Korean J. Chem. Eng., **27**(6), 1897-1900 (2010)
DOI: 10.1007/s11814-010-0299-z

Fast preparation of citrate-stabilized silver nanoplates and its nanotoxicity

Jinkyu Roh*, Eun-Jung Park, Kwangsik Park**, Jongheop Yi***, and Younghun Kim*,†**

*Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Korea
**College of Pharmacy, Dongduk Women's University, Seoul 136-714, Korea
***School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Korea
(*Received 12 January 2010 • accepted 26 March 2010*)

Abstract−Citrate-stabilized silver nanoplates (AgNPs) were prepared via a seed-mediated method without surfactants, such as cetyltrimethylammonium bromide (CTAB), in a short amount of time (15 min). Silver seeds with 3-4 nm in diameter were added to a growth solution containing $AgNO_3$, trisodium citrate (TSC) and L-ascorbic acid (AA). The size of the AgNPs depended on the concentration of the silver seed and TSC. The physical properties of the AgNPs were analyzed by transmission electron microscopy (TEM) and by an ultraviolet-visible (UV-vis) spectrophotometer. In addition, we tested the nanotoxicity of AgNPs prepared in TSC solution to the spleen of a rat, and found that AgNPs induced inflammation and white spots on the surface of the spleen.

Key words: Silver Nanoparticles, Nanoplates, Citrate, Nanotoxicity

INTRODUCTION

With the rapid growth of nanotechnology, various nanomaterials are being produced and used as raw materials for nano-consumer products. Nanomaterials have unique physicochemical properties that are dependent on their base material and morphology [1]. Therefore, many researchers are focused on novel synthesis methods and shape control of nanoparticles. In particular, gold and silver nanoparticles possess optical and electrical stability that can be applied to catalysts, sensors, medical devices and electrical material [2-5]. In 1951, Turkevich first invented a preparation method for gold nanoparticles via the reduction of gold precursors ($HAuCl_4$) [6]. Based on this research, Lee's group prepared silver nanoparticles using a citrate reduction method in 1982 [7], which used a plasmonic method [8-11] to control the shape and size of surfactants, polymers and biomaterials (such as DNA and proteins). A seed-mediated growth method was invented by Murphy and has been one of the most common methods for the preparation of gold and silver nanoparticles [12].

However, environmental, health and safety (EHS) risks of nanoparticles have recently emerged. It has come to light that nanoparticles might induce hazardous effects on bio-organisms by penetration into the cell membrane. In addition, the level of cytotoxicity is dependent on the unique physicochemical properties of the nanoparticles. For example, silver nanoparticles, which are a strong antibiotic material, were found to cause cell necrosis [13]. Therefore, *in-vivo* and *in-vitro* cytotoxicity testing of as-made nanomaterials has been necessary to quantify and qualify their nanotoxicity.

To exclude the effect of additives such as surfactants on cytotoxicity, silver nanoparticles should be prepared using non-toxic stabilizers [14,15]. The Organization for Economic Co-operation and Development (OECD) recommends using citrate for the stabilization of nanoparticles during silver nanotoxicity testing [16]. Citrate is a well-known non-toxic material that is used as a food additive and a blood anticoagulant. In the nanoparticle formation process, citrate was used to cap the particle surface, which changed the surface charge from positive to negative, thereby stabilizing the nanoparticles in solution.

Toxicity of silver nanosphere has being studied with live cells or mice by in-vivo or in-vitro tests [13]. Several studies had reported that silver nanosphere significantly induced necrosis or apoptosis of cells in several in vivo and in vitro tests. For example, silver nanosphere with 3 nm induced cytotoxicity in macrophages [17]. A decrease of cell viability was also observed in liver and neuron cells treated with silver nanoparticles [18,19]. However, nanotoxicity research for different-shaped silver nanoparticles was less reported. Thus, we want to know the nanotoxicity for silver nanoplates. Different shape of nanoparticles might be different behavior in bio-organism, due to morphological and physical differences. Therefore, in the present study, silver nanoplates (AgNPs, not silver nanosphere) were prepared using a seed-mediated growth method in the presence of citrate at room temperature. The shape and size of the AgNPs was changed using concentrations of citrate, silver seed, and aging time. The cytotoxicity of the AgNPs was verified by animal experimentation (rat).

EXPERIMENTAL SECTION

1. Synthesis of Silver Nanoplates (AgNPs)

Silver seed was prepared by a drop-wise method that added 0.6 ml of 0.01 M $NaBH_4$ (Sigma-Aldrich) solution to 1 ml of 0.018 M $AgNO_3$ (Sigma-Aldrich) in 20 ml of DI water with 0.1 ml of 0.017 M TSC (Sigma-Aldrich) under vigorous stirring. The color of the solution immediately changed to dark yellow after the $NaBH_4$ was added, which indicated particle formation. The particles in this solution were used as seeds within 2-5 h after preparation. Several growth solutions were prepared using different concentrations of TSC (0.025,

†To whom correspondence should be addressed.
E-mail: korea1@kw.ac.kr

0.050, 0.075 and 0.100 g) in 0.14 ml of 0.018 M $AgNO_3$, 0.1 ml of 0.1 M L-ascorbic acid (AA, Sigma-Aldrich) and 20 ml of DI water. Then, various amounts (0.50, 0.75, 1.00, 1.25 and 1.50 ml) of silver seed were added to the growth solution. In 15 min, the color of the solution changed quickly from colorless to orange/red, and, finally, to purple or blue, depending on the seed concentration. The silver nanoplates that were obtained were washed with DI water, followed by centrifugation at 12,000 rpm for 10 min. The morphology and UV-absorbance of AgNPs was analyzed by using transmission electron microscopy (TEM, JEM-1010, JEOL) and a UV-vis spectrometer (UV-1800, Shimadzu, Japan), respectively.

2. Nanotoxicity Test of AgNPs

For the nanotoxicity test, rats (SPF Sprague-Dawley rats aged 6 weeks, male) were intravenously injected with 4 mg/kg of prepared AgNPs. After 24 h, we observed a morphological change of the spleen surface of the controlled and tested rats.

RESULTS AND DISCUSSION

In the growing step of AgNPs, silver ions were gathered around silver seeds and citrates were capped onto the surface of the nanoparticles. Thus, the stability of nanoparticles in solution was improved and the transformation of shape was decreased, due to the dispersion force induced by the negative charge of the citrate-stabilized surface. The size of the nanoparticles was dependent on the concentration of silver seed. With a constant concentration of growth solution, when the amount of seed was increased, the particle size of the AgNPs was decreased. This was due to the comparative adsorption of silver ions onto the surface of the silver seeds. Therefore, we could easily control the size of the nanoparticles by controlling the amount of silver seed.

First, the size of the AgNPs was controlled by the concentration of TSC. AgNPs were synthesized by adding 0.5 ml of silver seed to the growth solution with various amounts of TSC. The AgNP solution showed a dark blue to a light blue color according to the concentration of TSC, as shown in the inset picture of Fig. 1. In the UV-vis spectra, the main peak (600 nm) of the AgNPs was red-shifted

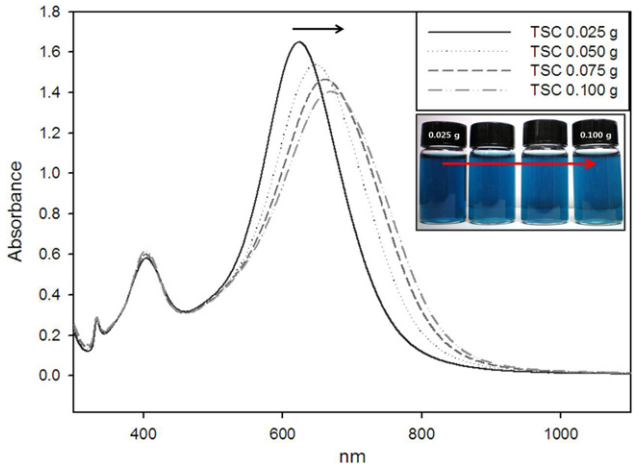

Fig. 1. UV-vis spectra of AgNP solutions in various concentrations of TSC (0.025, 0.050, 0.075 and 0.100 g). Inset: pictures of AgNP solutions according to concentration of TSC.

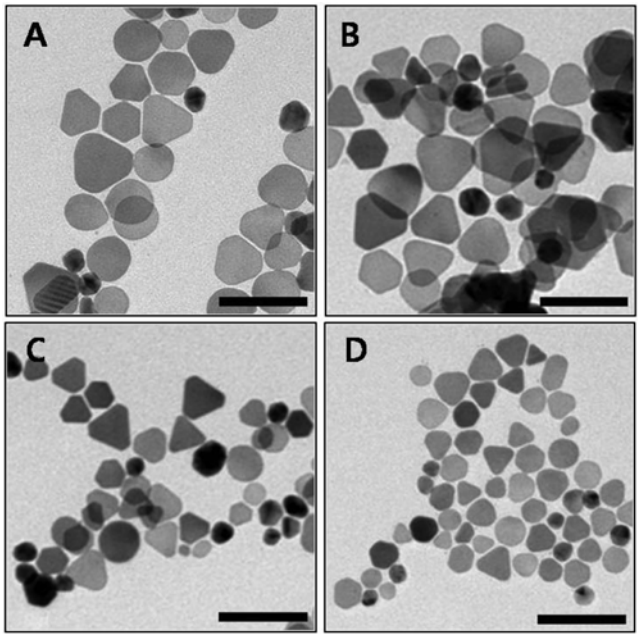

Fig. 2. TEM images of AgNPs: (A) TSC: 0.05 g and silver seed: 0.50 ml, (B) 0.10 g and 0.50 ml, (C) 0.10 g and 1.00 ml, and (D) 0.10 g and 1.50 ml (scale bar=100 nm).

with an increasing concentration of TSC. The three peaks shown in Fig. 1, weak, medium and strong, can be assigned to the out-of plane quadrupole resonance, in-plane quadrupole resonance and in-plane dipole plasmon resonance of the AgNPs, respectively [20, 21]. The in-plane dipole plasmon resonance was dependent on the edge length, and the third band among the three peaks showed only a peak shift. In UV-vis spectra, the first and second peaks were fixed at 333 nm and 404 nm, but the third peak made a red shift from 622 nm to 669 nm according to the amount of increased concentration of TSC. It was noted that AgNPs grew edge-directionally according to the amount of citrate, and confirmed by TEM data. AgNPs showed triangular or hexagonal shapes with an average diameter of 46±20 nm for TSC 0.05 g and 50±15 nm for TSC 0.10 g, as shown in Fig. 2(a) and 2(b), respectively. As-made silver nanoplates had {111} plane, which was similar with silver nanoplate prepared by photo-induced method [9]. However, as shown in Fig. 1, the third peak became broadened with the concentration of TSC. Peak broadening in UV spectrum was related to the volume of particles [22]. Width of peak for longitudinal plasmon resonance might be broadened, due to the high aspect ratio, heterogeneous particle sizes and mixture of different-shaped particle. This result showed that the excess concentration of TSC disturbed uniform growth of silver nanoplates. From these results, we concluded that the size of AgNPs could be controlled by control of the TSC concentration, but the change in size was not large enough to recognize the optical variation of the AgNPs.

Secondly, the size of the AgNPs could be adjusted by the concentration of the silver seed. Various amounts of silver seed (0.50, 0.75, 1.00, 1.25 and 1.50 ml) were added to the growth solution (0.10 g of TSC). The prepared AgNP solution showed various color changes according to the concentration of the silver seed; the colors ranged from blue to purple (or dark blue), as shown in the inset of

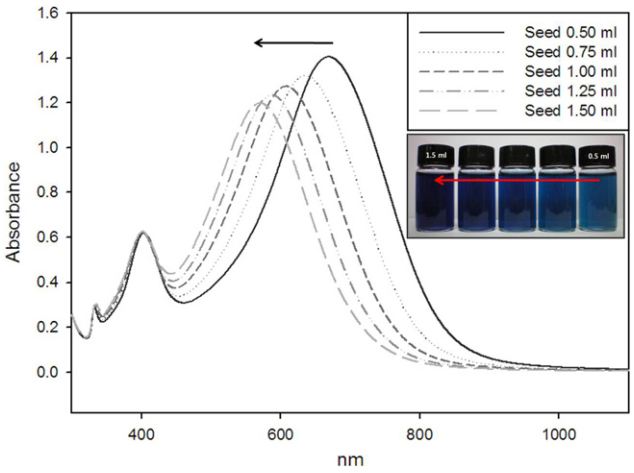

Fig. 3. UV-vis spectra of AgNP solutions in various concentrations of silver seed solutions (0.50, 0.75, 1.00, 1.25 and 1.50 ml). Inset: pictures of AgNP solutions according to concentrations of silver seed.

Fig. 3. The color of the nanoparticle solution was dependent on the size and shape of the nanoparticles, due to the plasmonic phenomena of the nanoparticles. The localized surface plasmon resonance (LSPR) was revealed when the incident photon oscillation frequency was resonant with the collective [21]. Therefore, we could expect the size of the AgNPs to be different, based on the various colors of the prepared AgNPs.

The UV-vis spectra (Fig. 3) of AgNPs showed three peaks; the first and second peaks were fixed at 333 nm and 404 nm, but the third peak was blue-shifted. When the concentration of the silver seed was higher, only the third peak was shifted from 669 to 571 nm. Namely, the color change was due to an edge-directional decrease in the nanoparticle size. In the TEM images (Fig. 2), the sizes of triangular and hexagonal AgNPs were 50±15, 41±7 and 28±9 nms when silver seed diameters were 0.5, 1.0 and 1.5 ml (Fig. 2(b), 2(c) and 2(d)), respectively. When the amount of silver seed was increased by 0.5 ml, the size of the nanoparticles was decreased by *ca.* 10 nm.

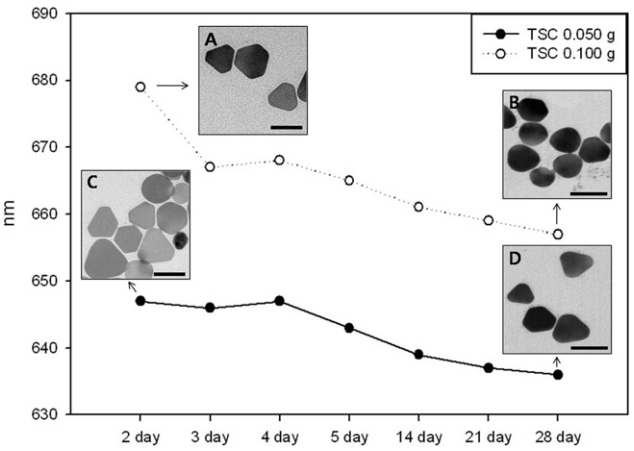

Fig. 4. Stability test of AgNPs during 4 weeks. Inset: TEM images of AgNPs at (A and C) day 2 and at (B and D) day 28 (scale bar=50 nm).

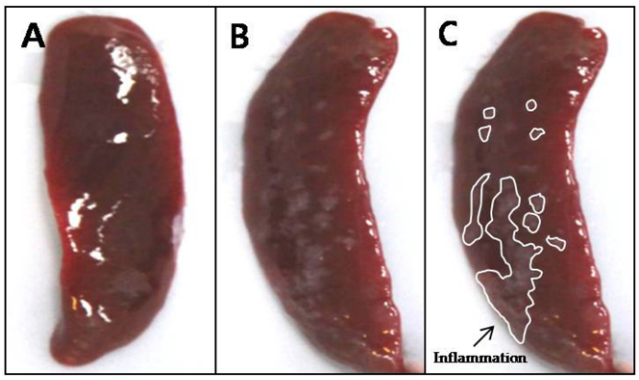

Fig. 5. Images of the spleens of AgNP-treated rats after 24 h: (A) control, (B) treated spleen, and (C) inflammations spots of (B).

In addition, we investigated the long-term stability of AgNPs, namely, shape variations with time, which was analyzed using UV-vis spectroscopy. Fig. 4 shows the change of the third peak in Fig. 1 from 2 to 28 days. Both points in Fig. 4 were decreased (blue-shift) from 647 to 637 nm for 0.05 g of TSC, and from 679 to 657 nm for 0.100 g of TSC, respectively. We can expect the size of AgNPs to decrease slightly by time through the effect of photo-oxidation. When citrate-coated AgNPs were exposed to light, the shape of the AgNPs was transformed due to the constant oxidization of the citrate on the surface of the nanoparticles. In particular, the carboxylic anion of citrate was easily oxidized by UV and then CO_2 was separated from the citrate; therefore, the eruption of silver ion was accelerated from the AgNPs because the citrate donated two electrons to the AgNPs [23]. As shown by the TEM images in the inset of Fig. 4, the shape of the AgNPs analyzed after 4 weeks had blunt edges. With the continuation of this process, we could expect the nanoplates to be transformed into nanodisks.

Citrate-coated AgNPs have long-term stability in the aqueous phase and are suitable for *in-vivo* cytotoxicity testing. Citrate is known as a non-toxic stabilizer, but it is not established whether citrate-stabilized silver nanoplates have a toxic or non-toxic feature in rats. In the present study, rats were treated with 4 mg/kg of AgNPs, and we observed the external appearance of the spleen after 24 h. The spleens of the AgNP-treated rats showed splenomegaly and cellular tissue damage, which meant that the function of the spleen was compromised when compared with the control group (Fig. 5(b)). When nanoparticles flow into human organs, the immune system activates. At this point, the spleen is very important for control of the immune system because it makes antibodies. The result with the tested rats suggests that the inflow of neutrophils, or lymphocytes, was not induced by macrophages ingesting impurities. In addition, our results support existing research that suggests that silver nanoparticles appear to exert their toxicity via a trojan-horse mechanism [24,25].

CONCLUSIONS

Citrate-stabilized AgNPs were prepared rapidly via a seed-mediated method and formed triangular and hexagonal nanoplates 20-50 nm of diameter. The sizes of AgNPs could be easily adjusted

by control of the TSC and silver seed. This preparation method of AgNPs had some advantages for simplicity and convenience, because the reaction could be conducted at room temperature and the reaction time was very short (15 min). The shape of the AgNPs was transformed by long-term photo-oxidation, but the shape stability was good because the transformation process was very slow. Generally, the citrate-coated silver nanoparticles with sphere type were known as less-toxic materials [25]. While, in this research, we can observe inflammation on the spleen of rat, which might be induced by injection of silver nanoplates. It is noted that platelet type was more toxic than spherical form. However, more detailed studies for the morphological cytotoxicity of nanoparticles should be investigated.

ACKNOWLEDGEMENT

This work was supported by the Research Grant of Seoul R&BD Program of Seoul City and Kwangwoon University in 2010. J. Yi is grateful for the MOE grants (No. 091-071-055).

REFERENCES

1. K. L. Kelly, E. Coronado, L. L. Zhao and G. C. Schatz, *J. Phys. Chem. B*, **107**, 668 (2003).
2. X. F. Wu, H. Y. Song, J. M. Yoon, Y. T. Yu and Y. F. Chen, *Langmuir*, **25**, 6438 (2009).
3. S. H. Wu, Y. S. Wu and C. H. Chen, *Anal. Chem.*, **80**, 6560 (2008).
4. C. K. Kim, P. Ghosh and V. M. Rotello, *Nanoscale*, **1**, 61 (2009).
5. H. H. Lee, K. S. Chou and K. C. Huang, *Nanotechnology*, **16**, 2436 (2005).
6. J. Turkevich, P. C. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, **11**, 55 (1951).
7. P. C. Lee and D. Meisel, *J. Phys. Chem.*, **86**, 3391 (1982).
8. J. L. H. Chau, M. K. Hsu, C. C. Hsieh and C. C. Kao, *Mater. Lett.*, **59**, 905 (2005).
9. J. An, B. Tang, X. Ning, J. Zhou, S. Xu, B. Zhao, W. Xu, C. Corredor and J. R. Lombardi, *J. Phys. Chem. C*, **111**, 18055 (2007).
10. G. Wei, H. Zhou, Z. Liu, Y. Song, L. Wang, L. Sun and Z. Li, *J. Phys. Chem. B*, **109**, 8738 (2005).
11. J. H. Fendler, *Korean J. Chem. Eng.*, **18**, 1 (2001).
12. C. J. Murphy, T. K. Sau, A. M. Gole, C. J. Orendorff, J. Gao, L. Gou, S. E. Hunyadi and T. Li, *J. Phys. Chem. B*, **109**, 13857 (2005).
13. P. V. AshaRani, G. L. K. Mun, M. P. Hande and S. Valiyaveettil, *ACS NANO*, **3**, 279 (2009).
14. A. M. Alkilany, P. K. Nagaria, C. R. Hexel, T. J. Shaw, C. J. Murphy and M. D. Wyatt, *Small*, **5**, 701 (2009).
15. V. K. Sharma, R. A. Yngard and Y. Lin, *Adv. Colloid Interface Sci.*, **145**, 83 (2009).
16. OECD, *Recommendations to OECD for common Nano-Ag testing materials*, Participants of the Joint NIST-ERDC Workshop on Nano Silver, Vicksburg, MS on April 7-8 (2009).
17. H.-J. Yen, S. Hsu and C.-L. Tsai, *Small*, **5**, 1553 (2009).
18. S. M. Hussain, K. L. Hess, J. M. Gearhart, K. T. Geiss and J. J. Schlager, *Toxicol. In Vitro*, **19**, 975 (2005).
19. S. M. Hussain, A. K. Javorina, A. M. Schrand, H. M. Duhart, S. F. Ali and J. J. Schlager, *Toxicol. Sci.*, **92**, 456 (2006).
20. S. Chen and D. L. Carroll, *Nano Lett.*, **2**, 1003 (2002).
21. R. Jin, Y. Cao, C. A. Mirkin, K. L. Kelly, G. C. Schatz and J. G. Zheng, *Science*, **294**, 1901 (2001).
22. W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, **79**, 4215 (2007).
23. R. L. Redmond, X. Wu and L. Brus, *J. Phys. Chem. C*, **111**, 8942 (2007).
24. E.-J. Park, J. Yi, Y. Kim, K. Choi and K. Park, *Toxico. In Vitro*, **24**, 872 (2010).
25. J. Abrega, S. R. Fawcett, J. C. Renshaw and J. R. Lead, *Environ. Sci. Technol.*, **43**, 7285 (2009).