Silver-doped Nanoclay with Antibacterial Activity

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ABSTRACT

The aim of this study was to synthesize Ag-nanoclay composite by solid ion exchange, then characterize, and evaluate the antibacterial activity. This newly introduced ion exchange is a simple and low cost technique, which provides a quicker method. Without chemical treatment, nanoclay was subjected to an ion exchange process in media containing molten salt of silver at 300 and 400 °C and at different periods. Scanning electron microscopy showed that the flakes' structure was opened by higher temperature and longer time. DRS graph revealed that Ag particles were successfully loaded, and that higher temperature and longer time increased the loading amount. Changes in basal reflection in XRD peak validated the presence of Ag. The Release test indicate that the materials do not present any risk for drinking water treatment since the leached metals were very small and in acceptable concentrations. The constant amount of release after four hours of sharp releasing. The antibacterial activity of the prepared composites was tested against gram-negative Escherichia coli and Staphylococcus aureus using the disc diffusion method. Presence of an inhibition zone clearly indicates the antibacterial effect of the composites. The results demonstrate that silver can be introduced onto nanoclay by single-step ion exchange.

Keywords: Solid ion exchange, silver-doped nanoclay, antibacterial activity.

1. Introduction

Considering the obvious consequences of bacterial infections, there is a growing need to develop new and powerful antibacterial agents. Nanoparticles in particular have been utilized in food preservation, burn dressings, safe cosmetics, medical devices, water treatment and a range of other products [1-8] Furthermore, inorganic antibacterial materials have several advantages over traditionally used organic agents, such as chemical stability, thermal resistance, user safety, and long-lasting action.

Nanoparticles' small size is the factor that favors their aggregation. Transforming nanoparticles causes changes in their chemical, physical and antibacterial properties. To cope with these challenges, appropriate supporting matrices can be used. However, the activity and stability of nanoparticles are influenced by the nature of the supports used in their immobilization. An ideal support should allow constant interaction among nanoparticles and bacteria, enabling them to retain their uniformity throughout coating processes. Antibacterial nanoparticles have been deposited on

glass, activated carbon, and metals to meet similar objective.[2,3,6-12] Recent studies on antibacterial activities of clay-based materials are mainly focused on immobilization of inorganic cations such as Ag^+, Cu^{2+} and Zn^{2+} onto clay minerals.[5-8]

Among many noble metal nanomaterials, silver nanoparticles (AgNPs) with size or shape-dependent physicochemical properties and antibacterial activities have been widely reported, and applied in catalysis, labeling tags, sensors and medical uses.[5, 13-17] Silver has long been known to have a broad spectrum of antimicrobial activities.

The antimicrobial activity of AgNPs apparently is dependent on release of Ag^+ ions and contact with the ions. According to previous findings, Ag^+ binds to electron donor groups in biological molecules containing sulfur, oxygen or nitrogen. [18] For example, interactions of Ag^+ ions with thiol groups in proteins may induce inactivation of bacterial enzymes, and DNA molecules may become condensed and unable to replicate. Another mechanism proposed for the antimicrobial activity of AgNPs was based on adherence to the cell surface, degradation of lipopolysaccharides and formation of pits in the membranes, largely increasing permeability.[19,20]

Methods of synthesizing metal nanoparticles generally involve both chemical reactions and physical means, including photo reduction, chemical reduction, vapor deposition and supercritical fluid technology.[21] Nanoparticles are usually synthesized via chemical reduction of suitable metal ions in a solution of sodium borohydride, ascorbates, citrates or carbohydrates. After reduction of metal ions, synthesized nanoparticles are often stabilized by coating with capping agents. Most antibacterial nanoparticles' chemical synthesis methods rely on the use of toxic reducing agents (e.g., sodium borohydride) and harmful organic solvents (e.g. N,N-dimethylformamide and tetrahydrofuran). These chemicals represent potential biological and environmental risks. Furthermore, adding these organic agents could adversely affect the surface activity of AgNPs due to the organic wrapping on the particle surface. [22]

After reviewing recent literature, we adopted the concept of nanocomposites that use nanoclays as the nanoscale supports to stabilize silver, while maintaining the silver surface without any organic contamination. Nanoclay is characterized by a layer 1–2 nm thick with lateral dimension of 100–200 nm, consisting of six octahedrally-arranged

aluminum ions sandwiched between two layers of four tetrahedral silicon atoms, balanced by 20 oxygen atoms and four hydroxyl groups. Under dry conditions, montmorillonite (MMT) clay crystals are stacked with cations (such as sodium ions) between neighboring layers to balance the negative charge of each clay layer.[5] Montmorillonite clay was recently reported to possess antibacterial properties, facilitated by the exchange of metal ions such as Ag^+ [21] and Cu^{2+}.[22] This research was done by immersion of clay into molten salt with alkaline ion, which was silver ion. This process is called alkaline ion exchange. Recently, some studies about using ion exchange molten salt were reported such as bentonite/ZnO,[6,7] bentonite/CuO.[8] Up to our knowledge, there is no any report on the synthesis of nanoclay/silver nanocomposites by solid ion exchange method.

The aim of this study was to synthesize nanocomposites by solid ion exchange and then characterize and evaluate the antibacterial activity. The differences are a) the reaction is in solid state, and solvent and reduction agents are not utilized; and b) given higher processing temperature, diffusion process is quite fast, and time is very short. Briefly, we introduce an easy and inexpensive approach to synthesize nanocomposites for industrial food packaging applications.

2. Experimental

Nanoclay montmorillonite K10 was obtained from Sigma-Aldrich. Silver nitrate and nutrient agar were purchased from Merck. The bacterial strains used for antibacterial activity were gram-negative E. coli (PTCC 1270) and gram-positive S.aureus (PTCC 1112), obtained from the Iranian Research Organization for Science and Technology.

2.1. Preparation of nanocomposites

Nanocomposites were prepared by immersion of nanoclay into molten $AgNO_3$ at 300 and 400 °C for 1, 2, 3 and 5 min. This step was conducted using 1 g of nanoclay and 1 g of $AgNO_3$. After the ion exchange process, the nanocomposite was adequately washed three times with distilled water and then sonicated. After filtration, the composites were dried overnight in an oven at 25°C.

2.2. Characterization of nanocomposites

X-ray diffraction (XRD) patterns were recorded using a Philips PW 1050 diffractometer

(The Netherlands). Diffuse Reflectance UV-Vis Spectroscopy (DR UV-Vis) of the powder samples was conducted in the wavelength range of 200–800 nm using a spectrophotometer (Scinco S4100, S. Korea). The morphology of samples was observed with a scanning electron microscope (LEO 1430VP, Germany).

The concentrations of Ag iones released from the nanocomposite suspension were determined by an atomic absorption spectrometer (AA800, PerkinElmer, USA). For each composite, 0.2 g of composite was immersed in 10 ml of distilled water and vigorously shaken in a shaking water bath (30 °C, 200 rpm) for 24 h. Supernatants from each test tube were collected by centrifugation at 4000 rpm for 10 min. Zinc ions released from the nanocomposites were qualitatively determined.

2.3. Antibacterial activity

The antibacterial activity of the Ag/nanoclay composites against both E. coli (gram-negative) and S. aureus (gram-positive) bacteria was tested by agar diffusion test. Samples were exposed to bacteria in solid media (nutrient agar), and the inhibition zone around each sample was measured and recorded as the antibacterial effect of copper nanoparticles. Agar plates were inoculated with 100 μL suspensions of bacteria. Composites with a diameter of 0.5 cm and 0.05 g weight were placed on agar disks and incubated at 37 °C for 24 h. The inhibition zone for bacterial growth was detected visually.

3. Results and discussion

Environmentally sustainable green one-step and solid-state synthesis of nanocomposites was carried out by alkaline ion exchange method without using chemicals. Appearance of brown color indicated the formation of nanocomposites (Fig.1). Changes in color are thought to be associated with the change in the state of silver (Ag^0 or Ag^+). The color of Ag^0 is yellow or brown/gray depending on the concentration of silver and size of silver nanoparticles in the matrix, while Ag^+ appears colorless.[23,24] Therefore, the coated silver is Ag^0.

High temperature used in the process resulted in a higher diffusion coefficient, and compared with the common ion exchange, the process time is much shorter. Furthermore, given the high temperature, highly energetic silver ions were able to penetrate into the layers, connect together and form larger particles. Here, the change in color validates our claim about coating the Ag0 form onto the clay. By increasing the process's time and temperature, more color change is seen, the reason for which is either the larger size or the greater amount of silver. Considering the color change from yellow to brown-gray, the nanoparticles' size had increased; therefore, increasing the temperature and time of ion exchange had a positive effect on nanoparticle size.

3.1. Scanning electron microscopy

From SEM images (Fig. 2) it can be observed that the nanoclays are composed of heterogeneous particles. The ion-exchanged samples represented flakes in layered morphology, and did not exhibit any recognized nanoparticles identified on the surfaces of the exchanged samples. The difference between images of parent nanoclay and nanoclay after ion exchange process is the swollen appearance of the flakes, which may state the presence of coated
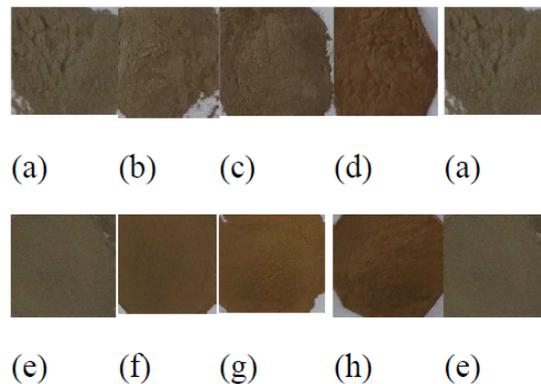

Fig. 1- The color of silver doped nano clay prepared at 300 °C for (a) 1, (b) 2, (c) 3 and (d) 5 min, and at 400 °C for (e) 1, (f) 2, (g) 3 and (h) 5 min.

silver on the clay. The SEM results also show that with increasing temperature and processing time, the sample's porosity increased, although there is no strong sign of coated nanoparticles. The reason may be the very small particles generated in our samples by this method.

On the other hand, this result is in agreement with findings by others in terms of the relationship between time and temperature, and porosity. At Magaña et al. [21] study, a container of nanoclay and $AgNO_3$ were left shaking for a week, so there was enough time for ion exchange and formation of larger particles. Another study by Rivera-Garza[24] reported coating silver on zeolite, claiming that
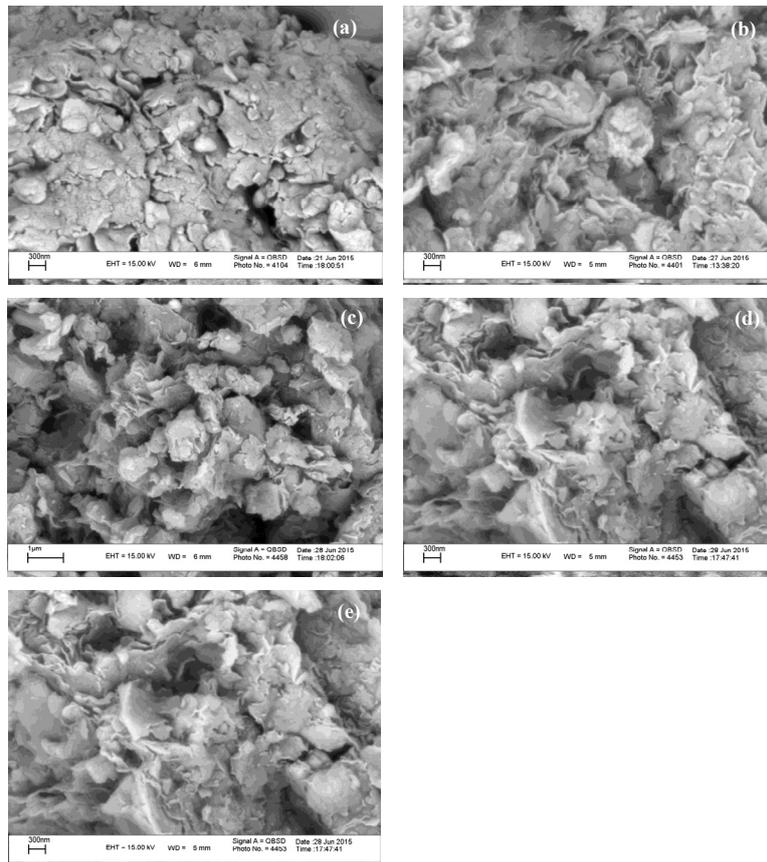

Fig. 2- SEM images of (a) parent nanoclay and ion exchanged nanoclay at 300 °C for (b) 1 and (c) 5 min, and at 400 °C for (d) 1 and (e) 5 min
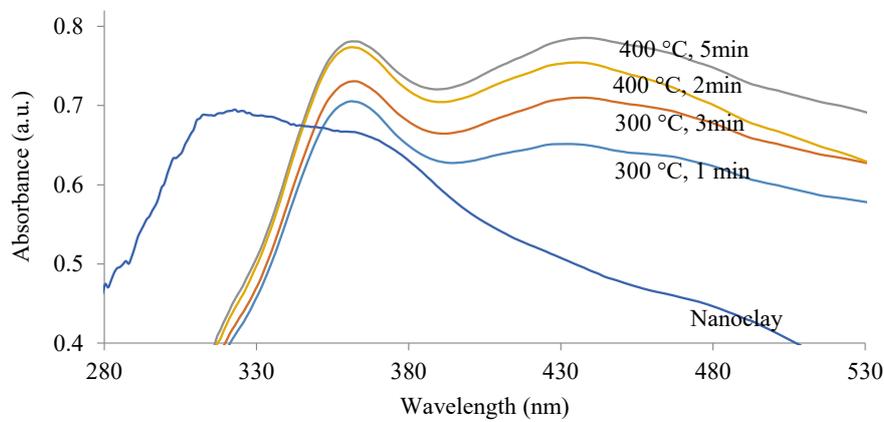

Fig. 3- The UV-visible spectrum of parent nanoclay and composites prepared at different deposition times and temperatures.

because of the light, exchanged Ag^+ converted to Ag^0.

3.2. UV-visible spectroscopy

The formation of nanocomposites could be monitored by characterizing the absorption of UV-Vis spectrum. Figure 3 shows different spectra for nanocomposites compared with parent nanoclay. Peaks at approximately 360 and 435 nm appeared in all nanocomposites. Other researchers have reported the absorption peak for silver to be around 435 nm.[25] Therefore, the sharp peak near 435 nm proved that Ag was doped successfully on nanoclay. The absorption peak shifted throughout the different times and temperatures of the process, implying the formation of different sizes of silver nanoparticles. A very small red shift in the absorption peak at 435 nm was observed with increasing ion exchange time and temperature. The red shift results from the increment of nanoparticle size due to higher temperature and longer

processing. Therefore, the changes in ion exchange temperature and time slightly affected nanoparticle size.

Alternatively, different peak heights represent different concentrations of Ag. Figure 3 shows a higher peak achieved when both processing time and temperature increased. This may be explained by loading and gradual conversion of silver nitrate to silver nanoparticles. Therefore, the nanoparticle count increased by incremental time and temperature. The finding here is in line with the color of nanocomposites (Fig. 1).

3.3. XRD analysis

Figure 4 displays the XRD pattern of clay composites. Clay characteristic peaks are shown at 2θ values around 6, 20 and 35° (these peaks are denoted with C on Fig. 4. The peaks are clearly seen in all patterns, consistent with other reported works.[26,27] Small quantities of quartz, at $2\theta = 26.59°$, and anorthite, at $2\theta = 21.85°$, 23.58°
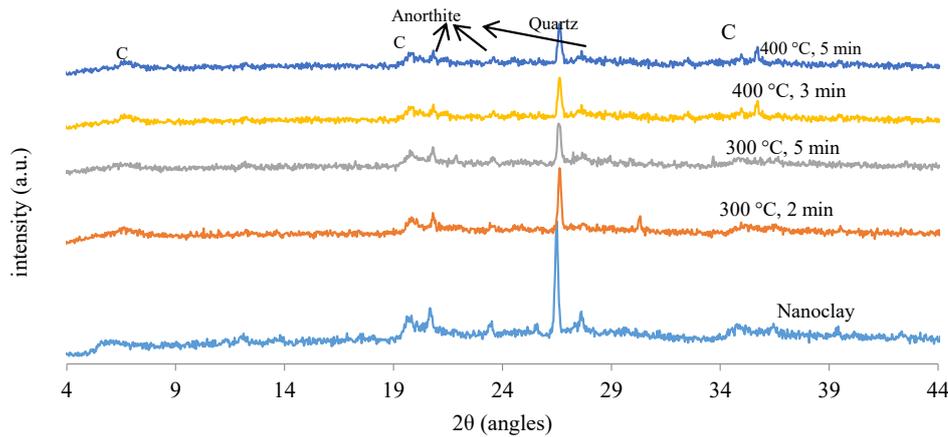

Fig. 4- The XRD spectrum of parent nanoclay and composites prepared at different deposition times and temperatures.
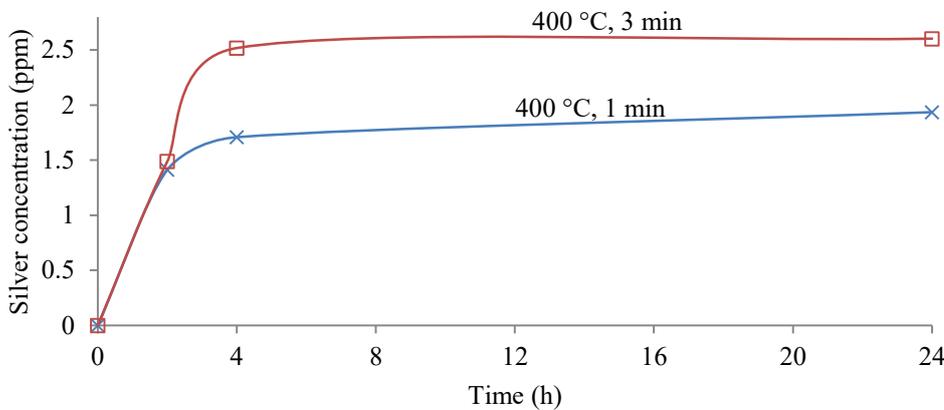

Fig. 5- The release of silver ions into deionized water after 24 h at 37 °C.

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and 27.75°, were also observed. As shown in Fig. 4, nanocomposites loaded with Ag and parent nanoclay have similar XRD patterns. However, the 001 basal reflection has shifted to a higher angle. The diffraction peak at $2\theta = 6.08°$ for parent nanoclay, which corresponds to the d-spacing of 1.45 nm, shifted to $2\theta = 6.33–6.84°$, corresponding to an average interlayer distance of 1.29–1.33 nm (Fig. 4). Thus, for the ion exchange samples, the (001) peak became narrower, its intensity increased, and the position shifts to a lower d-spacing. It has been reported that this structural change is caused by loss of water present in the interlayer. [27] Therefore, the d-spacing position and shape differences of the (001) reflection suggest the introduction of Ag+ ions within the structural interlayer.

3.4. Leaching tests

The stability in the water of these composites was tested because, ultimately, these composites are intended for drinking water treatment. Normally, water is treated in a fixed bed mode of operation, in which the flow conditions are nearly laminar when nanoparticles are embedded on certain substrates. In this study, vigorous agitation was adopted for the leaching test. This represented an extreme condition in terms of Ag+ release. However, the material must be kept stable under a calm flow. The advantage is that the antibacterial property of the composite would persist longer.

Figure 5 displays the release test result for two samples produced at 400 °C, for 1 and 3 min. Both samples released Ag+ into the water on a sharp slope during the first two hours. During the subsequent two hours, the release rates decreased for both samples. This is exactly the reverse of synthesizing action. Interestingly, from 4 to 24 hours, the amount of release was lower than 0.2 ppm. Another study about synthesized Ag/zeolite by ion exchange revealed the amount of Ag+ released into water increased because of immersion time. [24] However, even a low amount of Ag is poisonous, limiting its usage. Here, the constant amount of Ag+ in water after 4 hours is desirable. Consequently, within the first 4 hours, the surface-coated Ag+ as well as some that are physically attached are released

Table 1- Antibacterial activity of silver-doped nanoclay

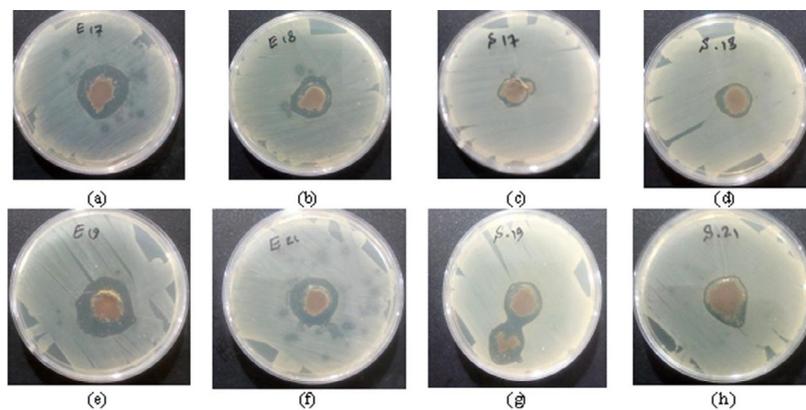| Ion exchange temperature (°C)	Ion exchange time (min)	Inhibition zones (mm)	
		E. coli	*S. aureus*
300	1	13.6 ± 0.5	4.0 ± 0.4
	2	12.0 ± 0.7	4.7 ± 0.9
	3	14.4 ± 0.8	5.9 ± 0.5
	5	14.9 ± 0.7	6.9 ± 0.8
400	1	12.1 ± 0.9	5.0 ± 0.4
	2	12.5 ± 0.5	5.2 ± 0.2
	3	13.9 ± 1.0	5.5 ± 0.7
	5	13.8 ± 0.8	5.6 ± 0.3

Fig. 6- Antibacterial activity of silver-doped nanoclay ion exchanged at 300 °C for (a) 3 and (b) 5 min against E. coli, for (c) 3 and (d) 5 min against S. aureus, and at 400 °C for (e) 3 and (f) 5 min against E. coli and for (g) 3 and (h) 5 min against S. aureus.

through the incomplete washing process. Through the further immersion time, Ag+ that was doped in the clay structure cannot be released. Therefore, Ag nanocomposite synthesis by molten salt ion exchange is more stable.

The result is comparative with previous researches about composites, which synthesized by Alkaline ion exchange such as bentonite/ZnO$_2$,[8] bentonite/CuO,[7] nanoclay/ZnO$_2$.[6] The antibacterial activity of nanoclay based composite reported higher than bentonite`s. However, no antibacterial activity was reported for bentonite/ZnO$_2$ and bentonite/CuO. The reason was more available surface of nanoclay compared with bentonite. On the other hand, the antibacterial activity of the produced nanoclay/silver is higher than the above mentioned nanocomposites. The higher antibacterial activity might be related to the large surface of nanoclay and the existence of pillared silver.

3.5. Antibacterial activity tests

The antibacterial activity of the prepared composites was tested against gram-negative E. coli (PTCC 1270) and gram-positive S. aureus (PTCC 1112) using the disc diffusion method (Fig. 6). Presence of an inhibition zone clearly indicates the antibacterial effect of the composites. The results reveal that parent nanoclay does not exhibit any antibacterial effect, while the composites do (Table 1). From the SEM images, it can be seen that the porosity of samples was increased by enhancing the ion exchange time and temperature. On the other hand, DRS results showed that the changes of time and temperature were not affected by the size of nanoparticles, while the amount of loaded particles increased. This relationship can also be explained by higher porosity and greater surface area, which result in a larger amount of loading. On the other hand, the distance between the layers is too low for coming into contact with the microbe and Ag. Samples synthesized at higher temperature and longer time show a little higher antibacterial activity. Generally, two mechanisms were reported for antibacterial activity: a) the release of Ag, and b) microbe contact with the Ag. It was reported that in an ion exchange system the main mechanism is the first method.[24,27] Our results show that within the first four hours, the first mechanism is prominent. After that, the release of Ag constants and the main mechanism bring the microbe into contact with the coated Ag. Our results are comparable

to those reported by Magaña et al. [17], which used montmorillonites ion exchanged with silver, where the inhibition zone had a mean diameter of 13.3–12.3 mm.

4. Conclusions

Nanoclay was subjected to ion exchange with silver. This newly introduced ion exchange is a simple and low cost technique which provides a quicker method. Scanning electron microscopy showed that the flakes' structure was opened by higher temperature and longer time. DRS graph revealed that Ag particles were successfully loaded, and that higher temperature and longer time increased the loading amount. Changes in basal reflection in XRD peak validated the presence of Ag+. Release test showed the constant amount of release after four hours of sharp releasing. Antibacterial activity showed good activity against gram-negative bacteria.

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