

Antibacterial silver-doped bioactive silica gel production using molten salt method

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Abstract Due to broad-spectrum antimicrobial activity, silver nanoparticles have great application potential in disinfection of contaminated water. The aim of this research was the introduction of a fast and simple method titled as “molten salt method” for the production of silver-doped bioactive silica gel (SG) nanocomposite. In this method, SG was imposed into the molten salt of silver nitrate at 150 and 300 °C for various times. Interestingly, molten salt method was not utilized any reducing reagent or other chemicals unless molten silver nitrate. The synthesis and fixing of nanoparticles into the support were done in <60 min. The prepared silver/SG nanocomposite was evaluated using scanning electron microscope (SEM), energy dispersive X-ray fluorescence, leaching test and antibacterial test. SEM images showed that the contact of SG with the molten salt caused the formation of nanoparticles on the SG. On the other hand, increasing the contact time, it led to a larger and increased number of particles. The antibacterial tests demonstrated that this composite is suitable for using as antibacterial material. The test of elution with water indicated that the prepared nanocomposite is stable and the amount of the released silver in the water was negligible.

Keywords Antibacterial · Silver · Silica gel · Molten salt method

Introduction

Silver nanoparticles have recently attracted great interest due to their distinctive properties such as large surface areas, unique physical, chemical and biological properties [1, 2]. It is widely known that materials containing silver show antibacterial property [3, 4]. Now, the silver nanoparticles are emerging as a new generation of antibacterial agent, which has been used in medical applications and antibacterial water filter [5]. The use of silver nanoparticles is particularly potential to treat drinking water, which is frequently infected by antibiotic resistant bacteria. Treatment of this water usually requires using high concentrated chlorine compounds, which may cause a high risk of human cancer [6].

However, aggregation of silver nanoparticles and leaching of Ag⁺ ions in aquatic system restricted the application of silver nanoparticles. Therefore, demand for the production of solid supported silver nanomaterials has been increased. Generally, the nanoparticle-doped materials have several advantages, such as high performance, low price (compared to pure silver), high chemical durability and low release silver ions for a long period of time [7, 8]. There are several methods for preparing silver-doped silica including multi-target sputtering [2, 9], sol-gel process [4] and ion exchange process [10, 11]. For example, spherical nanoparticles with a silver core and an amorphous silica shell were successfully fabricated using tetraethoxysilane as silica precursor and reducing silver nitrate with ascorbic acid. These nanoparticles had excellent antibacterial effects against *E. coli* and *S. aureus* [12]. In a similar approach,

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silver nanoparticles were immobilized onto the surface of magnetic silica composite to prepare magnetic disinfectant that exhibited enhanced stability and antibacterial activity [13].

However, the synthesis and fabrication of supported silver nanomaterial via these methods require extensive use of toxic and highly cost chemicals and organic solvents, which frequently raise health and safety issues [14]. This has led to increasing interest on the development of inexpensive preparation methods suitable for large economic industrial applications. This may significantly boost the industrial production of the inexpensive silver-doped silica products for various applications.

In this work, silver-doped bioactive silica gel (SG) was prepared by a new approach titled as “molten salt method”. To optimize the process condition while guaranteeing the antibacterial action, different synthesis conditions (time and temperature) were selected. The obtained samples were characterized by means of scanning electron microscope (SEM) and energy dispersive X-ray fluorescence (μ -EDXRF) observation. The effect of surface modifications on the antibacterial activity of samples has been investigated.

Methods

Materials

AgNO_3 , SG (Kieselgel 60, 0.063–0.200 mm), Mueller–Hinton broth and nutrient agar were purchased from the Merck Company (Tehran, Iran). All reagents were used without further purification. The bacterial strain used for the antibacterial activity was Gram-negative *E. coli* (PTCC 1270) and Gram-positive *S. aureus* (PTCC 1112) received from the Iranian Research Organization for Science and Technology.

Preparation of Ag/SG nanocomposite

This process was performed in the molten salt bath of AgNO_3 for different periods ranging from 1 to 30 min. AgNO_3 , after weighting, were grounded to get a homogeneous mixture. The temperature of the bath was maintained at about 150 and 300 °C. A quartz beaker was partially filled with the mixture of silver nitrate (AgNO_3) and SG (1:1, wt/wt), and placed in a furnace which was electrically heated up to the temperature of the process. The samples were then taken out of the molten beaker, and were cooled in air. Later, they were ultrasonically cleaned with distilled water. After molten salt process, a slight yellow coloration was observed.

Characterizations

The microstructures of the samples were observed by SEM (LEO 1430VP, Germany). μ -EDXRF analysis was performed using a XMF-104 X-ray Microanalyzer (Unisant S.A., Switzerland) equipped with a 50 W molybdenum tube and a high resolution two-stage Peltier-cooled Si-PIN detector. The samples were positioned in definite places and at a constant height of the holder base. The temperature was controlled between 32 and 34 °C throughout the experiments. The voltage and current were 30 kV and 300 μ A, respectively. Each μ -EDXRF analysis was performed in 50 s to obtain sufficient counts. For homogeneity tests, three different points of approximate constant orientation with respect to the nanocomposites were analyzed [15].

Water elution test

For each composite, 0.2 g of composite was immersed in 10 mL of distilled water and vigorous agitation in a shaking water bath (30 °C, 200 RPM) for 2, 6 and 24 h. Supernatants from each test tube were collected by centrifugation at 4,000 RPM for 10 min. Silver ions released from the nanocomposites were qualitatively determined by an atomic absorption spectrometer (AA800, Perkin Elmer). Water elution test was replicated twice.

Antibacterial activity

The antibacterial activity of the composites against both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) 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 composites. Agar plates were inoculated with 100 μ L suspensions of bacteria. The composites and parent SG were placed on agar disks and incubated at 37 °C for 24 h. The inhibition zone was measured at three different points. Antibacterial activity test was replicated twice.

Results

Characterization

After molten salt process, the color of the SG surface changed from pale yellow to bright yellow, amber and dark amber depending on the processing time and temperature, as shown in Fig. 1. For high temperature process, the coloring becomes darker. The coloration of the sample prepared at 150 °C for 30 min matches with the color of

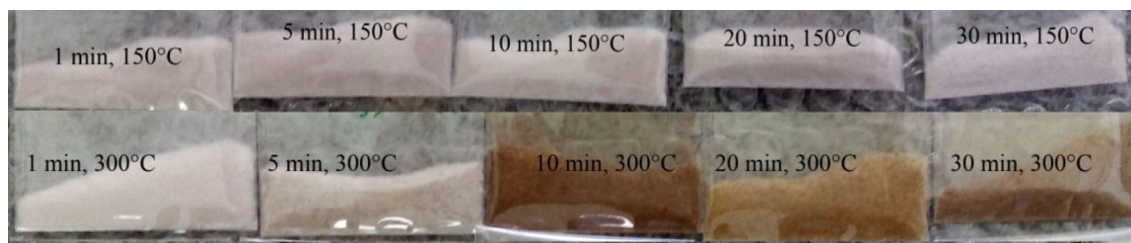


Fig. 1 The color of silver/silica gel nanocomposite at various temperatures and times

the sample processed at 300 °C for 5 min. The colors of the products obtained in this work were similar to previous reports [4, 11].

The un-embedded SG as well as the Ag^+ appears colorless. On the other hand, Ag^0 is yellow or brown/gray depending on the concentration and size of silver nanoparticles in the silica matrix [4]. The appearance and disappearance of colors is claimed to be associated with the change in the state of silver (Ag^0 or Ag^+) at the various preparation time and temperatures.

Figure 2 shows the morphology of producing SG and composites. One understands that there is no remarkable change on the surface after coating with silver when compared SG surface (Fig. 2b) with produced composites (Figs. 2c, e). The only difference is the developmental changes in the micro pores on the initial structure of SG. It seems that these pores have been filled during the molten salt process with very small particles of silver. For further investigation, composites were heated at 100 °C for 3 h. Based on the available reports, heating causes the movement of silver particles on the support and consequently their adhesion. The result of this action is the formation of larger particles [16]. The comparison of composites images before and after heating resulted that the heating caused the formation of larger particles on the surface (Fig. 2c with d and f with g). Inasmuch as it has not been added any silver particle to the system during the heating process, the formation of these particles is caused due to adhesion the loaded silver on the surface of SG during the molten salt process. In other words, during the process of molten salt at temperatures and times of utilized in this research, particles that formed on the surface had not enough time to move on the SG and join together and form larger particles. The heating of composites prepares this situation. Another point is the size and the amount of formed nanoparticles. In reference to Fig. 2d and e and two samples, which prepared at 300 °C, the increasing of the time of molten salt process caused to the formation of larger nanoparticles on the surface. These results correspond to the changes of formal colors of the composites (Fig. 1).

For the better investigation of the amount of fixed silver on the SG in the composites, μ -EDXRF was used (Fig. 3).

On the basis of the past researches, the intensity of the peak of the plot for each of the elements is directly related to their concentration [15]. The peak at the energy of 2.9 eV represents silver in the composite. With due attention to this energy and Fig. 3, in 300 and 150 °C, the amount of silver in the composite has been increased with increasing the time of molten salt process. On the other hand, at constant time, the increasing of temperature causes the same result. These results verify SEM results.

Water elution test

The results of water elution of silver/SG nanocomposites are given in Fig. 4. This nanocomposite has been prepared for bactericide action. The importance of this test is that whether this nanocomposite causes water pollution or not. On the contrary, the more stability of nanocomposites, the more aged. This test exerts more intense condition to the nanocomposites than industrial use condition. It is usually utilized laminar flow in industrial systems; therefore, it exerts less tension than turbulent flow on the nanocomposites which used in this experiment. As Fig. 4 indicates, the amount of releasing silver from all of the nanocomposites is negligible. On the other hand, during the first 2 h, the most amount of silver is released, but after this period the releasing of silver is decreased so that the amount of silver after 6 h becomes constant. Thus, one can claim that the stability of prepared nanocomposite is suitable. Also the increasing of time and temperature caused the increasing of released silver. The reason is mounted silver on the support, which has not strong binding with the support. It may be eluted better from the composites after preparation and this causes elution of these particles and decreases the released silver.

Antibacterial test

Table 1 shows the antibacterial activity of the nanocomposites against *E. coli* and *S. aureus*. Generally, all silver-doped products showed antibacterial activity, while the parent SG has no inhibitory effect. It can be seen that inhibition of sample prepared in 150 °C is less than

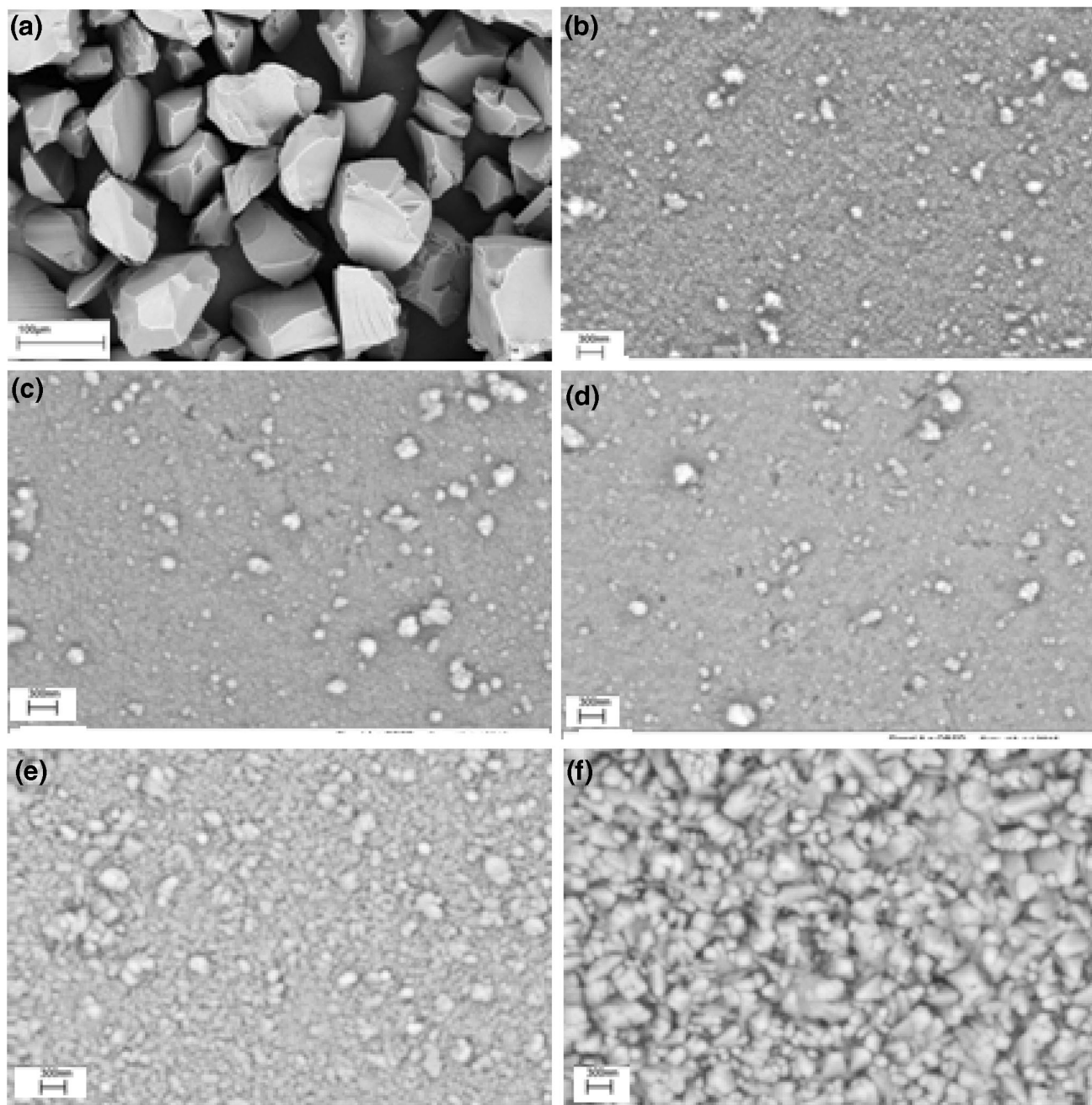


Fig. 2 The SEM micrographs of parent silica gel (a, b) and silver/silica gel nanocomposites prepared at 150 °C (c) and 300 °C (d) for 30 min then samples (c, d) annealed at 100 °C for 3 h (e, f), respectively

prepared samples in 300 °C (Table 1). In particular, for prepared samples in 300 °C, the zone of inhibition is almost similar for all silver-doped products.

The results of this research are comparable with the results of Hilonga et al. [4] study and our research has higher antibacterial effects relatively. They prepared the Ag/SG nanocomposite using the sol-gel method and in

several steps, however, their method is time consuming and more complicated. In 2011, Quang et al. [5] prepared Ag/SG nanocomposite via chemical reduction of silver nitrate and the obtained hollow against *E. coli* was equal to 6 mm. Referring to Table 1, the prepared samples in our study at 300 °C had hollows about 6 mm against *E. coli*.

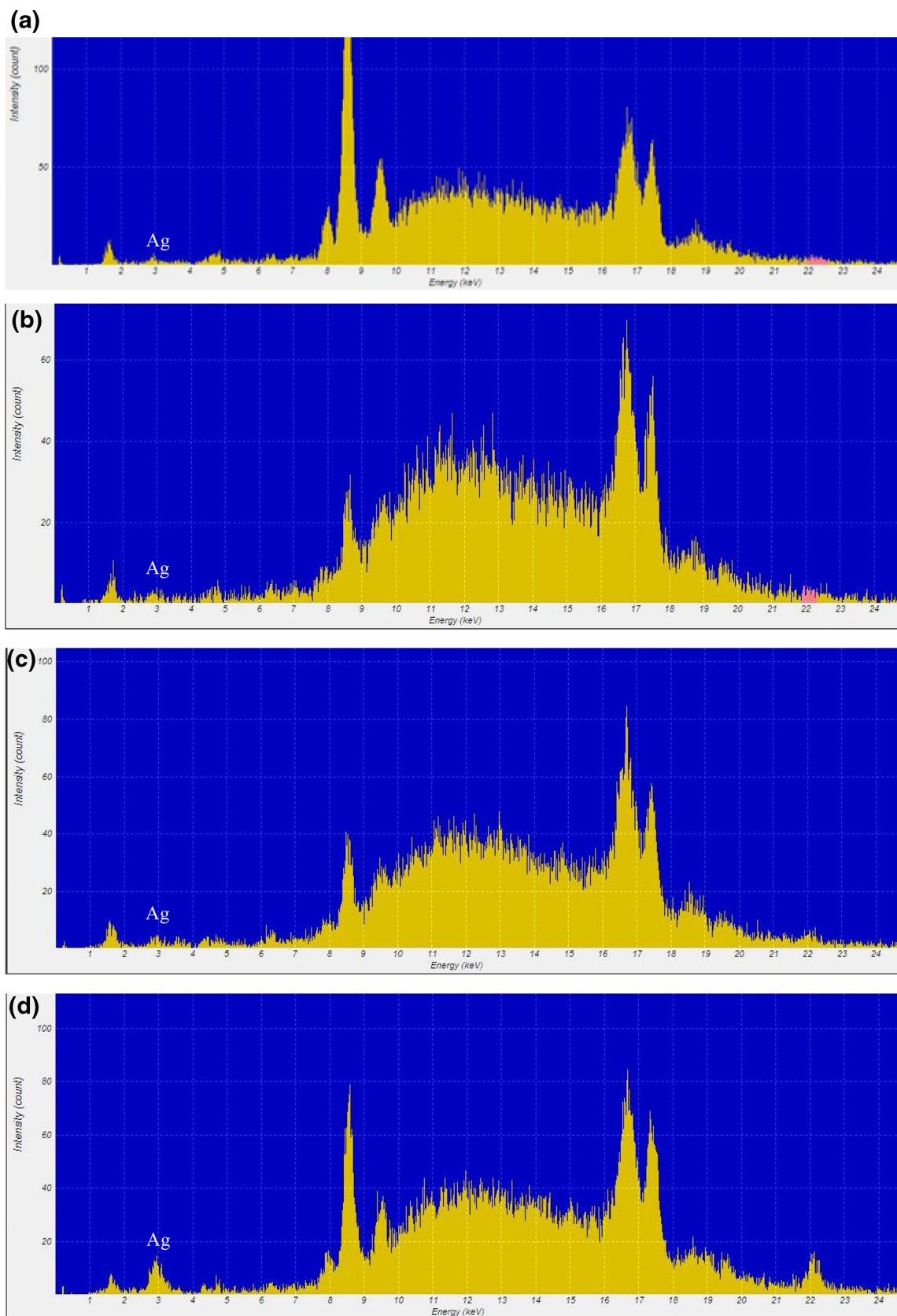


Fig. 3 The μ -EDXRF spectrum of composites, at deposition times of **a** 1 min and **b** 30 min at 100 °C and **c** 1 min and **d** 30 min at 300 °C

Fig. 4 The release of silver into deionized water after a 24 h period at 37 °C

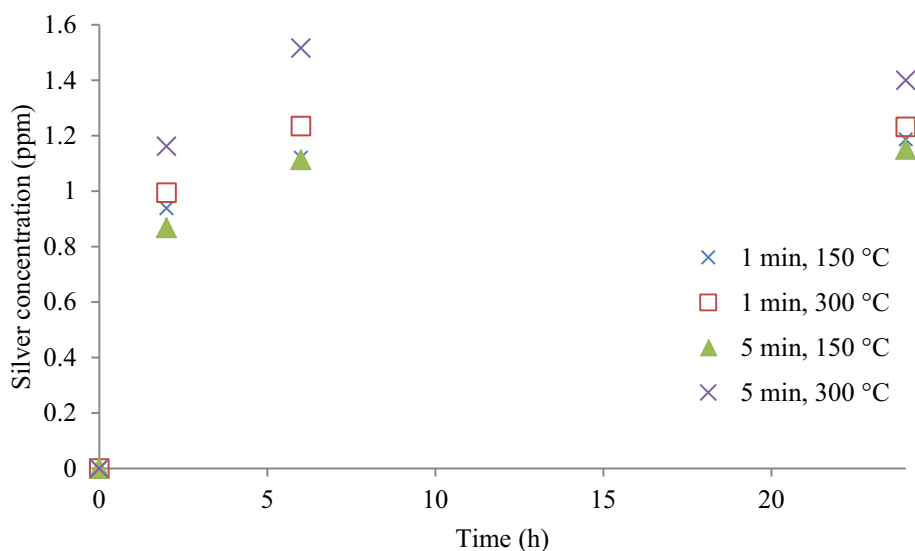


Table 1 Antibacterial activity of composites

| Temperature of process (°C) | Time of process (min) | Inhibition zones (mm) | |
|-----------------------------|-----------------------|-----------------------|------------------|
| | | <i>E. coli</i> | <i>S. aureus</i> |
| 150 | 1 | 0.7 ± 0.2 | 2.82 ± 0.6 |
| | 5 | 0.4 ± 0.2 | 2.05 ± 0.3 |
| | 10 | 0.9 ± 0.4 | 3.33 ± 0.4 |
| | 20 | 2.5 ± 1.1 | 3.10 ± 0.3 |
| | 30 | 4.1 ± 0.7 | 3.23 ± 0.8 |
| 300 | 1 | 6.0 ± 1.0 | 2.95 ± 1.1 |
| | 5 | 6.3 ± 1.4 | 7.07 ± 1.3 |
| | 10 | 5.85 ± 1.3 | 6.68 ± 1.5 |
| | 20 | 5.9 ± 1.7 | 6.49 ± 1.2 |
| | 30 | 5.7 ± 1.5 | 5.24 ± 2.0 |

Conclusions

An easy and new method was developed for the preparation of a bactericide nanocomposite. For the synthesis of the nanocomposite, SG was introduced into the molten salt of silver nitrate. After preparation of the nanocomposite, SEM and μ -EDXRF were utilized for its evaluation. The nanocomposite showed antibacterial properties. The prepared nanocomposite was stable during elution with water. There was negligible released silver during elution with water.

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