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Natural Amino Acid Based Phenolic Derivatives for Synthesizing Silver Nanoparticles with Tunable Morphology and Antibacterial Studies

V. Vinod Kumar, S. Nithya, Aswin Shyam, N. Sai Subramanian, J. Tennis Anthuvan,[†] and Savarimuthu Philip Anthony[*]

School of Chemical & Biotechnology, SASTRA University, Thanjavur-613401, Tamil Nadu, India
[*]E-mail: philip@biotech.sastra.edu
[†]Department of Chemistry, M. Kumarasamy College of Engineering, Tamil Nadu, India
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Silver nanoparticles (AgNPs) with spherical and prism morphologies were formed at room temperature depend on the amino acid attached with phenolic unit. Absorption studies showed 410-420 nm surface plasmon resonance absorption for spherical nanoparticles whereas prism morphology showed three absorption peaks (382, 452 and 523 nm). The formation of spherical and prism morphology was confirmed by scanning and high resolution transmission electron microscopy. Antibacterial studies of both the morphologies did not show any significant differences in the inhibition of bacterial growth.

Key Words : Silver nanoparticles, Tunable morphology, Green synthesis, Antibacterial activity, Phenols

Introduction

Nanostructures of noble metals particularly silver nanostructures with 1 to 100 nm size has attracted significant interest over the years because of the unique size dependent optical, electrical and magnetic properties.[1,2] Importantly those unique properties of nanostructures have been successfully integrated in various applications such as catalysis, optics, electronics, biotechnology, and biological imaging.[3-8] The intrinsic properties of silver nanostructures are generally controlled by size, shape, composition, crystallinity and structure.[9] Hence over the years, several methods including chemical, photochemical and biological method have been developed to synthesize silver nanostructures with controlled dimension and phase.[10-12] Of late concern of biological and environmental hazardness caused by the use and release of toxic chemicals in chemical method necessitated environmentally sustainable AgNPs synthetic protocols.

The design of a clean or green synthesis of AgNPs by which size, morphology and stability can be controlled for biological applications has become a major field of research.[13] Although, biological approach that uses microorganisms and plant extracts provided environmentally clean and sustainable way to synthesis AgNPs, controlling of morphology was rarely achieved.[14]

Developing new organic molecules that could act as reducing as well as stabilizing agents without the use of toxic reducing agents and organic solvents have gained importance in green chemistry point of view. In this context, amino acids and vitamins have already been reported as eco-friendly agents in the synthesis of metal nanoparticles.[15] Phenolic compounds have attracted significant interest from various researchers due to their powerful antioxidant, antibacterial properties as well as their potential contribution to defend against oxidative stress and protect against cancer and cardiovascular diseases.[16,17]

Herein, we report the amino acid attached phenolic compounds as eco-friendly agents in the synthesis of spherical and prism morphology of AgNPs at room temperature. Amino acids and phenols were chosen because of their benign nature, highly reactive hydroxyl group and ubiquitous present in human and plant cell along with structural versatility. Antibacterial studies of spherical and prism AgNPs showed almost similar growth inhibition against *E. coli.* The hydrophilic functional groups of amino acid would enhance water solubility with opportunity for AgNPs bioconjugation.

Experimental

Materials. Amino acids, ethanol and NaOH were obtained from Ranbaxy fine chemicals and used as received. Salicylaldehyde, $NaBH_4$ and $AgNO_3$ were obtained from Merck and used as-received. Amino acid attached phenolic compounds were synthesized according to the reported procedure.[18]

General Procedure for Synthesis of AgNPs using L1-L8. Typically amino acid based reduced Schiff base molecule (5×10^{-2} and 10^{-3} M) was dissolved in aqueous solution using equimolar amount of NaOH. Different volume of (2 mL, 4 mL, 7 mL) 10^{-3} M silver nitrate solution was added into 10 mL of L under stirring at room temperature. The immediate appearance of yellow or brown colour indicated the reduction of silver ion into AgNPs. The solution was allowed to stir at room temperature for another 10 min. All reactions were repeated at least three times to confirm the reproducibility of nanoparticles formation.

Characterization. Absorption of AgNPs was analyzed in a Perkin Elmer model UV–Vis double beam spectrophotometer from 250 to 800 nm, at the resolution of 1 nm. The reduction of silver ions in aqueous solution as a function of

time was monitored by periodic sampling of aliquots (0.2 mL) of the suspension, then diluting the samples with 2 mL de-ionized water and subsequently measuring UV–vis spectra of the resulting diluents. AgNPs morphology, size and crystallinity were analyzed using field emission scanning electron microscope (FE-SEM) (JSM-6701F, JEOL Japan INC) and high resolution transmission electron microscopy (HR-TEM, JEOL JEM-2100F).

Zeta Potential.

The Zeta Potential Measurement: Measurements were carried out using a Zetasizer ver.6.20. The aqueous suspension of silver nanoparticles was taken in a cuvette. Zeta potential is measured by the principle of Electrophoretic mobility created by applying an electric field across the dispersion media.

Biological Studies. *E. coli* cultures were obtained from MTCC, Chandigarh, India. Cultures were maintained in the form of 10% glycerol stocks in −70 °C and were revived by routine sub-culturing on LB agar plates. The silver nanoparticles synthesized using phenolic amines was tested for antibacterial activity by standard disc diffusion method. Muller Hinton Agar plates were seeded with 24 h broth culture of different bacteria. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 10 mm diameter were made on nutrient agar plates using gel puncture. Using micropipette, 50-200 µL of AgNPs solution was poured onto each well on all plates. After allowing for diffusion at room temperature for 2 h, the plates transferred to an incubation chamber maintained at 37 °C for 24 h.

Results and Discussion

Molecular structures of amino acid based phenolic derivatives that are used to prepare AgNPs are shown in Figure 1. Addition of silver nitrate ($AgNO_3$, 10^{-3} M) into aqueous solution of phenolic compounds (L1-L8) produced yellow or intense brown colour depends on the amino acid coupled with phenolic moiety and that confirms the reduction of silver ions into AgNPs. It is well known phenomena that AgNPs exhibit various colour depending on the size, morphology and self-assembly and these colors generally arises due to excitation of surface plasmon resonance (SPR) in the metal nanoparticles.[19]

Figure 2(a) shows absorption spectra of AgNPs synthesized by adding 2 mL of $AgNO_3$ (10^{-3} M) into aqueous solution of L1-L8 that exhibited SPR single absorption peak between 410 nm to 422 nm. AgNPs prepared using L1-L5 and L7 exhibited absorption around 420 nm. AgNPs-L8a (1:1 NaOH) also showed absorption at 420 nm whereas AgNPs-L8b (1:2 NaOH) exhibited blue shifted absorption at 410 nm. AgNPs obtained from phenylalanine based compound (L6) exhibited broad absorption with three humps at 383 nm, 452 nm and 523 nm. The appearance of AgNPs absorption around 550 nm might be attributed to the formation of different morphology in the solution.[20] Morphological investigation discussed below also confirms the formation of

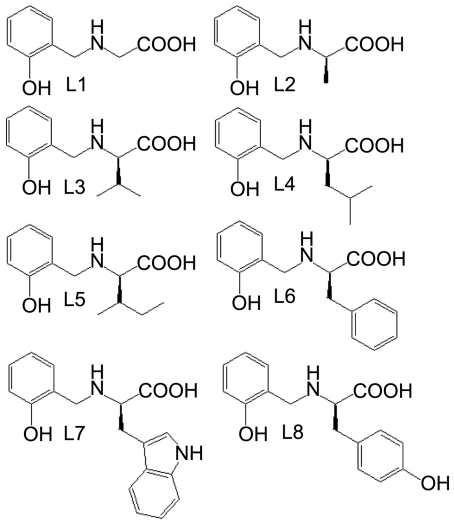


Figure 1. Molecular structures of the amino acid based phenolic molecules.

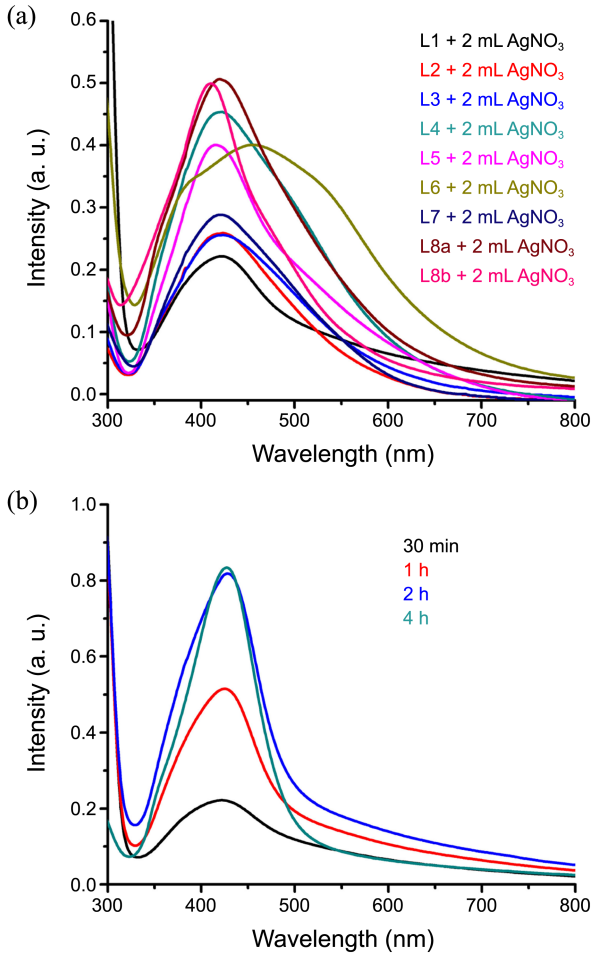


Figure 2. UV-Visible absorption spectra of AgNPs synthesized using L1-L8 (a) and time dependent absorption studies of AgNPs formation with L1 (b).

spherical as well as prism shaped AgNPs. Time dependent studies reveal that silver ion reduction by L1 has been robust and completed within 2 h and further increasing reaction

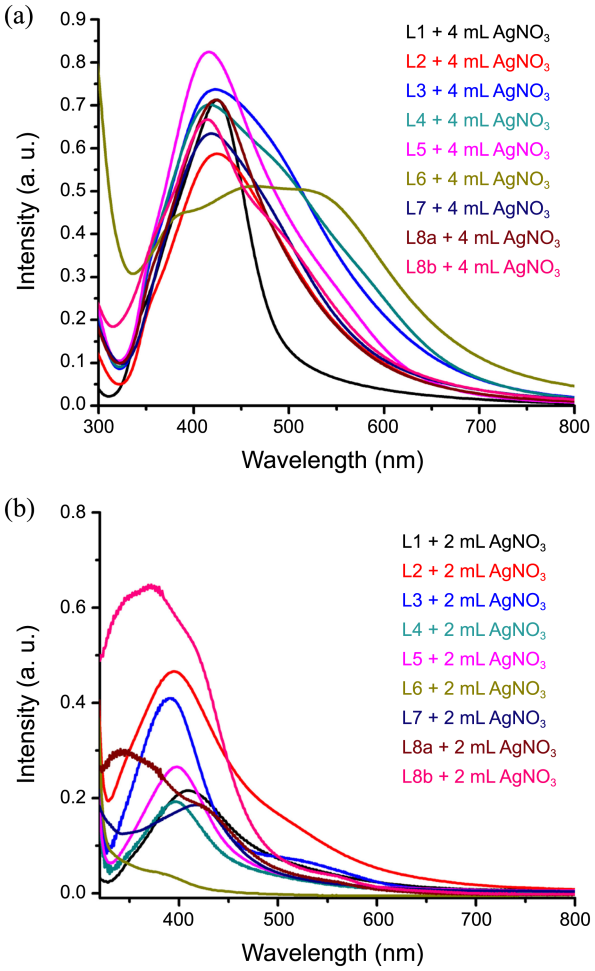
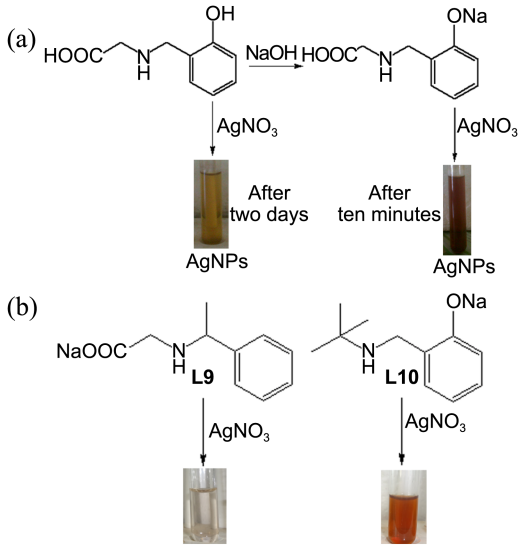


Figure 3. UV-Visible absorption spectra of AgNPs synthesized with L1-L8 using 4 mL (a) and 7 mL (b) of $AgNO_3$.

time did not show much change on the peak position of AgNPs absorption profile (Fig. 2(b)). AgNPs synthesized using higher volume of $AgNO_3$ (4 mL, 10^{-3} M) also showed almost similar absorption spectra except small red shift (Fig. 3(a)). AgNPs obtained with higher concentration of L1-L8 (10^{-2} M) showed blue shifted absorption from 420 nm to around 395 nm almost for all compounds (Fig. 3(b)). Blue shifting of absorption indicates the formation of smaller NPs that could be possible since the availability of more number of capping molecules.

Phenols are known to reduce silver ions into AgNPs via electron transfer that converts phenols to quinone.[21] Phenolates are stronger and faster reducing agents for silver ions than phenols due to easy ionization.[22] L1 which dissolves in water without NaOH showed yellow colour formation with silver nitrate after two days whereas L1 with NaOH produced immediate yellow colour and confirms the faster reduction (Scheme 1(a)). To further confirm the role of phenolic OH in the silver ions reduction, L9 and L10 were synthesized and investigated for AgNPs formation. L9 that has only COOH in the structure did not show significant colour change with silver nitrate but L10 showed immediate yellow colour formation and confirms importance of



Scheme 1. UV-Visible absorption spectra of AgNPs synthesized with L1-L8 using 4 mL (a) and 7 mL (b) of $AgNO_3$.

Table 1. Zeta potential of synthesized AgNPs

Compound	Zeta Potential (mV)	Compound	Zeta Potential (mV)
L1	10.3	L5	21.2
L2	15.2	L6	25.3
L3	16.7	L7	20.1
L4	20.3	L8a	16.4

phenolic OH in the silver ions reduction (Scheme 1(b)). It is noted that both L9 and L10 were dissolved with 1:1 ratio of NaOH. The synthesized AgNPs zeta potential was measured to get the insight of molecular structure (L1-L8) and NPs stability that suggested moderate stability of AgNPs. However, it showed a trend that increasing side chain carbon atom of amino acids enhanced the AgNPs stability (Table 1).

The phase structure of the prepared AgNPs was charac-

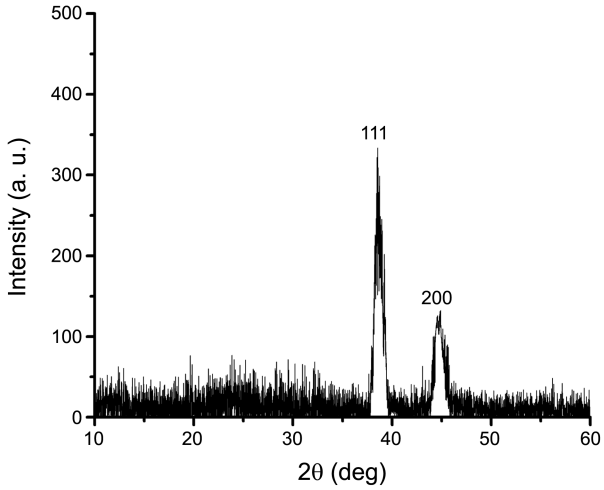


Figure 4. Representative PXRD pattern of AgNPs obtained with L4.

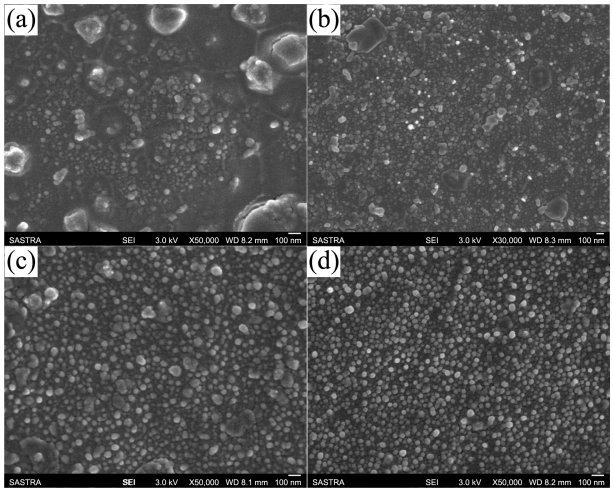


Figure 5. FE-SEM images of AgNPs synthesized using (a) L1, (b) L2, (c) L3, and (d) L4.

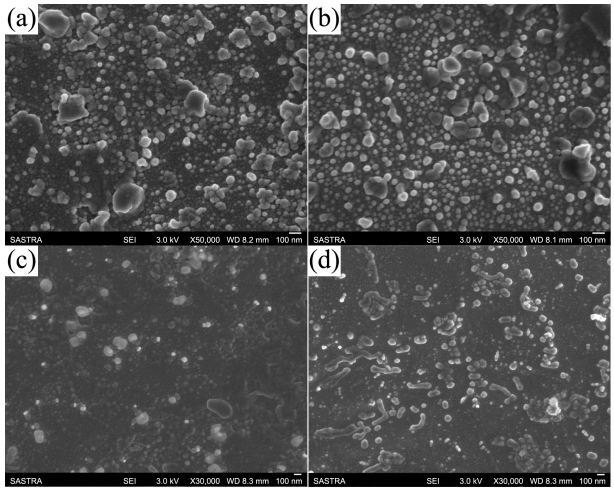


Figure 6. FE-SEM images of AgNPs synthesized using (a) L7, (b) L8, (c) L6, and (d) L1 with 7 mL of $AgNO_3$.

terized by powder X-ray diffraction (XRD). Figure 4 shows the representative case for AgNPs prepared by using L4 and peak at 38.2 and 44.1 agree well with the (111) and (200) diffraction of face centered cubic (fcc) silver (JCPDS file no. 04-0783). The other two peaks assigned to the glass matrix. The peak broadening is due to the formation of smaller sized AgNPs.

Morphology of the synthesized AgNPs was investigated using field emission scanning electron and high resolution transmission electron microscope. Spherical shaped AgNPs formation was observed in all samples with broad size distribution (Figs. 5, 6, 7) and L4-AgNPs appeared to be more uniform sized spherical NPs (Fig. 5(d)). AgNPs obtained by mixing 7 mL of $AgNO_3$ (10^{-3} M) which showed two absorption also showed poly-dispersed NPs with different morphology (Fig. 6(d)). HR-TEM studies further confirmed the poly-dispersed spherical crystalline NPs formation with phenolic derivatives (Fig. 7(a)). L6-AgNPs that showed three absorption peaks clearly reveals the formation of

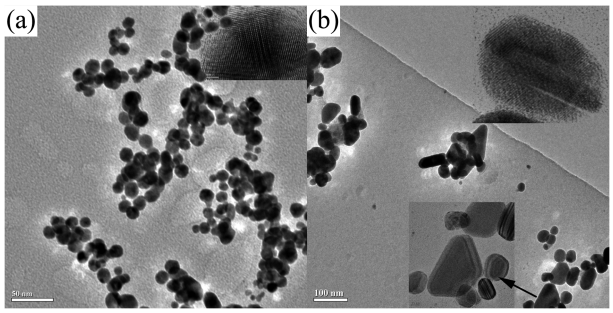


Figure 7. HR-TEM images of AgNPs synthesized using (a) L2 and (b) L6 with 4 mL of 10^{-3} M $AgNO_3$. High resolution image is shown in the inset that indicates the single crystallinity of AgNPs.

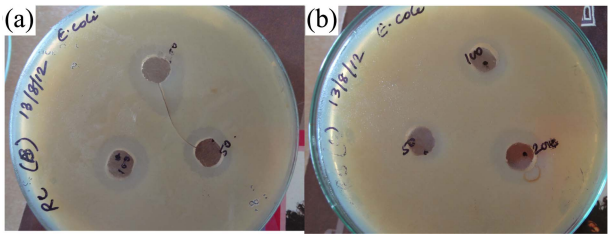


Figure 8. Antibacterial studies of (a) L4-AgNPs (b) L6-AgNPs.

different morphology of NPs including triangular prism (Fig. 7(b)). However, the mechanism of triangular prism formation with L6 is not clear.

Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications.[23] AgNPs with phenol and amino acid might be interesting materials for antimicrobial activities because of inherent antioxidant properties of phenol and natural availability of amino acids. L4- and L6-AgNPs were investigated for growth inhibition against *E. coli* and compared effect of spherical and prism NPs (Fig. 8). It is clear from the images that AgNPs with prism morphology are showing slightly better antibacterial activities compared to spherical NPs. It was observed that phenolic compounds alone did not show any growth inhibition. In the earlier reports, it was shown that AgNPs do not compromise membrane integrity but might instead cause cell wall pitting by binding to cell wall.[24] The systematic antimicrobial studies of other samples and the effect of tuning AgNPs morphology are currently under progress.

Conclusion

We have showed that amino acid based phenolic compounds can act as reducing as well as stabilizing agents for the synthesis of AgNPs in eco-friendly condition at room temperature. Structural variations in the amino acids lead to the formation of spherical and prism morphology. The formation of different morphology of AgNPs was clearly supported by the absorption spectra that showed two absorption peaks. The prepared AgNPs with phenolic compounds showed good inhibition of *E. coli* growth. The structural versatility of amino acid is expected to offer enhanced water solubility

with opportunity for bioconjugation of AgNPs.

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References

1. Li, L.; Hu, J.; Alivistos, A. P. *Nano Lett*. **2001**, *1*, 349.
2. Rao, C. N. R.; Cheetham, A. K. *J. Mater. Chem*. **2001**, *11*, 2887.
3. Prashant, K. J.; Huang, X.; El-Sayed, I. H.; El-Sayed, M. A. *Acc. Chem. Res.* **2008**, *41*, 1578.
4. Dahl, J. A.; Maddux, B. L. S.; Hutchison, J. E. *Chem. Rev.* **2007**, *107*, 2228.
5. Hutchison, J. E. *ACS Nano* **2008**, *2*, 395.
6. Haes, A. J.; Haynes, C. L.; McFarland, A. D.; Schatz, G. C.; Van Duyne, R. P.; Zou, S. *MRS Bull*. **2005**, *30*, 368.
7. Hutter, E.; Fendler, J. H. *Adv. Mater*. **2004**, *16*, 1685.
8. Evanoff, D. D.; Chumanov, G. *J. Phys. Chem. B* **2004**, *108*, 13948.
9. Mallick, K.; Witcomb, M. *Mater. Chem. Phys.* **2006**, *97*, 283.
10. McFarland, A. D.; Van Duyne, R. P. *Nano Lett*. **2003**, *3*, 1057.
11. Zhang, J.; Malicka, J.; Gryczynski, I.; Lakowicz, J. R. *J. Phys. Chem. B* **2005**, *109*, 7643.
12. Nickel, U.; Castell, A. Z.; Pöppl, K.; Schneider, S. *Langmuir* **2000**, *16*, 9087.
13. Mukherjee, P.; Ahmad, A.; Mandal, D.; Senapati, S.; Sainkar, S. R.; Khan, M. I.; Parishcha, R.; Ajayakumar, P. V.; Alam, M.; Kumar, R.; Sastry, M. *Nano Lett.* **2001**, *1*, 515.
14. Mohanpuria, P.; Rana, N. K.; Yadav, S. K. *J. Nanopart. Res.* **2008**, *10*, 507.
15. Vemula, P. K.; Aslam, U.; Mallia, V. A.; John, G. *Chem. Mater.* **2007**, *19*, 138.
16. Antolovich, M.; Prenzler, E. D.; Patsalidas, E.; McDonald, S.; Roboards, K. *Analyst* **2002**, *127*, 183.
17. Robbins, R. J. *J. Agric. Food Chem*. **2003**, *51*, 2866.
18. Koh, L. L.; Ranford, J. O.; Robinson, W. T.; Svensson, J. O.; Tan, A. L. C.; Wu, D. *Inorg. Chem.* **1996**, *35*, 6466.
19. Mulvaney, P. *Langmuir* **1996**, *12*, 788.
20. Pastoriza-Santos, I.; Liz-Marzán, L. M. *J. Mater. Chem*. **2008**, *18*, 1724.
21. Jacob, J. A.; Mahal, H. S.; Biswas, N.; Mukherjee, T.; Kapoor, S. *Langmuir* **2008**, *24*, 528.
22. Swami, A.; Selvakannan, P. R.; Pasricha, R.; Sastry, M. *J. Phys. Chem. B* **2004**, *108*, 19269.
23. Kaviya, S.; Santhanalakshmi, J.; Viswanathan, B.; Muthumary, J.; Srinivasan, K. *Spectrochim. Acta Part A* **2011**, *79*, 594.
24. Landsdown, A. B. G. *J. Wound Care* **2002**, *11*, 125.