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Research Article

Synthesis, Characterization and Comparison of Antibacterial Activity of Chitosan Coated Silver and Dithiocarbamate Chitosan Coated Silver Nanoparticles

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Abstract

Silver nanoparticles are frequently used in biomedical applications due to their antimicrobial activity, especially in the production of wound dressing materials. In this study, dithiocarbamate chitosan compound, a derivative of chitosan, was synthesised. The synthesised dithiocarbamate chitosan was characterised by scanning electron microscopy, energy dispersive X-ray spectrometry and C^{13}-nuclear magnetic resonance spectroscopy analyses. Silver nanoparticles coated with dithiocarbamate chitosan were successfully synthesised for the first time in this study. Chitosan-coated silver nanoparticles were also synthesised for comparison. The synthesised silver nanoparticles were characterised by ultraviolet-visible region spectrophotometry, size analysis and zeta potential measurements. The antibacterial activities of the synthesised silver nanoparticles against *Escherichia coli* and *Staphylococcus aureus* bacteria were tested using liquid medium dilution method. Characterisation results show that dithiocarbamate chitosan was successfully synthesised. The sizes of silver nanoparticles coated with chitosan and dithiocarbamate chitosan were determined as 175.20±2.98 and 158.00±1.27 nm and zeta potentials as 29.70±4.00 and 33.40±0.26 mV, respectively. From the results of antibacterial activity, it was determined that the synthesised nanoparticles showed a high antibacterial activity against bacteria. As a result, it was determined that dithiocarbamate chitosan coated silver nanoparticles obtained for the first time in this study have a great potential for use in the production of wound dressing materials due to their stability and high antibacterial effect.

Keywords

Dithiocarbamate chitosan, chitosan, silver nanoparticle, wound dressing material.

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1. Introduction

Nanoscience can be defined as all studies related to the production of materials with nano size. Nanosized particles have many different properties compared to those encountered in normal bulk molecules. For this reason, nanotechnology offers many different areas for human application such as medical, energy, optics, electronics, space, food industry. Especially in recent years, nanoparticles have been used in many medical fields such as imaging systems, biosensors, design of new drugs, release systems, diagnosis and treatment of diseases. (Priya et al. 2020; Thakkar et al. 2010; Nagarajan & Arumugam Kuppusamy 2013; Rajeshkumar et al. 2012)

Nanomaterials can be produced from many elements such as gold, copper, zinc or silver. Nanoparticles can be synthesised by many different chemical, physical, and biological methods. Among these particles, silver nanoparticles (AgNP) are widely used in coating of the food, catalysing reactions, electronic applications, water treatment, textile applications. In addition, silver nanoparticles produced can exhibit very good anticancer, antibacterial, antifungal, anti-inflammatory and antiviral activities that can expand their use in the medical field. Researchers believe that the high antibacterial activity of AgNPs against normal bacteria and even antibiotic-resistant bacteria is due to their large surface/volume ratio (Priya et al. 2020; Abou El-Nour et al. 2010; Mira et al. 2015; Naganthran et al. 2022).

The skin has many important features such as protecting the body's natural balance, protecting from homeostasis, acting as a barrier against pathogenic microorganisms and managing metabolic processes. When the skin is damaged, this important balance is disrupted, and harmful microorganisms can colonise the wound site and cause serious bacterial infections, especially when our immunity decreases. Prevention of microbial infection is one of the most basic requirements during the period until the skin regains its former state. For this reason, the most important criterion sought in the development of wound dressing materials is high antimicrobial properties (Zhou et al. 2021, Church et al. 2006; Jahromi et al. 2018).

In particular, silver has been widely investigated to improve the antibacterial properties of materials to be used as wound dressings. In recent years, AgNPs have attracted great interest with their large surface-to-volume ratio compared to bulk silver molecules. This property has led AgNPs to have highly effective antibacterial properties. Compared to silver ion, AgNPs are more favourable in terms of achieving a prolonged and controlled release at the wound site. These properties will reduce the frequency of dressing changes, prevent tissue damage, increase patient compliance and reduce care costs (Zhou et al. 2021; Atiyeh et al. 2007; Gravante et al. 2009).

AgNPs can be synthesised by many different methods. Most of these approaches allow the particle size and morphology of the synthesised AgNPs to be adjusted as desired. However, the use of toxic organic solvents and toxic reducing agents during production increases environmental risks, which limits the use of AgNPs in medical fields. Therefore, researchers are looking for an easily applicable and environmentally risk-reduced method for synthesising AgNPs that can be used in medical fields (Yan et al. 2021).

In particular, the synthesis of monodispersed AgNPs by preventing thermally induced aggregation has been carried out in the presence of polysaccharides such as chitosan (Priya et al. 2020), alginate (Chen et al. 2020) and gelatin (Ye et al. 2019) used as reducing and/or stabilising agents. Chitosan is one of the most commonly used polysaccharides for this purpose (Priya et al. 2020; Shehabeldine et al. 2022; Dara et al. 2020).

Chitin is the most common polysaccharide on Earth after cellulose. Chitin is found as a structural material in the cell walls of fungi and algae, and in the exoskeletons of insects and shellfish. However, its insolubility in commercial solvents limits its use (Ogawa & Okuyama 2004). Therefore, water-soluble derivatives of chitin have been produced. The most important of these is chitosan. Chitosan is obtained by deacetylation of chitin and is known to be the only natural cationic polysaccharide (Hamed et al. 2016). The open molecular structure of the polymer of chitosan is shown in Figure 1.

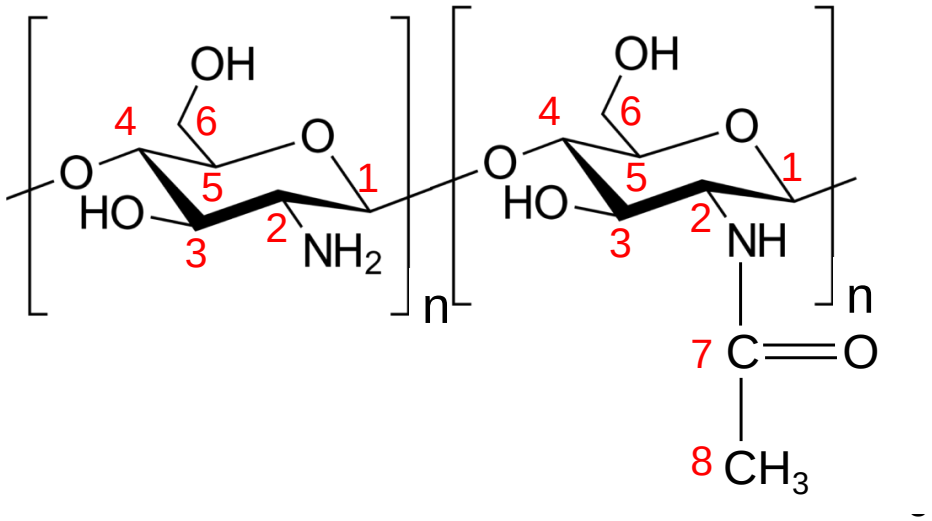


Figure 1. Open molecular structure of chitosan polymer (numbering refers to carbon atoms)

The properties of chitosan such as high-water retention capacity, biocompatibility, biodegradability, low cytotoxicity, low cost, and haemostatic properties expand its application areas for medical applications. Chemically modified chitosan can not only improve the physical and chemical properties of chitosan, but also expand its application areas by preserving the unique properties of chitosan. Modified chitosan derivatives have been reported to retain the pharmacological effects of the original chitosan such as antibacterial, anticancer and antiviral properties. Today, chitosan derivatives are widely used in both medical materials and biomedical fields. With the advancement of nanotechnology, chitosan derivatives are used as nanomaterials, nanoparticles, hydrogels and microspheres, as well as targeted carriers for drugs (Zhou et al. 2021; Lu et al. 2017; Li et al. 2017; Wang et al. 2020).

Among the chitosan derivatives, dithiocarbamate chitosan (DTCC) chemistry has been developing rapidly in recent years. Dithiocarbamate chitosan is a chitosan derivative in which amino groups on the 2nd carbon atom is converted into dithiocarbamate groups. The open molecular structure of the DTCC polymer is shown in Figure 2. The molecular structure of DTCCs contains sulfur atoms with metal atom chelating properties. Due to this unique molecular structure, it is potentially an excellent metal ion complexing agent. Thanks to this feature, it is useful in various industrial and environmental applications, especially in the removal of metal ions and reduction of heavy metal pollution (Qin et al. 2012; Liu et al. 2021; Muzzarelli et al. 1982).

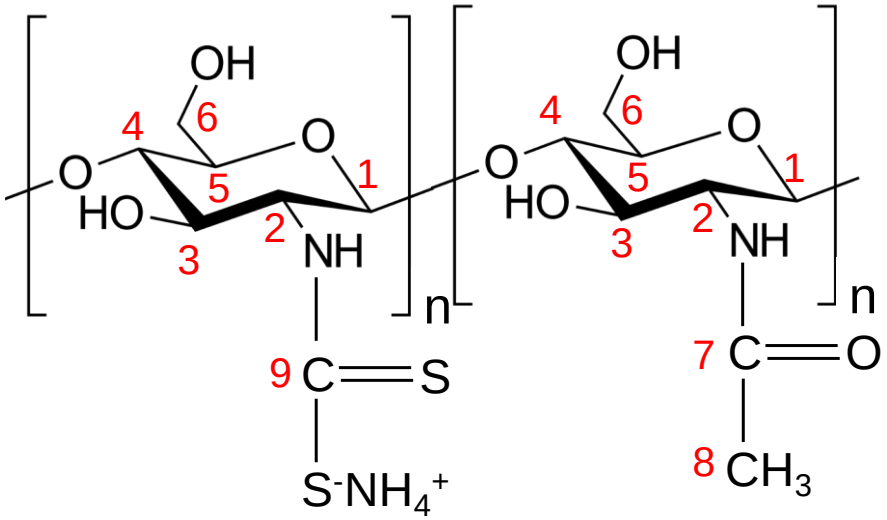


Figure 2. Open molecular structure of DTCC polymer (numbering refers to carbon atoms)

The dithiocarbamate derivative of chitosan is a modified natural polymer that has been used in many different fields for many years. In this study, it was aimed to use AgNPs coated with DTCC in order to expand the area of use of AgNPs as wound dressing material and to prevent cytotoxic effect as a result of rapid release. For this reason, AgNPs coated with DTCC, which do not exist in the literature, were synthesised for the first time in this study. The synthesised AgNPs were compared with chitosan-coated AgNPs, which have been available in the literature for many years, and their potential for use was evaluated on the basis of their antibacterial activity.

2. Material and Method

2.1. Synthesis of Dithiocarbamate Derivative of Chitosan

In order to increase the affinity of chitosan for metal ions, it was modified with carbon disulfide (CS_2). Dithiocarbamate chitosan can be obtained by reacting chitosan with CS_2 under alkaline conditions. The synthesis was carried out by slightly modifying the procedure of Yin et al. (Yin et al. 2021).

Chitosan was dispersed in a flask by adding ethanol. Then ammonium hydroxide was added dropwise. After mixing for half an hour, CS_2 was added and left to mix for 24 hours. At the end of 24 hours, it was filtered with a glass filter, washed with methanol and left to dry at 50 ºC in vacuum. The synthesised dithiocarbamate chitosan was characterised by scanning electron microscopy (SEM), energy dispersive X-ray spectrometry (EDAX) and C^{13}-nuclear magnetic resonance spectroscopy (C^{13}-NMR) analysis.

2.2. Preparation and Characterisation of Chitosan and Dithiocarbamate Chitosan Coated Silver Nanoparticles and Antibacterial Effect Studies

The synthesis of silver nanoparticles was carried out by combining the methods of Suteewong et al. (Suteewong et al. 2019) and Kumar-Krishnan et al. (Kumar-Krishnan et al. 2015). Dithiocarbamate chitosan (DTCC) was taken and 1% (v/v) acetic acid was added. It was homogenised in a tube with a homogeniser at 15,000 rpm for 15 minutes. 20 mM $AgNO_3$ dissolved in water was added dropwise. This mixture was stirred for 30 minutes, taken into a flask and continued to be stirred at 90 ºC for 20 hours. Then centrifuged at 5000 rpm for 15 minutes. The tubes were combined and stored for experiments. Chitosan coated AgNPs were synthesised by the same procedure. The obtained nanoparticles were characterised by ultraviolet-visible region (UV-VIS) spectrophotometry, particle size analysis and zeta potential measurements.

2.3. Antibacterial Activity Studies of Chitosan and Dithiocarbamate Chitosan Coated Silver Nanoparticles

The antibacterial activity of the obtained chitosan coated AgNPs (Chi-Ag) and DTCC coated AgNPs (DTCC-Ag) was investigated against gram positive *Escherichia coli* (E. coli) and gram negative *Staphylococcus aureus* (S. aureus) bacteria selected as pathogenic microorganisms. As a method, the liquid medium dilution method used by Wiegand et al. (Wiegand et al. 2008) was slightly modified. For this purpose, nutrient broth liquid media were prepared and bacteria were added to the prepared media with the help of a swab. Then, the bacterial culture was pre-grown at 37 ºC for 24 hours and added to nutrient broth media. Various amounts of 20-fold diluted (1 mM) Chi-Ag and DTCC-Ag were added to the media. Media containing only bacteria without AgNP were used as control group. Bacteria were allowed to grow at 37 ºC for 24 hours. The cell densities of the bacteria were determined using UV-VIS spectrophotometer at 600 nm wavelength. The inhibition percentages of AgNPs for bacteria were determined by the following equation;

$$\text{İnhibisyon (\%)} = \frac{A_C - A_S}{A_C} x100 \quad (1)$$

Here, A_C and A_S were taken as absorbance values of the control group and the sample, respectively.

3. Results

3.1. C^{13}-NMR Analysis of Synthesised DTCC

Nuclear magnetic resonance of carbon atoms in the solid state is a valuable technique not only for the components of the main skeleton but also for confirming the structural properties of side chains attached to chitosan. The spectra of chitosan and dithiocarbamate chitosan are shown in Figures 3 and C^{13}-NMR results are shown in Table 1. For chitosan, six different peak sequences can be attributed to C4/C1, C6, C3/C5 and C2 at 85.3/ 63.2/ 55.1 and 37 ppm, respectively (see Figure 1 for the numbers of carbon atoms). In addition, two more signals at 3.43 (C8) and 153.2 (C7) ppm can be attributed to methyl and carbonyl carbons present in non-deacetylated chitosan. As expected, the chemically modified chitosan shows the characteristic peaks of the precursor chitosan and an additional peak attributed to the methylene carbon (C9) of the dithiocarbamate group between 194-198 ppm depending on the DTCC moiety (Khan et al. 2011).

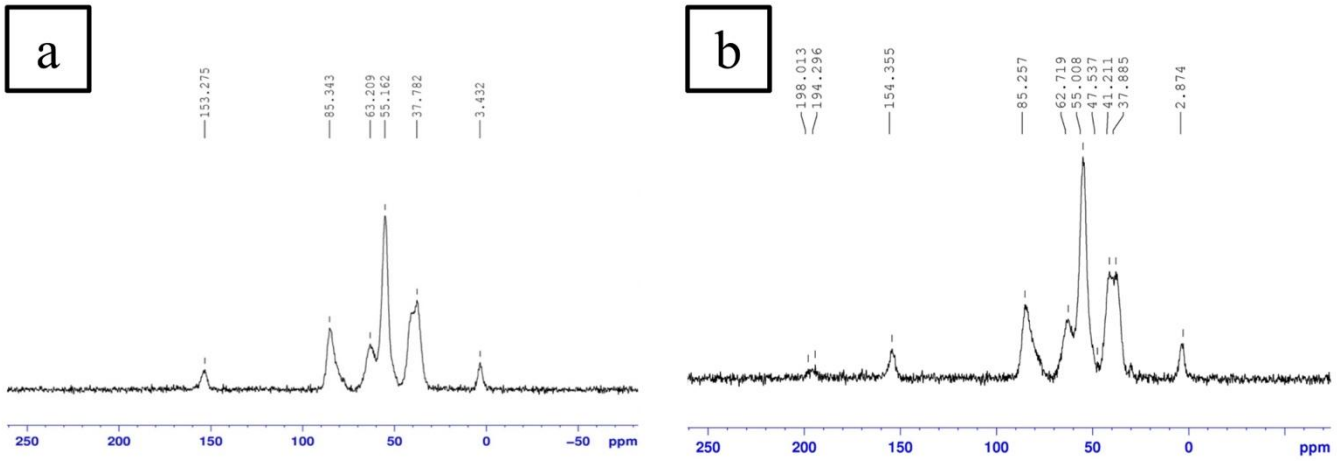


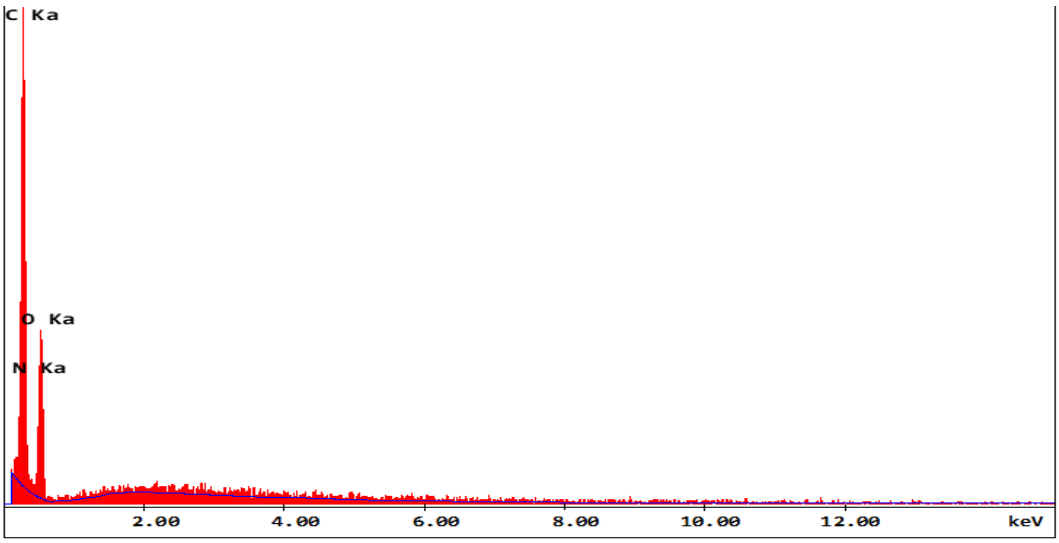
Figure 3. (a) C^{13}-NMR results of chitosan **(b)** C^{13}-NMR results of DTCC

Table 1. C^{13}-NMR results table

Carbon Atom Number	Chitosan	DTCC
C-1	85.3	85.2
C-2	37-41	37-41
C-3	55.1	55.0
C-4	85.3	85.2
C-5	55.1	55.0
C-6	63.2	62.7
C-7	153.2	154.3
C-8	3.43	2.87
C-9	-	194-198

3.2. SEM and EDAX Results of Synthesised Dithiocarbamate Chitosan

The EDAX results of chitosan and synthesised DTCC are given in Figure 4 and 5 and SEM results are given in Figure 6, respectively. When the EDAX results are examined, the presence of sulfur atoms with modification is clearly seen.

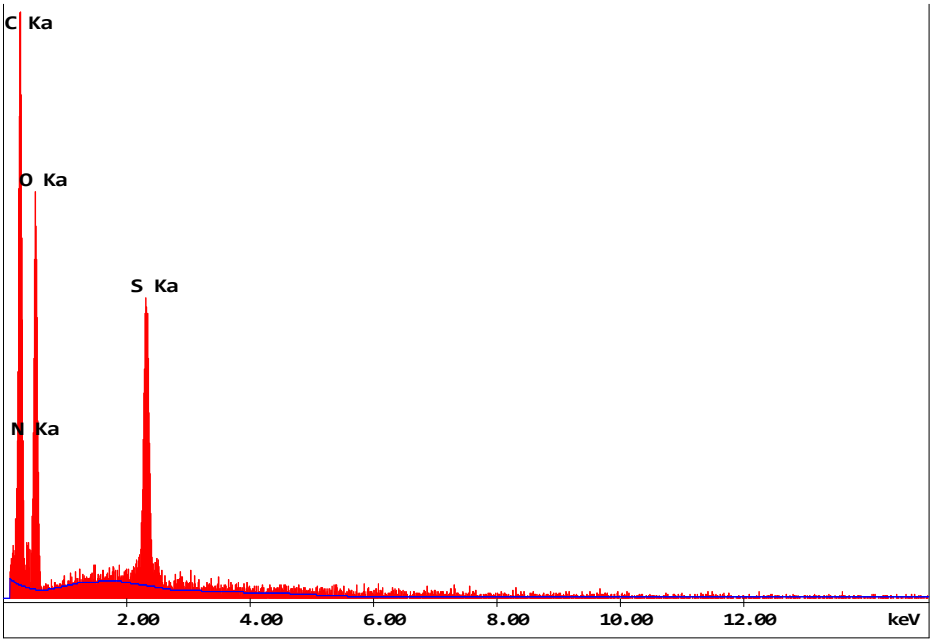


EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	62.71	68.63	0.3951	1.0048	0.6268	1.0003
N K	6.26	5.88	0.0063	0.9974	0.1012	1.0006
O K	31.02	25.49	0.0441	0.9907	0.1436	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	118.04	5.36	2.02	22.02
N K	2.70	3.32	23.81	0.81
O K	43.82	1.77	3.31	24.73

Figure 4. EDAX results of chitosan



EDAX ZAF Quantification (Standardless)
Element Normalized
SEC Table : Default

Element	Wt %	At %	K-Ratio	Z	A	F
C K	53.70	60.74	0.2308	1.0070	0.4266	1.0004
N K	10.36	10.05	0.0115	0.9995	0.1105	1.0007
O K	32.87	27.91	0.0469	0.9929	0.1436	1.0000
S K	3.07	1.30	0.0278	0.9528	0.9473	1.0000
Total	100.00	100.00				

Element	Net Inte.	Bkgd Inte.	Inte. Error	P/B
C K	149.49	4.09	2.21	36.54
N K	10.61	3.33	10.31	3.19
O K	100.82	2.70	2.69	37.28
S K	114.27	5.96	2.59	19.16

Figure 5. EDAX results of DTCC

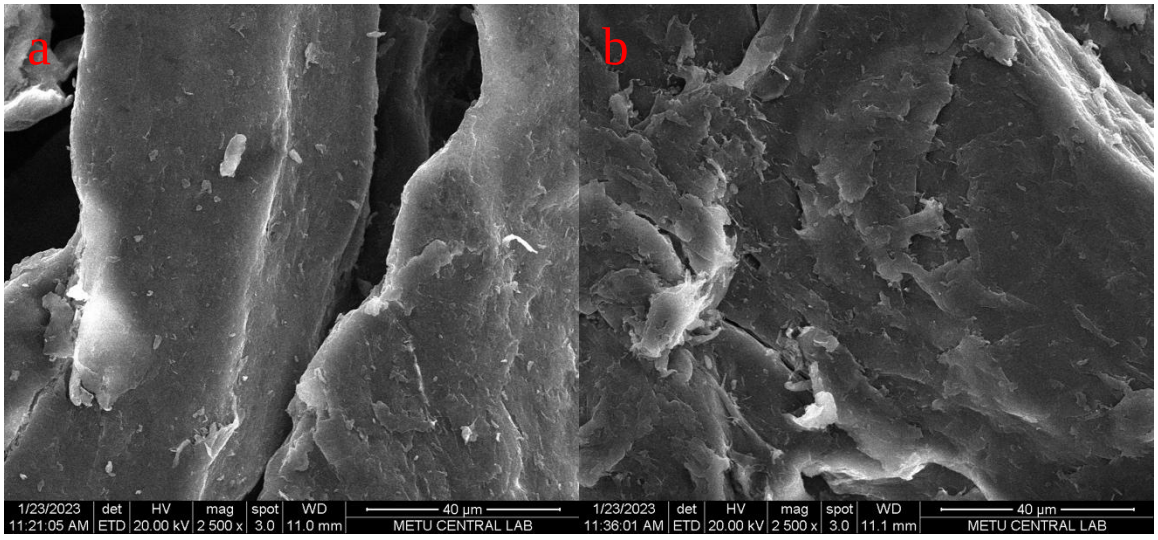


Figure 6. SEM results of **(a)** chitosan and **(b)** DTCC (Magnification rate x2500)

When Figure 6a is analysed, it is seen that chitosan exhibits a dense and flat morphology with very little roughness. This appearance can be attributed to the high intermolecular hydrogen bond density of chitosan. DTCC (Figure 6b), on the other hand, show a rougher

surface morphology due to the dithiocarbamate groups partially located on chitosan. It is thought that modification of chitosan breaks some of the intermolecular hydrogen bonds, thus increasing the roughness (Qin et al. 2012).

3.3. Characterisation of Chitosan and DTCC Coated Silver Nanoparticles

The obtained nanoparticles were characterised by UV-VIS spectrophotometer analysis, particle size distribution and zeta potential measurements. The results of absorbance scanning with UV-VIS spectrophotometer are given in Figure 7.

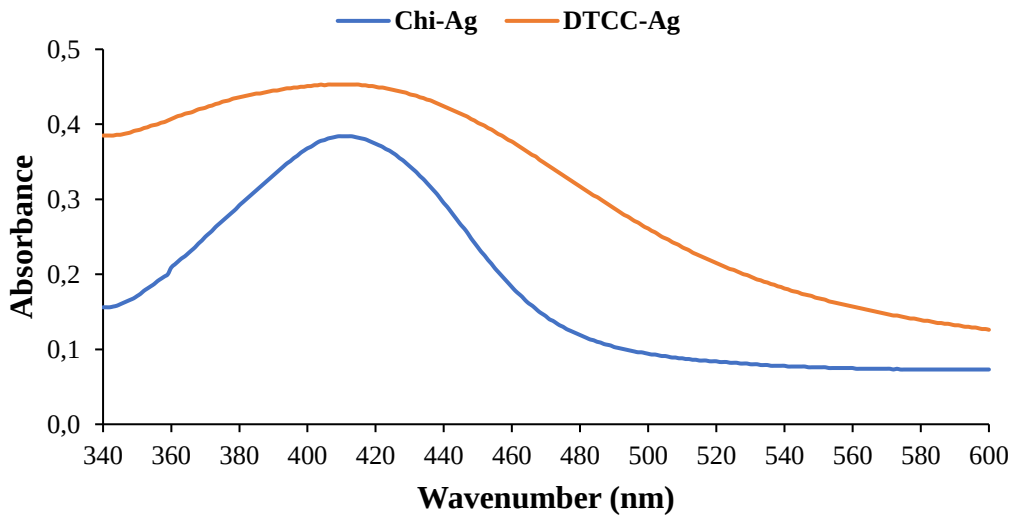


Figure 7. UV-VIS Spectrophotometer analyses of the obtained silver nanoparticles

The synthesised Chi-Ag and DTCC-Ag were first characterised by UV-VIS spectrophotometer. The spectra of the NP solutions were taken using a UV-VIS spectrophotometer in the range 340-600 nm. The transformation of the colourless chitosan or DTCC solutions to light yellow and then yellowish brown over time is evidence of the reduction of Ag ions. Under the influence of the reducing agent, Ag ions gradually agglomerate into metallic silver and then into oligomeric clusters. Finally, these aggregates are transformed into metallic colloidal AgNPs. Chitosan and DTCC biopolymers used in this study were used as protective agents. These polymers protect the particle surfaces from agglomeration or loss of surface properties and keep them in a stable state. The peak exhibited by both AgNPs at approximately 410 nm is due to the excitation of the surface plasmon resonance of $AgNO_3$. The surface plasmon resonance band at 410 nm for Chi-Ag is characteristic for it and is an indication of the interaction between the amino and hydroxyl groups on the chitosan polymer and Ag ions. Likewise, DTCC-Ag peaked at a similar point, but the graph was observed much more diffuse due to the interaction between Ag ions and sulfur in carbamate groups on DTCC (Yan et al. 2021; Dara et al. 2021).

The hydrodynamic diameter, multiple dispersion index and zeta potential results of the synthesised Chi-Ag and DTCC-Ag are given in Table 2.

Table 2. Hydrodynamic diameter, multiple dispersion index and zeta potential results of the obtained silver nanoparticles

AgNP	Hydrodynamic diameter (nm)	Multiple dispersion index (PDI)	Zeta potential (mV)
Chi-Ag	175.20±2.98	0.337±0.051	29.70±4.00
DTCC-Ag	158.00±1.27	0.294±0.007	33.40±0.26

When the results were analysed, it was found that the particle size was below 200 nm. The particle size of DTCC-Ag was slightly smaller and the PDI values were slightly lower. The smaller particle size of DTCC-Ag is due to the stronger interaction between silver ions and sulfur atoms on DTCC molecules. The zeta potentials of both NPs were around 30 mV. The high zeta potentials indicate that the nanoparticles are highly stable.

3.4. Antibacterial Effect Study of Chitosan and DTCC Coated Silver Nanoparticles

Silver ions are well known for their antibacterial properties and are often used in nanoparticle form. Compared to the direct use of bulk silver or silver ions, AgNPs with their large surface area are generally recognised as an ideal antibacterial agent with high efficiency and sustained antibacterial activity (Yan et al. 2021). In order to reveal the potential use of both synthesised NPs in wound dressing materials and to compare their efficiency with each other, the bactericidal activities of AgNPs against common bacteria (*E. coli* and *S. aureus*) were evaluated in liquid culture medium. AgNPs at different concentrations were kept in bacterial cultures for 24 h and bacterial growth was measured as optical density at 600 nm using UV-VIS spectrophotometer and the results are given in Figures 8 and Figure 9 for both microorganisms and both NPs.

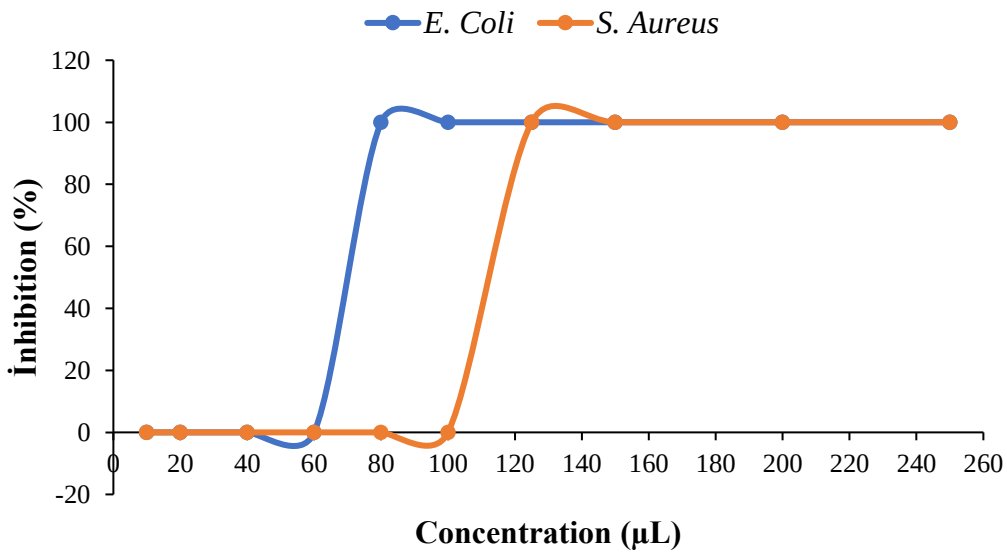


Figure 8. Percentage inhibition test results of Chi-Ag on *E. coli and S. Aureus*

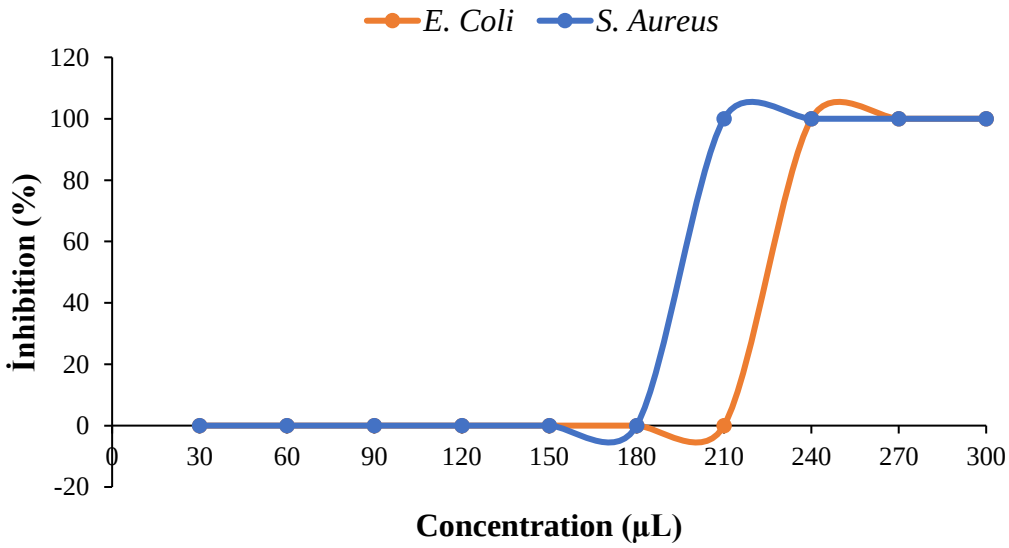


Figure 9. Percentage inhibition test results of DTCC-Ag on *E. coli and S. aureus*

When the results were analysed for both NPs, no inhibition occurred up to a certain concentration value and after a certain concentration, 100% inhibition occurred. This study showed a very different inhibition pattern compared to many AgNP studies. For this reason, only 100% minimum inhibition values (MIC100) of NPs could be calculated. MIC 100 values for each microorganism and NP were calculated and given in Table 3.

Table 3. MIC100 Values for Chi-Ag and DTCC-Ag

AgNP	*E. Coli*	*S. Aureus*
Chi-Ag	1.73 µg/mL	2.70 µg/mL
DTCC-Ag	5.18 µg/mL	4.54 µg/mL

When the values are analysed, it is seen that the bactericidal activity of Chi-Ag is higher. MIC 100 values of DTCC-Ag particles were approximately 3 times higher for *E. coli* and 2 times higher for *S. aureus* than Chi-Ag. This result is due to the fact that DTCC interacts much more tightly with Ag ions than pure chitosan and releases silver ions more difficult.

Again, although the values were not very different within themselves, it was determined that Chi-Ag exhibited better antibacterial activity against *E. coli* and DTCC-Ag exhibited better antibacterial activity against *S. aureus*. It is known that various factors such as the structure and amount of reducing agent used in nanoparticle synthesis, the type of metal salt, temperature and pH have determinative effects on nanoparticle formation as well as its size and shape. For this reason, the shape, size, surface area, structural and chemical properties of the nanoparticles obtained cause differences in their antimicrobial activity (Dara et al. 2020). The results obtained from our study also show that the structure of AgNPs obtained by modification of chitosan changes, which affects its bactericidal activity.

In a study conducted by Pansara et al. (2019) with Chi-Ag, MIC values were found to be 2.64 µg/mL for *S. aureus* and 1.32 µg/mL for *E. coli*. In another study conducted by Rodríguez-Argüelles et al. (2011) with Chi-Ag, MIC values were found to be 2.5 µg/mL for *S. aureus* and 1.3 µg/mL for *E. coli*. The results obtained in our study were found to be very compatible with the literature.

4. Discussion

In this study, DTCC, a derivative of chitosan, was first synthesised and the resulting polymer was characterised by SEM, EDAX and C^{13}-NMR analyses. Then, in the second part of the study, AgNPs coated with chitosan and DTCC were synthesised by heating reduction method. The synthesised AgNPs were characterised by particle size and zeta potential measurements. The characterised Chi-Ag and DTCC-Ag showed effective antibacterial activity against both gram-negative and gram-positive bacteria. MIC 100 values for *E. coli* and *S. aureus* were found to be 1.73 and 2.70 µg/mL for Chi-Ag and 5.18 and 4.54 µg/mL for DTCC-Ag, respectively. It was determined that AgNPs synthesised using DTCC for the first time really have a very high antibacterial activity. As a result, it is thought that the developed DTCC coated AgNPs can provide antimicrobial formulations in the development of wound dressing materials.

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References

Abou El-Nour, K. M., Eftaiha, A. A., Al-Warthan, A., & Ammar, R. A. (2010). Synthesis and applications of silver nanoparticles. Arabian journal of chemistry, 3(3), 135-140. doi: 10.1016/j.arabjc.2010.04.008

Atiyeh, B. S., Costagliola, M., Hayek, S. N., & Dibo, S. A. (2007). Effect of silver on burn wound infection control and healing: review of the literature. burns, 33(2), 139-148. doi: 10.1016/j.burns.2006.06.010

Chen, K., Wang, F., Liu, S., Wu, X., Xu, L., & Zhang, D. (2020). In situ reduction of silver nanoparticles by sodium alginate to obtain silver-loaded composite wound dressing with enhanced mechanical and antimicrobial property. International journal of biological macromolecules, 148, 501-509. doi: 10.1016/j.ijbiomac.2020.01.156

Church, D., Elsayed, S., Reid, O., Winston, B., & Lindsay, R. (2006). Burn wound infections. Clinical microbiology reviews, 19(2), 403-434. doi: 10.1128/cmr.19.2.403-434.2006

Dara, P. K., Mahadevan, R., Digita, P. A., Visnuvinayagam, S., Kumar, L. R., Mathew, S., Ravishankar, R.N. & Anandan, R. (2020). Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Chi-Ag-NPs): In vitro studies on antioxidant and antibacterial applications. SN Applied Sciences, 2, 1-12. doi: 10.1007/s42452-020-2261-y

Gravante, G., Caruso, R., Sorge, R., Nicoli, F., Gentile, P., & Cervelli, V. (2009). Nanocrystalline silver: a systematic review of randomized trials conducted on burned patients and an evidence-based assessment of potential advantages over older silver formulations. Annals of plastic surgery, 63(2), 201-205. doi: 10.1097/SAP.0b013e3181893825

Hamed, I., Özogul, F., & Regenstein, J. M. (2016). Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. Trends in food science & technology, 48, 40-50. doi: 10.1016/j.tifs.2015.11.007

Jahromi, M. A. M., Zangabad, P. S., Basri, S. M. M., Zangabad, K. S., Ghamarypour, A., Aref, A. R.,Karimi, M. & Hamblin, M. R. (2018). Nanomedicine and advanced technologies for burns: Preventing infection and facilitating wound healing. Advanced drug delivery reviews, 123, 33-64. doi: 10.1016/j.addr.2017.08.001

Khan, A., Badshah, S., & Airoldi, C. (2011). Dithiocarbamated chitosan as a potent biopolymer for toxic cation remediation. Colloids and Surfaces B: Biointerfaces, 87(1), 88-95. doi: 10.1016/j.colsurfb.2011.05.006

Kumar-Krishnan, S., Prokhorov, E., Hernández-Iturriaga, M., Mota-Morales, J. D., Vázquez-Lepe, M., Kovalenko, Y., Sanchez, I.C. & Luna-Bárcenas, G. (2015). Chitosan/silver nanocomposites: Synergistic antibacterial action of silver nanoparticles and silver ions. European Polymer Journal, 67, 242-251. doi: 10.1016/j.eurpolymj.2015.03.066

Li, Q., Lu, F., Zhou, G., Yu, K., Lu, B., Xiao, Y., Dai, F., Wu, D. & Lan, G. (2017). Silver inlaid with gold nanoparticle/chitosan wound dressing enhances antibacterial activity and porosity, and promotes wound healing. Biomacromolecules, 18(11), 3766-3775. doi: 10.1021/acs.biomac.7b01180

Liu, J., Hao, J., Dong, W., & Zeng, Y. (2021). Depression mechanism of environment-friendly depressant dithiocarbamate chitosan in flotation separation of Cu-Zn sulfide. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 615, 126290. doi: 10.1016/j.colsurfa.2021.126290

Lu, Z., Gao, J., He, Q., Wu, J., Liang, D., Yang, H., & Chen, R. (2017). Enhanced antibacterial and wound healing activities of microporous chitosan-Ag/ZnO composite dressing. Carbohydrate polymers, 156, 460-469. doi: 10.1016/j.carbpol.2016.09.051

Mira, A. K., Yousef, A. S., & Abdullah, A. (2015). Biosynthesis of silver nanoparticles by cyanobacterium Gloeocapsa sp. IJERSTE, 4(9), 60-73. https://www.researchgate.net/profile/Mira-Al-Katib/publication/362826406_Biosynthesis_of_Silver_Nanoparticles_by_Cyanobacterium_Gloeocapsa_sp/links/63013bd9e3c7de4c346f093c/Biosynthesis-of-Silver-Nanoparticles-by-Cyanobacterium-Gloeocapsa-sp.pdf

Muzzarelli, R. A. A., & Tanfani, F. A. B. I. O. (1982). N-(o-carboxybenzyl) chitosan, N-carboxymethyl chitosan and dithiocarbamate chitosan: new chelating derivatives of chitosan. Pure and Applied Chemistry, 54(11), 2141-2150. doi: 10.1351/pac198254112141

Nagarajan, S., & Arumugam Kuppusamy, K. (2013). Extracellular synthesis of zinc oxide nanoparticle using seaweeds of gulf of Mannar, India. Journal of nanobiotechnology, 11, 1-11. doi:10.1186/1477-3155-11-39

Naganthran, A., Verasoundarapandian, G., Khalid, F. E., Masarudin, M. J., Zulkharnain, A., Nawawi, N. M., Karim. M., Abdullah. C.A.C. & Ahmad, S. A. (2022). Synthesis, characterization and biomedical application of silver nanoparticles. Materials, 15(2), 427. doi: 10.3390/ma15020427

Ogawa, K., Yui, T., & Okuyama, K. (2004). Three D structures of chitosan. International Journal of Biological Macromolecules, 34(1-2), 1-8. doi: 10.1016/j.ijbiomac.2003.11.002

Pansara, C., Chan, W. Y., Parikh, A., Trott, D. J., Mehta, T., Mishra, R., & Garg, S. (2019). Formulation optimization of chitosan-stabilized silver nanoparticles using in vitro antimicrobial assay. Journal of pharmaceutical sciences, 108(2), 1007-1016. doi: 10.1016/j.xphs.2018.09.011

Priya, K., Vijayakumar, M., & Janani, B. (2020). Chitosan-mediated synthesis of biogenic silver nanoparticles (AgNPs), nanoparticle characterisation and in vitro assessment of anticancer activity in human hepatocellular carcinoma HepG2 cells. International journal of biological macromolecules, 149, 844-852. doi: 10.1016/j.ijbiomac.2020.02.007

Qin, Y., Liu, S., Xing, R., Yu, H., Li, K., Meng, X., Li, R. & Li, P. (2012). Synthesis and characterization of dithiocarbamate chitosan derivatives with enhanced antifungal activity. Carbohydrate polymers, 89(2), 388-393. doi: 10.1016/j.carbpol.2012.03.018

Rajeshkumar, S., Kannan, C., & Annadurai, G. (2012). Green synthesis of silver nanoparticles using marine brown algae Turbinaria conoides and its antibacterial activity. International Journal of Pharma and Bio Sciences, 3(4), 502-510. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=770c42f5f0389d4a5298fe8693645dcc3f2c44d3

Rodríguez-Argüelles, M. C., Sieiro, C., Cao, R., & Nasi, L. (2011). Chitosan and silver nanoparticles as pudding with raisins with antimicrobial properties. Journal of colloid and interface science, 364(1), 80-84. doi: 10.1016/j.jcis.2011.08.006

Shehabeldine, A. M., Salem, S. S., Ali, O. M., Abd-Elsalam, K. A., Elkady, F. M., & Hashem, A. H. (2022). Multifunctional silver nanoparticles based on chitosan: Antibacterial, antibiofilm, antifungal, antioxidant, and wound-healing activities. Journal of Fungi, 8(6), 612. doi: 10.3390/jof8060612

Suteewong, T., Wongpreecha, J., Polpanich, D., Jangpatarapongsa, K., Kaewsaneha, C., & Tangboriboonrat, P. (2019). PMMA particles coated with chitosan-silver nanoparticles as a dual antibacterial modifier for natural rubber latex films. Colloids and Surfaces B: Biointerfaces, 174, 544-552. doi: 10.1016/j.colsurfb.2018.11.037

Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2010). Biological synthesis of metallic nanoparticles. Nanomedicine: nanotechnology, biology and medicine, 6(2), 257-262. doi: 10.1016/j.nano.2009.07.002

Wang, W., Meng, Q., Li, Q., Liu, J., Zhou, M., Jin, Z., & Zhao, K. (2020). Chitosan derivatives and their application in biomedicine. International journal of molecular sciences, 21(2), 487. doi: 10.3390/ijms21020487

Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols, 3(2), 163-175. doi: 10.1038/nprot.2007.521

Yan, K., Xu, F., Wei, W., Yang, C., Wang, D., & Shi, X. (2021). Electrochemical synthesis of chitosan/silver nanoparticles multilayer hydrogel coating with pH-dependent controlled release capability and antibacterial property. Colloids and Surfaces B: Biointerfaces, 202, 111711. doi: 10.1016/j.colsurfb.2021.111711

Ye, H., Cheng, J., & Yu, K. (2019). In situ reduction of silver nanoparticles by gelatin to obtain porous silver nanoparticle/chitosan composites with enhanced antimicrobial and wound-healing activity. International journal of biological macromolecules, 121, 633-642. doi: 10.1016/j.ijbiomac.2018.10.056

Yin, Z., Qiu, D., & Zhang, M. (2021). Molecular level study of cadmium adsorption on dithiocarbamate modified chitosan. Environmental Pollution, 271, 116322. doi: 10.1016/j.envpol.2020.116322

Zhou, L., Zhao, X., Li, M., Yan, L., Lu, Y., Jiang, C., Liu, Y., Pan, Z. & Shi, J. (2021). Antibacterial and wound healing–promoting effect of sponge-like chitosan-loaded silver nanoparticles biosynthesized by iturin. International journal of biological macromolecules, 181, 1183-1195. doi: 10.1016/j.ijbiomac.2021.04.119