Section 1. Chemistry

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THE COMPLEX OF CHITOSAN-SILVER FROM THE DEAD BEES APIS MELLIFERA AND THEIR PHYSICAL, CHEMICAL AND ANTIBACTERIAL PROPERTIES

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Abstract

This article examines the physicochemical and antibacterial properties of a chitosan-silver complex synthesized from dead bees *Apis Mellifera*. The chitosan-silver complex demonstrated potent antimicrobial effectiveness, making it an effective agent against various pathogenic microorganisms due to its high biocompatibility and biodegradability. The research highlights the complex's strong antibacterial activity against pathogens such as Staphylococcus Aureus and Pseudomonas Aeruginosa.

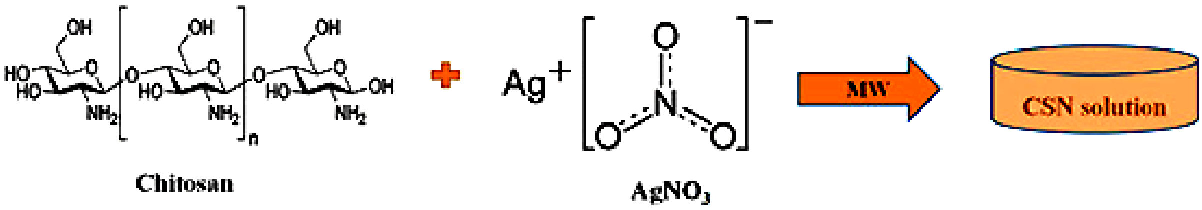
Keywords: *Chitosan, Silver nanoparticles, Apis Mellifera, Antimicrobial properties, Physicochemical properties, Bactericidal effect*

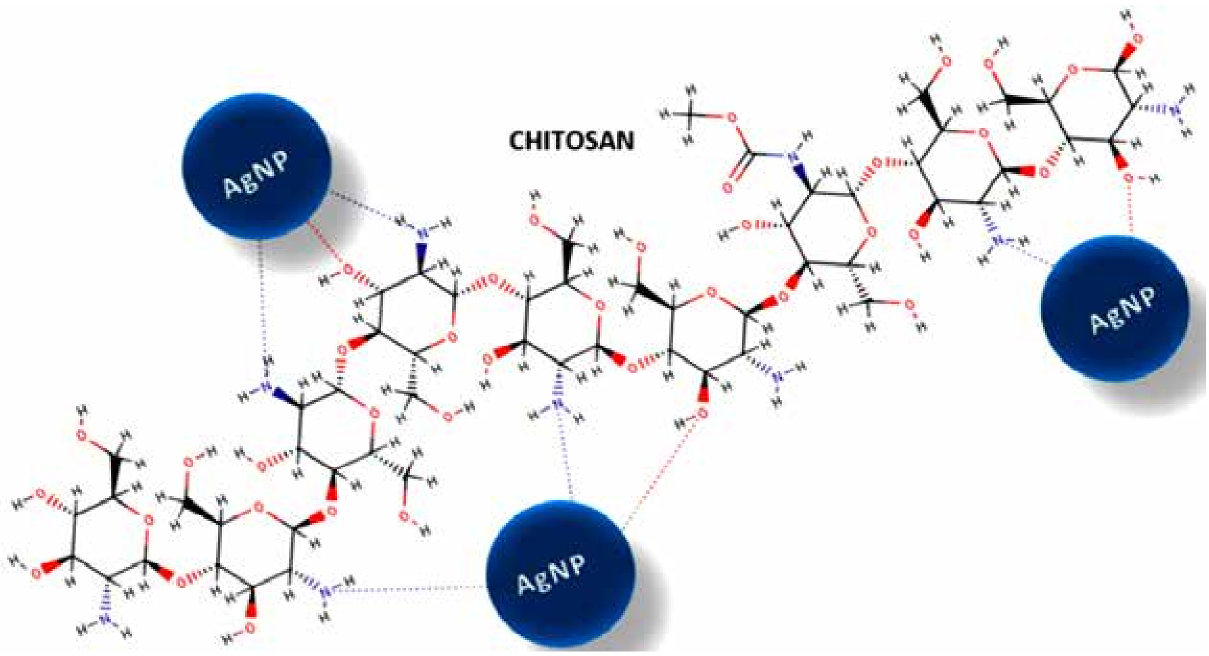
Introduction

In recent years, the development of efficient and green routes to synthesise metal nanoparticles has gained considerable attention in various areas of nanotechnology. Among metal nanoparticles, silver nanoparticles (AgNPs) have attracted much interest because of their potential antimicrobial agents and they are widely applied in many biological and medical fields. Concerning Ag-based nanocomposites, chitosan-silver nanoparticles (Ch-AgNPs) represent an emerging group of bio-nanostructured hybrid materials due to their biocompatibility and biodegradability (Mohamed E. I., Badawy, Tesby M. R. Lotfy Samar M. S., 2019). Today, biopolymers of chitosan and its derivatives, mainly their complexes with silver ions, are of great importance. In particular, complexes of chitosan with silver ions are studied by scientists all over the world. It is known that chitosan and silver as well as their derivatives have very good bactericidal properties (Govindan S., Nivethaa E. A.K., Saravanan R., Narayanan V., Stephen A., 2012; Lok C. N., Ho C. M., Chen R., He Q. Y., Yu W. Y., Sun H., Tam P. K.H., Chiu J. F., Che C. M., 2007; Shahverdi A. R., Fakhimi A., Shahverdi H. R., Minaian S., 2007). Given their properties, the chitosan-silver complex can also be used as an antiviral and antibacterial agent. Studies show that the antibacterial properties of the complex of silver ions with chitosan are higher than that of chitosan (Sattarova D. M., 2019; Mourya V. K., Inamdar N. N., 2008). Films based on chitosan and its compounds containing silver ions or silver nanoparticles contain antibiotics and exhibit more fungicidal properties than composites. The effect of molecular weight on the bactericidal properties of chitosan and its derivatives is not the same. This is due to different levels of polymerization of the polymer molecule, which exhibits maximum antibacterial properties at different pH values of the medium. For example, in an acidic environment, high-molecular chitosan has relatively better bactericidal properties, since in an environment with a pH above 6–6.5, its amino groups lose their charge and the polymer precipitates

Object and method of research

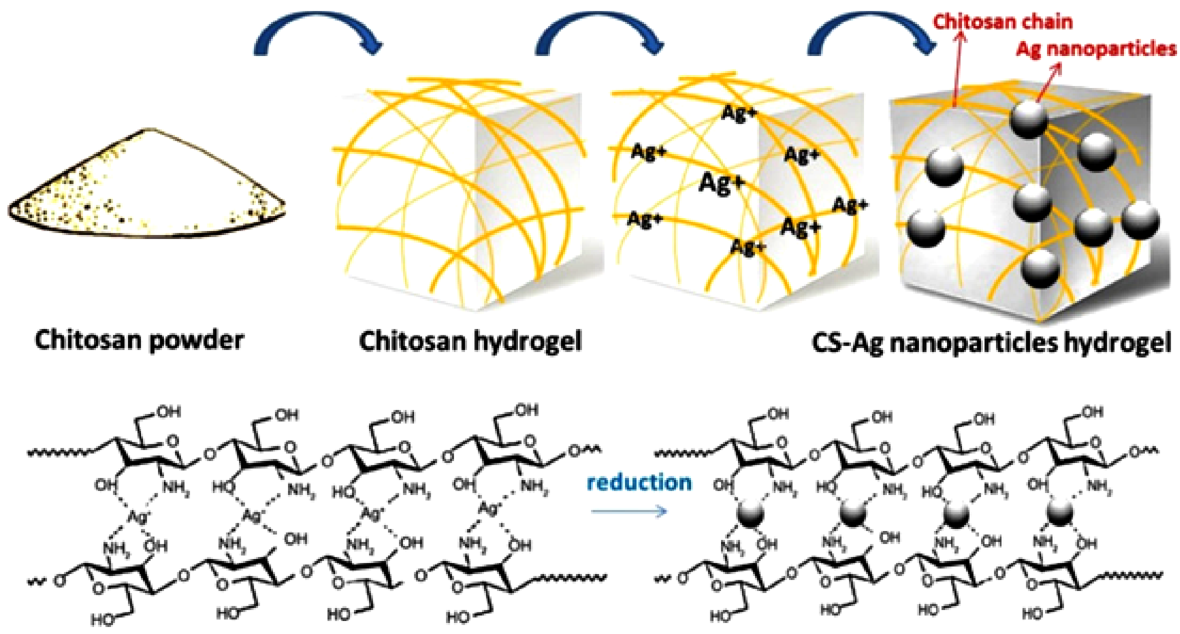
Chitosan synthesis was carried out in two ways. Chitosan was obtained from chitin using the usual method and cryogenically in liquid nitrogen. We synthesized a chitosan-silver complex obtained from a bee subscore, and a film based on this complex was obtained to determine its physical and chemical properties. The reaction follows the following mechanism.





Chitosan-silver nanocomposites are a powerful and promising material for reducing the pathogenicity associated with infections caused by microorganisms without harming mammalian cells (Ikhtiyarova G. A., Isomitdinova D.S, Saidov J. E., 2023). One of the unique biological activities of chitosan is its induction of disease resistance in plants and inhibition of pathogens. The antimicrobial properties of silver have been known for a very long time. Silver ions have a broad-spectrum effect; they prevent the development of pathogenic bacteria, viruses and fungi. Chitosan-stabilized silver nanoparticles with antibacterial and antifungal activity were synthesized. UV spectra showed that with increasing chitosan concentration in the reaction, the efficiency of nanoparticle formation increases and the particle size decreases proportionally. X-ray diffraction patterns showed that Ag nanoparticles have a spherical crystalline shape. The addition of stabilized silver nanoparticles to the nutrient medium containing chitosan inhibits the growth of pathogenic microorganisms (Jian D., You-Lo H., 2008).

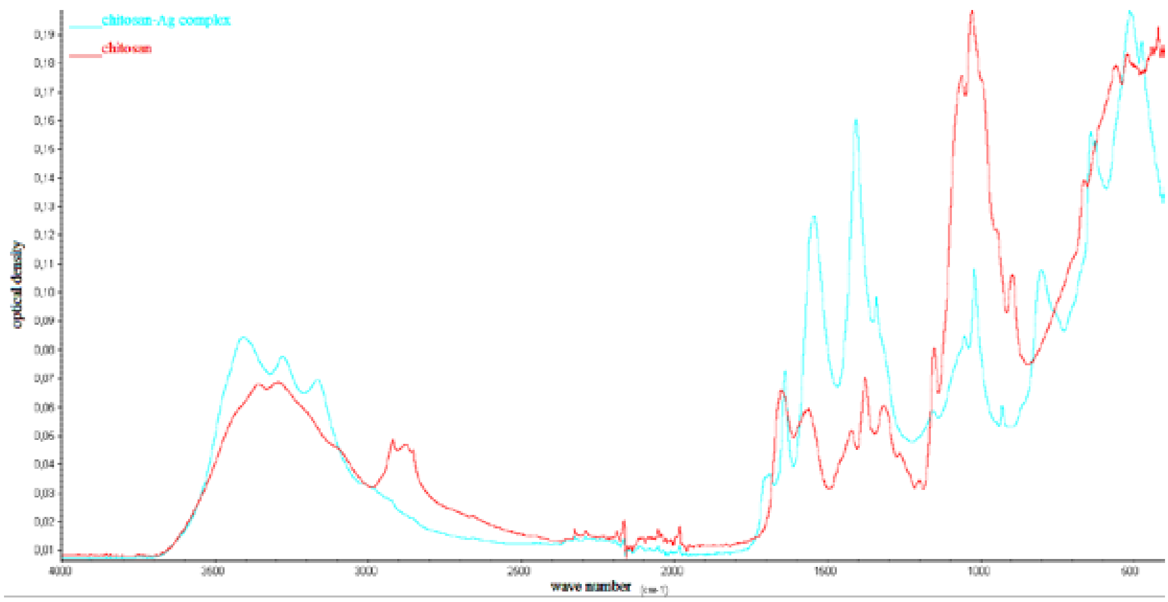
Figure 1. *Mechanism and structure of chitosan reaction with silver ion*



Experimental method

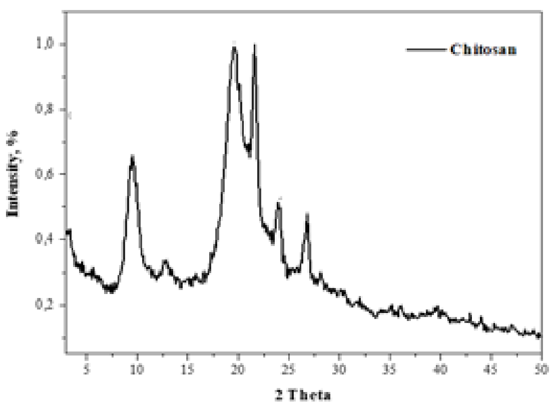
Preparation of CS-Ag nanoparticles Chitosan-silver nanoparticles stabilized by adding 0.5% chitosan solution to silver nanoparticle solution by reducing $AgNO_3$ salt were obtained A 0.5% chitosan solution was prepared by adding 0.5 µm of 0.005 M $AgNO_3$ salt solution and then reduced with $NaBH_4$. 10 ml of the prepared chitosan solution was added to the reaction mixture and stirred vigorously (rotation speed 800 times per minute) in a magnetic stirrer. Then the reaction product was filtered and placed in petri dishes to form a film. CS-Ag films were dried at room temperature for 4 days, and the physicochemical properties of the formed film were studied.

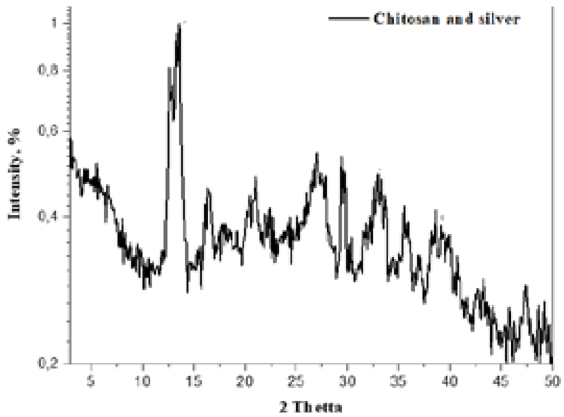
Figure 2. *IR spectrum of: A) chitosan and B) chitosan-silver complex*



Vibrational lines in the chitosan spectrum at 3439 cm^{-1} and 2885 cm^{-1} indicate the presence of amide bonds (A and B). The amide bond A is formed by valence vibrations in the O-H and N-H bonds. The amide bond is mainly due to the valence vibrations of CH-aliphatic bonds. In addition, C = O (Amid I), deformed vibrations of N-H (Amid II) and vibrations of amide III can be seen in the areas of 1655 cm^{-1}, 1558 cm^{-1} and 1383 cm^{-1}. The IR spectrum of the Fur of the chitosan-silver complex shows several changes compared to the spectrum of chitosan. The amide line A is divided into two parts at 3397 and 3164 cm^{-1}, and the amide line B has a length of 2928 cm^{-1}. These changes may indicate a relationship between the primary amino and amide groups of chitosan and silver. The lines of amides A and B in the chitosan-silver complex are not as wide as in chitosan, which indicates the appearance of hydrogen bonds. The lines of amides I, II, and III in the chitosan-silver complex in the region of 1636 cm^{-1}, 1558 cm^{-1}, and 1399 cm^{-1}, however, the intensity of these three lines also decreased, and the amino and hydroxyl groups of silver ions in chitosan may indicate the formation of chelate. The connections between Ag, O and N are due to the presence of intersecting lines and low-intensity peaks. Absorption lines in the 1072 cm^{-1} and 1083 cm^{-1} regions provide information about the C-O-C bond in the polysaccharide fragment. Peaks in the range of 1000–450 cm^{-1} indicate the deformation of the CH and -OH groups in the chitosan-silver complex. All five main lines in the chitosan-silver complex prove that amino and hydroxyl groups form coordination bonds with the Ag ion.

Figure 3. *X-ray diffractograms of CS and CS/Ag complex films synthesized from Apis Mellifera dead bees*



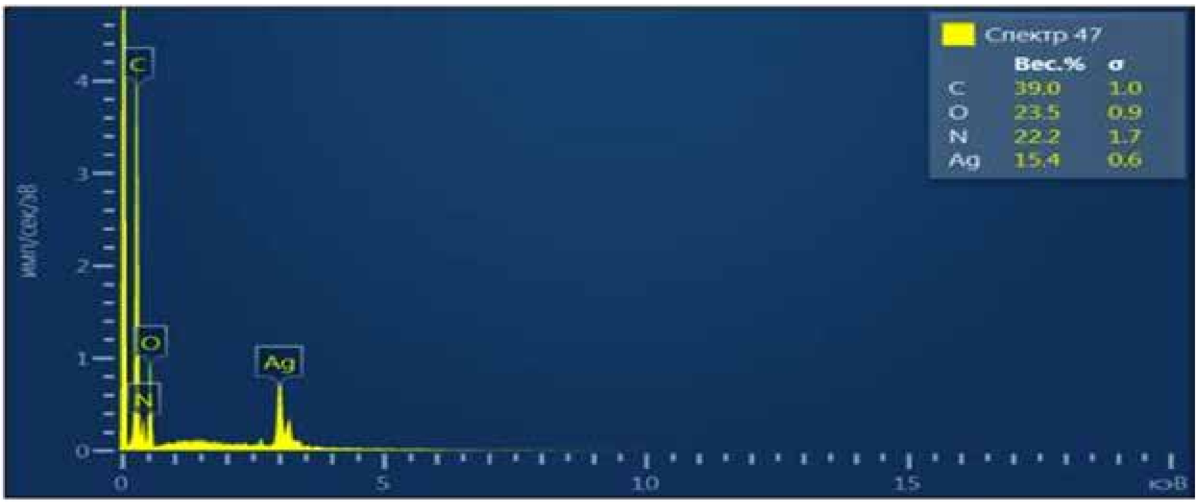


X-ray diffraction analysis is often used for the experimental study of particle sizes and shapes, which has very important information. The X-ray diffraction patterns of CS and CS/Ag metal complex films show that nanoparticles affect the polymer structure. The diffractogram of Apis Mellifera chitosan (Figure 3) shows two characteristic peaks at 10 and 20° corresponding to chitosan, one at 2 A strong peak at 2θ = 20° and a weak peak at 2θ = 10° (Ikhtiyarova G. A., Isomitdinova D.S, Saidov J. E., 2023) we can see a sharp decrease in intensity. This may be due to the amorphous supramolecular structure. Peaks corresponding to XZ/Ag were shown in the 2nd =28.5 (210), 35 (214), and 38 (311) sectors in the diffractogram (Egamberdiev, E., Ergashev, Y., Khaydullayev, K., Husanov, D., Rahmonberdiev, G., 2022).

The results of electron-dispersion spectroscopic analysis of the chitosan-silver complex were also studied in the Fig. 4.

The film of the chitosan-silver complex was brought to the same mass using alcohol by mixing starch, salicylic acid and the complex in various proportions. Then it is evenly distributed on a hard surface and shaped. When the initial product was obtained in a ratio of 2:1:10, the resulting film had a small defect after drying, and the defects of the film increased as the amount of the complex decreased. The film based on the chitosan-silver complex is yellowish-light brown in colour, and as the amount of the complex increases, the colour of the film becomes darker.

Figure 4. *EDS-electron dispersion spectroscopic analysis of the chitosan-silver complex*



Elemental analysis of Chitosan with silver ions annealed in different ratios Element Mass. % Sigma Mass.% Element Mass. % Sigma Mass.% C 39.0 1.0 C 16.25 0.61 N 22.2 1.7 N 2.74 1.74 O 23.5 0.9 O 21.85. Further, the antibacterial properties of the films based on the complex formed by chitosan and silver ions were studied on Staphylococcus Aureus and Pseudomonas aeruginosa. Staphylococcus aureus is one of the pathogenic bacteria that cause various diseases. It is a type of Staphylococcus bacteria. Under a microscope, Staphylococcus bacteria look like a bunch of grapes. There are more than 30 types of staphylococcal bacteria, but Staphylococcus aureus is the most common. The diameter of this bacterium is 0.7–1.2 microns. Does not form a dispute and does not move. The optimal temperature for bacterial growth is 37 °C, but they can multiply at 20–25 °C (Ikhtiyarova G. A., Turabdjanov S. M., Mengliyev A. S., Usmanova G. A., Axmadjonov A. N., Haydarova Ch.Q., 2020).

Pseudomonas aeruginosa is a liquid spore-forming bacterium with a complex protein structure, the length of which is approximately 1–3 microns, and the width is 0.5–1 microns. equal. This bacterium is found mainly in water, plants, sewage and moist soil. The bacterium Pseudomonas aeruginosa grows well at 37–41 °C. It can be found in medical institutions and even in surgical instruments. This bacterium causes infections of the skin, respiratory and urinary tract. This bacterium is dangerous due to its resistance to various antibiotics and the ability to form new defence mechanisms. To assess the bactericidal properties of the chitosan-silver complex, it was stored in a laboratory at 37 °C in a thermostat for 24 hours for bacterial growth. Bacteria Staphylococcus Aureus and Pseudomonas Aeruginosa were grown in an agarized Muller-Hinton medium (Fig. 5).

Figure 5. *Effect of chitosan and chitosan-silver complex films on Pseudomonas Aeruginosa and Staphylococcus Aureus strains*

a) b)

It was cut from a chitosan-silver film measuring 10 mm. Then the surface of the bacteria in the Petri dish was covered with a film. The active bactericidal part of the film, i.e. the bacterial growth suppression zone, had a radius of 5–6 mm. At the same time, silver ions penetrate the cell through the bacterial membrane, disrupting its structural composition. In addition, silver ions block the activity of several bacterial enzymes that transfer electrolytes and regulate energy metabolism. Replication also stops due to the loss of enzymatic activity. Eventually, silver ions attach to the walls of the bacterial cell, weakening the structure and protection of the cell, which leads to defects in the inner protective layer and accelerates the death of the bacterium (Ikhtiyarova G. A., Hazratova D. A., Umarov B. N., Seytnazarova O. M., 2020; Ikhtiyarova G. A., Xolto'rayeva N.R., Turabdjanov S. M., Aliyeva M. T.; Ikhtiyarova G. A., Mamatova Sh.B., Temirov F. F. Holturaeva N. R., 2022; Ikhtiyarova G. A., Isomitdinova D.S, Saidov J. E., 2023).

In the case of Staphylococcus Aureus and Pseudomonas Aeruginosa, we also studied the bactericidal properties of the film obtained from chitosan and compared the results with the bactericidal properties of the film based on the chitosan-silver complex. The bacteria Staphylococcus Aureus and Pseudomonas Aeruginosa grown in the laboratory were cut 10 mm from chitosan film.

Conclusion

Chitosan also has antibacterial properties, which reduce the growth of bacteria under the influence of the film. However, the radius of its bacterial growth retardation zone was less than 3 mm. Based on the results, it can be concluded that films obtained from the chitosan-silver complex are an effective means for medical use for further use as a biopolymer film, which makes it possible to use such films against some different bacteria and viruses that can quickly develop resistance to antibiotics.

The fact that such a film can dissolve even a drop of water allows it to be used in medicine. In addition, the fact that Apis Mellifera chitosan has a lower molecular weight of chitosan than chitosan obtained from other sources also simplifies the use of such films in medicine. The antibacterial properties of the film have been thoroughly studied, and it has been found that such films can inhibit the growth of active bacteria Staphylococcus Aureus and Pseudomonas aeruginosa.

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