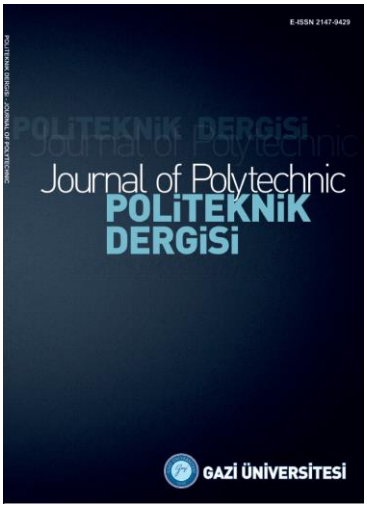

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Synthesis and characterization silver nanoparticles and coating with chitosan

Gümüş nanopartikül sentezi, karakterizasyonu ve kitosan ile kaplanması

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Synthesis and Characterization of Silver Nanoparticles and Coating with Chitosan

Highlights

- *Ag nanopartiküller sentezlendi ve karakterize edildi/Ag Nanoparticles were synthesized and characterized*
- *Ag nanopartiküller doğal polimer kitosan ile kaplandı/Ag Nanoparticles were coated with chitosan*
- *Ag nanopartiküllerin yapısını bozmadan kitosan ile kaplama işlemi başarıldı/ Coating with chitosan was achieved without damaging the structure of Ag nanoparticles.*

Graphical Abstract

Ag nanoparticles were chemically synthesized in a laboratory environment. They were successfully coated with a natural biopolymer Chitosan, without damaging their structure.

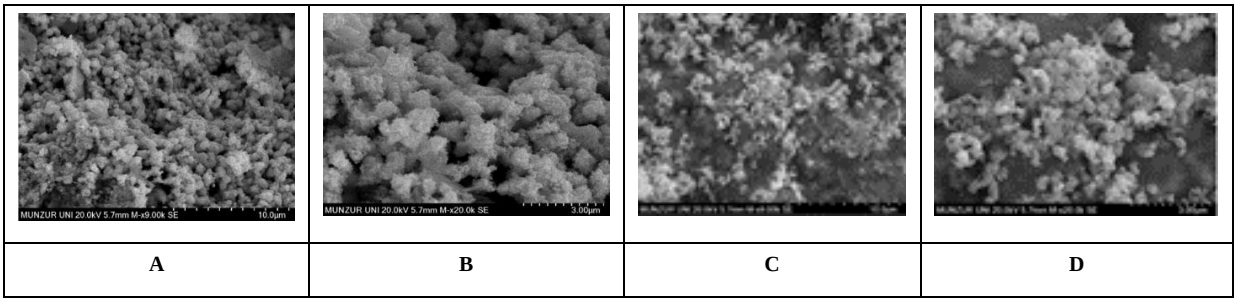

Figure. *SEM image results of both Ag-NPs (A-B) and chitosan-coated Ag-NPs (C-D).*

Aim

Synthesis of silver nanoparticles by chemical means in the laboratory environment and coating of the formed particles with natural chitosan polymer without damaging their structure.

Design & Methodology

Extraction of Silver nanoparticles from $AgNO_3$ solution with a reducing agent. Coating the particles formed by preparing the aqueous solution of the natural polymer.

Originality

This study is original for coating silver nanoparticles with toxic properties in a moderate environment with a biocompatible polymer.

Findings

Silver nanparticles were characterized by UV-Vis, SEM, TEM and XRD. Characterization of coated nanoparticles was also carried out using the same methods. Pure and polymer-coated particles were found to be nanoscale.

Conclusion

Synthesis of Silver nanoparticle coated with Chitosan, a natural and biocompatible polymer, was carried out successfully in an aqueous medium.

Declaration of Ethical Standards

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Gümüş Nanopartikül Sentezi Karakterizasyonu ve Kitosan ile Kaplanması

Araştırma Makalesi / Research Article

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ÖZ

Nanoteknolojideki son gelişmeler, oldukça çok küçük partiküllerin üretilmesi ve kullanılması sebebiyle artan bir ilgiyle takip edilmektedir. Özellikle benzersiz özellikleriyle, metal nanoparçacıklara ayrı bir ilgi vardır. Gümüş nanopartiküller (Ag-NP), benzersiz elektriksel, optik ve fizikokimyasal özelliklerinin yanı sıra biyomedikal uygulamalarından dolayı ayrıca bir ilgi çekmektedir. Ag-NP'ler, antibakteriyel etkinliklerinden dolayı en hızlı gelişen nano ölçekli materyallerdir. Şimdi yeni nesil antimikrobiyaller olarak kabul edilirler. Günümüzde, dezenfektan özelliklerinden dolayı Ag-NP'ler tıbbi cihazların ve ev aletlerinin dezenfekte edilmesi, boyalar, optik cihazlar, plastikler, tekstil ürünleri, sabunlar ve çamaşır deterjanları, kozmetikler, sağlık bakım ürünleri de dahil olmak üzere birçok tüketici ürününde, gıda ambalajlarında ve gıda ürünlerinde koruyucu ve topaklanma önleyici maddeler olarak yaygın olarak kullanılırlar. Yaygın kullanımlarına rağmen Ag-NP katkı maddeleri içeren ürünler, toksisiteleri nedeniyle ciddi bir çevre ve insan sağlığı sorunudur. Hücre ve bakteri kültürleri üzerine yapılan çalışmalar Ag-NP'lerin toksik olduğunu göstermiştir; ve toksik etkileri, esas olarak Ag-NP'lerin su içine saldıkları çözünmüş Ag iyonlardan kaynaklanmaktadır. Sayılan kriterler göz önüne alınarak; bu çalışmada, Laboratuvar ortamında kimyasal olarak Ag-NP' lerin sentezlenmiş ve doğal, zararsız bir polimer olan kitosan ile kaplanmıştır. Gümüş nanoparçacıkları karakterize etmek için UV-Vis spektroskopisi, Transmisyon elektron mikroskobu, Taramalı elektron mikroskobu, X-ışını kristalografisi ile Zeta potansiyel analizleri yapılmıştır.

Anahtar Kelimeler: Gümüş, nanopartikül, sentez, biyopolimer, kaplama.

Synthesis and Characterization of Silver Nanoparticles and Coating with Chitosan

ABSTRACT

Recent advancements in nanotechnology have garnered increasing attention due to the improvements in producing and utilizing particles whose sizes are extremely small. There is particular interest in metal nanoparticles due to their distinctive properties. Silver nanoparticles (Ag-NPs) have been of particular interest owing to their unique electrical, optical and physicochemical properties as well as their biomedical applications. Ag-NPs are the fastest growing class of nanoscale materials because of their antibacterial activities. They are now considered to be the next generation antimicrobials. Today, owing to their disinfectant properties, Ag-NPs are extensively used in numerous consumer products, including disinfecting medical devices and home appliances, paints, optical devices, plastics, textiles, soaps and laundry detergents, cosmetics, health-care products, as well as in food packing and food products as preservative and anti-caking agents. Despite their widespread usage, the products containing Ag-NP additives are a serious environmental and human health concern because of their toxicity. Studies on cell and bacterial cultures have shown that Ag-NPs are toxic; and the toxic effects are mainly due to the dissolved Ag ions when Ag-NPs degrade in water. In this study, considering the criteria outlined above, the purpose of the study and Ag-NPs were chemically synthesized in the laboratory environment and modified as chitosan coating. UV-Vis spectroscopy, transmission electron microscopy, scanning electron microscopy, X-ray crystallography and zeta potential analysis were used to characterize the silver nanoparticles.

Keywords: Silver, nanoparticle, synthesis, biopolymer, coating.

1. INTRODUCTION

Nanotechnology is an interdisciplinary science related to nanoscale technology and research in terms of material science, physics, chemistry, biology, bioengineering and pharmacology. The concept of "nano" is expressed dimensionally as the measure of one in billion pieces of the matter (10^{-9} m) in other words nanometer (<100 nm) [1]. Technological developments in nanoscale materials have a variety of applications insociety, from medical treatments and health care products to water treatment technologies. The unique features and biological effects of metal-based nanoparticles (NPs) indicate that they can be potentially used as an alternative treatment in the various diseases. Silver (Ag), a metal NP, has become one of the fastest growing product categories in the nanomaterial sector and turned into the first product to be commercialized [1, 2]. Due to their antibacterial effects, Ag-NPs grow rapidly among the nanoscale material group. As a result of their unique features, they are

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considered as the new generation anti-microbial [3, 4]. Anti-microbial applications of Ag-NPs include their use as preservatives in medical and optical devices, household appliances, healthcare products optical devices as well as dye, plastic, textile, soap and laundry detergents, cosmetics and various food industries for disinfection purposes [5 -7].

Colloidal instability of Ag-NPs created a major concern for the aquatic environment and biological safety. Over the years, Ag-NPs have been intensively researched in order to understand their potential risk for humans and ecosystems [7-13]. Evidence from *in-vivo* and *in-vitro* studies using model aquatic species and microorganisms has shown that Ag-NPs are toxic. Although its effect size is different on various biological organisms, it is found out that Ag-NPs cause toxicity in mosses, plants, fungi [6, 14, 12], zebra fishes [15], invertebrates (*C. elegans*) [16], bacteria (*E. coli* and *P. putida*) [17, 18], mammalian root cells [19] and human cells (skin keratinocytes, lung fibroblast cells and glioblastoma cells) [20, 15].

It has been argued that the toxicity is caused by Ag^+ ions in the solution. It has also been observed that the coating agents which are used for the size, shape and stability of Ag-NPs, are also causing toxicity [6, 12, 21]. Smaller Ag-NPs (1-15 nm) are more toxic than larger Ag-NPs due to their ability to bind to the cellular structure more easily and to convert to Ag^+ ions in the aqueous medium more rapidly [6, 11, 20]. In some other studies on direct uptake of Ag-NPs, it has been reported that Ag^+ ions are not the only reason for toxic effect [6, 22, 23]. Current up-to-date information regarding to toxicity of Ag-NPs point out a complex phenomenon that is affected by various chemical, biological and environmental conditions, including ultraviolet light radiation. Many studies have shown that some of the Ag-NPs are highly toxic. Gubbins et al. have stated that Ag-NPs are toxic even at very low doses and Ag-NPs can be a potential risk for the environment [10]. Furthermore, studies on the toxic effects of these NPs have revealed similar results for many different species and NPs.

Particle aggregation, solubility and ion release are important factor that affects the toxicological behavior of Ag-NPs. Ag-NPs are stabilized either during or after the synthesis by various surface coatings in order to ensure physical and chemical stability. Polymeric structures such as carboxylic acids (citric acid, oleic acid and fatty acids) [6,13,20,24-27] polyvinylpyrrolidone (PVP) [13,20,25,27-29], polyethylene glycol (PEG) [25,30,29], polyacrylic acid (PAA) and polymethacrylate or methyl methacrylate (PMA, PMMA)[28-30], polyvinyl alcohol (PVA) [29-31], polystyrene sulfonate (PSS) [20] and polyethylenimine (PEI) [27] are used in the coating process. These surface coatings play vital role for the prevention of Ag-NPs aggregating in the colloidal solution.

In contrast to bulk materials, Ag-NPs have high surface energy values due to small sized NPs [32]. For that reason, the selection of a suitable coating agent is often a prerequisite for the stabilization of NPs [33]. The selection of the chemical agent or material to be used as a coating for NPs is very important because the coating-forming agent generally affects various properties of the NPs, including the size, shape and interaction with the surrounding solvent [34]. Therefore, the selection of materials which will be used in the coating of NPs produced by chemical methods, is significant. Because, all the coating materials affect the toxic features of NPs. Natural coaters can be modified and used as the key factors in the design and production of commercially available Ag-NPs as to reduce risk of exposure to toxic Ag^+ ions for all living organisms and environment. The natural coaters prevent the ion release from Ag-NPs and thus produced materials with less toxic features.

Chitosan, a natural biopolymer material, has attracted the particular a particular interest of researchers in recent years. Chitosan, which can be obtained in abundant quantities from natural sources, is superior to other biopolymers in terms of its non-toxic behavior for the living organisms, biodegradability, biocompatibility as well as its chemical and physical features. In this study, Ag-NPs were synthesized by chemical synthesis method and Ag-NPs were produced by using chitosan as a natural coating agent. Characterization analysis techniques were used to identify chemically produced Ag-NPs as well as chitosan-coated Ag-NPs.

2. MATERIAL and METHOD

2.1. Chemical Materials

All chemical materials, notably Sodium nitrate ($AgNO_3$), tri-sodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$), ethanol (C_2H_5OH), sodium hydroxide (NaOH) and chitosan (poly-[β-(1,4)-2-amino-2-deoxy-β-D-glucopyranose]) were supplied from Sigma-Aldrich. All chemicals were analytical reagent grade and were used without any sterilization or purification.

2.2. Synthesis of the Silver Nanoparticles

Ag-NPs were synthesized by making some modifications to internationally published chemical procedures [25]. To achieve a more homogeneous particle size distribution in the study sodium citrate was preferred over $NaBH_4$ as the reducing agent. 40 mg $AgNO_3$ dissolved in 100 mL ultra-pure water. This solution was heated to the boiling temperature in a nitrogen atmosphere. A previously prepared 20 mL 1% (w/w) sodium citrate solution was added drop by drop to the hot solution. Formation of Ag-NPs is observed when the solution color turns yellow. The solution was kept warm until a stable dark brown-green color was obtained. Heating was stopped and stirring was continued for 15 minutes. When the mixture reached room temperature, it was centrifuged at 4000 rpm for 90 minutes and then the supernatant was removed, the pellet was washed twice with ethyl alcohol and dried in oven.

2.3. Chitosan Coating Silver Nanoparticles

For the coating process, 1 gram of 75-85% deacetylated and medium molar massed (200-500 kDa) chitosan was weighed and dissolved in a solution containing 100 mL of 0.5% (w/w) acetic acid. Then, 80 mg of Ag-NPs which has suspended in 50 mL of ethyl alcohol was added to this solution. This mixture was stirred with magnetic stirrer for 24 hours at high intensity [35]. The mixture was centrifuged at 4100 rpm for 90 minutes. After that, the supernatant was removed and the pellet was washed twice with ethyl alcohol, dried in oven and stored in a dark and cool place for further work.

2.4. Characterization

Synthesized and chitosan-coated Ag-NPs were characterized using various techniques. The UV-Vis spectrophotometer (Optima, SP-3000 Nano) was used for the UV-visible spectra of Ag-NPs and the absorption mode was determined at 300-500 nm wavelength [36]. The size and shape of the particles were characterized using a JOEL-1011 TEM instrument which provided 0.2 nm resolution at 50-106 magnification under a voltage of 40-100 kV accelerated by Transmission Electron Microscopy (TEM) [37]. Dynamic light scattering (DLS) analysis for the real size distribution of Ag-NP in the aquatic environment and zeta patellar analysis for determination of the surface load and value were performed with the Zetasizer device (Malvern Nano ZS) [38,31]. The crystal structures of Ag-NPs were characterized with X-Ray Diffraction (XRD) (D8 Enhanced X-ray diffractometer, Bruker, Germany) and were scanned with 2.2 kW copper anodic radiation produced in 1.54A^{o} wavelength by Ceramic X-Ray tube [39].The crystal formation was determined from the diffraction pattern and the crystallite size was calculated with the Scherrer formula. In addition, the morphology of Ag-NPs was analyzed at 25 kV by using KYKY-EM3200 instrument pursuant to the standard analysis method specified in the Scanning Electron Microscope (SEM) procedures [40].

3. RESULTS AND DISCUSSION

3.1. UV-Vis spectroscopic analysis

UV-Vis absorption spectroscopy is an essential technique for evaluating the formation of stable Ag-NPs. Generally, it appears that Ag-NPs show an intense absorption peak at 390-425 nm due to the surface plasmon resonance phenomenon (co-oscillation of surface electrons). According to Mie's theory [41,42], only one surface plasmon resonance band is expected during the absorption spectrum of metal spherical NPs. On the other hand, two or more surface plasmon resonance bands are expected for anisotropic NPs depending on the specific form of the particles. In addition, it is observed that, when the number of surface plasmon resonance peaks increase, NPs symmetries decrease. Chemically synthesized Ag-NPs showed maximum absorption peaks between 395 and 420 nm Fig.1. Depending on the absorbance values, it shows the formation of relatively small NPs (<80 nm) with narrow size distributions.

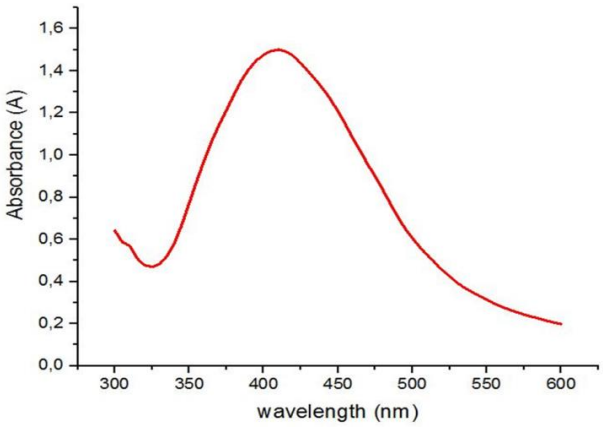

Fig.1. UV-Vis absorbance values of synthesized Ag-NPs

3.2. Transmission Electron Microscope Analysis

TEM images of the Ag-NPs which are synthesized in laboratory and coated with chitosan are given in Fig.2. It is known that although they have a distinctive character, Ag-NPs tend to aggregate in the colloidal structure (aquatic environment) [43]. In this study, similar results were obtained. Furthermore, when the shape of the Ag-NPs is examined, it is observed that most of them have a round or spherical structure. It is seen that Ag-NPs are synthesized in two different dimensions, they have round or round-like structures and almost all of them show homogeneous distribution. The chitosan-coated Ag-NPs have decent appearance and their particles are smaller than 100 nm.

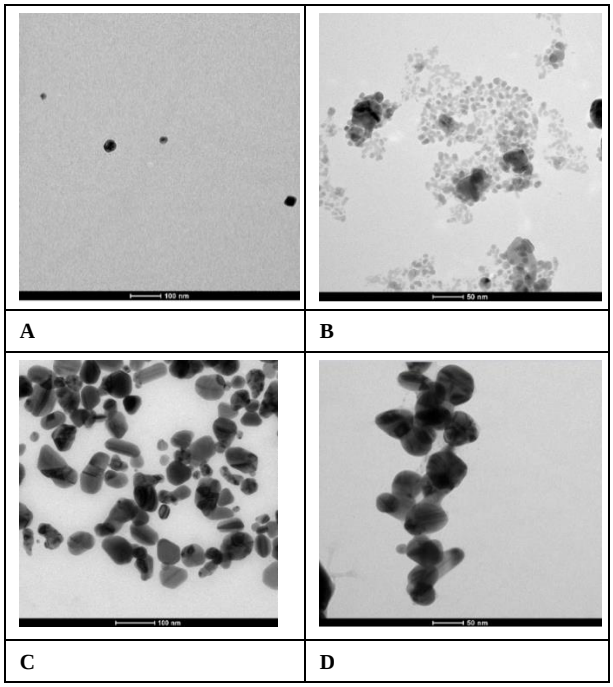

Fig. 2. TEM images of synthesized Ag-NPs (A-B) and chitosan-coated Ag-NPs (C-D).

3.3. Dynamic Light Scattering Analysis

DLS is often used to size colloidal solution environment in which NPs are present and to determine aggregations of Ag-NPs in the suspensions. The diameter value measured with DLS shows how a particle moves in the liquid. This is called hydrodynamic diameter. The hydrodynamic diameter is complementary to other size measurements, such as TEM, because it provides information on aggregation status of NP solutions. DLS results of Ag-NPs produced in our study are provided in Fig.3 below. When the DLS results are examined, it is seen that all NPs showed some growth from few to 10-100 times comparing to their actual size. This is a general characteristic feature of Ag-NPs and they usually show some growth in aqueous environments. It is also known that it is very difficult for Ag-NPs to remain stable in aqueous environments and tend to form aggregations in the aqueous environments as long as they remain in it. Therefore, DLS results are obtained in µm levels depending on the concentration (mg/L).

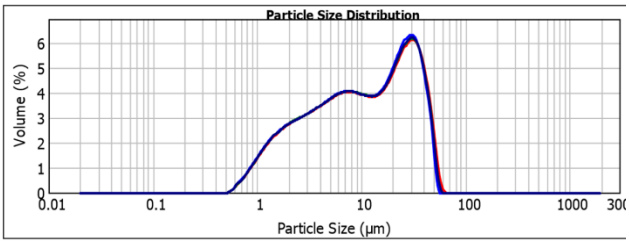

Fig. 3. Particle size distributions of Ag-NPs.

3.4. Zeta Potential Analysis

Zeta potential is an important parameter for evaluating the stability of Ag-NPs in aqueous suspensions. The results of the Zeta Potential Analysis; the surface charge (-/+) and the values (mV) of chemically synthesized Ag-NPs are provided in Table-1. When the results of zeta potential analysis are examined, it is found out that Ag-NPs show a negative (-) surface charge. Particles with a positive zeta potential can bind to negatively charged surfaces or vice versa. The magnitude of the zeta potential provides information about particle stability, the higher potentials indicate increased electrostatic repulsion and hence increased stability. For instance, 0-5 mV particles tend to aggregate whereas 5-20 mV particles are minimally stable and 20-40 mV particles are moderately stable. If they are bigger than 40+ mV, the particles are extremely stable. Accordingly, the zeta potential value of the Ag-NPs produced in this study is 30.1 ± 6.8 (mV), so they are moderately stable. The results obtained are very close to the information reported in the literature [44,45]. It has been suggested that the cytotoxicity of Ag-NPs is altered by the surface potential of NPs and the positively charged ones are most biocompatible whereas the negatively charged ones are most toxic [46].

Table 1. Surface charge and size distributions of the Ag-NPs in aqueous environment

Nanoparticles	DLS size [Z-Ave (d.nm)]	PdI	Pk 1 Mean Int (d.nm)	Zeta Potential (mV)
Ag-NPs	1416 ± 92	0,4	1146,9	+30.1±6.8

3.5. X-Ray Diffraction Analysis

The results of the XRD analysis of Ag-NPs and chitosan-coated Ag-NPs are provided in Fig. 4. Accordingly, when the XRD results are examined, both samples showed 4 peak points at 10 to 80 2θ degree and their positions are very closely related to the information reported in the literature [47,48]. In both spectra, the four high scattering peaks seen at 38.1°, 44.2°, 64,3° and 77.3° are (111), (200), (220) and (311) respectively. These indices confirm the Bragg scattering surfaces and silver-centered cubic crystal structure. It is understood from the high similarity between the X-ray spectrum of both samples that the coating with chitosan does not change the crystal structure of Ag-NPs.

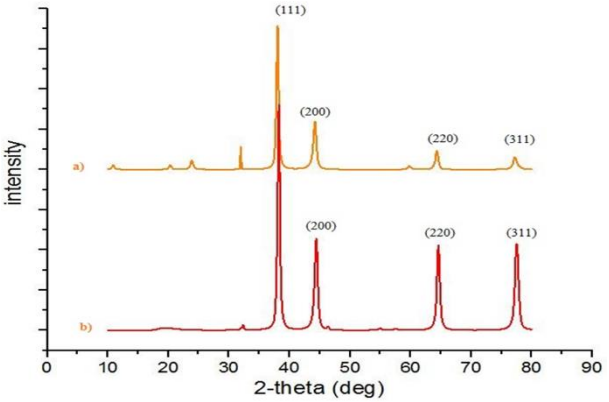

Fig. 4. Results of the X-Ray Diffraction of **a)** Ag-NPs **b)** Chitosan-coated Ag-NPs.

3.6. Scanning Electron Microscope Analysis

SEM analysis was performed in order to obtain the morphology results of Ag-NPs and chitosan-coated Ag-NPs, and these results are provided in Fig. 5. When the SEM results of both samples are examined, it is observed that the uncoated Ag-NP crystals show moderate homogeneity and spherical shape along with high aggregation tendency. These results are also proved with DLS values. On the other hand, aggregation of the chitosan-coated Ag-NPs show high homogeneity along with its amorphous particles and spherical shapes. Most of them seem to be homogeneously distributed and spontaneously linked [49]. Although both samples form aggregations, it is observed that there is less aggregating in chitosan-coated Ag-NPs. The sizes of Ag-NPs and chitosan-coated are close to the values determined by TEM analysis. SEM and XRD analyses proved the homogeneity and crystallinity of them. SEM images have

shown that both Ag-NPs have an average size ranging from 20 to 80 nanometers and crystalline character.

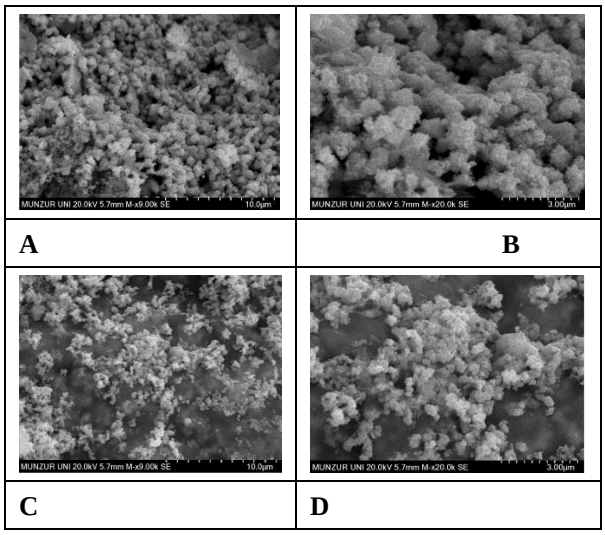

Fig. 5. SEM image results of both Ag-NPs (A-B) and chitosan-coated Ag-NPs (C-D).

6. CONCLUSION

The characteristic features of nanomaterials vary from their structures to the processes in which they are used. Nanostructured materials can have very different characteristics comparing to their macroscopic forms. Especially for the metal based and nanosized materials; the toxicological features of material should be biologically tested, physical and chemical features of the material should certainly be determined and it should be indicated whether the material is suitable for the purpose of the study. In order to reduce the toxic effect of NPs, either a suitable method of synthesis should be applied or NPs should be modified by using appropriate natural coaters with high biocompatibility. In this context, it is shown that coating agents play a significant role for the interaction of Ag-NPs with bacterial cells and effecting the bacterial cells with gingival fibroblast cytotoxicity [50-52]. Despite the widespread usage of Ag-NPs as oral medicines in the recent years, there are uncertain risks associated with the exposure of xenobiotics for local cells and tissues [53-55] In another study, it is found out that the Ag-NPs coated with polyethylene glycol are less toxic than the uncoated NPs [56]. Toxicity difference in the coated and modified Ag-NPs have been studied and it is found out that the coating material is a significant factor affecting the toxicity of Ag-NPs. It has also been shown that some nanoparticle coating agents may reduce the toxicity of NPs. Furthermore, both extraneous and PEG-coated iron oxide NPs were significantly less toxic than the uncoated NPs in terms of endothelial cells [57]. Interestingly, DeBrosse et al. [58] have shown that surface functionalization of gold nanorods results in a significant cytotoxicity which was observed in human keratinocyte cell line. All these studies have proved that Ag-NPs, which have toxic characteristic, can be encapsulated with suitable coating agents, and this is very important as NPs are very useful in biological and engineering fields.

In this research, Ag-NPs are synthesized and then coated with chitosan biopolymer. The synthesis of Ag-NPs is verified by the 4 characteristic peaks (111), (200), (220), and (311) in the X-ray scatter spectrum with absorption observed between 395 and 420 nm in the UV-Vis spectrometer. It is also found out from the zeta potential analyzes that the produced silver nanoparticles are stable. It was also confirmed by TEM and SEM analyzes that the synthesized particles were in nanoscale as intended. From the XRD analyzes, it is understood that the coating of Ag-NPs has occurred without destroying the structure and from the SEM analysis it is found out that coating of Ag-NPs made with chitosan biopolymer reduced in aggregation tendency of Ag-NPs. SEM and TEM analyses revealed that the applied coating has increased the NP size at negligible level. The coating of the toxic featured Ag-NPs with biocompatible chitosan polymer without destroying their structure is significant. In this way, it is obvious that the Ag-NPs' area of usage, in particular, its application within living organisms will increase considerably. As a result of this study, it is expected that the chitosan coated nano-sized Ag particles will lead to a new researches in biocompatibility. In this context, it is very significant to discover new synthesis methods that reduce the toxic features of metal based nano materials and to identify new approaches for using the engineered nanomaterials in areas which affect human life, especially in bioengineering fields.

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DECLARATION OF ETHICAL STANDARDS

The author(s) of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

AUTHORS' CONTRIBUTIONS

Mehmet Ateş: Performed the experiments and analyse the results.

Ersen YILMAZ: Perofrmed the experiments and analyse the results.

Bülent KAR: Wrote the manuscript.

İlknur KARS DURUKAN : Wrote the manuscript.

ÇIKAR ÇATIŞMASI (CONFLICT OF INTEREST)

There is no conflict of interest in this study.

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