Journal of Medicinal Chemistry & Toxicology

OMMEGA Publishers
Answering Intelligence
www.ommegaonline.com

Research Article Open Access

Antibacterial Activities of Nanoparticles of Titanium Dioxide, Intrinsic and Doped with Indium and Iron

Teklit Gebregiorgis Amabye[1*], Abrham Birara[2]

[1]Department of Chemistry, Mekelle University College of Natural and Computational Science, Ethiopia
[2]Department of Biology, Mekelle University, Ethiopia

***Corresponding authors:** Teklit Gebregiorgis Amabye, Department of Chemistry, Mekelle University College of Natural and Computational Science, Ethiopia, E-mail: teklitgeb@gmail.com

Abstract

The need for new antimicrobial compounds has been attention on developing new and emerging materials based on nanoparticles with antimicrobial activity. The aim of this research was to evaluate the antibacterial activities of nanoparticles of titanium dioxide, intrinsic and doped with Indium and Iron in *Escherichia coli* and *Staphylococcus aureus*. The bacteriostatic effect of TiO_2 nanoparticles (two samples), TiO_2:Fe and TiO_2:In with concentrations of 50, 250 500 µg/ml. It was observed by optical density measurements. The bactericidal effect was determined by plate count agar Muller-Hinton, where they were incubated for 12 hours: 50 µl of bacterial suspension (concentration 1.5 x 10^6 bacteria/ml), concentration 50 µl of nanoparticles suspended at concentrations between 39 and 2500 µg/ml. Then, to 10 - 6 dilutions were made and plated on agar for colony counts. There were significant decreases $P < 0.05$ in the bacterial optical densities with respect to control, using TiO_2 nanoparticles prepared with different contents of acetic acid at concentrations of 250 and 500 µg/ml versus *E. coli* and *S. aureus*. In the plate counts, there was a significant reduction, $P < 0.05$ in the number of CFU of *E. coli* using TiO_2 nanoparticles (50% AcAc) in concentrations 39 to 2500 µg/ml; in the case of *S. aureus* decrease seen in concentrations 78 to 2500 µg/ml. In both bacteria, we observed decreased bacterial growth with TiO_2 nanoparticles at concentrations of 156 - 2500 µg/ml. The two variants of preparing TiO_2 nanoparticles have higher intrinsic activity against *E. coli* and *S. aureus*, while nanoparticles doped with Indium and Iron, not power the antibacterial effect.

Keywords: Dope; Nanoparticles; Titanium oxide

Received Date: June 20, 2016
Accepted Date: July 5, 2016
Published Date: July 12, 2016

Citation: Amabye, T.G, et al. Antibacterial Activities of Nanoparticles of Titanium Dioxide, Intrinsic and Doped with Indium and Iron. (2016) J Med Chem Tox 1(1): 8- 14.

DOI: 10.15436/2575-808X.16.977

Introduction

The term nanoparticles (NP) is used to define particles smaller than 100 nm in diameter, which originate from two sources: primary (natural) and secondary or artificial (synthesized compounds), which may be organic or inorganic[1,2]. The Nanoparticles have different forms (helix, zigzag, belt, sphere, oval, prism, cube, helical or pillar) and can form agglomerations or be dispersed[3]. As NP become smaller, the percentage of surface atoms increases in relation to the total number of atoms[4], its properties change considerably and have a melting point lower than that of a larger mass of the same composition[5].

Actually, the nanoparticles are used in various areas such as medicine, pharmaceuticals, textiles and the electronics industry with the purpose of improving the quality of life[6]. Because of its photocatalytic activity they are used in the separation of water, energy production, air and water purification, sterilization of surfaces, synthesis of organic compounds and in the reduction environmental pollution. The NP used in medicine as metal oxide for coating of biomedical devices, such as prostheses, to prevent bacterial colonization and proliferation, with its catalytic activity[7]. Also, the NP apply for the preparation of drugs, protein detection and pathogen, treatment of tumors, separation and purification of biological molecules and cells[8].

The organic NP have been used as bactericides, but its antibacterial properties are reduced at high temperatures[9] and the inorganic type may have a general mechanism of toxicity against bacteria[10].

One of the materials used in the last decades has been titanium dioxide (TiO_2) because it is not toxic, is easy to produce

and cheap[11]. The morphological properties of titanium dioxide NPs greatly influence their applications[12]. TiO_2 (occurs in nature) in three different forms: rutile, anatase and brookite. Of the three structures mentioned, the anatase NPs, are the most commonly used for photocatalysis. TiO_2 nanomaterials are of interesting in a wide range of applications such as photocatalysis, dye sensititized solar cells, gas sensors, photochromic devices, photo degradation of organic compounds, deactivation of microorganisms, organic synthesis and cells culture[13].

Metals such as platinum (Pt), silver (Ag), gold (Au), nickel (Ni) and copper (Cu) have been added to TiO_2 NPs. These combinations have been very effective in improving photocatalysis[14]. TiO_2 NPs combined with silver (Ag) in the presence of UV light have been showing greater effect against the growth of *E. coli* compared with TiO_2 NPs without Ag[15].

The inhibitory activity of the NPs, generally can be along two main pathways that are related to each other and in many cases occur simultaneously: 1) disruption of membrane potential and integrity and 2) production of reactive oxygen species (ROS), also known as oxygen-free radicals, the NPs acting as nanocatalysts[16]. The membrane damage occurs when NPs electro statically bind to the bacterial cell wall and membranes, leading to alteration of membrane potential, membrane depolarization and loss of integrity which, in turn, result in an imbalance of transport, impaired respiration, interruption of energy transduction and/or cell lysis, and eventually cell death. The distortion of the cell structure and expansion could cause destabilization of the membrane and increase membrane fluidity, which in turn increases the passive permeability and is manifested as a leak of several vital intracellular components such as ions, ATP, nucleic acids, sugars, enzymes and amino acids[17].

The free radicals are induced indirectly due to respiratory chain disruption or directly by NPs themselves. A burst of ROS cause, via severe oxidative stress, damage the all the cell´s macromolecules, leading to lipid peroxidation, alteration of proteins, inhibition of enzymes, and RNA and DNA damage. At high concentrations the ROS leading to cell death and low doses cause severe DNA damage and mutations[18]. In some cases where ROS production is induced by visible or UV light toxicity of the particles is photocatalytic[19]. It was observed that the TiO_2 NP combined with Ag plus UV light can be used for sterilization of vegetative cells of *Bacillus*, while TiO_2 nanoparticles in the presence with UV light are effective against spores[20].

In the present investigation we were evaluated the antimicrobial activity of nanoparticles of titanium dioxide intrinsic and doped with indium and iron against Gram-positive and Gram-negative pathogenic bacteria to assess the possibility of their being used as a new antibacterial strategy and environmental health.

Material and Methods

Nanoparticles TiO_2, TiO_2 : In and TiO_2 : Fe nanoparticles was prepared by the technique of Sol-Gel without reflux, using a mixture of titanium propoxide, acetic acid and Tween80R on propanol, with the following molar proportions 4 : 24 : 1 : 180; based on the results of the synthesis of Choi *et al*[21] (2006). For doping with indium and iron we used in appropriate amounts $InCl_2$ and $FeCl_3$ in propanol, for obtaining 0.5% and 1% indium and 1.25% iron for the titanium ions. Also, a sample prepared with half acetic acid, half TiO_2 (50% AcAc), that is to say 4 : 12 : 1 : 180. The samples were stirred until they became too viscous to continue. After drying in air at 200°C for 2 hours and calcined in air at 400°C for 8 hours in a muffle (Barnstead 1100). The powders obtained were ground in an agate mortar and reserved for tests of antibacterial activity. For comparison we used commercial TiO_2 (P25Degussa).

Antibacterial activity: Bacterial growth curves were performed in order to observe the effect of different nanoparticles on strains of *E. coli* ATTC 25922 and *Staphylococcus aureus* ATCC 29213, growth following a 12 hour interval. The strains were seeded in MacConkey agar and mannitol salt agar respectively and incubated at 37°C until colonies have visible. From one or two colonies of bacteria suspended in 3ml of 0.9% saline solution prepared to a concentration of 0.5 McFarland (1.5 x 10^8 bacterial cells/ml). The flasks were prepared for each layer containing 20 ml of nutrient broth with concentrations of 0 µg/ml, 50 µg/ml, 250 µg/ml and 500 µg/ml of TiO_2 of nanoparticles, respectively. The flasks were inoculated with 100 µl of the bacterial suspension of each strain and incubated at 37°C under continuous stirring to 250 r.p.m. for 24 hours. The Optical Density (OD) of the cultures at 600 nm, an initial determination and readings every 2 hours was measured for 12 hours to monitor bacterial growth[9].

Plate count: Bactericidal activity was evaluated and nanoparticles in bacterial strains were tested in bacterial growth curves. To determine the number of bacterial colonies growing with different concentrations of nanoparticles. From a dilute suspension 1: 100 concentration of 0.5 McFarland nephelometer (1.5 x 10^8 bacteria), 50 µl of this dilution was incubated in Eppendorf tubes, mixed with 50 µl of suspensions of nanoparticles of TiO_2, TiO_2 (50% AcAc) and P25D at concentrations of 39 to 2500 µg/ml and incubation at 37°C for 12 hrs, during this time the tubes were shaken every hour in vortex. To perform the plate count preparations were diluted to 10 - 6 and plated on Mueller Hinton agar. After incubation for 24 hrs colony counting was performed, the results of which were multiplied by the dilution factor to obtain the total number of CFU per 100 ml.

Analysis of results: Experiments were performed in triplicate. Statistical analysis was performed using STATA v.12.0 software, determining mean and standard deviation of the O.D determined between repetitions of the experiments; also p-values were determined using the Student t test between different experimental groups over control. The graphics were made in the program GraphPad PRIMS.

Results

The nanoparticles synthesized in the laboratory were characterized using SEM images. The particle size of TiO_2 is approximately of 50 nm (Figure 1). Antibacterial activity of 6 samples of TiO_2 nanoparticles were evaluated on the growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 in liquid medium. The decrease in O.D of experimental cultures (nanoparticles/bacteria) from the control cultures (bacteria without nanoparticles) was the indicator of the effect of nanoparticles. The following conversion factors of O.D was used CFU/ml: 1 O.D 600 nm *E. coli* = 1 x 10^9 CFU/ml[22] and 1 O.D 600 nm *S. aureus* = 1.5 x 10^8 CFU/ml[23].

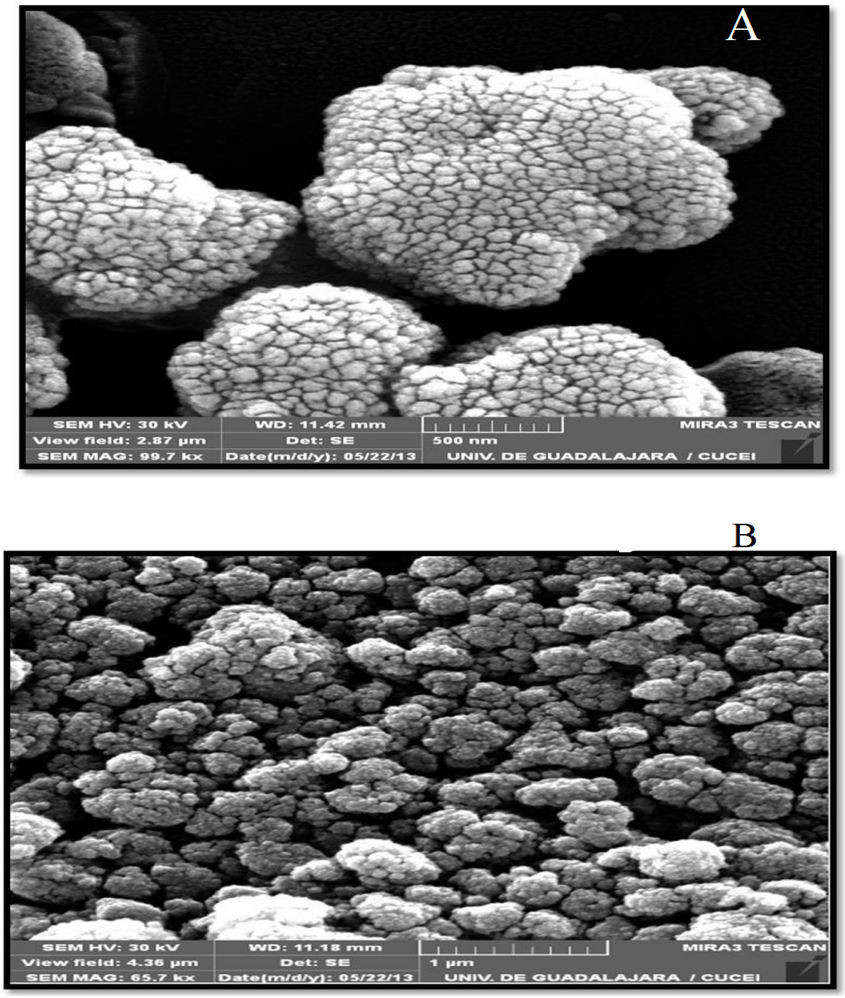
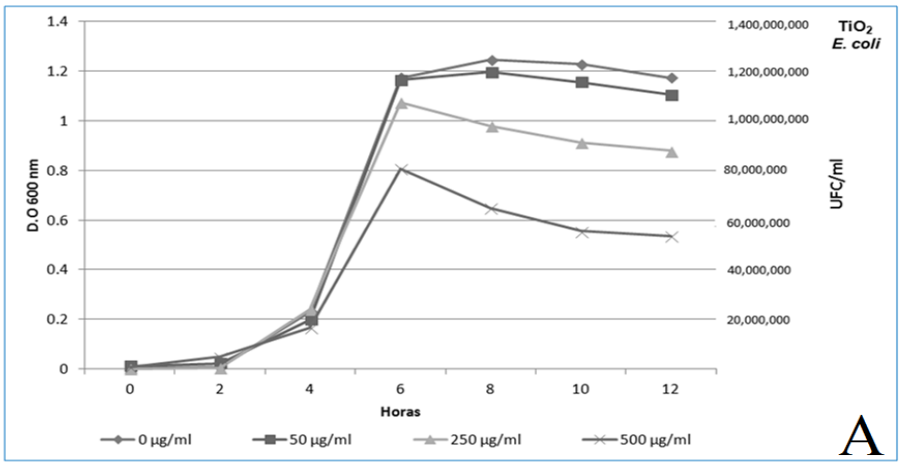


Figure 1: A) Nanoparticles of TiO_2 B) Nanoparticles of TiO_2 (50% AcAc)

In the figure 2 the bacterial growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 is observed in the presence of TiO_2 NPs. *E. coli* had a decrease of optical density in the exponential phase between 4 to 6 hours using concentrations of 250 and 500 µg/ml, and which became more evident after 6 hours of growth. For *S. aureus*, using concentrations of 50, 250 and 500 µg/ml showed an altered decreasing growth between 4 to 8 hours.



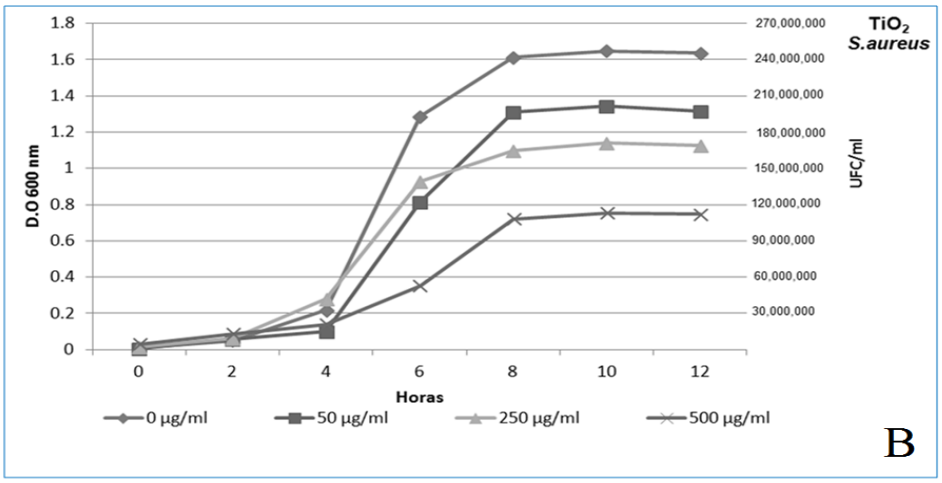


Figure 2: Bacterial growth with different concentrations of nanoparticles TiO_2.
(A) *Escherichia coli* ATTC 25922 (B) *Staphylococcus aureus* ATCC 29213.

With the nanoparticles of TiO_2 (50% AcAc) we observed an antibacterial effect on *E. coli* using concentrations 250 and 500 µg/ml in the exponential phase between 4 and 8 hours of growth. While for *S. aureus* only with concentrations of 500 µg/ml was noticeable antibacterial activity between 6 to 12 hours of growth. With commercial nanoparticles Degussa P25, a decrease in optical density of *E. coli* and *S. aureus* between 4 and 6 hours of growth was observed using the three concentrations, but the effect it was greater at concentrations of 250 and 500 µg/ml compared with TiO_2 prepared in this study.

By employing doped nanoparticles of TiO_2: In (1%), TiO_2:In (0.5%), TiO_2: Fe (1.25%) there was no significant decrease in optical density with the three concentrations used for *E. coli*. However, a significant decrease was observed in *S. aureus* at concentrations of 500 µg/ml of the nanoparticles of TiO_2:In (0.5%).

In order to compare the antimicrobial effect of nanoparticles studied using growth curves *p* value were calculated. The effect of the nanoparticles at a concentration of 500 µg/ml showed antibacterial activity, which was more evident in nanoparticles of TiO_2, TiO_2 (50% AcAc) and P25, a significant value ($P < 0.05$) in *E. coli*. The concentration of TiO_2:In (0.5%) also had an effect on *S. aureus* (Figure 3).

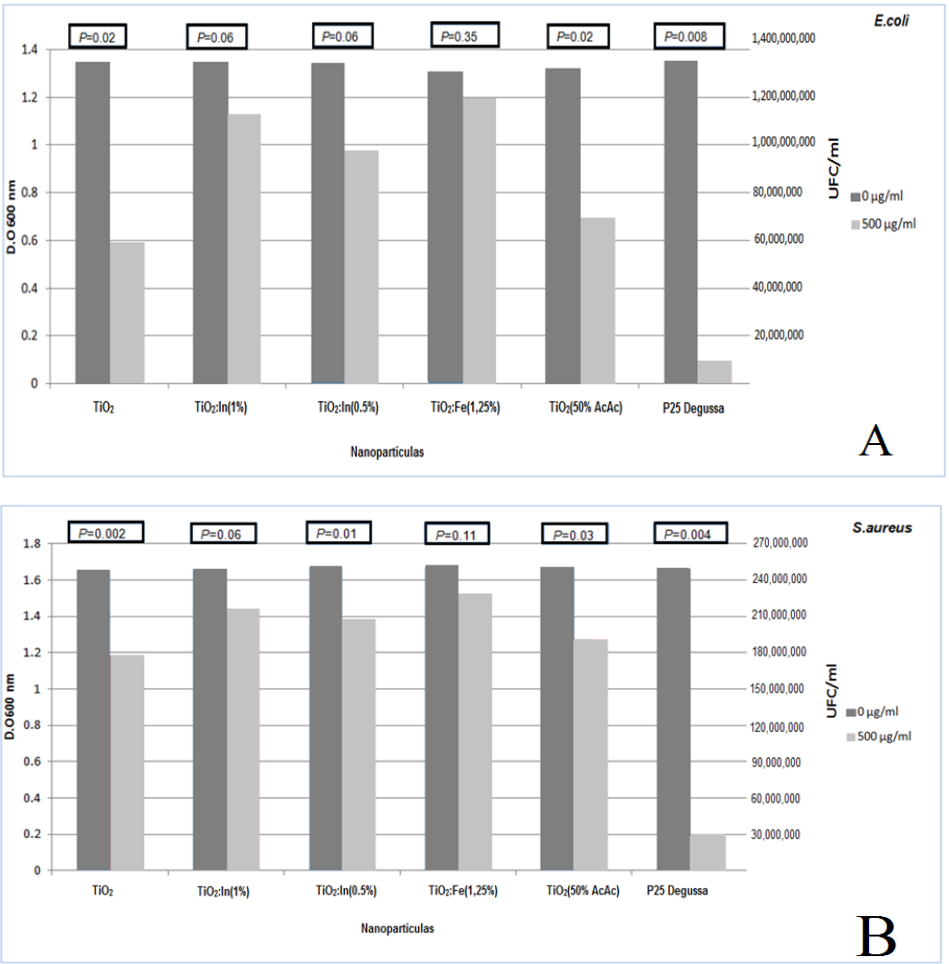


Figure 3: Optical density and Colony Forming Units / ml of bacterial growth after 12 hours incubation with a concentration of 500 µg/ml, of intrinsic and doped nanoparticles.
A) *Escherichia coli* ATTC 25922 B) *Staphylococcus aureus* ATCC 29213

The bactericidal activity of the nanoparticles in the studied bacterial strains was determined by the bacterial colonies growing number being challenged at different nanoparticles concentrations. We counted in a range of 30 - 200 colonies by dilutions made. In controls without nanoparticles of *E. coli* and *S. aureus*, we determined 179 x 10^8 UFC/ml; we compared with viable counts of various concentrations of nanoparticles and obtained the p value. The Table 1 shown p values of different concentrations for *E. coli*, the P25 commercial nanoparticle showed significant values low ($P < 0.05$) at all concentrations. p values against *S. aureus* are shown in Table 2.

Table 1: Values of p plate count against *E. coli*

Nanoparticle oncentration (µg/ml)	TiO_2 p value	TiO_2 (50% AcAc) p value	P25 p value
2500	0.0118	0.0055	0.0055
1250	0.0131	0.0089	0.0089
625	0.0232	0.0097	0.0097
312.5	0.0259	0.0146	0.0146
156.25	0.0406	0.0187	0.0187
78.125	0.0515	0.0240	0.0240
39.062	0.0760	0.0374	0.0374

Table 2: Values of p plate count against S.aureus

Nanoparticle concentration (µg/ml)	TiO_2 p value	TiO_2 (50% AcAc) p value	P25 p value
2500	0.0133	0.0095	0.0026
1250	0.0148	0.0102	0.0029
625	0.0202	0.0150	0.0031
312.5	0.0315	0.0165	0.0135
156.25	0.0489	0.0251	0.0119
78.125	0.0752	0.0435	0.0202
39.062	0.1257	0.0552	0.0374

Discussion

Actually the search for new alternatives for application in biomedicine such as the elimination of bacterial infections, mainly nosocomial type, has caused a significant interest in the development of nanomaterials with antimicrobial capacity. During this study the intrinsic antibacterial properties of TiO_2 nanoparticles were evaluated and doped TiO_2: Fe and TiO_2: In at different concentrations to determine which presented the best antibacterial activity against the growth in culture of *E. coli* (ATCC 25922) and S. *aureus* (ATCC 29213); used as test organisms for conducting experiments.

The carrying out growth in a liquid medium helped us to evaluate the bacteriostatic effect of various nanoparticles. The most significant results were obtained on both strains using TiO_2 and TiO_2 (50% AcAc) nanoparticles at concentrations of 500 mg/ml. The quantitative test plate count allowed us to estimate the antibacterial activity through the survival ratio calculated from the number of viable cells which formed colonies on Muller Hinton agar plates (CFU µg/ml). The results were relevant to the two intrinsic synthesized nanoparticles that were used TiO_2 and TiO_2 (50% AcAc).

The TiO_2 nanoparticles (50% AcAc) showed the best antibacterial activity; even surpassing control nanoparticles, when using these nanoparticles, smaller amounts CFU/ml were obtained. This shows that it has a greater bactericide effect on the bacterial species, a fact which can also be directly related to the percentage of acetic acid used in the synthesis of these nanoparticles. The use of 50% acetic acid favored the bactericide effect of TiO_2 unlike doping of TiO_2 nanoparticles with iron and indium. In a study by Hernandez Enriquez *et al.*,[24] in 2008, a synthesis of TiO_2 nanoparticles were made, with varied amounts of acetic acid to analyze the physicochemical properties of nanoparticles and it was found that when using 1.125 ml of acetic acid the nanoparticles reached a size of 7.2 nm, smaller than the 16 nm commercial nanoparticles. Therefore, the amount of acetic acid influences nanoparticles size. It should be noted that compared to bacterial growth in liquid medium in the presence of TiO_2 and TiO_2 nanoparticles (50% AcAc) in 3 different concentrations (50, 250 and 500 µg/ml), lower growth was observed in *E. coli* than *S. aureus*. This indicates increased resistance of the latter due to the large amount of peptidoglycan that it has, which gives greater protection; whereas *E. coli* has only a thin layer of peptidoglycan and lipopolysaccharide allowing greater interaction of the nanoparticles with these bacterial components.

The antibacterial activity of the nanoparticles used may be related to the binding of the nanoparticles to the outer membrane of *E. coli* causing inhibition of active transport and eventually inhibiting RNA, DNA and protein synthesis, leading to cell

death. Research has been conducted indicating the possible mechanisms involved in the interaction of nanoparticles with biological macromolecules, which indicate that bacteria have a negative charge, while the metal oxide nanoparticles have a positive charge. This causes an attraction between bacteria-nanoparticles and leads to oxidation of the bacteria. The nanoparticles react with the thiol group (-SH) of the proteins in the bacterial cell wall, causing inactivation of transport proteins nutrients, reducing cell permeability and causing death[25].

These mechanisms of action are proposed as being responsible for the antibacterial activity of the NP which is analyzed in this paper. Most researchers have demonstrated so far the mechanism of photocatalytic elimination of different microorganisms using titanium dioxide with UV or sunlight. This is the only mechanism of action resulting in the loss of structural integrity of the cells membranes in the presence of TiO_2 nanoparticles, although lipid peroxidation by the release of reactive oxygen species such as hydroxyl radicals and superoxide has also been proposed[26].

The results obtained in this study are compared with two studies; the first made in 2010, for Reazaei-Zarchi *et al*[9], which determine the antibacterial activity of TiO_2 nanoparticles against E. coli, without the presence of UV rays, using concentrations of 500 and 1000 µg/ml; obtaining a value of $p < 0.05$ at these concentrations. This result was the same as that obtained in the present work, since a significant bacterial reduction ($p = 0.02$) was observed with TiO_2 nanoparticles at a concentration of 500 µg/ml against the same bacteria.

A second study, by Mohammed Sadiq *et al* in 2010[27], where the effect of TiO_2 nanoparticles on the growth of *E. coli*, *Pseudomonas aeruginosa* and *Bacillus* subtilis was studied and mentions that at a concentration of 100µg/ml there was an inhibitory effect for 3 bacteria, because logarithmic growth phase showed a marked decrease in bacterial optical density, similar to the effect observed in this work on *E. coli*, but at concentrations of 250 µg/ml and 500 µg/ml.

In the case of the nanoparticles doped with Indium (0.5% and 1%) decreased antibacterial activity. In the study conducted in 2011 by Martínez and Gómez[28] it is mentioned that there must be an optimum doping in the synthesis of nanoparticles that causes a phase transition from anatase to rutile, resulting in a decrease in the size of these nanoparticles and promoting their properties. So it is possible that in future studies increasing or decreasing doping it may be possible find the right ratio that favors their antibacterial effect.

In the case of nanoparticles of TiO_2: Fe (1.25%), there was not a significant decrease in optical density for *E. coli* and *S. aureus*, and p values greater than 0.05 obtained at the three concentrations used. As doping with Fe 1.25% decreases the antibacterial activity of nanoparticles of TiO_2. The null antibacterial activity by these nanoparticles could relate to the assimilation of Fe used in the metabolism of bacteria, since iron is an essential micronutrient for normal growth of most microorganisms. It should be mentioned that the bacteria have different mechanisms to capture environmental iron or a host. In Gram negative bacteria, the cell possesses outer membrane complex receptors that supply the energy required for iron transport (Ton complex) which allow the iron to pass through the outer membrane and is internalized into the cytoplasm via the cell membrane. In Gram positive bacteria, the iron binding to lipoproteins anchored in the cytoplasmic membrane, thus must traverse this membrane and peptidoglycan[29]. Therefore doping with iron nanoparticles appears not to be a favorable option, at least when used as an antibacterial agent.

One limitation to study the antibacterial activity of nanoparticles is the precipitation of these. But with this work forms a basis for assessing the antibacterial activity of nanoparticles with potential biomedical applications.

Conclusion

In conclusion nanoparticles of TiO_2 and TiO_2 (50% AcAc) have a higher activity against *E. coli* and *S. aureus*, and doping TiO_2 with Indium and Iron do not significantly enhance the antibacterial effect. The concentration of 250 and 500 µg/ml the intrinsic nanoparticles significantly alter bacterial growth in the exponential phase and have a greater antibacterial effect on *E. coli* and *S. aureus*; TiO_2 (50% AcAc) nanoparticles have a greater bactericide effect.

References

1. Kaluza, S., Balderhaar, J.K., Orthen, B., et al. Literature Review - Workplace exposure to nanoparticles. (2009) EU-OSHA-European Agency for Safety and Health at Work.
2. Sanvicens, N., Marco, M.P. Multifunctional nanoparticles – properties and prospects for their use in human medicine. (2008) Trends Biotechnol 26(8): 425–433.
3. Buzea, C., Pacheco, I.I., Robbie, K. Nanomaterials and nanoparticles: Sources and toxicity. (2007) Biointerphases 2(4): 17–71.
4. Li, W., Zeng, T. Preparation of TiO_2 anatase nanocrystals by $TiCl_4$ Hydrolysis with Additive H_2SO_4. (2011) PloS one 6(6): e21082.
5. Grande, A.H. Nanotecnología y nanopartículasmagnéticas: La física actual enlucha contra la enfermedad. (2007) Rev R Acad Cienc Exact Fís Nat 101(2): 321–327.
6. Tsuzuki, T. Commercial scale production of inorganic nanoparticles. (2009) Int J Nanotech 6(5): 567–578.
7. Visai, L., De Nardo, L., Punta, C., et al. Titanium oxide antibacterial surfaces in biomedical devices. (2011) Int J Artif Organs 34(9): 929-946.
8. Wang, J., Byrne, J.D., Napier, M.E., et al. More effective nanomedicines through particle design. (2011) Small 7(14): 1919-1931.
9. Rezaei-Zarchi, S., Javed, A., Ghani, M.J., et al. Comparative Study of Antimicrobial Activities of TiO_2 and CdO Nanoparticles against the Pathogenic Strain of *Escherichia coli* (2010) Iran J Pathol 5(2): 83–89.
10. Taylor, E., Webster, T.J. Reducing infections through nanotechnology and nanoparticles. (2011) Int J Nanomedicine 6: 1463-1473.
11. Xie, Y., Heo, S.H., Yoo, S.H., et al. Synthesis and Photocatalytic Activity of Anatase TiO_2 Nanoparticles-coated Carbon Nanotubes. (2009) Nanoscale Res Lett 5(3): 603–607.

12. Yan, M., Chen, F., Zhang, J., et al. Preparation of controllable crystalline titania and study on the photocatalytic properties. (2005) J Phys Chem B 109(18): 8673–8678.
13. Banerjee, A.N. The design, fabrication, and photocatalytic utility of nanostructured semiconductors: focus on TiO_2-based nanostructures. (2011) Nanotechnology Sci Appl 4: 35–65.
14. Gupta, S.M., Tripathi, M. A review of TiO_2 nanoparticles. (2011) Chin Sci Bull 56: 1639-1657.
15. Ashkarran, A.A., Aghigh, S.M., Farahani, N.J. Visible light photo-and bioactivity of Ag/TiO_2 nanocomposite with various silver contents. (2011) Current Applied Physics 11(4): 1048-1055.
16. Beyth, N., Houri-Haddad, Y., Domb, A., et al. Alternative Antimicrobial Approach: Nano-Antimicrobial Materials. (2015) Evidence-Based Complementary and Alternative Medicine 1-16.
17. Díaz-Visurraga, J., Gutiérrez, C., von Plessing, C., et al. Metal nanostructures as antibacterial agents. (2011) Science against microbial pathogens: communicating current research and technological advances 210-218.
18. Sondi, I., Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. (2004) J Colloid Interface Sci 275(1): 77–182.
19. Park, S.M., Kim, H.S., Yu, T.S. Effect of titanium ion and resistance encoding plasmid of *Pseudomonas aeruginosa* ATCC 10145. (2006) J Microbiol 44(3): 255-262.
20. ThiTuyetNhung, L., Nagata, H., Takahashi, A., et al. Sterilization effect of UV light on *Bacillus* spores using TiO_2 films depends on wavelength. (2012) J Med Invest 59(1-2): 53–58.
21. Choi, H., Stathatos, E., Dionysiou, D.D. Synthesis of nanocrystalline photocatalytic TiO_2 thin films and particles using sol–gel method modified with nonionic surfactants. (2006) Thin Solid Films 510(1-2): 107-114.
22. Chang, Y.C., Yang, C.Y., Sun, R.L., et al. Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles. (2013) Sci Rep 3: 1863
23. Ausubel, F., Brent, R., Kingston, R.E., et al. Short protocols in Molecular Biology. (2002) 5th ed Editorial While, United Stated of America.
24. Hernández Enríquez, J.M., García Serrano, L.A., ZeifertSoares, B.H., et al. Síntesis y Caracterización de Nanopartículas de N-TiO_2–Anatasa. (2008) Superficies y Vacío 21(4): 1-5.
25. Zhang, H., Chen, G. Potent antibacterial activities of Ag/TiO_2 nanocomposite powders synthesized by a one-pot sol-gel method. (2009) Environ Sci Technol 43(8): 2905–2910.
26. Allahverdiyev, A.M., Abamor, E.S., Bagirova, M., et al. Antimicrobial effects of TiO_2 and Ag_2O nanoparticles against drug-resistant bacteria and *Leishmania* parasites. (2011) Future Microbiol 6(8): 933–940.
27. Mohammed Sadiq, I., Chandrasekaran, N., Mukherjee, A. Studies on effect of TiO2 nanoparticles on growth and membrane permeability of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. (2010) Current Nanoscience 6(4): 381–387.
28. Martínez, L.M.T., Gómez, M.A.R. Estudio de las propiedadesestructurales, texturales y catalíticas de TiO_2 dopado con indio y niquel. (2011) Ingenierias 53(14): 23 -34.
29. Köster, W. ABC transporter-mediated uptake of iron, siderophores, heme and vitamin B12. (2001) Res Microbiol 152(3-4): 291–301.