Evaluation, Preparation, and Characterization of Chitosan/ZnO Nanocomposite and Antibacterial Activity Against Pathogenic Microbial Strains

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ABSTRACT: *Objective (s): Background: Nanotechnology is used as a tool to develop advanced therapies and control the fight against infections. The aim of this study was to evaluate the physicochemical properties such as morphological analysis of the chitosan nanocomposite on oxide composite through a simple method and to investigate their anti-bacterial properties of them. Materials and Methods: The study method in this study was experimental and the chitosan-zinc oxide nanocomposite was chemically precipitated after preparation of the Chitosan/ZnO nanocomposite physicochemical properties and antibacterial activity against pathogenic microbial strains were investigated. The nanocomposite was evaluated using SEM, FT-IR techniques, XRD X-ray diffraction, and DLS particle size distribution. The antimicrobial effect of this nanocomposite was evaluated on the bacteria Staphylococcus aureus and Micrococcus luteus. In this study, the antimicrobial effect of Chitosan/ZnO nanocomposite ZnO nanoparticles loaded in chitosan was investigated by MIC method on microorganisms (Candida albicans, Microscotus luteus, and Staphylococcus aureus). Results: The results showed that the concentration of zinc oxide nanoparticles affected the antimicrobial activity of chitosan nanocomposite. In this study, the antimicrobial behavior of the Chitosan/ZnO nanocomposite zinc oxide was determined against pathogenic microbial strains of bacteria including Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, and Klebsiella pneumoniaagainst E.Coli was investigated and the results show that zinc oxide has an antimicrobial effect against Escherichia coli and also the use of two types of dispersants (peg/pvp) on antimicrobial activity of zinc with MIC (Minimum Inhibitory Concentration) approach. The ZnO nanoparticles oxide has no effect and only the Chitosan/ZnO nanocomposite increases the stability of the suspensions. SEM analysis shows that synergistic effect on the destruction of the bacterial wall. The nanoparticles on the oxide damage the bacterial wall. Conclusion: Based on the results, the synthesized compounds have an antimicrobial effect and the antimicrobial effect has increased with increasing polymer (chitosan) concentration. The antimicrobial effect has been seen on gram-positive and gram-negative bacteria.*

KEYWORDS: *ZnO/chitosan nanocomposites; Gram-positive and negative bacteria; Antimicrobial properties.*

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1021-9986/2021/1/1119-1125 7/$/5.07

INTRODUCTION

Nanotechnology is an emerging technology. Fields such as chemistry, physics, biology, engineering, and medical sciences provide accumulated knowledge of nanotechnology. Nanoparticles are currently used in drug delivery, protein, gene, vaccine, polypeptide, nucleic acid, etc [1-3]. Among the most promising nanoparticles, metal nanoparticles, show an increase in chemical activity due to the ratio of surface to high volume and crystal surface structure [4-8]. Connecting polymeric materials with metal ions by combining polymeric hydrogels with nanoparticles (metals, metal oxides) is a simple and effective approach to obtaining a multifunctional system with various capabilities [9-12]. Despite the fact that we are in advanced age and innovative technology to discover the underlying mechanisms of disease and molecular design of new drugs, infectious diseases are still one of the biggest health challenges around the world [13-15].

Drug resistance injects higher concentrations of antibiotics and often causes intolerable toxicity and the development of new antibiotics [16-18]. Improper use of antibiotics is a major risk factor for developing drug resistance [19-22]. Pharmaceutical resistance is created by the absorption of genetically resistant microbes, followed by the expression of resistant genes, and then the choice to express resistant microbes. Bacteria can also obtain resistant genes by spontaneous mutations in existing genes [23]. Bacteria that grow fast are more sensitive to antibiotics than bacteria that grow at low speeds. The mechanism of toxicity of nanoparticles against bacteria is such that nanoparticles are able to join the bacterial membrane and destroy the integrity of the bacterium [24]. The toxicity mechanism of nanoparticles depends on the modification of the level of intrinsic and bacterial properties, and also the antibacterial activity of nanoparticles depends on factors such as bacterial type and physical and chemical properties of nanoparticles [25]. Nanoparticles can be used for biological and cellular imaging applications and also for heat treatment applications based on their optical properties [26]. Chitosan is a biodegradable polymer obtained by N-deacetylation from chitin can be used as an antimicrobial agent [27]. Chitosan has been used as an element in the pharmaceutical industry. In the form of sprays, direct tablets were controlled as tablet-destroying agents to produce dose formulations or used to improve drug dissolution. Compared to biological toxins, chitosan has superior properties, especially flexibility in its use [28-31]. Chitosan polysaccharide is a natural polycation line that is derived from chitin. The low solubility of chitosan in neutral and alkaline solutions limits its use. However, chemical modification to composites or hydrogels brings new functional properties to various applications. Due to its non-toxicity, low allergenicity, environmental compatibility, and biodegradation, chitosan is known as a biologically stable polymer. Most polysaccharides are usually acidic, neutral, or negative. This feature allows chitosan to be loaded to form electrostatic aggregates, multilayer structures, and other negative synthetic or natural polymers. Interesting features of chitosan such as biocompatibility, non-toxicity, low allergenicity, and biodegradation allow it to be used in various applications.

EXPERIMENTAL SECTION

Among the materials used in this research are $Zn(CH_3CO_2)_2.2H_2O$ and chitosan powder which was purchased from Merck company. The NaOH used in this study was purchased from Dr. Abidi's Laboratory Materials Company. The distilled water used was prepared by Abban distilled water company. In order to investigate the properties of nanostructures, Kashan University X-ray machine with specifications (Rigaku D - max C III X-ray) was used. Also, a scanning electron microscopy device with specifications (LEO 1455VP) was used at the Razi Metallurgical Research Center for imaging nanostructures. The tests for the synthesis of the nanoparticles were performed in Dr. Ranjbar's nanomaterials and nanostructures laboratory, as well as antibacterial tests in Dr. Moshafi's microbiology laboratory at Kerman University of Medical Sciences.

Preparation of chitosan/ZnO nanocomposite

In order to carry out this project, first one gram of $Zn(CH_3CO_2)_2.2H_2O$ was added to a certain volume and the amount of 10 mL of it was heated for 30 minutes, then 2mL NaOH, 2M was added, and then placed in the reflux system for 45 minutes and 5 mL of NaOH 2M was added every 5 minutes. The solution was then placed in the microwave. The water and ethanol solution was added to zinc oxide in a ratio of 2: 1 and placed in the autoclave for 6 hours at 150 °C for nucleation.

In the second step for synthesis of the chitosan nanoparticles, 3 different concentrations of chitosan

Table 1. Polymer to the metal composition ratio

Metal(mg) Weight	Chitosan Concentration (mg/mL)
0.4	0.05
0.06	0.1
0.1	0.5

Table 2: Polymer concentration of microwave irradiation.

Chitosan Concentration (mg)	Power (watt)
0.05	300
0.1	150
0.5	100

Table 3. Specifications of the microorganism used.

Grouping	PTTCC	The name of the germ
Gram-negative bacilli	1330	*Ecsherchia coli*
Gram-negative bacilli	1074	*Pseudomonas aerogios*
Gram-negative bacilli	1621	*Serratia marcescens*
Gram-negative bacilli	1053	*Klebsiella pneumoniae*
Gram-positive cocci	1112	*Staphylococcus aureus*
Gram-positive cocci	1023	*Bacillus subtillis*
Gram-positive cocci	1110	*Micrococuus luteus*

(0.05, 0.1, 0.5 mg) were made with water solution and DMF in a ratio of 1: 2 at a specified temperature and time, then 0.05g lactulose was added to the chitosan under the reflux system, then the mixture was exposed to microwave irradiation. In order to prepare zinc oxide-chitosan nanocomposites, the zinc oxide nanoparticles were first dried in an oven and the polymer and metal were mixed in a ratio of 5 to 1.

Nanoparticles on oxide and chitosan with a solution of water and ethanol were placed in the microwave at a ratio of 2: 1.

The resulting nanoparticles were then poured into a falcon tube and the resulting centrifuge and sediment were removed. Then, to check the antimicrobial properties, the minimum precipitation inhibition concentration was placed at 120 °C for 5 hours before the method. In this study, the antibacterial effects of seven microbes were investigated, with four gram-negative bacteria and three gram-positive bacteria. The table shows the scientific name and PTCC number of the microbial strains used in the experiment. Once every three days, these bacteria are re-prepared.

Preparation of Muller Hinton agar and Muller Hinton broth culture medium

In this study, the dilution method was used in a solid medium. Different concentrations of the desired compound with the agar-containing medium were mixed in the tube and poured into the plate when it was melted. The lowest concentration of the substance that inhibits the growth of microbes was considered MIC (8). To preparation of the culture medium with Mueller-Hinton Agar and Mueller Hinton Broth 7.6 g of powder was poured into 200 ml of distilled water and dissolved by heat, and the heating was continued until the solution was completely clear. It was then removed 18 ml by pipette and poured into large coiled tubes and sterilized by autoclave at a temperature of 121 °C and a pressure of 15 pounds per square inch for 15 minutes. After sterilization, the medium was mixed with two milliliters of the synthesized mixture, which was prepared by the environment of Mueller Hinton Broth in different dilutions.

To prepare the culture medium of Mueller Hinton Broth 1/2 gram of the powder into 100 mL of distilled water and stir until completely dissolved, then 2mL of the prepared medium into small coiled tubes and sterilized them for 15 minutes by autoclaving at 121 °C and a pressure of 15 pounds per square inch. Some of the synthesized samples were poured separately into a joule balloon, then dissolved with the lowest amount of dimethyl sulfoxide solvent and sterilized with distilled water to a volume of 5 mL to maintain a concentration of 1280 µg / ml. Then 8 small tubes of sterile capillaries were removed and 2 ml of Mueller Hinton Broth culture medium was poured into each. Then 2 ml of the storage solution was added to the first tube and after mixing, 2 mL was removed from the first tube and added to the second tube. He continued this process until he was finally 2 mL away from the last pipe. Thus, 8 consecutive dilutions are obtained from the sample, and concentrations of 5, 10, 20, 40, 80, 160, 320, and 640 micrograms per milliliter are obtained. Finally, negative control and positive control were allocated for the investigation of antibacterial properties (9). To transfer the microbes to the surface of the pre-prepared plates, a 2.5 microliter sterilized sterilizer was used in the

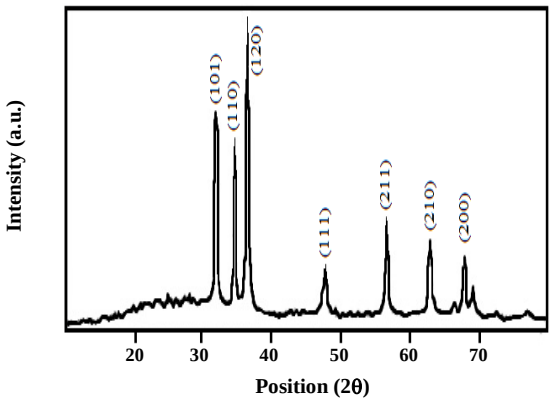

Fig. 1: X-ray diffraction pattern of ZnO/Chitosan nanocomposite.

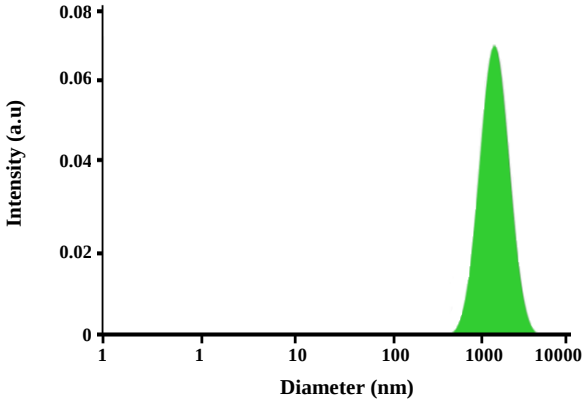

Fig. 2: DLS diagram of ZnO/Chitosan nanocomposite.

autoclave. In this way, 2.5 µL of microbial suspension was transferred to the agar surface each time. After absorbing the microbial suspension to the agar surface, the plates were inverted in the incubator 37 °C for 24 h. Each plate was then examined for growth or non-growth of the tested microbe. The lowest concentration of the active ingredient that can inhibit the growth of the microbe is the MIC of the female against the microbe.

RESULTS AND DISCUSSION

The X-ray pattern of the sample with the best morphology

The X-ray pattern of the sample with the best morphology was examined under standard conditions and room temperature. The X-ray patterns display that strong peaks are centered at 2θ =31.6°, 2θ =34.8°, 2θ =36.1°, 2θ =47.3°, 2θ=57.4°, 2θ=63.2°, 2θ=67.8°, and 2θ =69.4° respectively. Using the Debye Scherer equation, the particle size can be calculated using the following equation:

$$D_c = K\lambda / \beta.\cos\theta \quad (1)$$

Where θ is the scattering angle of the scattering of the X-ray, Kl is the present wavelength of the radiation wave at the device constant, the value of which is 0.9.

In order to check the exact size of nanostructures, the particle size of Dynamic Light Scattering (DLS) light dynamics is used. In this analysis, the interaction of light with a particle size of particles is accurately obtained. The results of the particle size distribution of the nanoparticle dynamics light diffusion are shown in Fig. 2, confirming the approximate size of the nanoparticles in the range of 800 to 900 nm.

The results of imaging using electron microscopy for samples, A_1, A_2, and A_3 are given in Fig. 3a, 3b, and 3c, respectively.

SEM images show that the ZnO/Chitosan nanostructures have a uniform distribution and in some areas due to the increase in the surface-to-volume ratio of nanoparticles, adhesions are observed. The results of the FT-IR infrared spectroscopic spectroscopy show that the wavelengths displayed in the 3650 1/cm area are related to the O-H group hydroxide bonds in the chitosan polymer structure and 2 water molecules in the zinc acetate structure. C-C bonds in the chitosan polymeric structure appear as a polymeric base at wavelengths of about 2924 1/cm and 2853 1/cm. The C-O-C bonds present in the polymeric base structure of the cytosolic acid are shown in the area of 1625 1/cm and 1558 1/cm in the FT-IR infrared spectroscopic spectrum. The presence of metallic bonds on the oxide on the polyethylene chitosan bed in the area below 1000 cm^{-1} appears as weak peaks.

The results of the FT-IR spectroscopy show that the functional groups related to ZnO nanoparticles and chitosan polymers have appeared in the final ZnO /Chitosan nanostructures. The MIC method was used to investigate the antimicrobial effects of the synthesized compounds. In the MIC results tables, a positive sign indicates growth and a negative sign indicates a lack of bacterial growth.

Discussion

In order to investigate the antimicrobial effect of ZnO/Chitosan nanocomposite and considering that the

Table 4: The antimicrobial effects of A_3 nanoparticles on the tested bacteria in different dilutions.

Strain \ Cons	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	2 µg/ml	1 µg/ml	0.5 µg/ml
E. coli	-	-	-	-	-	-	-	-
K. pneumoniae	-	-	-	-	-	-	-	-
S. marcescens	-	-	-	-	-	-	-	-
M. luteus	-	-	-	-	-	-	-	-
B. sabtilis	-	-	-	-	-	-	-	-
S. aureus	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	-	-	+	+

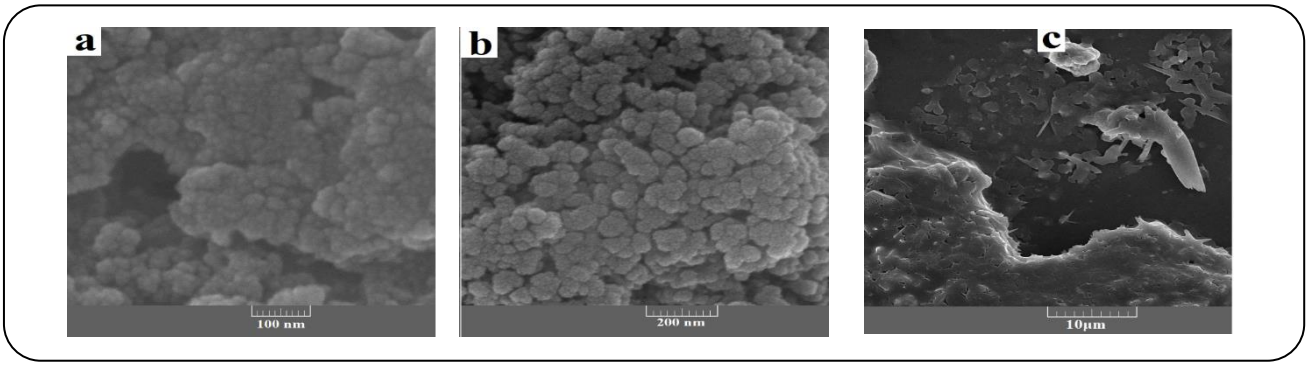

Fig. 1: SEM images for A_1, A_2 and A_3 samples of the ZnO /chitosan nanostructures.

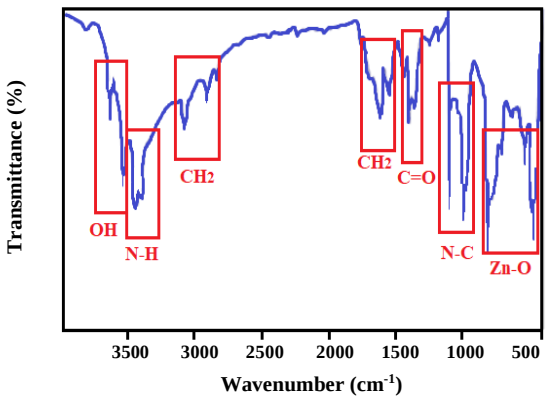

Fig. 4: FT-IR spectroscopy of crystal nanostructures derived from ZnO /Chitosan nanostructures.

antimicrobial effects of chitosan and zinc oxide have been proven, and the studies related to nanocomposites are mentioned in this section. In this study, the antimicrobial effect of ZnO nanoparticles loaded in chitosan was investigated by MIC method on microorganisms (*Candida albicans*, *Microscotus luteus*, and *Staphylococcus aureus*). For this purpose, in the present study, the sedimentation synthesis method has been used and the proof of antimicrobial properties on *Staphylococcus aureus* and *Lotus micrococcus* indicates the accuracy of the present study and confirms the work done in the mentioned article. ZnO nanoparticles due to their synergistic effect on chitosan polymers can increase the antibacterial effect. The precursors used as well as the type of solvents mentioned in the research work were the same as in the present work, and in the study we did, the particle size was more homogeneous and the structures formed had better distribution. Various studies and observations show that when the safe polyethylene polymer was added to zinc oxide nanoparticles, the antimicrobial effect should be increased on drug-resistant gram-negative bacteria than when the nanoparticles alone were present. The temperature conditions used and also the type of solvents in the mentioned research work were the same as in the present work. In this study, the particle size was more homogeneous and the formed structures had a more suitable dispersion. In another study, *Escherichia coli* and *Staphylococcus aureus* were used to study the antimicrobial activity of chitosan with different molecular weights.

Another study looked at the degree of cytosine depletion and its effect on antimicrobial activity. The antimicrobial effect of chitosan varies and depends on the species of microorganisms. In general, the lower the degree of sterilization, the greater the antimicrobial effect [12[. In this study, the antimicrobial behavior of zinc oxide against *E. Coli* was investigated and the results show that zinc oxide has an antimicrobial effect against Escherichia coli and also the use of two types of dispersants (peg/pvp) on the antimicrobial activity of zinc. The oxide has no effect and only increases the stability of the suspensions. SEM analysis shows that nanoparticles on oxide damage the bacterial wall [13]. In another study, ZnO/Chitosan nanoparticles were synthesized by microwave heating and showed an antimicrobial effect on *E. coli* and *S. aureuse* bacteria, and with increasing energy, the size of nanoparticles also increased [14].

CONCLUSIONS

Based on the results obtained, the ZnO/Chitosan nanocomposites with uniform distribution and high purity percentage had an antimicrobial effect and the antimicrobial effect increased with increasing polymer concentration (chitosan). Due to the antibacterial effects of chitosan and zinc oxide, which have been proven in articles, it is necessary to perform in vitro tests with these compounds. These nanostructures are predicted which have a high potential for use in various applications as pharmaceutical and antibacterial carriers and also antibacterial activity against pathogenic microbial strains.

Acknowledgments

The authors are grateful to the council of Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran. Local ethical and tracking code 98000319 was approved by the ethical committee of Kerman University of Medical Sciences. The ethics approval Code is IR.KMU.REC.1398.237.

Received : Dec. 20, 2020 ; Accepted : May 17, 2021

REFERENCES

[1] Zahin N., Anwar R., Tewari D., Kabir M.T., Sajid A., Mathew B., Mathew B., Uddin Md. S., Leya L., Ablel-Daim M.M., Nanoparticles and its Biomedical Applications in Health and Diseases: Special Focus on Drug Delivery, *Environ. Sci. Pollut. Res. In.t,* **12**: 1-18 (2019).

[2] Morones J.R., Elechiguerra J.L., Camacho A., Holt K., Kouri JB, Ramírez J.T., The Bactericidal Effect of Silver Nanoparticles, *Nanotechnology*, **16**: 2346 (2005).

[3] Wahid F., Zhong C., Wang H.-S., Hu X-H., Chu L.-Q., Recent Advances in Antimicrobial Hydrogels Containing Metal Ions and Metals/Metal Oxide Nanoparticles, *Polymers*, **9**: 636-347 (2017).

[4] Huh A.J., Kwon Y.J., Nanoantibiotics: A New Paradigm for Treating Infectious Diseases Using Nanomaterials in the Antibiotics Resistant Era, *J. Control Rel.* **156**: 128-45 (2011).

[5] Bronzwaer S.L., Cars O., Buchholz U., Mölstad S., Goettsch W., Veldhuijzen I.K., Kool J.L., Sprenger M.J.W., Degener J.E., The Relationship between Antimicrobial Use and Antimicrobial Resistance in Europe, *Emerging Infect. Dis.*, **8**: 278-281 (2002).

[6] Bradley G., Juranka P.F., Ling V., Mechanism of Multidrug Resistance, *Bio Chim. et Biophy. Acta (BBA)-Rev. Cancer*, **948**: 87-128 (1988).

[7] Pelgrift R.Y., Friedman A.J., Nanotechnology as a Therapeutic Tool to Combat Microbial Resistance, *Adv. Drug Deli. Rev.*, **65**:1803-1815 (2013).

[8] Ventola C.L., The Antibiotic Resistance Crisis: Part 1: Causes and Threats, *Pharmacol Ther,* **40**: 277-289 (2015).

[9] Hajipour M.J., Fromm K.M., Ashkarran A.A., de Aberasturi D.J., de Larramendi I.R., Rojo T., Serpooshan V., Parak W.J., Mahmoudi M., Antibacterial Properties of Nanoparticles, *Trends Biotechnol.*, **30**: 499-511 (2012).

[10] Gupta N., Rai D.B., Jangid A.K., Kulhari H., Use of Nanotechnology in Antimicrobial Therapy, *Nanotechnology,* **46**: 143-156 (2019).

[11] Heiligtag F.J., Niederberger M., The Fascinating World of Nanoparticle Research, *Mater. Today* **16**: 262-271 (2013).

[12] Khan I., Saeed K., Khan I., Nanoparticles: Properties, Applications and Toxicities, *Arabian J. Chem.*, **12**: 908-931 (2017).

[13] El-Nour K.M.A., Eftaiha A., Al-Warthan A., Ammar R.A., Synthesis and Applications of Silver Nanoparticles, *Arabian J. Chem.*, **3**: 135-0 (2010).

[14] Tiwari J.N., Tiwari R.N., Kim K.S., Zero-Dimensional, One-Dimensional, Two-Dimensional and Three-Dimensional Nanostructured Materials for Advanced Electrochemical Energy Devices, *Prog. Mat. Sci.*, **57**:724-803 (2012).

[15] Rabea E.I., Badawy M.E.-T., Stevens C.V., Smagghe G., Steurbaut W., Chitosan as Antimicrobial Agent: Applications and Mode of Action, *Biomacromolecules*, **4**: 1457-65 (2003).

[16] Ilium L., Chitosan and Its Use as a Pharmaceutical Excipient, *Pharm Res*, **15**:1326-31 (1998).

[17] Cheung R., Ng T., Wong J., Chan W., Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications, *Mar. Drugs*, **13**: 5156-5186 (2015).

[18] Naskar S., Sharma S., Koutsu K., Chitosan-Based Nanoparticles: an Overview of Biomedical Applications and its Preparation, *J. Drug. Delivery. Sci. Technol.*, **49**: 66-81 (2018).

[19] Qi L., Xu Z., Jiang X., Hu C., Zou X., Preparation and Antibacterial Activity of Chitosan Nanoparticles, *Carbohydrate Res.*, **339**: 2693-2700 (2004).

[20] Dai T., Tanaka M., Huang Y.-Y., Hamblin M.R., Chitosan Preparations for Wounds and Burns: Antimicrobial and Wound-Healing Effects, *Expert Rev. Anti-Infect Ther.*, **9**: 857-579 (2011).

[21] Petkova P., Francesko A., Fernandes M.M., Mendoza E., Perelshtein I., Gedanken A., Tzanoy T., Sonochemical Coating of Textiles with Hybrid ZnO/Chitosan Antimicrobial Nanoparticles, *ACS Appl. Mat. & Int.*, 6:1164-72, 2014.

[22] Verlee A., Mincke S., Stevens C.V., Recent Developments in Antibacterial and Antifungal Chitosan and Its Derivatives, *Carbohydr Polymers*, **164**: 268-283 (2017).

[23] Palza H., Antimicrobial Polymers with Metal Nanoparticles, *Int. J. Mol. Sci.*, **16**: 2099-2116 (2015).

[24] Zhang L., Jiang Y., Ding Y., Povey M., York D., Investigation into the Antibacterial Behaviour of Suspensions of ZnO Nanoparticles (ZnO Nanofluids), *J. Nanoparticle Res.*, **9**: 479-489 (2007).

[25] Yamamoto O., Influence of Particle Size on the Antibacterial Activity of Zinc Oxide, *Int. J. Inorganic Mat.*, **3**: 643-646 (2001).

[26] Jones N., Ray B., Ranjit K.T., Manna A.C., Antibacterial Activity of ZnO Nanoparticle Suspensions on a Broad Spectrum of Microorganisms, *Microbiology Lett*, **279**:71-76 (2008).

[27] Pauzi N., Zain N.M., Yusof N.A.A., "Microwave-Assisted Synthesis for Environmentally ZnO Nanoparticle Synthesis". *Proceedings of the 10th National Technical Seminar on Underwater System Technology*; Springer, 541-6, (2019).

[28] Akbar A., Sadiq M., Ali I., Muhammad N., Rahman Z., Najam Khan M., Synthesis and Antimicrobial Activity of Zinc Oxide Nanoparticles Against Foodborne Pathogens Salmonella Typhimurium and Staphylococcus Aureus, *Biocatal. Agric. Biotechnol.*,**17**: 36-42 (2018).

[29] Zak A.K., Razali R., Majid W.A., Darroudi M., Synthesis and Characterization of a Narrow Size Distribution of Zinc Oxide Nanoparticles, *Int. J. Nanomed*, **6**:139-149 (2011).

[30] Sirelkhatim A., Mahmud S., Seeni A., Kaus N.H.M., Ann L.C., Bakhori S.K.M., Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism, *Nano-Micro Lett.*, **7**: 219-242 (2015).

[31] Zhang Y., R. Nayak T., Hong H., Cai W., Biomedical Applications of Zinc Oxide Nanomaterials, *Curr. Mol. Med.*, **13**: 1633-1645 (2013).