining the bacterial cultures.

Synthesis of ZnO nanoparticle

In this paper the ZnO powders were synthesized by solid state pyrolytic reaction process [14]. A typical synthesis is as follows: equal amount of Zn $(CH_3COO)_2.2H_2O$ and $NaHCO_3$ are mixed at the room temperature. Then the mixture is initially heated at 1600^oC, and then kept in pyrolytic temperature. The Zn $(CH_3COO)_2.2H_2O$ is changed into ZnO nanoparticles, while the $NaHCO_3$ is changed into CH_3COONa and eventually washed away with de-ionized water. Consequently, white ZnO nanoparticles are obtained.

Characterization of synthesized ZnO nanoparticle

- **X-ray diffraction**

The XRD technique was used to determine the crystal structure of ZnO nanoparticles. We used a Bruker AXS D8-advance diffract meter using Cu kα radiation and λ=1.5406 nm. The structure of the products was first characterized by using XRD; the XRD patterns of the powders measured for corresponding samples were investigated. Finally, XRD diffraction patterns (Figure 1) stabled the size of nanoparticle. Moreover, by Scherrer formula demonstrated the size nanoparticle was 4.97 nm.

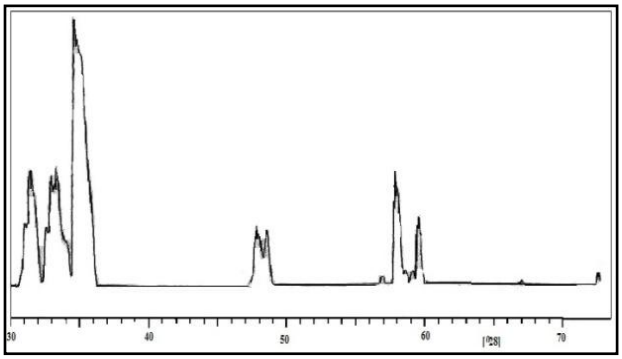

Fig.1.XRD diffraction spectra of ZnO nanoparticles powder for sample 2hr-160^oC-2-180 ^oC.

Also X-ray powder diffraction was used to investigate the ZnO powders. We applied a Bruker AXS D8-advance diffractometer using cuk radiation and =1.5406 nm

The structure of the products was first characterized by using XRD; the XRD patterns of the powders measured for corresponding samples were investigated. The lattice constants calculated from diffraction peaks and three pronounced ZnO peaks, corresponds to planes of crystallinity (100), (002) and (101), appears at respective 2θangles, which is indicative of single phase. In comparison with JCPDS data card, the diffraction patterns can be well indexed to hexagonal wurtzite structure of ZnO [12-13, 14].

Moreover XRD is used to characterize the size of ZnO nanoparticles by Scherrer formula. The formula is,

$$D=K\lambda/FWHM.COS\theta^{*}$$

The size of nanoparticles were measured by investigations of first pronounced XRD peak corresponds to (100) plane of crystallinity. Moreover, the average size of nanoparticles demonstrated to be 4.97 nm.

- **Scanning electron microscopy**

The scanning electron microscopy (SEM) images were taken by a scanning electron microscope Model XL30- Philips Company, operated at 30Kv. SEM provided images of the particles by magnification of about one million times greater.The assembly was attached with a computer software programming to analyze the mean size of the particles in sample. It should be noted that the particle diameter is always overestimated due to the distortion of SEM images [15].The results of nanoparticle size measurement of samples by XRD & SEM indicate that the size nanoparticle is about 5 nm.

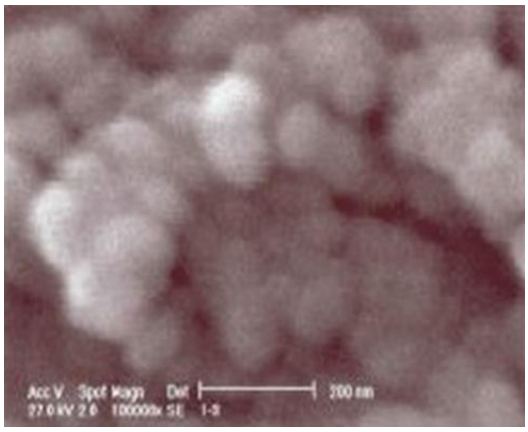

Fig.2. SEM image of ZnO particle for corresponding sample 2hr-160°C-2-180°C

- **UV-Visible absorption spectroscopy**

The model Cary UV-visible Spectrometer was used to study the absorption of the samples. The peak position of absorption spectra corresponds to the most probable size distribution of nanoparticles in sample.

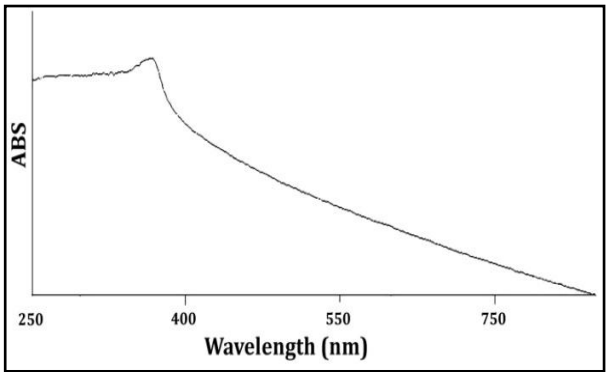

Fig.3. Absorption spectra of ZnO nanoparticle; thermal for sample 2hr-160°C-2-180 °C

Bactericidal susceptibility to nanoparticles

To examine the susceptibility of *S.dysenteriae* type 1 to the experimental nanoparticles, four different estimation methods were used with four tile-repetitions.

Bactericidal growth in the presence of nanoparticles in liquid broth

During the first experiment, bactericidal cells were grown in nutrient broth (NB). To start the growth, 2 ml of the overnight-cultured *S.dysenteriae* stock was added to 100 ml NB containing 0.12% glucose with and without 0.01,

*D: Diameter of the particle K: Shape factor is 0.9 for sphere
λ: Wavelength of X-ray in Angstrom
FWHM: Full Width at Half Maximum in radian

0.5 and 1% nano-ZnO separately. The bacteria were aerobically cultured at 30ºC for 24 hours. OD measurements were taken at 600 nm to monitor the bactericidal concentration.

Bactericidal killing in the presence of nano-ZnO in liquid broth

In the second study, the culture solution was centrifuged, the cells were washed and re-suspended in distilled water, reaching a final concentration of 6.3 log CFU/ml in each of the sample flasks and incubated at 4ºC. The final concentration of the *S.dysenteriae* suspensions was made in 100 ml distilled water. Different amounts of nano-ZnO (0.01, 0.5 and 0.1%) were then separately added to keep in contact with the bactericidal cells and shaken at 4ºC for 48 hours. OD was measured to obtain the results. Aliquots of 0.1 ml of the growth mixtures (water + bactericidal cells + nanoparticles) were sampled every two hours and the number of resulting bactericidal cells was noted at the end of each incubation. Bactericidal number was determined by measuring the OD) at 600 nm (The OD values were converted into the *S.dysenteriae* concentration as log CFU/ml.

Bactericidal growth in the presence of nano-ZnO in agar medium

In the third experiment, 10 µl (one loop) of *S.dysenteriae* cells was transferred from the stock solution to Petri plates, containing nutrient agar medium. With 0.12% glucose and 2% agar. 1% nano-ZnO was added to the plates, representing the experimental groups. Bacterial cells were grown at 30ºC for 48 hours. Afterwards, the plates were visually estimated and bactericidal colonies counted. The pictures were taken by a digital camera. The data obtained in all tests were compared with those of control. Student's t-test was used to evaluate the significance of experimental results ($p<0.05$).

RESULTS AND DISCUSSION

Effect of nano-ZnO on the growth of S. dysenteriae in NB

During the first experiment, the effect of different concentrations of nanoparticles was evaluated on the *S.dysenteriae* cultures in NB. The optical density of the medium was investigated as the number of bactericidal cells after contact with nanoparticles. Figure 4 demonstrated the effect of 0.01, 0.5 and 1% nano-ZnO on the growth and killing kinetics of *S.dysenteriae*.

The effect of different concentrations of nano-ZnO was also evaluated on the growth of S. dysenteriae (Figure 4). As demonstrated in this Figure, 0.01% nano-ZnO did not have antibactericidal effect while 0.5 and 1% nano-ZnO were highly efficient in inhibiting the bactericidal growth as compared to the control group. This Figure shows that the presence of 0.5% nano-ZnO caused a 2.2 times decrease in the optical density of bactericidal cultures ($p<0.05$) as compared to the control. The results of first experiment show that nano-Zno has more efficient antibactericidal property against *S.dysenteriae*.

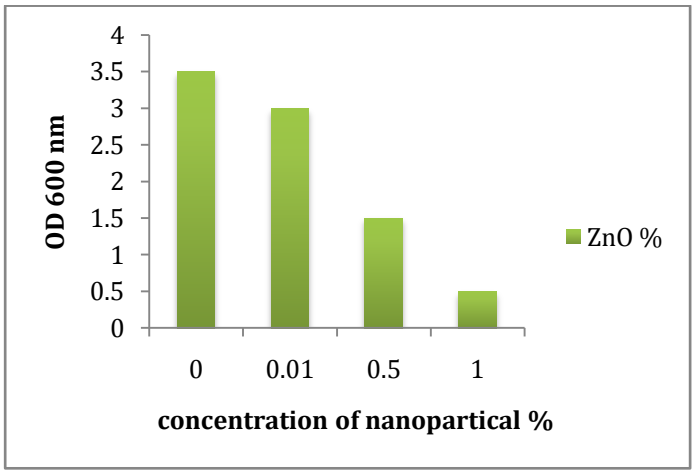

Fig.4.*S.dysenteriae* concentration dependence upon different concentrations of nano-ZnO in the culture medium

Bactericidal effect of nano-ZnO S.dysenteriae in liquid medium

In the second study, estimation of the number of viable *S.dysenteriae* cells in contact with 1% nano-ZnO was carried out in water at 4ºC for different contact time intervals. Our result showed the reduction of *S.dysenteriae* cells from 6.3 log CFU/ml to undetectable levels after 12 days (Data have not been shown), while, upon the addition of these nano-materials to the bactericidal culture showed decreased survival rate within 2 days as compared to that of 12-day experiment for control group.

Figure 5 represents the number of viable *S.dysenteriae* cells in contact with 1% nano-ZnO, separately, suspended in water at 4ºC for different contact times. From the figure, it can be clearly observed that nano-ZnO exhibited different antibactericidal properties. After the *S.dysenteriae* were suspended in water along with ZnO, the number of microbial cells reached zero after 22 hours. These results demonstrate a stronger antibactericidal effect of nano-ZnO on *S.dysenteriae* as compared to the control group where survival was seen up to a culture period of 12 hours. The administration of nano-materials to the bactericidal cultures killed the bacteria in less than 2 days. These results demonstrate that nano-ZnO have a high antibactericidal efficiency against *S.dysenteriae*.

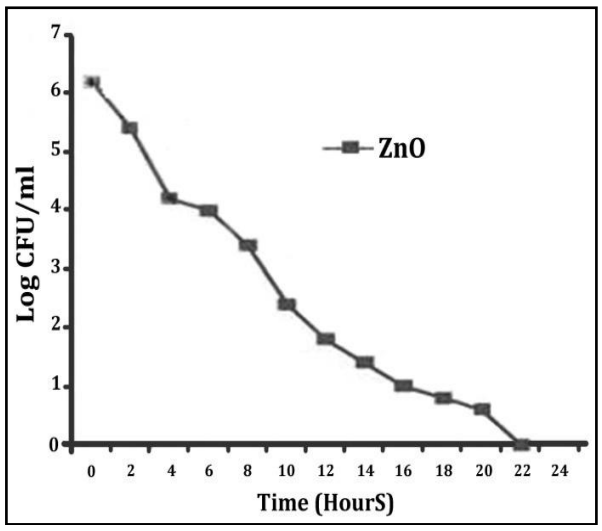

Fig.5. Comparative killing kinetics of 1% ZnO (■) on the *S.dysenteriae* cultures

Effect of nano-ZnO on the S.dysenteriae growth in agar medium

In the third investigation, *S.dysenteriae* was grown on agar medium without (control) or with 1% nano-ZnO separately. Distinct bactericidal colonies were observed in 105 times dilution. The visual estimation and bactericidal colony counts were performed at this dilution. In Figure 6, we can see smaller number of *S.dysenteriae* colonies on the agar medium with nano-ZnO (plat B), as compared to the control group (plate A). In the control plates, 802±75 bactericidal colonies were obtained while in the experimental plates with 1% nano-ZnO, 140±32 bactericidal colonies were seen. Thereby, nano-ZnO suppresses the bactericidal growth 15 times compared to the control plate ($P<0.05$).

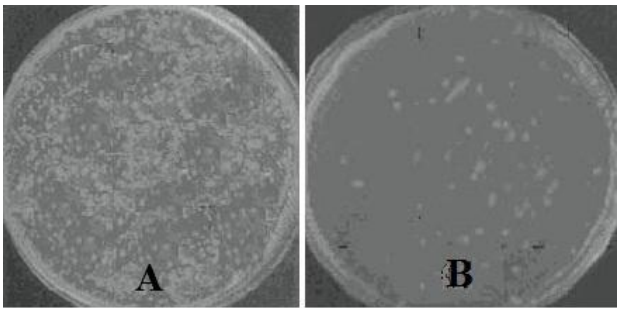

Fig.6.*S.dysenteriae* growing on the agar medium without ZnO nano-particles (A) and with 1% nano-ZnO (B)

The antibactericidal activities of different concentrations of nano-ZnO were investigated in this study. *S.dysenteriae* (ATCC 1020) was used as the test organism during the experiments. Good growth-inhibition results were observed when the bactericidal cells were incubated with both kinds of samples during the liquid and solid cultures. The quantitative examination of bactericidal activity was estimated by the survival ratio as calculated from the number of viable cells, which formed colonies on the nutrient agar plates. Another study states the nano-ZnO is a strong and effective bactericidal agent [16]. The present data demonstrate that a formulation made with the biologically stabilized ZnO nanoparticles can be useful in the treatment of infectious diseases caused by *S.dysenteriae*. A strong binding of nanoparticles to the outer membrane of *S.dysenteriae* causes the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis,

which finally leads to the cell lysis as was seen for *S.dysenteriae* during the study. Such effective and less-time consuming formulations can be useful in the clinical practices where *S.dysenteriae* causes urinary tract infections (UTIs). It has been known that nano-materials exhibit strong inhibitory effects towards a broad spectrum of bactericidal strains.

In this study, different concentrations of nano-scale ZnO were tested to find out the best concentration that can have the most effective antibactericidal property against the *S.dysenteriae* culture. Our data is in accordance with the previous studies, dealing with the antibactericidal effects of nano-materials [17]. If concentration of nano-ZnO increases in culture medium, interaction between oxygen and dehedrogenase enzyme increases too [17]. A previous study has reported the negative and positive effects of nanoparticles on the plant. According to some studies, ZnO nanoparticles prevented of photosynthesis and nitrogen metabolism [18].

In another study, ZnO nanoparticles were observed to have impact on the DNA damage. Collectively, a huge number of researchers have studied the effect of nanoparticles on the gram-negative and gram-positive bacteria such as *E.coli* and Staphylococcus aureus as well as human immune cells. But the results of these studies have demonstrated that the nanoparticles had harmful effects on bactericidal strains and humans T-lymphocytes [19].

Several investigations have suggested the possible mechanisms involving the interaction of nano-materials with the biological macromolecules. It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an "electromagnetic" attraction between the microbe and treated surface. Once the contact is made, the microbe oxidizes and dies instantly. Generally, it is believed that nano-materials release ions, which react with the thiol groups (-SH) of the proteins present on the bactericidal cell surface. Such proteins protrude through the bactericidal cell membrane, allowing the transport of nutrients through the cell wall. Nano-materials inactivate the proteins, decreasing the membrane permeability and eventually causing the cell death. Nano-materials also retard the bactericidal adhesion and bio-film formation [20]. Antimicrobial modification to prevent the growth of detrimental microorganisms is a highly desired objective. Microbial cell growth and colonization result in the formation of a compact bio-film matrix, capable of protecting the underlying microbes from antibiotics and host defence mechanisms. Such microbial infestation can cause serious infections [21-22]. The metal ion-based nanomaterials exhibit broad-spectrum biocidal activities towards different bacteria, fungi, and viruses [22]. Nanomaterials are known to deactivate cellular enzymes and DNA by coordinating with the electron-donating groups such as thiols, carboxylates, amides, imidazoles, indoles, hydroxyls, and so forth. They create pits in bactericidal cell walls, leading to increased permeability and eventually the cell death [23].

CONCLUSION

The present study reveals that the characteristics of antibacterial effect of nano-ZnO, given the fact that the nano-composition of ZnO or nano-covers of ZnO can be used in Bio-medicineproduct, consequently contained more antibacterial active sites than other antibacterial agents.

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REFERENCES

[1] Yadav, A., Prasad, A.,Virendra, Kathe A., Raj, S., Yadav, D., Surdamoorthy, C., Vigneshwaran, N. (2006).Functional finishing in cotton fabrics using zinc oxide nanoparticles. *Bull.Mater.Sci*., 29(6), 641-645.

[2] Kumar, A., Jakhmola, A.(2007). RNA-mediated fluorescent Q-PbS nanoparticles. *J. Langmuir*, 23, 2915–2918.

[3] Aslan, K., Geddes, CD. (2005). Metal-enhanced fluorescence: An emerging tool in biotechnology. *J. Curr. Opn. Biotech*., 16,55–62.

[4] Alivisatos, P. (2004).The use of nanocrystals in biological detection. *Nat. Biotechnol*., 22,47–52.

[5] Adams, L., Lyon D.Y., Alvarez P.J.J. (2006). Comparative eco-toxicity of nanoscale TiO2,

SiO2, and ZnO water suspensions. *Water Res*, 40, 3527–3532.

[6] Li, P., Li, J., Wu, C., Wu, Q., Li, J. (2005). Synergistic antibactericidal effects of lactam antibiotic combined with silver nanoparticles. *J. Nanotechnol*., 5(16),1912–1917.

[7] Te-Hsing, W., Yi-Der, T., Lie-Hang, S.(2007).The novel methods for preparing antibactericidal fabric composites containing nano-material. *Solid State Phenomena*, 124,1241-1244.

[8] Shiga, K.(1998).Ueber den Erreger der Dysenterie in Japan, Vorlaufige Mitteilung Zentralbal BakteriolMicrobiol Hyg, 23,599-600.

[9] Shiga, K. (2002).Obsevation on the epidemiology of dysentery in Japan, Philippine.*J.of Sci*., 1, 485-500.

[10] Hale., T. L. (1991). Virulence phenotype and genetic characteristics of the T32-1STRATI Shigellaflexneri 2a vaccine strain.*Vaccine*, 9,358-363.

[11] Sungkaworn, T., Triampo, W., Nalakarn, P., Triampo, D., Tang, I.M., Lenbury, Y., Picha, P. (2007). The effects of ZnO nanoparticles on tumor cell colonies: fractal dimension and morphological properties. *Int. J. Biomed. Sci*., 2,67-74.

[12] Soon, J., Kwan, G., Yu, J.H., Hyeon, T.(2005). Synthesis of ZnO nanocrystals with cone, hexagonal cone, and rod shapes via non-hydrolytic ester elimination sol-gel reactions. *J. Adv. Mater*., 17, 1873-1877.

[13] Lee, J., HKo, K.H., Park, B.O. (2003) Electrical and optical properties of ZnO transparent conducting films by the sol-gel method", *J. Cryst. Growth*, 247, 119-125.

[14] Zhijian, W., Haiming Z., Ligong H., Jinshan Y., Shenggang Y., Chunyan W.(2003). Low-temperature synthesis of ZnO nanoparticles by solid-state pyrolytic reaction. *J. Nanotechnology*, 14,11-15.

[15] Lu, J.G., Ye, Z., Huang, J., Zhu, L., Zhao, B., Wang, Z., Fujita. S .(2006). ZnO quantum dots synthesized by a vapour phase transport process, *Appl. Phys. Lett*., 88, 063110.

[16] Fortner, J., Lyon, D.Y., Sayes, C., Boyd, A., Falkner, J., Hotze, E., Alemany, L., Tao, Y., Guo, W., Ausman, K., Colvin, V., Hughes, J. (2005). C-60 in water: Nanocrystal formation and microbial response. *Environ. Sci. Technol*., 39, 4307-4316.

[17] Hranisavljevic, J., Dimitrijevic, N., Wurtz, G., Wiederrecht, G. (2002). Photoinduced charge separation reactions of J-aggregates coated on silver nanoparticles. *J. Am. Chem. Soc*., 124, 4536–4537.

[18] Clement, J., Jarrett, P.(1994). Antibacterial Silver. Met. Based. *Drugs, J.of Sci*., 1,467-482.

[19] Aslan, K., Huang, J., Wilson, G., Geddes, C. (2006). Metal-enhanced fluorescence-based RNA sensing. *J. Am. Chem. Soc*., 128, 4206–4207.

[20] Zhang, H., Chen, G. (2009).potent antibacterial activities of Ag/TiO_2nanocomposite powders synthesized by a one-pot sol-gel method. *Environ.J. Sci Technol*, 43(8), 2905-2910.

[21] Jones, G., Muller, C., O'Reilly, M., Stickler, D. (2006). Effect of triclosan on the development of bacterial biofilms by urinary tract pathogens on urinary catheters. *J Antimicrob Chemother*, 57(2), 266−272.

[22] Greenberg, C., Steffek, C. (2005). Bio-adhesion to thin films in relation to cleaning. Thin Solid Films.*J. Cryst. Growth*, 484(2), 324−327.

[23] Holt, K., Bard, A. (2005). Interaction of silver (I) ions with the respiratory chain of Escherichia coli: An electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag. *J. Biochemistry*, 44(39),13214−13223.