JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 16, No. 1 - 2, January - February 2014, p. 182 - 187

Gelatinous silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity

M. DARROUDI[*], M. B. AHMAD[a], M. MASHREGHI[b]
Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[a]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, Selangor, Malaysia
[b]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

The major method used for synthesis of silver nanoparticles (Ag-NPs) is the chemical wet reduction method. The problem with the chemical reduction method is that the synthetic process is expensive and can also have toxic substances absorbed onto them. To overcome this, the “green” method provides a feasible alternative. This study demonstrated a green and facile approach for one-step synthesis of Ag-NPs at ambient temperature. It was found that gelatin can play double roles in the proposed synthesis, including a stabilizer and a reducing agent. Silver ions can be rapidly reduced by gelatin at room temperature due to its dominant reducing capacity. The effects of temperature and reaction times on particle size of Ag-NPs were studied. The capability in size control can be evidenced by TEM images. The antibacterial properties of the Ag-NPs were tested against different gram-positive and gram-negative bacteria. This approach reveals that green mediated method is a promising facile route for the size-controlled synthesis of gelatinous colloid Ag-NPs in aqueous solution with good antibacterial properties for different applications (e.g., in medical applications).

(Received February 11, 2013; accepted January 22, 2014)

Keywords: Green synthesis, Silver nanoparticles, Gelatin, Antibacterial

1. Introduction

Synthesis of nanoscale-sized materials with the desired quality and properties is one of the key issues in current nanotechnology [1]. Today, the “green” synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technologies in materials science [2]. Well-dispersed and ultrafine metal nanoparticles, especially transition metals, have great interest due to their distinctive physicochemical and thermodynamic properties, which have made them suitable for use in various fields, such as catalysis [3], optics [4], and medicine [5]. In particularly, metal nanoparticles such as gold, silver, and copper nanoparticles can show intense surface Plasmon resonance (SPR) absorption band in UV-vis and near infrared (NIR) region [6-8]. Most methods have been used to prepare Ag-NPs with desirable SPR by chemical reduction method, in which silver ions are reduced by chemically e.g., borohydride [9], hydrazine [10], ascorbic acid [11], glucose [12], citrate acid [13], ultrasonic [14], or photochemical method [15,16].

The use of environmentally friendly chemicals and solvents is central to materials synthesis and processing, considering the green chemistry rules. Natural materials such as gelatin, chitosan, proteins, and starch are all interesting materials for medical applications because they are biodegradable and bioabsorbable with degradation products that are non-toxic [17-19]. Gelatin is the protein from collagen and has a three-chain helical structure in which individual helical chains are stranded in a super-helix about the common molecular axis [20,21]. Gelatin contains positively and negatively charged as well as hydrophobic domains folded into a stable, nonnative state that minimizes hydrophobic interactions with water (when dissolved in it). Colloidal Ag-NPs, displaying very strong antibacterial activity against both gram-positive and gram-negative bacteria including multi-resistant strains [22-24]. The antibacterial effect of Ag-NPs as well as colloidal Ag-NPs or Ag nanocomposites has been seriously investigated recently [25-28], mostly due to the growing bacterial resistance to common antibiotics. Bactericidal activity of Ag-NPs may help in reducing infections when treating burns [29] as well as in preventing bacterial colonization of prostheses and catheters [30,31]. In this work, we have reported on the synthesis of Ag-NPs with gelatin as a reducing agent and capping agent. One of the advantages of this method is being able to use renewable materials like gelatin, which is eco-friendly agent.

2. Materials and methods

2.1 Chemicals

All reagents in this work were analytical-grade and were used as received without further purification. $AgNO_3$ (99.98%) was used as a silver precursor, and was provided by Merck, Germany. Gelatin (type B) was used as a stabilizer and a reducing agent for the preparation of Ag-NPs and was purchased from Sigma-Aldrich, MO, USA. All solutions were freshly prepared using double distilled water and kept in the dark to avoid any photochemical reactions. All glassware used in experimental procedures were cleaned in a fresh solution of HNO_3/HCl (3:1, v/v), washed thoroughly with double distilled water, and dried before use.

2.2 Green synthesis of colloidal silver nanoparticles

For synthesis of Ag-NPs, 1.0 g gelatin was added to 95 ml H_2O in a flask, and the solution was stirred and heated at 40°C to obtain a clear solution. To obtain the Ag^+/gelatin solution, the silver solution (5 ml, 1.0M) was added to the gelatin solution with continuous stirring. The prepared solution was distributed into 3 cuvettes, and were stirred and maintained at different temperatures (i.e., 28, 40, and 60 ˚C). Throughout the reduction process (48 hours), all solutions were kept in the dark to avoid any photochemical reactions. For investigation of reaction times on size of prepared Ag-NPs, the solution of Ag^+/gelatin (100 ml), was distributed into 5 cuvettes, and the prepared solutions were stirred and maintained for different periods of time: 1 (S1), 3 (S2), 6 (S3), 24 (S4), and 48 (S5) hours, respectively.

2.3 Antibacterial assay of Ag-NPs

Antibacterial effect of gelatinous Ag-NPs was evaluated using disk diffusion method against common Gram positive bacteria, i.e., *Staphylococcus aureus* (PTCC 1431), *Bacillus subtilis* (PTCC 1420) and Gram-negative bacteria, i.e., *Escherichia coli* (PTCC 1399), *Pseudomonas aeruginosa* (PTCC 1074) according to standard methods (NCCLS, 2000). Overnight culture of bacterial suspension was adjusted to 0.5 McFarland equivalents to 1.5×10^8 colony-forming units/ml. Dried surface of Muller–Hinton agar (MHA) inoculated with above bacterial suspension by streaking the swab three times and then 6 mm discs impregnated with 15 µl of gelatinous Ag-NPs solution were placed on inoculated agars. Besides discs impregnated with gelatin (0.1% v/v) considered as negative control. All plates were incubated at 37 □C for 24 hours. Gentamicin and Streptomycin were used as antibacterial standards against all pathogens. All experiments were performed in triplicate.

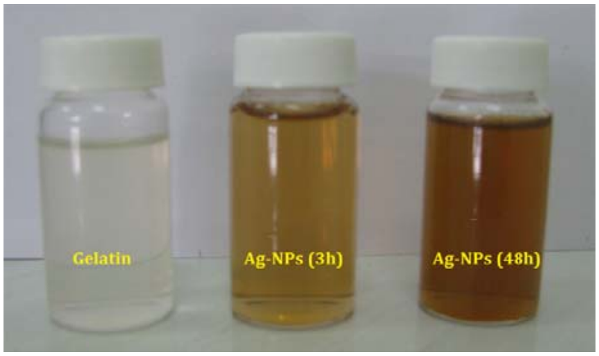
2.4 Characterization

The prepared colloidal Ag-NPs were characterized by using ultraviolet-visible (UV-vis) spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM). To ensure the formation of Ag-NPs, the particles were tested for their optical absorption property using a Lambda 35-Perkin Elmer UV-vis spectrophotometer over the range of 300 to 700 nm.

The crystalline structure of Ag-NPs was characterized with an XRD (Philips, X'pert, Cu K_α). The XRD patterns were scanned at a speed of 2°/min. The TEM observations were performed with a Hitachi H-7100 electron microscopy, and the particle size distributions were determined using UTHSCSA Image Tool software (Ver. 3.00).

3. Results and discussion

Depending on temperature, the reaction solutions display a spectrum of colorless to dark brown colors, indicating the formation of Ag-NPs in the gelatin solution (Scheme 1).



Scheme 1. Photography scheme of the gelatinous colloid Ag-NPs synthesized at different reaction times.

Gelatin is a polypeptide including praline, glutamic acid, lysine, etc., can be used as reducing and stabilizing agent in synthesis of noble metallic nanoparticles. The functional group of gelatin such as $-NH_2$, $-SH$, and $-COOH$ endow it as reducing and stabilizing agent to reduce transition metal ions. The gradual reduction of silver ions and formation of Ag-NPs was investigated by UV-vis spectroscopy, which has proven to be a useful spectroscopic method for the detection of prepared Ag-NPs in solution. In UV-vis spectra, the Ag-NPs can be shown by a SPR peak at around 400-450 nm, but a small shift (blue-shift or red-shift) in the wavelength of the peak could be related to obtaining Ag-NPs in different shapes, sizes or solvent dependences of prepared Ag-NPs.

After reaction at 28˚C, the obtained Ag-NPs showed a broad SPR band, a characteristic UV-vis absorption peak for Ag-NPs, centered at 445 nm. As shown in Fig. 1, the intensity of the SPR peak increased as the reaction temperature increased, which indicated the continued reduction of the silver ions, and the increase of the absorbance with the reaction temperature indicates that the concentration of Ag-NPs increases [32].

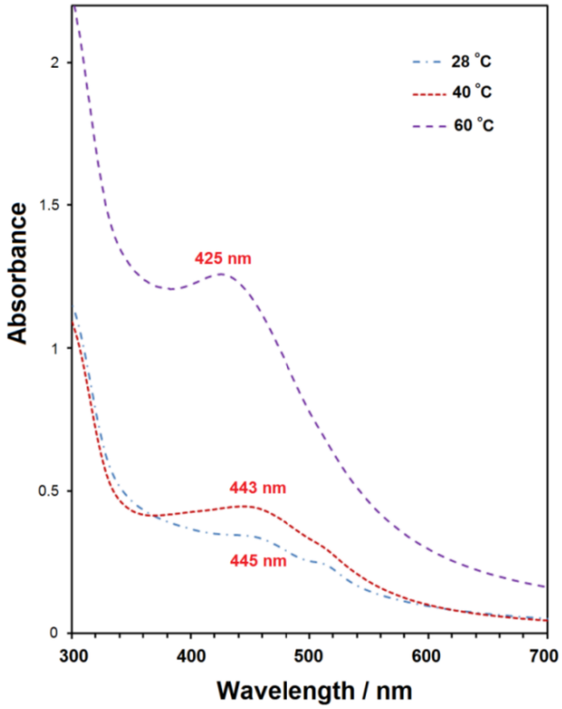


Fig. 1. UV-vis absorption spectra of gelatinous colloid Ag-NPs synthesized at different reaction temperatures (48 hours).

When the reaction temperature reached 40 ˚C the absorbance was increased, and the λ_{max} value was slightly blue-shifted to 443 nm. In reaction temperature of 60 ˚C, the absorbance was considerably increased and blue-shifted to 425 nm. This phenomenon indicated that the size of particles was decreased because the absorbance peak due to the SPR of metal nanoparticles shows the blue-shift with decreasing in the particle size [33]. At the initial stage of the reaction, the Ag-NPs formed with a broad size distribution, which led to a SPR peak at about 445 nm. After this stage, the Ag-NPs could dissociate due to heating to form smaller particles stabilized by the amine pendant groups on the gelatin, which leads to the formation of gelatin-stabilized stable Ag-NPs [34]. The effect of reaction times on size of synthesized Ag-NPs was investigated via UV-vis spectra. Gradually longer reaction times, from 1 to 48 hours, increase the corresponding SPR peak intensities (Fig. 2), with concomitant blue shifts from 455 to 425 nm. It can be seen that the smaller Ag-NPs was obtained under longer reaction times because the SPR of metal nanoparticles displays the blue shift with decreasing in the particle size [32].

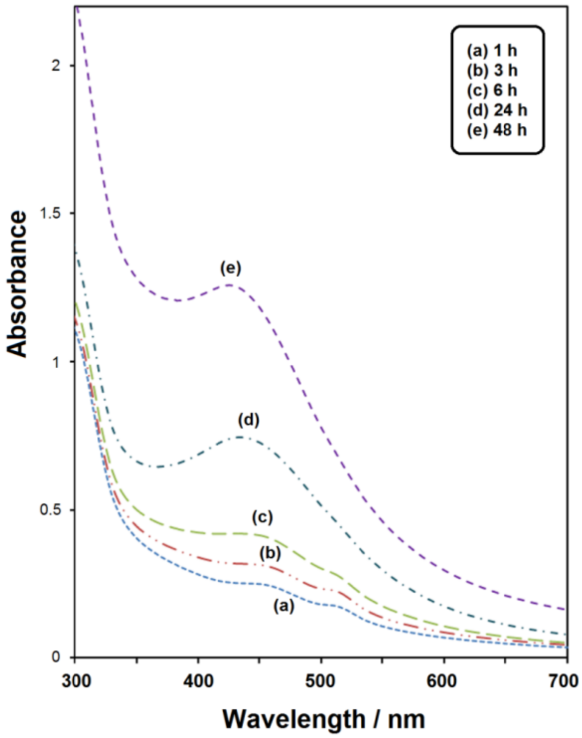


Fig. 2. UV-vis absorption spectra of gelatinous colloid Ag-NPs synthesized at different reaction times (60˚C).

The TEM images demonstrate the formation of Ag-NPs at different periods of time. Fig. 3 shows TEM images and the corresponding particle size distribution of the prepared Ag-NPs at different times. The TEM results indicate that the samples obtained over a longer time period retained a narrower particle size distribution; the average size of all prepared Ag-NPs was less than 10 nm; and a smaller average size (about 3.5 nm) was obtained for S5.

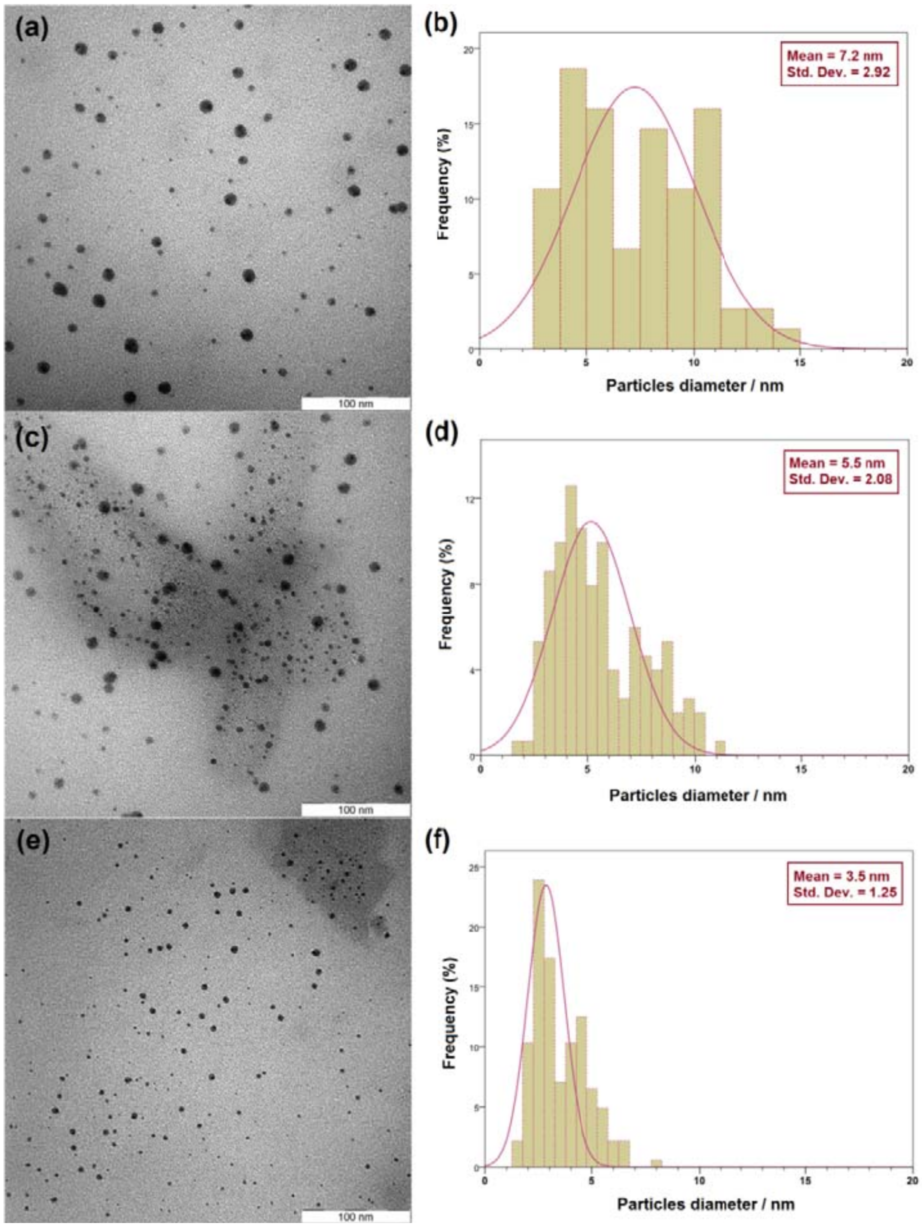


Fig. 3. TEM images and corresponding particles size distributions of gelatinous colloid Ag-NPs at different reaction times; 3 (a, b), 24 (c, d), and 48 (e, f) hours.

Fig. 4 shows the XRD patterns of Ag-NPs formed in S5, which indicates the formation of the silver crystalline structure. The XRD peaks at 2θ degrees of 38.1, 46.2, 64.6, and 77.3 can be attributed to the (111), (200), (220), and (311) crystalline planes of face-centered-cubic (fcc) crystalline structure of metallic silver, respectively (JCPDS file no. 00-004-0783).

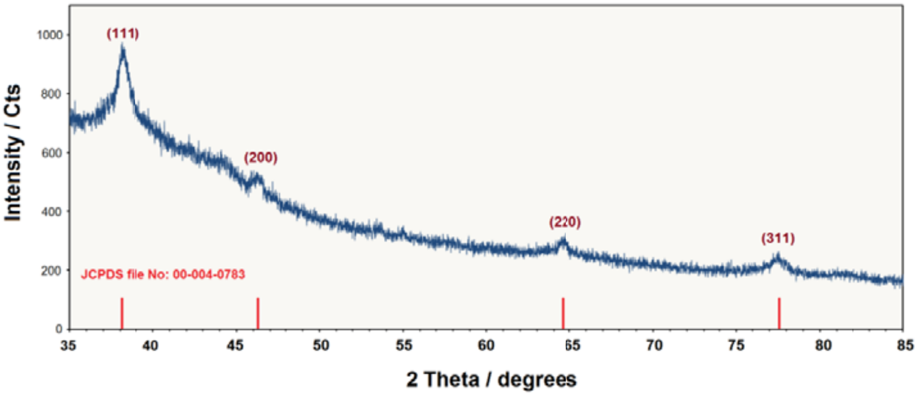


Fig. 4. XRD pattern of synthesized gelatinous colloid Ag-NPs (S5).

The antibacterial activity of the Ag-NPs/gelatin against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* was tested by disk diffusion test. Inhibition zone values represented as averages in Table 1 and Fig. 5. However, results showed the antimicrobial activity of gelatinous Ag-NPs against some of common gram positive and gram negative bacteria.

Table 1. Average inhibition zone and standard deviation for synthesized gelatinous colloid Ag-NPs at 60˚C for 48 hours ([a]NA= Not appear, [b]GM= Gentamicin, [c]S = Streptomycin).

Bacteria	Inhibition zone (mm)			
	Test	**Control negative**	**Control positive**	
	(Gelatinous Colloid Ag-NPs)	**Gelatin**	**GM[b]**	**S[c]**
S. aureus	10.3 ± 1.1	NA[a]	15	15
B. subtilis	9.3 ± 1.5	NA	19	14
E. coli	8.6 ± 0.6	NA	16	15
P. aeruginosa	12.6 ± 0.5	NA	16	13

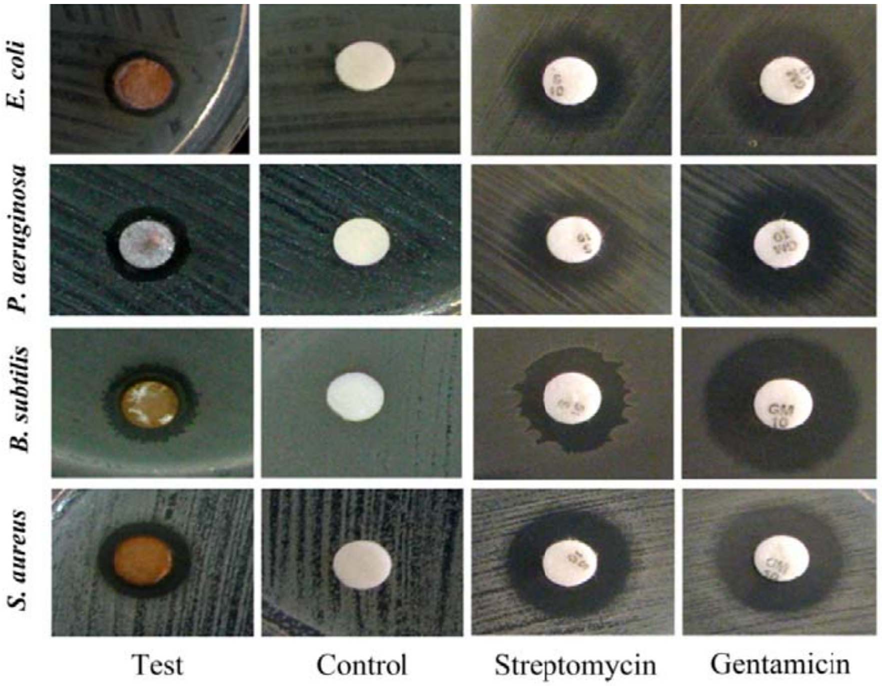


Fig. 5. Antibacterial activity of synthesized gelatinous colloid Ag-NPs (60˚C, 48 hours).

4. Conclusion

We demonstrate a facile and effective approach for the synthesis of antibacterial Ag-NPs by green chemistry method. By this method, the Ag-NPs with diameter of below 10 nm can be synthesized through a one-step procedure at ambient conditions. The results reveal that the gelatin molecules can exhibit double-functional role in our proposed approach, including a reducing and a capping agent. This preparation method is eco-friendly and may be extended to other noble metals, such as Au, Pd, and Pt, and may possibly find different additional medicinal and industrial applications.

References

[1] D. Bhattacharya, R. K. Gupta, Crit. Rev. Biotechnol. **25**, 199 (2005).
[2] M. Darroudi, M. B. Ahmad, A. H. Abdullah, N. A. Ibrahim, Int. J. Nanomed. **6**, 569 (2011).
[3] R. Narayanan, M. A. El-Sayed, J. Phys. Chem. B **109**, 12663 (2005).
[4] A. Eychmuller, J. Phys. Chem. B **104**, 6514 (2000).
[5] O. V. Salata, J. Nanobiotechnol. **2**, 3 (2004).
[6] M. A. El-Sayed, Acc. Chem. Res. **34**, 257 (2001).
[7] M. Darroudi, A. K. Zak, M.R. Muhamad, N. M. Huang, M. Hakimi, Mater. Lett. **66**, 117 (2012).
[8] R. Zamiri, Z. Aazmi, M. B. Ahmad, M. Darroudi, K. Shameli, M. A. Mahdi, J. Optoelectron. Adv. Mater. **12**, 1083 (2010).
[9] K. Shameli, M. B. Ahmad, W. Z. W. Yunus, N. A. Ibrahim, M. Darroudi, Int. J. Nanomed. **5**, 743 (2010).
[10] C. Aimé, I. B. Rietveld, T. Coradin, Chem. Phys. Lett. **505**, 37 (2011).
[11] X. Jiang, A. Yu, J. Nanosci. Nanotechnol. **10**, 7643 (2010).
[12] M. Darroudi, M. B. Ahmad, A. H. Abdullah, N. A. Ibrahim, K. Shameli, Int. J. Mol. Sci. **11**, 3898 (2010).
[13] G. Zhoua, M. Lu, Z. Yanga, H. Zhanga, Y. Zhoua, S. Wanga, S. Wanga, A. Zhanga, J. Cryst. Growth **289**, 255 (2006).
[14] M. Hakimi, M. Darroudi, A. Khorsand Zak, M. R. Muhamad, H. A. Hosseini, J. Optoelectron. Adv. Mater. **6**, 840 (2012).
[15] M. Darroudi, M. B. Ahmad, K. Shameli, A. H. Abdullah, N. A. Ibrahim, Solid State Sci. **11**, 1621 (2009).
[16] M. Darroudi, M. B. Ahmad, A. K. Zak, R. Zamiri, M. Hakimi, Int. J. Mol. Sci. **12**, 6346 (2011).
[17] M. R. Mozafari, Nanomaterials and Nanosystems for Biomedical Applications, Springer, Dordrecth, 2007.
[18] J. Jiao, X. Liu, W. Gao, C. Wang, H. Feng, X. Zhao, L. Chen, Solid State Sci. **11**, 976 (2009).
[19] K. Raja, A. Saravanakumar, R. Vijayakumar, Spectrochim. Acta A, **97**, 490 (2012).
[20] C. N. Likos, K. A. Vaynberg, H. Lowen, N. J. Wagner, Langmuir **16**, 4100 (2000).
[21] Y. Ikada, Y. Tabata, Adv. Drug Deliv. Rev. **31**, 287 (1998).
[22] A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, J. Phys. Chem. B **110**, 16248 (2006).
[23] M. B. Ahmad, K. Shameli, M. Darroudi, W. M. Z. W. Yunus, N. A. Ibrahim, A. A. Hamid, M. Zargar, Res. J. Biol. Sci. **4**, 1156 (2009).
[24] J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramirez, Nanotechnology **16**, 2346 (2005).
[25] E. Falletta, M. Bonini, E. Fratini, A. L. Nostro, G. Pesavento, A. Becheri, J. Phys. Chem. C **112**, 11758 (2008).
[26] S. Shrivastava, T. Bera, A. Roy, G. Singh, P. Ramachandrarao, D. Dash, Nanotechnology **18**, 225103 (2007).
[27] W. C. Chiang, C. Schroll, L. R. Hilbert, P. Moller, T. Tolker-Nielsen, Appl. Environ. Microbiol. **75**, 1674 (2009).
[28] S. Pal, Y. K. Tak, J. M. Song, Appl. Environ. Microbiol. **73**, 1712 (2007).
[29] B. S. Atiyeh, M. Costagliola, S. N. Hayek, S. A. Dibo, Burns **33**, 139-148 (2007).
[30] J. Hardes, H. Ahrens, C. Gebert, A. Streitbuerger, H. Buerger, M. Erren, Biomaterials **28**, 2869 (2007).
[31] M. E. Rupp, T. Fitzgerald, N. Marion, V. Helget, S. Puumala, J. R. Anderson, Am. J. Infect. Control **32**, 445 (2004).
[32] C.F. Bohren, D.R. Huffman, Wiley, New York, 1983.
[33] J. R. Heath, Phys. Rev. B **40**, 9982 (1989).
[34] J. J. Zhang, Anal. Chem. **81**, 6641 (2009).

---

[*] Corresponding author: darroudim@mums.ac.ir, majiddarroudi@gmail.com