MATERIALS BASED ON CHITOSAN ENRICHED WITH ZINC NANOPARTICLES FOR POTENTIAL APPLICATIONS ON THE SKIN

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Abstract

Chitosan as a nontoxic, biodegradable, and biocompatible biopolymer with film-forming properties can also be modified to improve its parameters. Modification of polymer films by the addition of nanoparticles is an increasingly common solution due to the higher efficiency of products at the nanoscale compared to the macroscale. In this work, thin chitosan films enriched with biogenic zinc oxide nanoparticles (ZnONPs) from Fusarium solani IOR 825 were obtained by the solvent evaporation method. The influence of nanoadditive on the physicochemical, mechanical, and antimicrobial properties of the polymeric matrix was evaluated. Two different concentrations of ZnONPs were added to the chitosan solution. Spectrometric measurements, mechanical tests, microscopic imaging, and microbiological tests were performed for nanoparticles-modified and control samples. Analysis revealed that ZnONPs influence the properties of chitosan films. FTIR spectroscopy showed changes that are the result of interactions between polymer matrix and the additive. Modified samples were characterized by increased values of Young's modulus and tensile strength. SEM analysis combined with energy-dispersive X-ray spectrometry confirmed the presence of zinc in the modified films. The addition of nanoparticles slightly affected the surface morphology of the tested samples, and an increase in roughness was observed. Microbiological tests showed the biostatic activity of the films containing ZnONPs. The obtained films based on chitosan with the addition of ZnONPs can be considered easy-to-obtain biomaterials with potential use as cosmetic and biomedical products.

Keywords: *chitosan, zinc oxide nanoparticles, polymer films, antimicrobial activity*

Introduction

Chitosan is a well-known biopolymer with valuable physicochemical and biological properties. It is a chitin derivative that is soluble in dilute acid solutions. It is biodegradable and biocompatible, nontoxic, and has film-forming properties, as well as antioxidant and antimicrobial activity [1,2]. The source of chitosan is usually marine organisms, but also fungi and insects. Chemical or enzymatic deacetylation of chitin results in a product composed of β-glucosamine and N-acetylglucosamine units. Depending on the process parameters used and the quality of the starting material, a product with a different degree of deacetylation (DD) and a different molecular weight can be obtained.

Skin applications of chitosan are important, especially in cosmetic and biomedical fields. The biological activity of chitosan includes antioxidant and antimicrobial effects, as well as anti-inflammatory properties [3,4]. The antioxidant properties are the result of the presence of free functional groups (amino, amino acetyl, and hydroxyl groups) in the polymeric chain. These groups can react with reactive oxygen and nitrogen species [5]. In cosmetic formulations, it acts as an emulsion stabilizer and thickener, and thanks to its antimicrobial activity, it can reduce the number of preservatives used. In addition, it has a beneficial effect on skin and hair, moisturizing them and preventing the loss of water through the creation of an occlusive layer. Chitosan not only improves the condition of the skin in terms of care but can also help in wound treatment. It is typically used in dermatology in the form of active dressing. There are several stages in the wound healing process: hemostasis, inflammation, proliferation, and remodeling [6]. Chitosan-based active dressing participates in three phases of this process. First of all, its polycationic molecules bind to negatively charged thrombocytes and erythrocytes, supporting the blood coagulation process [3,6]. In the next step, it helps fight inflammation by cleansing the wound of bacteria. The last phase involves stimulating the production of granulation tissue, which accelerates skin proliferation [6]. Additionally, it helps to maintain good wound moisture and can easily absorb exudate [3].

Chitosan can be used in many different forms, but for applications on the skin, the film form seems to be one of the best choices. The film-forming properties of this carbohydrate polymer are extremely useful in products for surface applications, particularly in the biomedical, cosmetic, food, and packaging industries. Depending on the application, the mechanical properties of such films are important. Chitosan-based films are often characterized by relatively low tensile strength and brittleness, while cross-linking improves these properties [7,8]. Modifications of chitosan films are carried out not only to improve mechanical properties but also to obtain a material with completely new activity, such as biological. In chitosan-based products for biomedical or food applications, additives are often used to increase the antimicrobial potential, e.g. drugs, zinc oxide, or essential oils [9-12]. Lian et al. obtained chitosan/starch films incorporated with zinc oxide nanoparticles (ZnONPs) and the results of their work showed positive antibacterial activity on *S. aureus* and *E. coli* compared to the pure films [13].

[Engineering of Biomaterials 167 (2022) 24-31]

doi:10.34821/eng.biomat.167.2022.24-31

Submitted: 2023-04-22, Accepted: 2023-05-24, Published: 2023-05-30

The use of nanoadditives has recently become widely applied in various fields, including the one discussed. Nanotechnologies involve precise molecular and atomic precision techniques, and nanotechnology products exhibit two key characteristics: at least one dimension within the range of 1-100 nm and properties dependent on the characteristic dimension. Nanostructures often demonstrate distinct physicochemical properties compared to their macroscale counterparts, encompassing chemical, optical, mechanical, or magnetic traits [14]. Among nanoparticles (NPs), different groups can be distinguished, such as metallic NPs, ceramic NPs, and polymeric NPs [15]. At the nanoscale, numerous materials can readily interact with biomolecules at cellular and subcellular levels, making biomedical applications a specialized domain for nanotechnology products. Metal nanoparticles, notably silver (AgNPs) or zinc oxide nanoparticles (ZnONPs), are one of the most interesting for enhancing polymer films with nanoadditives [16-20]. The purpose of incorporating such additives is to enhance mechanical properties, boost antimicrobial activity, and improve parameters like conductivity and water retention capacity [18,19]. These parameters are particularly vital for dressing products and food packaging.

Comparison of zinc on a macro and nanoscale

Zinc, with an atomic number of 30, is a naturally occurring element. It presents as a brittle metal with a blue-white hue, possesses relatively low melting and boiling points, reacts with acids and bases, and undergoes passivation in the air [21]. In living organisms, it functions as with multifaceted role. Within the human body, zinc contributes to skin regeneration, the proper functioning of the cardiovascular system, and plays vital roles in immune and metabolic mechanisms. Several functions of zinc arise from its role as a cofactor of enzymes with antioxidant activity and its involvement in DNA transcription ('zinc fingers'), cell proliferation and differentiation, and more [22,23]. Approximately 2 g of zinc are distributed throughout the entire human body, with the highest concentrations found in skeletal muscle, bone, skin, brain, and kidney, in descending order. In its natural state, zinc is not found in free form but primarily exists as zinc oxide, zinc carbonate, and zinc sulphide [21].

Zinc nanoparticles exhibit antimicrobial, photocatalytic, and radioprotective properties. Due to their increased surface area-to-volume ratio, nanoparticles display distinct reactivity, making them more reactive thank bulk materials. This heightened reactivity is desirable for applications such as antimicrobial purposes. However, in other applications like radioprotective formulations, may pose a risk to the user due to possible phototoxic effects [24]. Zinc ions are essential for cells function but become harmful at higher concentrations. Nanoformulated zinc appears to be a more effective antibacterial agent than traditional forms of zinc [25]. The antibacterial mechanism may involve the release of ions that penetrate cells and induce toxicity [25] or the production of reactive oxygen species that breach microorganism cell membrane and cause bacterial death [26]. In addition, zinc nanoforms operates through a distinct mechanism compared to conventional antibiotics, making them more effective against resistant strains [26]. Concerning the enhancement of antibacterial activity through the addition of ZnONPs to chitosan films, the mechanism may involve electrostatic interactions between the positively charged amino groups of chitosan and the negatively charged bacterial cell wall, facilitating the penetration of zinc ions [13]. Furthermore, the incorporation of zinc oxide nanoparticles into the biopolymer matrix can enhance its structural integrity, creating a more compact structure.

The method of nanoparticle synthesis significantly impacts their physicochemical properties [27]. Three main methods can be distinguished for the synthesis of nanoparticles: chemical, physical, and biological [14,21]. In recent years, biological methods, considered environmentally friendly alternatives, have gained popularity. Biogenic nanoparticles, in contrast to chemically and physically synthesized ones, are believed to be less toxic because their synthesis does not require toxic reagents or high energy consumption and stabilization process, which often use toxic reagents, and do not produce toxic wastes. To stabilize biogenic nanoparticles, they are coated with biomolecules of natural origin [28,29]. Microorganisms, plant extracts, or enzymes are used as substrates in these methods [30,31].

In the present study, we investigated the effect of the addition of mycogenic ZnONPs on the properties of films prepared from low molecular weight chitosan. A series of tests were carried out to characterize the polymer films obtained, including microscopic analysis, infrared spectroscopic analysis, and microbiological studies. The use of different nanoparticle concentrations was compared.

Materials and Methods

Materials

Low molecular weight chitosan ($M_v = 7.74 \cdot 10^5$ (g/mol), deacetylation degree 82.90%) was purchased from the POLAURA company (Dywity, Poland). Glycerol, acetic acid, zinc sulphide, and sodium hydroxide were acquired from POCH (Gliwice, Poland).

ZnONPs synthesis

Zinc oxide nanoparticles (ZnONPs) were synthesized using a fungal extract from *Fusarium solani* IOR 825, $ZnSO_4$, and NaOH in a ratio of 1:1:1 (v/v/v) and heated for 15 min at 40°C. They were further characterized for physical and chemical activities, as previously described by Trzcińska-Wencel et al. [29].

Preparation of initial solution

2% (w/v) solution of chitosan (CS) was prepared in 0.1 M acetic acid. The mixture was stirred on a magnetic stirrer until complete dissolution.

Preparation of chitosan films

Films were obtained using a solvent evaporation method. A 30 g of chitosan solutions were weighed out and 1%(w/w) of glycerol (G) was added to each solution. The solutions thus prepared were stirred for 3 h. Glycerol (G) was used as a plasticizer. After stirring, one solution with glycerol was poured onto a polystyrene plate (10 x 10 cm) as well as a pure chitosan solution. 0.1% (w/w) and 0.2% (w/w) ZnONPs in powder form were added to subsequent solutions with glycerol, respectively. The solutions were stirred to dissolve the additive and then poured onto polystyrene plates (10 x 10 cm). The solution with 0.2% additive was additionally homogenized before pouring. They were left at room temperature to dry completely.

FTIR spectroscopy

The interactions between the polymer and the additive were evaluated by Fourier transform infrared spectroscopy (FTIR) using Nicolet iS10 equipment with an ATR (attenuated total reflection) accessory and a diamond crystal (Thermo Fisher Scientific, Waltham, MA, USA). All spectra were recorded in absorption mode, with a resolution of 4 cm^{-1} with 64 scans.

Mechanical properties

Mechanical tests were carried out using a mechanical testing machine (Z.05, Zwick and Roell, Ulm, Germany). Young Modulus, tensile strength, and elongation at break were evaluated. The samples were cut in the shape of paddles (width 4 mm in the center). The parameters of the test program were as follows: the speed starting position was 50 mm/min, the speed of the initial force was 5 mm/min, and the initial force was 0.1 MPa. Data were collected using the TestXpert II 2017 program and the results were presented as average values with standard deviation.

Scanning electron microscopy (SEM-EDX)

Surface imaging of the tested polymer samples was carried out using a scanning electron microscope (SEM) manufactured by LEO Electron Microscopy Ltd. (Model 1430 VP). In addition, an EDX Quantax 200 X-ray spectrometer with a Bruker AXS XFlash 4010 detector was used for spot analysis of the chemical composition of the samples to confirm the presence of zinc.

Atomic force microscopy (AFM)

The polymer samples were analyzed using atomic force microscope (MultiMode Scanning Probe Microscope NanoScope IIIa; Digital Instruments Veeco Metrology Group, Santa Barbara, CA).

Microbiological tests

The antimicrobial activity study of chitosan films with the additive of biogenic ZnONPS was performed using the diffusion method against selected strains of bacteria: *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Listeria monocytogenes* PCM 2191, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella enterica* PCM 2565, *Salmonella infantis* SES, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, and yeasts of *Candida albicans* ATCC 10231. Strains were purchased from the American Type Culture Collection (ATCC; Manassas, Virginia, United States), the Polish Collection of Microorganisms (PCM; Wrocław, Poland), or obtained from Sanitary-Epidemiology Station (SES) in Toruń, Poland. Briefly, the films were cut into 10 x 10 mm pieces and placed on the surface of tryptic soy agar (TSB, Becton Dickinson, USA) in the Petri plates inoculated with microorganisms. Microbial inoculum was prepared from strain grown in tryptic soy broth (TSB, Becton Dickinson, USA) for 24 h at 35°C ± 2°C under shaking conditions at 120 r.p.m. Culture was used to prepare microbial suspension in sterile deionized water at a density of 0.5 units on the McFarland scale ($1.5 \cdot 10^8$ CFU mL^{-1}) measured using a densitometer (Biosan, Latvia). The microbial inoculum was diluted with sterile deionized water to final concentrations of $1.5 \cdot 10^5$ CFU mL^{-1} before spreading onto the TSA medium in the Petri plates using a sterile swab. The plates with placed films were incubated for 24 h at 37°C and evaluated for antimicrobial activity.

Results and Discussions

Physicochemical properties

Chitosan films for testing were obtained using the solution casting method. After drying, the obtained films were easily removed from the plate. Control samples and the sample with 0.1% ZnONPs additive were smooth and homogeneous, whereas the film with 0.2% additive showed lumps (FIG. 1).

The spectra of pure chitosan samples and films with additives are presented in FIG. 2. The FTIR spectrum obtained for a pure sample of chitosan is characteristic of this polysaccharide, showing four main bands with the highest intensity. The broad visible band with a maximum at 3355 cm^{-1} comes from the O-H and N-H stretching vibrations, which overlap. In addition, this band is the result of intramolecular hydrogen bonding of chitosan molecules [32]. The bands at about 3400 cm^{-1} may additionally indicate the presence of water in the tested samples [33]. The band at 2868 cm^{-1} confirms the presence of C-H bonds from $-CH_3$ and $-CH_2$ groups. The bending vibration coming from the N-H group appears at a wavelength of 1653 cm^{-1}. In the 1200-1000 cm^{-1} spectral region, vibrations of the C-O-C groups can be observed, confirming the presence of the O-glycosidic bond. The 1200-900 cm^{-1} region is typical of polysaccharides and represents stretching vibrations of C-C and C-O bonds and C-H bending vibrations [32].

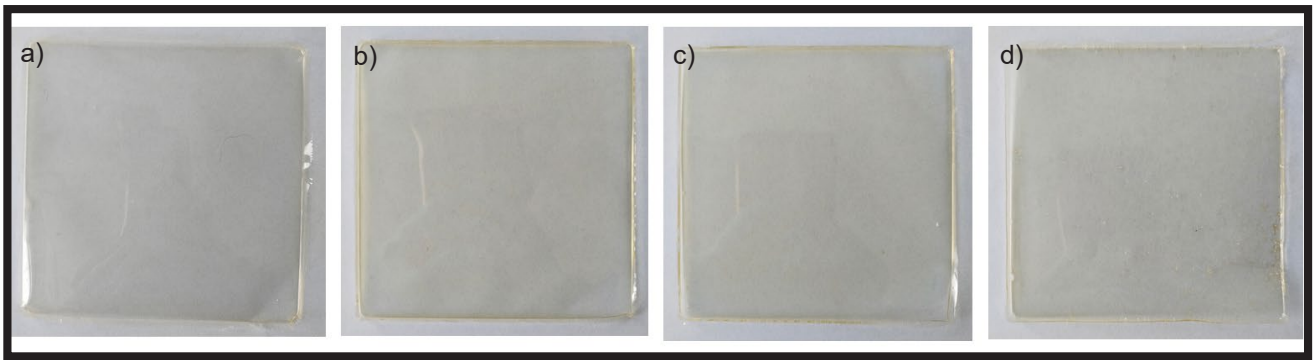

FIG. 1. Pictures of chitosan films: a) chitosan film (CS); b) chitosan film with 1% glycerol (CS/G); c) chitosan film with 1% glycerol and the addition of 0.1% zinc oxide nanoparticles (CS/G+ZnONPs(0.1%)); d) chitosan film with 1% glycerol and the addition of 0.2% zinc oxide nanoparticles (CS/G+ZnONPs(0.2%)).

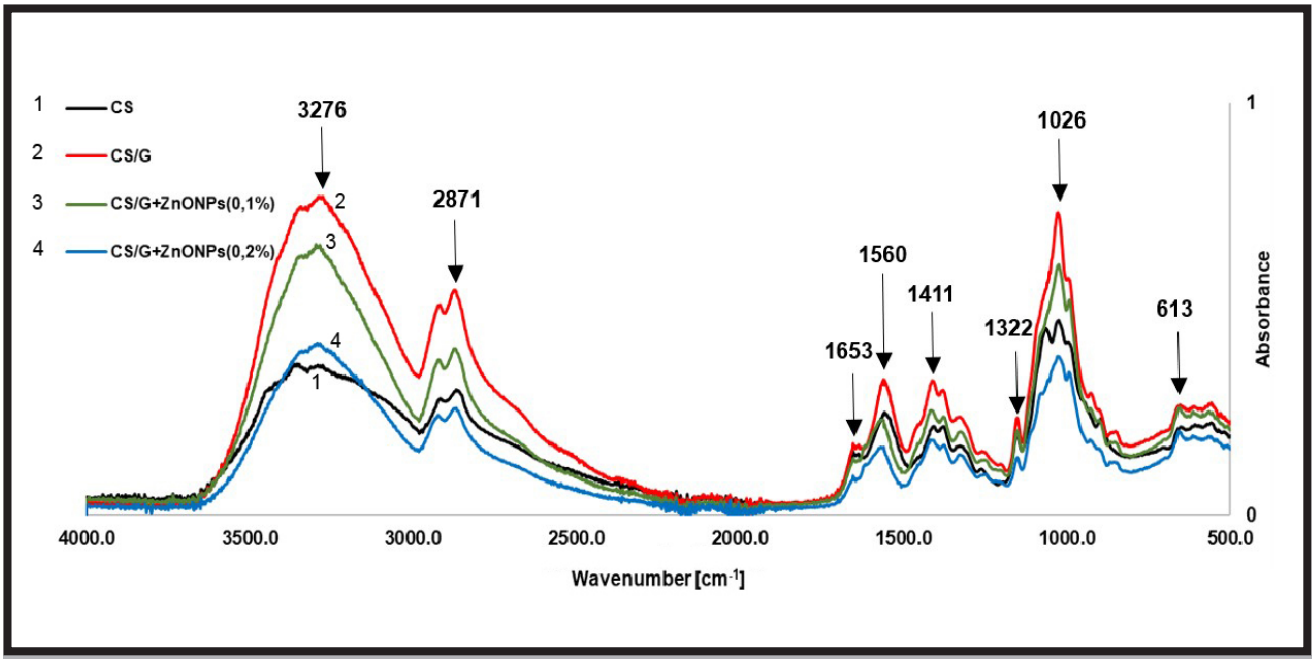

FIG. 2. IR spectra of polymer films.

Interactions that occur in a particular system can be uniquely reflected in the wavelengths present for the peaks in the spectrum [34]. The FTIR spectrum for the sample with the plasticizer added is similar to the spectrum of the original sample in terms of the appearance of individual bands. However, the intensity of the bands has been changed and a significant increase can be observed. First of all, the increased intensity of the bands around 3200-3500 cm^{-1} and the sharpening of the peak indicate the interaction of the polymer with the plasticizer, in particular the formation of many new intermolecular hydrogen bonds. Small shifts were observed from 3355 cm^{-1} (for the initial sample) to 3276 cm^{-1} (sample with the addition of the plasticizer - glycerol), confirming that the addition of glycerol promotes the formation of hydrogen interactions in the tested system [32].

IR spectra for samples with the addition of ZnONPs show significant changes in the intensity of individual bands. These changes correspond to the amount of nanoparticles added; the higher the concentration of additive, the lower the intensity of the bands. In the case of the band corresponding to the -OH and -NH_2 groups, this can be explained by the reduction of existing hydrogen bonds with the introduction of nanoparticles into the chitosan matrix [18,35]. The formation of new hydrogen bonds between the matrix and zinc oxide is indicated by slight shifts at a wavelength of about 3300 cm^{-1}.

Changes for samples with the addition of nanoparticles in the band 560-619 cm^{-1} confirm the presence of ZnO, reflecting the Zn-O stretching vibrations [18,36]. The positions of individual bands are presented in TABLE 1.

TABLE 1. Wavenumbers in IR spectra that occur in chitosan films with and without additives.

Functional group vibrations	Wavenumber [cm^{-1}]			
	CS	CS/G	CS/G+ZnONPs(0.1%)	Ch/G+ZnONPs(0.2%)
O-H stretching	3355	3276	3359	3292
N-H stretching	3355	3276	3359	3292
C-H stretching	2868	2871	2971	2868
N-H bending	1653	1653	1649	1653
amide II C=O	1556	1560	1565	1568
O-H deformational	1407	1411	1415	1416
C-N stretching	1326	1322	1326	1328
C-O-C stretching	1024	1026	1024	1025
Zn-O	-	-	613	614

Mechanical properties

The results of the tensile tests of thin chitosan films prepared with and without additives are shown in FIG. 3 and TABLE 2. It can be seen that the addition of glycerol and ZnONPs has a significant effect on the parameters tested.

Films with glycerol are characterized by a much lower Young's modulus value compared to pure chitosan films. The same applies to the tensile strength. Glycerol films are much more flexible and achieve a higher value of elongation at break. The use of a plasticizer (glycerol) allowed the nanoparticle films to be completely removed from the polystyrene plates and analyzed.

The addition of ZnONPs affects the properties of the chitosan-based film. The samples become more brittle, which is reflected in the values of the parameters tested, especially in the increase in the value of the Young modulus. It can be observed that the higher the additive content, the higher the tensile strength and the lower the elongation at break.

Surface morphology

The surface morphology was studied by SEM-EDX and AFM. SEM and AFM pictures of the obtained films are shown in FIG. 4. EDX analysis was carried out to study the elemental composition of the films and to confirm the presence of zinc in the films. This analysis showed a homogeneous distribution of the elements in the film, indicating that the solutions were well mixed before the films were obtained (FIG. 5).

It can be observed that all films, both without and with additives, have a uniform structure with a fairly smooth surface morphology. The pure chitosan film (without additive) has a low roughness parameter. The presence of zinc nanoparticles in 0.1% concentration increases the roughness of the sample. This parameter is also influenced by the addition of glycerol (plasticizer) (TABLE 3).

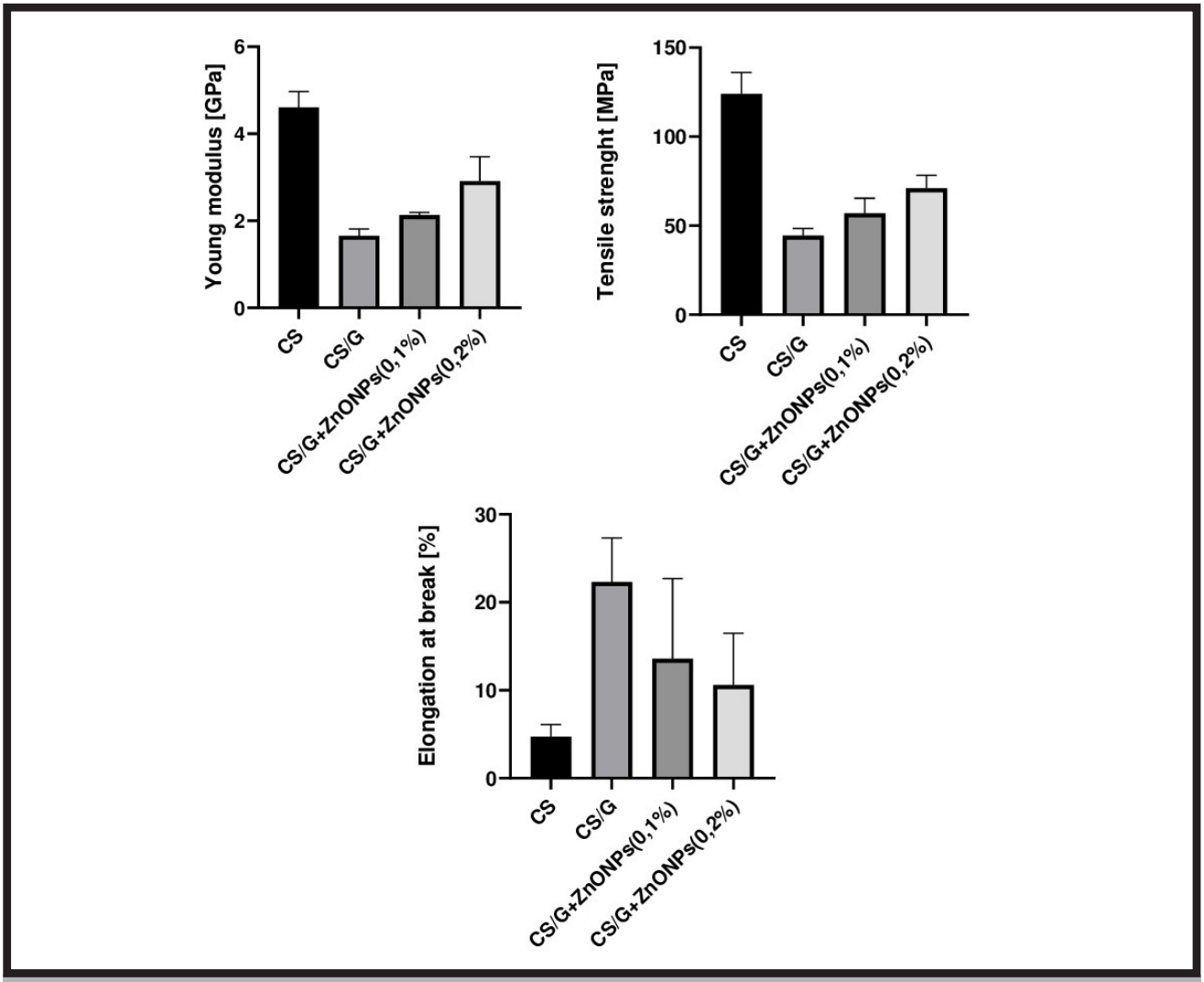

FIG. 3. Mechanical properties of chitosan films; error bars represent standard deviation (SD).

TABLE 2. Mean values of the mechanical parameters of the chitosan films with standard deviation (SD).

Parameter	CS	CS/G	CS/G+ZnONPs(0.1%)	CS/G+ZnONPs(0.2%)
Young Modulus [GPa]	4.61 ± 0.37	1.66 ± 0.16	2.13 ± 0.06	2.91 ± 0.56
Tensile strength [MPa]	124 ± 12.10	44.40 ± 3.96	56.90 ± 8.47	71.10 ± 7.24
Elongation at break [%]	4.70 ± 1.40	22.3 ± 5.00	13.60 ± 9.10	10.60 ± 5.90

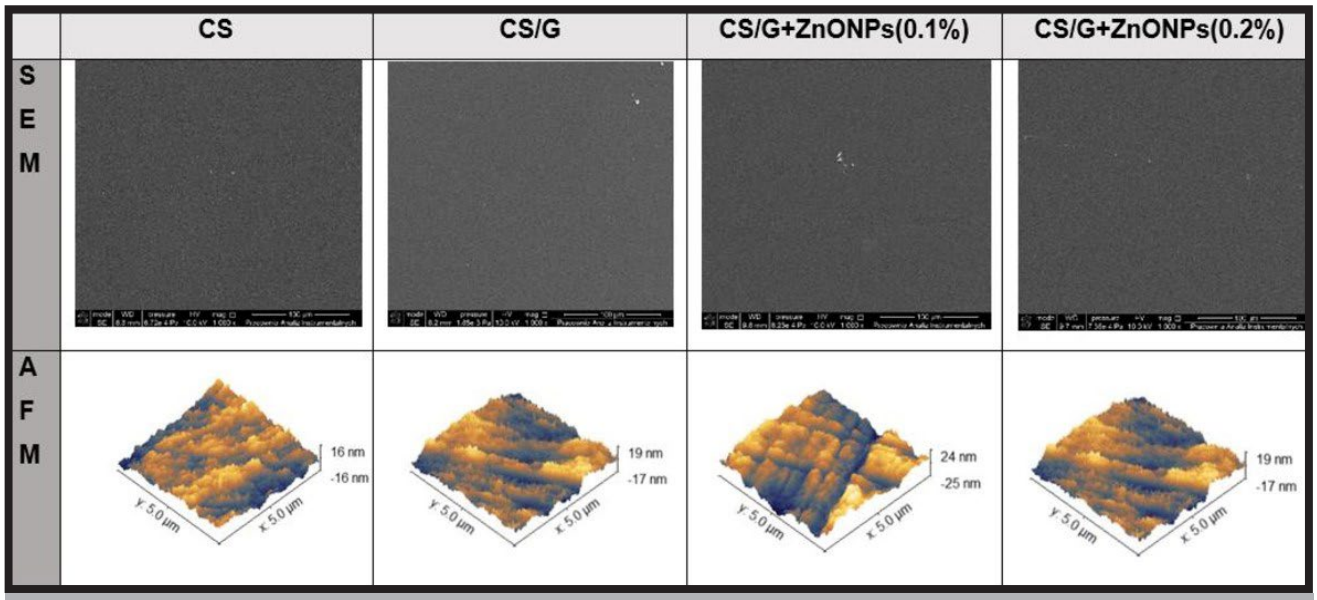

FIG. 4. SEM and AFM images of polymer films (SEM magnification 1000x).

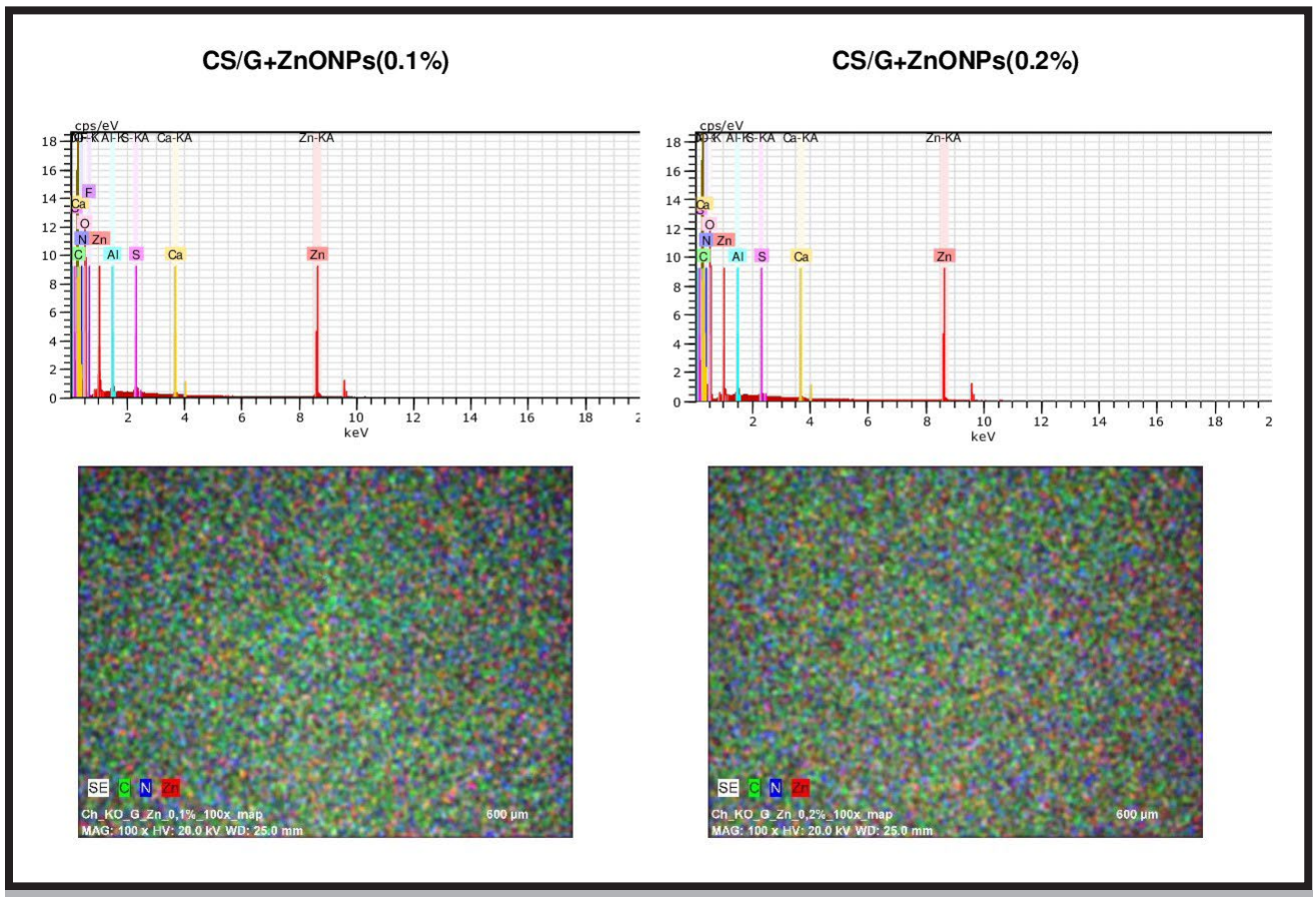

FIG. 5. Analysis (SEM-EDX) of the elemental composition of the samples with the addition of zinc oxide nanoparticles with mapping.

TABLE 3. Roughness parameters of chitosan films with and without additives.

Sample	CS	CS/G	CS/G+ZnONPs(0.1%)	CS/G+ZnONPs(0.2%)
R_q [nm]	4.80 ± 1.13	6.85 ± 0.46	7.06 ± 0.67	4.45 ± 0.56
R_a [nm]	3.815 ± 0.84	5.48 ± 0.42	5.62 ± 0.72	3.57 ± 0.45

TABLE 4. Influence of chitosan films with the addition of various concentrations of ZnONPs on inhibition of test bacteria growth.

Tested strain	Diameter of inhibition zone [mm ± SD]		
	CS/G	CS/G+ZnONPs(0.1%)	CS/G+ZnONPs(0.2%)
Staphylococcus aureus ATCC 25923	ND	ND	ND
Staphylococcus aureus ATCC 6538	ND	ND	ND
Salmonella infantis SES	ND	ND	ND
Salmonella enterica PCM 2565	ND	ND	ND
Pseudomonas aeruginosa ATCC 10145	ND	+	+
Listeria monocytogenes PCM 2191	ND	ND	ND
Escherichia coli ATCC 8739	ND	ND	ND
Escherichia coli ATCC 25922	ND	ND	ND
Klebsiella pneumoniae ATCC 700603	ND	ND	ND
Candida albicans ATCC 10231	ND	ND	ND

*ND – antimicrobial activity not detected in the tested ZnONPs content in the film. +; growth inhibition

Microbiological tests

The sensitivity of microorganisms to different contents of ZnONPs in the films was estimated by the inhibition growth zones under samples placed on inoculated agar plates. Results shown in TABLE 4 are the average of three separate experiments. The films with 0.1% and 0.2% content of ZnONPs showed antibacterial activity against *Pseudomonas aureginosa* ATCC 10145. No antimicrobial activity has been observed against any of the other strains. Interestingly, Li et al. [37] found pure chitosan films to be active against bacteria of *Alicyclobacillus acidoterrestris, Staphylococcus aureus, Escherichia coli*, and *Salmonella* sp., which was in contrast to the findings of the present study. The authors also showed that antibacterial activity of the films increased with increasing content of ZnONPs (0.2, 0.4, and 0.6%). It was discussed that inhibition of bacterial growth of pure chitosan films resulted from antimicrobial properties of chitosan which forms porous structures on the surface of Gram-positive bacterial cells, binds to the cell membrane and disturbs its barrier function while in gram-negative bacteria chitosan penetrates into cells to adsorb its ionic substances and affects metabolism. These mechanisms could lead to the inhibition of bacterial growth. The additive of ZnONPs increased the antibacterial effect of chitosan films. In this case, ZnONPs enhance the positive charges of the chitosan amino group, which intensify interactions with the negatively charged bacterial cell walls. This may potentially exert a synergistic effect in ZnONPs composite films [37]. In the present study, the antibacterial effect of the nanocomposite films was not significant, but the ZnONPs content in the films was low. Zhang et al. [38] tested chitosan/ZnONPs films with different concentrations (0.1, 0.2, and 0.3%) and size (5, 50, and 100 μm) of ZnONPs for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* The nanocomposite chitosan film containing 0.3% of 50 nm zinc oxide particles revealed the highest inhibition of bacterial growth indicating that smaller ZnONPs have better antibacterial activity. In the present study, ZnONPs had an irregular shape and showed an average size of 117.79 ± 4.71 nm and a size ranging from 54.44 to 209.69 nm [31], which could affect lower penetration of nanoparticles into cells. However, the antibacterial activity of films with the additive of mycogenic ZnONPs against *Pseudomonas aeruginosa* strain is very promising, as the isolates of this species belong to ESKAPE pathogens, the multidrug resistant ones, and are one of the leading causes of the nosocomial infections throughout the world [39].

Conclusions

The results of our research show that by adding zinc oxide nanoparticles (ZnONPs) to the chitosan solution, it is possible to obtain modified chitosan films in a simple way. The additives used in this research modify the properties of chitosan films. Spectrometric measurements confirmed changes in chitosan samples with the additive, resulting from interactions between the polymer and the nanoparticles. The addition of nanoparticles reduces the intensity of the bands and causes very slight shifts, which suggests a reduction in the number of intermolecular hydrogen bonds within the polymer matrix and the formation of new ZnONPs-chitosan hydrogen bonds. The Young modulus and tensile strength of the CS films increase when ZnONPs are added. To obtain films suitable for mechanical testing, the addition of a plasticizer was required. There was a slight change in the roughness parameters of the nanoparticle-enriched films compared to the pure chitosan film. All tested samples had a smooth surface morphology. The additives used allowed the properties of the films to be modified, particularly in terms of antimicrobial activity. The films show antibacterial activity against the *Pseudomonas aureginosa* strain even at low concentrations of ZnONPs. This is a particularly desirable feature for topical application of this material on the skin. The proposed combination could potentially have applications in cosmetics and biomedical applications such as wound dressing.

Acknowledgements

Statutory activity of KChBiK WCh UMK in Toruń.

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References

[1] Muthu M., Gopal J., Chun S., Devadoss A.J.P., Hasan N., Sivanesan I.: Crustacean waste-derived chitosan: Antioxidant properties and future perspectives. Antioxidants 10 (2021) 228.
[2] Younes I., Rinaudo M.: Chitin and chitosan preparation from marine sources. Structure, properties and applications. Marine Drugs 13 (2015) 1133-1174.
[3] Kulka K., Sionkowska A.: Chitosan Based Materials in Cosmetic Applications: A Review. Molecules 28 (2023) 1817.
[4] Guzman E., Ortega F., Rubio R.G.: Chitosan: A Promising Multifunctional Cosmetic Ingredient for Skin and Hair Care. Cosmetics 9 (2022) 99.
[5] Ferreira P.G., Ferreira V.F., Silva F., Freitas C.S., Pereira P.R., Paschoalin V.M.F.: Chitosans and Nanochitosans: Recent Advances in Skin Protection, Regeneration, and Repair. Pharmaceutics 14 (2022) 1307.
[6] Sheokand B., Vays M., Kumar A., Srivastava C.M., Bahadur I., Pathak S.R.: Natural polymers used in the dressing materials for wound healing: Past, present and future. Journal of Polymer Science 61 (2023) 1389-1414.
[7] Dodero A., Scarfi S., Mirata S., Sionkowska A., Vicini S., Alloisio M., Castellano M.: Effect of crosslinking type on the physical-chemical properties and biocompatibility of chitosan-based electrospun membranes. Polymers 13 (2021) 831.
[8] Wahba M.I.: Enhancement of the mechanical properties of chitosan. Journal of Biomaterials Science, Polymer Edition 31 (2020) 350-357.
[9] Machado A.H.S., Garcia I.M., Motta A., Leitune V.C.B., Collares F.M.: Triclosan-loaded chitosan as antibacterial agent for adhesive resin. Journal of Dentistry 83 (2019) 33-39.
[10] Preethi S., Abarna K., Nithyasri M., Kishore P., Deepika K., Ranjithkumar R., Bhuvaneshwari V., Bharathi D.: Synthesis and characterization of chitosan/zinc oxide nanocomposite for antibacterial activity onto cotton fabrics and dye degradation applications. International Journal of Biological Macromolecules 164 (2020) 2779-2787.
[11] Rajasekaran P., Santra S.: Hydrothermally treated chitosan hydrogel loaded with copper and zinc particles as a potential micronutrient - Based Antimicrobial Feed Additive. Frontiers in Veterinary Science 2 (2015) 62.
[12] Zhang X., Ismail B.B., Cheng H., Jin T.Z., Qian M., Arabi S.A., Liu D., Guo M.: Emerging chitosan-essential oil films and coatings for food preservation – A review of advances and applications. Carbohydrate Polymers 273 (2021) 118616.
[13] Lian R., Cao J., Jiang X., Rogachev A.V.: Physicochemical, antibacterial properties and cytocompatibility of starch/chitosan films incorporated with zinc oxide nanoparticles. Materials Today Communications 27 (2021) 102265.
[14] Matatkova O., Michailidu J., Miskovska A., Kolouchova I., Masak J., Cejkova A.: Antimicrobial properties and applications of metal nanoparticles biosynthesized by green methods. Biotechnology Advances 58 (2022) 107905.
[15] Khan I., Saeed K., Khan I.: Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry 12 (2019) 908-931.
[16] Ahmed F., Prashanth S., Sindhu K., Nayak A., Chaturvedi S.: Antimicrobial efficacy of nanosilver and chitosan against Streptococcus mutans, as an ingredient of toothpaste formulation: An in vitro study. Journal of Indian Society of Pedodontics and Preventive Dentistry 37 (2019) 46-54.
[17] Fatoni A., Yessica H.S., Aldilah, Almi M., Rendowaty A., Romsiah, Sirumapea L., Hidayati N.: The Film of Chitosan-ZnO Nanoparticles-CTAB: Synthesis, Characterization and In Vitro Study. Science and Technology Indonesia 7 (2021) 58-66.
[18] Mujeeb Rahman P., Abdul Mujeeb V.M., Muraleedharan K., Thomas S.K.: Chitosan/nano ZnO composite films: Enhanced mechanical, antimicrobial and dielectric properties. Arabian Journal of Chemistry 11 (2018) 120-127.
[19] Soubhagya A.S., Moorthi A., Prabaharan M.: Preparation and characterization of chitosan/pectin/ZnO porous films for wound healing. International Journal of Biological Macromolecules 157 (2020) 135-145.
[20] Thomas V., Yallapu M.M., Sreedhar B., Bajpai S.K.: Fabrication, characterization of chitosan/nanosilver film and its potential antibacterial application. Journal of Biomaterials Science, Polymer Edition 20 (2009) 2129-2144.
[21] Ali A., Phull A.R., Zia M.: Elemental zinc to zinc nanoparticles: Is ZnoNPs crucial for life? Synthesis, toxicological and environmental concerns. Nanotechnology Reviews 7 (2018) 413-441.
[22] Sharma P., Reddy P.K., Kumar B.: Trace Element Zinc, a Nature's Gift to Fight Unprecedented Global Pandemic COVID-19. Biological Trace Element Research 199 (2021) 3213-3221.
[23] Skrajnowska D., Bobrowska-Korczak B.: Role of zinc in immune system and anti-cancer defense mechanisms. Nutrients 11 (2019) 2273.
[24] Ginzburg A.L., Blackburn R.S., Santillan C., Trulong L., Tanguay R.L., Hutchison J.E.: Zinc oxide-induced changes to sunscreen ingredient efficacy and toxicity under UV irradiation. Photochemical and Photobiological Sciences 20 (2021) 1237-1285.
[25] Cuajungco M.P., Ramirez M.S., Tolmasky M.E.: Zinc: Multidimensional effects on living organisms. Biomedicines 9 (2021) 1-25.
[26] Slavin Y.N., Asnis J., Hafeli U.O., Bach H.: Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology 15 (2017) 65.
[27] Czyżowska A., Barbasz A.: A review: zinc oxide nanoparticles – friends or enemies? International Journal of Environmental Health Research 32 (2022) 885-901.
[28] Singh A., Singh N.Á., Afzal S., Singh T., Hussain I.: Zinc oxide nanoparticles: A review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants. Journal of Materials Science 53 (2018) 185-201.
[29] Trzcińska-Wencel J., Wypij M., Terzyk A.P., Rai M., Golińska P.: Biofabrication of novel silver and zinc oxide nanoparticles from *Fusarium solani* IOR 825 and their potential application in agriculture as biocontrol agents of phytopathogens, and seed germination and seedling growth promoters. Frontiers in Chemistry 11 (2023) 1235437.
[30] Bandeira M., Giovanela M., Roesch-Ely M., Devine D.M., Silva Crespo J.: Green synthesis of zinc oxide nanoparticles: A review of the synthesis methodology and mechanism of formation. Sustainable Chemistry and Pharmacy 15 (2020) 100223.
[31] Mirzaei H., Darroudi M.: Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics International 43 (2017) 907-914.
[32] Liu H., Adhikari R., Adhikari B.: Preparation and characterization of glycerol plasticized (high-amylose) starch-chitosan films. Journal of Food Engineering 116 (2013) 588-597.
[33] Jakubowska E., Gierszewska M., Szydłowska-Czerniak A., Nowaczyk J., Olewnik-Kruszkowska E.: Development and characterization of active packaging films based on chitosan, plasticizer, and quercetin for repassed oil storage. Food Chemistry 399 (2023) 135617.
[34] Yin Y.J., Yao K.D., Cheng G.X., Ma J.B.: Properties of polyelectrolyte complex films of chitosan and gelatin. Polymer International 48 (1999) 429-433.
[35] Khung K., Ibupoto Z.H., AlSalhi M.S., Atif M., Ansari A.A., Willander M.: Fabrication of Well-Aligned ZnO Nanorods Using a Composite Seed Layer of ZnO Nanoparticles and Chitosan Polymer. Materials 6 (2013) 4361-4374.
[36] Dobrucka R., Dugaszewska J.: Biosynthesis and antibacterial activity of ZnO nanoparticles using *Trifolium pratense* flower extract. Saudi Journal of Biological Sciences 23 (2016) 517-523.
[37] Li Y., Zhou Y., Wang Z., Cai R., Yue T., Cui L.: Preparation and Characterization of Chitosan-Nano-ZnO Composite Films for Preservation of Cherry Tomatoes. Foods 10 (2021) 3135.
[38] Zhang X., Zhang Z., Wu W., Yang J., Yang Q.: Preparation and characterization of chitosan/Nano-ZnO composite film with antimicrobial activity. Bioprocess Biosystems Engineering 44 (2021) 1193-1199.
[39] Santajit S., Indrawattana N.: Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. Biomed Research International 2016 (2016) 2475067.