Digest Journal of Nanomaterials and Biostructures Vol. 6, No 1, January-March 2011, p. 149 - 154

ANTIMICROBIAL ACTIVITY OF COLLOIDAL SILVER NANOPARTICLES PREPARED BY SOL-GEL METHOD

N. LKHAGVAJAV[a], I. YAŞA[b], E. ÇELİK[c], M. KOIZHAIGANOVA[a*], Ö. SARI[a]
[a]*Leather Engineering Department, Engineering Faculty, Ege University, Izmir, Turkey*
[b]*Industrial Basic Microbiology Department, Basic Science Faculty, Ege University, Izmir,Turkey*
[c]*Metallurgical and Material Science Engineering Department, Engineering Faculty, Dokuz Eylul University, Izmir, Turkey*

In this study, the antimicrobial activity of colloidal silver nanoparticles prepared by the sol-gel method was investigated, and the turbidity, viscosity and pH of the colloidal solutions were determined. The size of the silver nanoparticles was measured by Atomic Force Microscopy (AFM). The minimum inhibitory concentration (MIC) value of the colloidal solution resistance to test microorganisms *Escherichia coli, Staphylococcus aureus, Candida albicans, Bacillus subtilis, Salmonella typhimurium, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was carried out by the Broth Microdilution Method. It was found that the silver nanoparticles inhibited the growth and multiplication of the tested microorganisms, including the fungus *C. albicans*. The antimicrobial activity was observed against all tested microorganisms at a very low concentration of 2-4 μg/ml of nano silver.

(Received December 12, 2010; accepted January 15, 2011)

Keywords: Colloidal Ag nanoparticles, Antimicrobial activity

1. Introductıon

Among of noble metals, silver nanoparticles have been extensively used in various fields. Widespread studies are being conducted on silver nano-sized particles for their antibacterial efficiency and they may have potential commercial application in areas such as medical tools, appliances and health care products [1, 2]. Composites formed by silver nano-clusters embedded in ceramics or glass has found applications in optoelectronics [3], as catalysts [4] and as antibacterial materials [5].

Silver or silver ions have long been known to possess strong inhibitory and bactericidw effects as well as a broad spectrum of antimicrobial activities [6, 7]. Several propositions have been developed to explain the inhibitory effects of silver ion/silver metal on bacteria. It is generally believed that heavy metals react with proteins by combining the thiol (– SH) groups, which leads to the inactivation of the proteins [8]. Recent microbiological and chemical experiments have revealed that the interaction of silver ions with thiol groups plays an essential role in bacterial inactivation [9].

The mechanism of the bactericidal effect of silver colloid nanoparticles is not very wellknown. Silver nanoparticles may attach to the surface of the cell membrane and disturb such properties as permeability and respiration [10]. It is reasonable to assume that the binding of the particles to the microorganisms depends on the surface area available for interaction. Smaller particles having the larger surface area available for the interaction will give a greater bactericidal effect than larger particles [11].

*Corresponding author: meruyert_k@hotmail.com

In the present study, we investigated the MIC value of colloidal silver solutions against seven microorganisms: *Escherichia coli, Staphylococcus aureus, Candida albicans, Bacillus subtilis, Salmonella typhimurium, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.* The colloidal solutions were prepared by the sol-gel method and characterized using a Rheometer, a pH meter, a Turbidimeter and Atomic Force Microscopy (AFM). We used the sol–gel method for the preparation of the silver colloidal solution because it has the advantages of good homogeneity, ease of composition control, low processing temperature and good optical properties. In particular, the sol–gel process is efficient in producing thin, transparent and multi-component oxide layers of many compositions on various substrates [12].

2. Experimental

Preparation of the silver colloids: Silver colloids were prepared using sol-gel processing. Silver nitrate ($AgNO_3$, 99.9% pure, Merck) was used as a precursor and glucose ($C_6H_{12}O_6$, 99.95% pure, Merck) as a reducing agent. The reducing agent and the precursor, $AgNO_3$, were dissolved in distilled water in different containers under the same conditions. Next, the two solutions were mixed and stirred until a colorless or transparent aqueous solution, which contained nAg particles, was formed. The nano-sized silver colloidal solutions were prepared at 0.5 μg/ml, 1 μg/ml, 2 μg/ml, 3μg/ml, 4 μg/ml and 5 μg/ml Ag concentrations according to the following reaction [13]:

$$2\ AgNO_3 + C_6H_{12}O_6 + H_2O \rightarrow 2\ Ag^0 + C_6H_{12}O_7 + 2\ HNO_3$$

To determine the solution characteristics which affected nanoparticle formation, the turbidity, pH values and rheological properties of the prepared solutions were measured and the size of the silver particles was determined. The turbidity properties of the solutions were measured to use as standard solutions for the coating process using a Scientifica TB 1 Velp Turbidimeter (Usmate, Milano, Italy) according to the ISO 7027 nepheometric method. The sample was placed in a vessel with a diameter of 25 mm and a height of 50 mm. Formazin was used because it has been recognized as the only true primary standard available for turbidity calibration. The measurements were taken in the range of 0–1000 nephelometric turbidity units. After the preparation of the transparent solutions, the pH values of the solutions were measured to determine their acidic and basic characteristics with a standard pH meter (WTW Inolab, Weilheim, Germany). Additionally, the rheological behavior of the solutions, including the viscosity, was obtained with a CVO 100 digital rheometer (Bohlin Instrument, Worcestershire, UK). Silver nano particles sizes were determined by XE-100 AFM (Atomic Force Microscope). Atomic Force Microscopy is an instrument that is used to study the surface structure of a sample by measuring the force between atoms.

Determination of Minimum Inhibitory Concentration (MIC): The Microdilution broth method was used to obtain the MIC of colloidal nanoAg solution [14]. The microorganisms utilized for the test were *Escherichia coli* ATCC 12228*, Staphylococcus aureus* ATCC6538-P*, Bacillus subtilis* ATCC 6633*, Salmonella typhimurium* CCM 5445, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* CCM 2318 and a unicellular yeast *Candida albicans* ATCC 10239. Microbial inoculums were prepared by subculturing microorganisms into Muller Hinton Broth (MHB) at 37 ^{0}C for 18 h and were diluted to approximately 10^5 to 10^6 of organisms/ml in twofold MHB. A stock solution of the 10 μg/ml nano Ag was prepared in ultra pure distilled water. Further serial dilutions were made in the range 10 μg/ml to 1 μg/ml of nano Ag. A 100 μl aliquot of each dilution and 100 μl of MHB containing each test microorganism were added to the wells of a microplate [15-17]. The microplates were then incubated at 37 ^{0}C for 24 h. After incubation, 40 μL of Triphenyl tetrazolium chloride (0.02 mg/ml, TTC) were added to each microplate well. Color changes of TTC in the microplate from colorless to red were accepted as microbial growth [18]. The MIC was determined as the lowest concentration that inhibited the visible growth of the test microorganism.

3. Results and discussion

We analyzed nanoAg in a colloidal state, taking into account the possible applications of these nanoparticles in different fields such as medical devices, water filters or textiles. Turbidimetric measurements were carried out to reveal the complete dissolution of the powder-based precursors in the solutions. Turbidity values of the transparent silver solutions prepared were in the range of 4.29 – 4.74 nephelometric units, indicating that the powder based chemical precursors were completely dissolved in the solutions. It is important to note that the pH value of silver sols is a factor which influences for the formation of the polymeric three-dimensional structure of the gel during the gelation process; it should be taken into consideration when preparing solutions. While ramified structure is randomly formed in acidic conditions, separated clusters are formed from solutions showing basic characters as mentioned in [5]. The pH values of silver based sols have a mildly acidic pH value of 4.1 to 4.6.

The dependence of the viscosity on the shear rate or test time is known as a characteristic feature of much sol - gel solutions. Fig 1 depicts the measured viscosity for Ag solution as a function of the test time. The viscosity curve illustrates the viscosity as a function of increasing test time. However, when the subsequent decline in the test time was probed (not shown here), no significant hysteresis effects were detected; that is, the up-ramp curve and down-ramp curve practically coincided. The decrease in viscosity with increasing test time could be attributed to the breakup of association complexes or network junctions; that is, the rate of disruption of the complexes exceeded the rate at which associations were re-formed. It was determined that the viscosity of the Ag solution was approximately equal to 2.55 mPa.s. The viscosity value of the solution was a key factor in controlling the film thickness. Because the thin films were formed from diluted solutions, these results were reasonable for sol – gel processing. In our case, Ag films were obtained with low-viscosity diluted solutions.

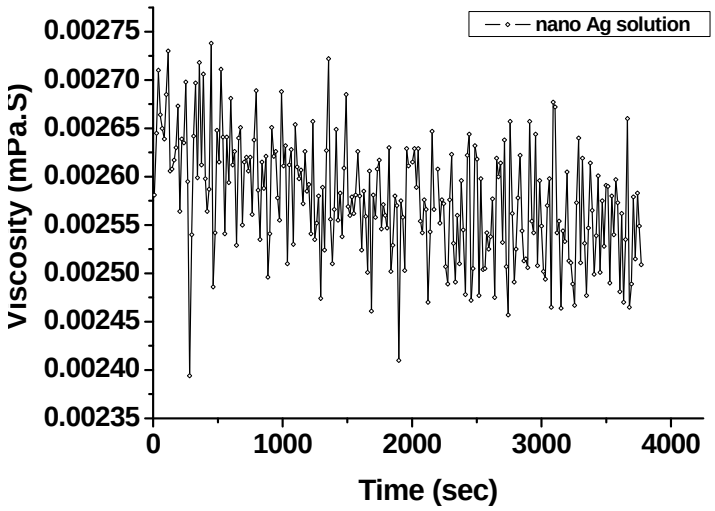

Fig. 1. Viscosity value of the prepared Ag transparent solution.

Contrary to the bactericidal effects of ionic silver, the antimicrobial activity of colloid silver particles are influenced by the dimensions of the particles; the smaller the particles, the greater the antimicrobial effect [19]. Inasmuch as particle size of Ag nanoparticles is a significant issue in killing microorganisms, surface topography was conducted on films obtained from colloidal silver solutions by Atomic Force Microscopy in order to show morphological changes and to determine the size of the nanoparticles on the surface. Almost all of the silver nanoparticles determined as small particles were equally spread and uniformly distributed (Fig. 2).

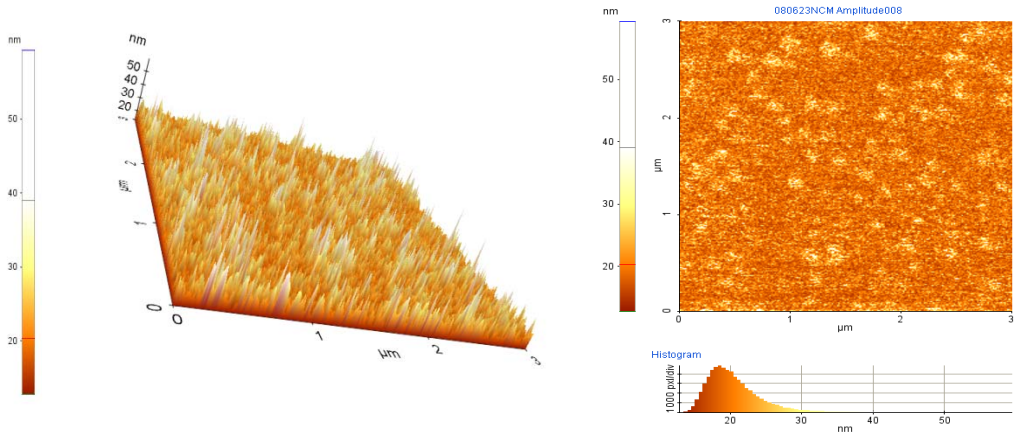

Fig. 2. AFM images of the nano Ag film surface.

The size of particles was in the range ~20–45 nm. Smaller sized Ag nanoparticles have many positive attributes, such as good conductivity, chemical stability, and catalytic and antibacterial activity, which would make them suitable for many practical applications.

The antimicrobial effects of the colloidal nano silver solutions were measured by determining the minimum concentration needed to inhibit the growth of tested microorganisms. MIC values of the colloidal nano silver solutions against test microorganisms are given in Table 1. Table 1 shows that all tested microorganisms were completely inhibited at the concentration of 2–4 µg/ml of nano silver. The colloidal nano silver solution at the concentration of 3 µg/ml of nano Ag showed inhibition kinetics against *E .coli, B. subtilis, S. thryphimurium* and *C. albicans* while 2 µg/ml of nano Ag was found to be the most effective against *P. aeruginosa*. The MIC of nano silver against *K. pneumoniae* and *S. aureus* was also 4 µg/ml. Thus, we can conclude from the results of this study that the colloidal silver nanoparticles inhibited the growth and multiplication of all the tested microorganisms, including the fungus *C. albicans.*

Table 1. MIC of colloidal nano silver solutions against tested microorganisms (µg/ml).

	S.aureus	*P.aeruginosa*	*B.subtilis*	*C.albicans*	*S.thyphimurium*	*E.coli*	*K.pneumoniae*
MIC of nano Ag solution (µg/ml)	4	2	3	3	3	3	4

A great many studies carried out recently demonstrate the antimicrobial activity of aqueous suspensions of silver nanoparticles. Cho et al., 2005 [20], investigated the antimicrobial activity of silver nanoparticles prepared using various stabilizers, such as sodium dodecylsulfate (SDS) and poly-(N-vinyl-2-pyrrolidone) (PVP) and found that MIC values of Ag nanoparticles against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) were 5 µg/mL and 10 µg/mL respectively. SEM investigations of the morphology changes of those bacteria showed that the surfaces of the cell walls of both bacteria were disrupted by Ag nanoparticles. MIC results obtained in our study resembled those reported by Cho et al. For PVP stabilized silver nanoparticles in the case of *S. aureus*.

Raffi et al., 2008 [21], reported that silver nanoparticles (mean size 16 nm) synthesized by the inert gas condensation (IGC) method were an effective bactericide against *Escherichia coli* at a concentration of 60 µg/mL and higher. Jain et al., 2009 [22], synthesized silver nanoparticles by a proprietary process that involved photoassisted reduction of Ag+ to metallic nanoparticles and

their biostabilization. The MIC90 values of the silver nanoparticles obtained (size range 7-20 nm) were 6.25 μg/ml for the bacterial cultures tested of *E. coli*, *P. aeruginosa*, *S.typhimurium* and *B. subtilis* and 12.5μg/ml for *S. aureus* and *C. albicans*. The reported MIC results are higher then those obtained by us in the present study, which suggests that the antimicrobial activity of nano silver may be influenced by preparation method as well as by particle size. In support of this, in a study of the synthesis of silver nanoparticles of varying sizes using glucose, galactose, maltose and lactose, it was declared that the antimicrobial property of these nanoparticles against many Gram-positive and Gram-negative bacteria including multidrug-resistant strains was depended on the size of the particles [11]. The silver nanoparticles obtained in that study by the reduction of a silver ammoniac solution with glucose were 44 nm in size and exhibited MIC of 6.75 μg/ml against *S. aureus*, and 27 μg/ml against *E. coli, K. pneumonae and P. aeruginosa*. Although our particles were approximately the same size (20-45 nm), the difference in MIC results against the test microorganisms might be due to the strains used.

4. Conclusıons

In summary, silver nanoparticles with mean sizes of 20-45 nm were successfully synthesized using the sol-gel method. Turbidity values of the prepared silver transparent solutions were in the range of 4.29-4.74 nephelometric units. The pH values of silver-based sols are mildly acidic with a pH value of 4.1 to 4.6. It was determined that the viscosity of the Ag solution was approximately equal to 2.55 mPa.s.

MIC results have demonstrated that synthesised colloidal dispersions show a pronounced antibacterial effect, as evidenced by a very low concentration of 2–4 μg/ml of nano silver against all tested microorganisms. This very high activity could be related to the advantages induced through the sol-gel method since we can obtain very small nanoparticles without significant contamination of their surfaces, which makes them very active against microorganisms. Under this framework, further studies are focusing on the investigation of the antimicrobial performance of colloidal nano silver on leather material.

Acknowledgement

We would like to thank TÜBITAK (The Scientific and Technological Research Council of Turkey) for the financial support (Project №107M201).

References

[1] M. Kokkoris, C. C. Trapalis, S. Kossionides, R. Vlastou, B. Nsouli, R. Grotzschel et al., Nucl. Instr. Methods Phys. Res. B **67,** 188 (2002).
[2] M. Kawashita, S. Tsuneyama, F. Miyaji, T. Kokubo, H. Kozuka, K. Yamamoto, Biomaterials**,** **21**, 393. 2000
[3] G. De, L. Tapfer, M. Catalano, G. Battaglin, F. Caccavale, F. Gonella et al., J. Appl. Phys. Lett. **68,** 3820 (1996).
[4] Y. Cao, W. L. Dai, J. F. Deng, Mater. Lett. **50,** 12 (2001).
[5] C. J. Brinker, G. W. Scherer, Sol–gel Science: The Physics and Chemistry of sol–gel Processing, Academic Press, San Diego (1990).
[6] M. Uchida, Chem. Ind. **46,** 48 (1995).
[7] N. Grier, Silver and Its Compounds in Disinfection, Sterilization and Preservation edited S.S. Block, Lea & Febiger, Philadelphia, p.375 (1983).
[8] A. Lehninger, D. Nelson, M. Cox, Principles of Biochemistry, second ed., Worth, New York (1993).
[9] S. Liau, D. Read, W. Pugh, J. Furr, A. Russell, Lett. Appl. Microbiol. **25,** 279 (1997).
[10] K. Yliniemi, M. Vahvaselka, Y.V. Ingelgem, K. Baert, B.P. Wilson, H. Terryn et al., J. Mater. Chem. **18,** 199 (2008).

[11] A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova et al., J. Phys. Chem. **B 110,** 16248 (2006).
[12] D. J. Kim, S. H. Hahn, S. H. Oh, E. Kim, J. Mater. Lett. **57,** 355 (2002).
[13] J. Yan, J. Cheng, United States Patent 6979491, Beijing, China (2005).
[14] National Committee for Clinical Laboratory Standards, Wayne, Pa (1993).
[15] C. R.Olson, T. Balasubramaniam, J. Shrum, T. Nord, P.L. Taylor, R.E. Burrell, Novel Antimicrobial Activity of Nanocrystalline Silver Dressings, Int. Congress on MEMS (2005).
[16] S. Sarkar, A. D. Jana, S. K. Samanta, G. Mostafa, J. Polyhedron **26,** 4419 (2007).
[17] F. B. Holetz, G. L. Pessini, N. R. Sanches, D. A. Garcia Cortez, C. V. Nakamura, B. P. Dias Filho, Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases, Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol.97(7): p.1027-1031 (2002).
[18] V. Navarro, M. L. Villareal, G. Rojas, X. Lozoya, J. Ethnopharmacol. **53,** 143 (1996).
[19] A. R. Shahverdi, A. F. Pharm, H. R. Shahverdi, S. Minaian, Nanomedicine: Nanotechnology, Biology, and Medicine **3,** 168 (2007).
[20] K. H. Cho, J. E. Park, T. Osaka, S. G. Park, Electrochimica Acta **51**, 956 (2005).
[21] M. Raffi, F. Hussain, T. M. Bhatti, J. I. Akhter, A. Hameed, M. M. Hasan, J. Mater. Sci. Technol. **24,** 192 (2008).
[22] J. Jain, S. Arora, J. M. Rajwade, P. Omray, S. Khandelwal, K. M. Paknikar, Molecular Pharmaceutics **6,** 1388 (2009).