Electronic Supplementary Material (ESI) for RSC Advances


# Electronic Supplementary Information

# Size-controlled silver nanoparticles synthesized over the range 5-100 nm using the same protocol and their antibacterial efficacy

**Shekhar Agnihotri[a], Soumyo Mukherji[a,b,c], and Suparna Mukherji*[a,d]**

[a] *Centre for Research in Nanotechnology and Science, Indian Institute of Technology-Bombay, Powai, Mumbai 400076, India*
[b]*WRCBB, Department of Biosciences and Bioengineering, Indian Institute of Technology-Bombay, Powai, Mumbai 400076, India*
[c]*Centre of Excellence in Nanoelectronics, Indian Institute of Technology-Bombay, Powai, Mumbai 400076, India*
[d]*Centre for Environmental Science and Engineering, Indian Institute of Technology-Bombay, Powai, Mumbai 400076, India;*

*Corresponding Author: mitras@iitb.ac.in

## S1. Determination of MIC and MBC

To examine the minimum inhibitory concentration (MIC), silver nanoparticles at various concentration (5-200 μg $ml^{-1}$) were introduced into sterile flasks each containing 50 ml nutrient broth and was sonicated for 10 minutes to avoid their aggregation in the test solution. The flasks were then inoculated with a freshly prepared bacterial suspension and an initial bacterial concentration of $10^5$-$10^6$ CFU $ml^{-1}$ was maintained for all the experiments. The culture media was incubated for 24 hours (37°C) in an orbital shaker at high rotation speed (200 rpm) to avoid agglomeration of silver nanoparticles during incubation and to prevent underestimation of their antibacterial effects. The MIC was determined by visual observation of turbidity of the culture broth before and after 24 hours incubation.

The minimum bactericidal concentration (MBC) can be defined as the lowest concentration of silver nanoparticles that kills 99.9% of the bacteria, and was determined from the batch culture studies done for assessing MIC values. The sample flasks that appeared to have no or little bacterial growth were selected and a loop full from each flask was plated on fresh solid media (2% agar in nutrient broth) and incubated at 37°C for 24 hours. The nanoparticle concentration causing bactericidal effect was selected based on the absence of colonies on agar plate and the lowest concentration causing bactericidal effect was reported as MBC. For assessing MIC/MBC values, all studies were done in triplicate.

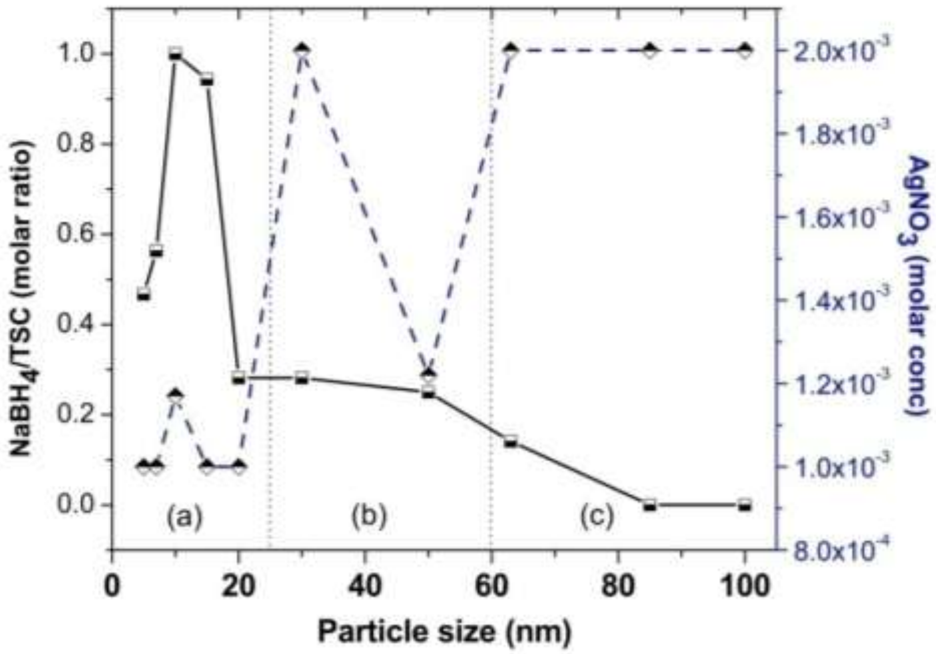


**Fig.S2** Variation in $NaBH_4$/TSC molar ratio with size of nanoparticles. Secondary axisshows the corresponding silver nitrate (molar conc.) in the final mixture. The graph has 3 zones divided by two dashed line (a) Particle size below 25 nm is governed by a higher $NaBH_4$/TSC ratio, i.e. AgNPs are predominantly formed by sodium borohydride mediated reduction, which lead to rapid reduction of Ag ion to Ag nuclei and favor an instant nucleation, where all nuclei have the opportunity to grow at relatively constant rate. (b) Particles in the size range 25-60 nm is governed by co-reduction of both $NaBH_4$ and TSC. (c) Particle size >60 nm is governed mainly by TSC mediated reduction, where a lower $NaBH_4$ amount may lead to sufficient generation of silver nuclei, which later acts as the reduction sites subsequently for growth of nanoparticles.

## S3 Effect of Temperature on Synthesis of Different Sized Silver Nanoparticles

The choice of thermal treatment was based on the temperature favouring reducing ability of $NaBH_4$ and TSC. $NaBH_4$ mediated reduction is normally done at a lower temperature, which causes longer nucleation process and produces particles with varying size. In contrast, TSC shows its reducing property only at the high temperatures (> 75ºC), and produces particles with different size and shape. For synthesis of AgNPs illustrated in Figure S3, the initial temperature for $1^{st}$ stage reduction process was optimised by varying the temperature from room temperature (25ºC) to near boiling temperature (95ºC).

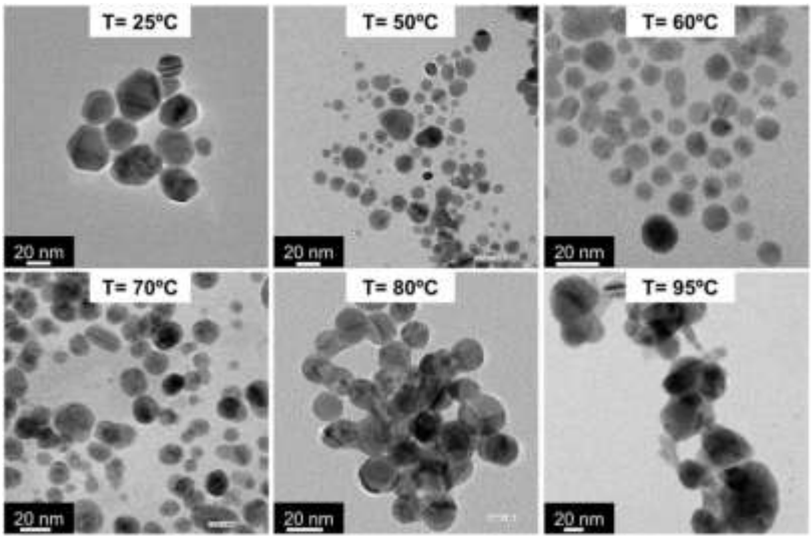


**Fig. S3:** Effect of temperature (initial) on the synthesis of silver nanoparticles (10 nm AgNPs) keeping other reaction conditions same throughout the protocol. As the temperature was raised from 25º to 60ºC, silver nanoparticles became smaller, spherical in shape with lower dispersivity and lower aggregation tendency. Further increase in temperature, i.e. 70º to 90ºC resulted in formation of larger AgNPs with variable size, shape and clustering/agglomeration tendency. Analyzing the AgNP morphologies at various temperatures, 60º C appeared to be the most favourable temperature for 1st stage reduction process. In a similar way, the second stage temperature was also optimized to be 90ºC, which facilitated the synthesis of size-controlled AgNPs with tunable size ranges.

## S4 Effect of pH on Synthesis of Different Sized Silver Nanoparticles

The two stage reduction process may also be optimized by variation in pH of the “final sol” in the second stage by NaOH addition. We hypothesized three different pH zones i.e., 5-7, 8-11, and 12-13 to represent ‘slow’, ‘controlled’, and ‘high’ reducing property of trisodium citrate, respectively.

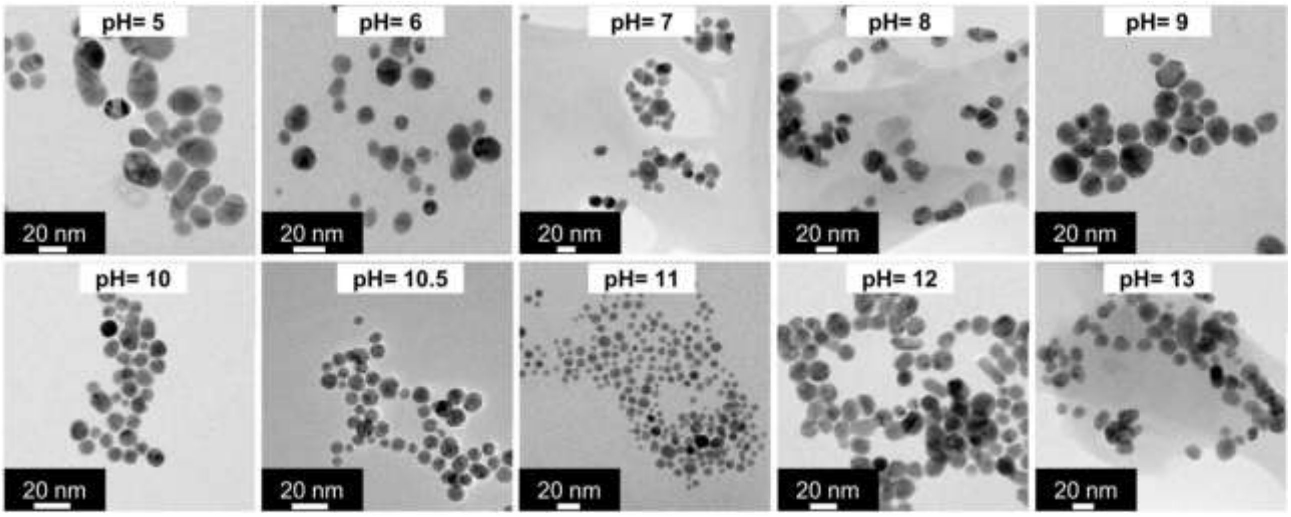


**Fig. S4** Effect of pH on synthesis of silver nanoparticles (10 nm AgNPs), keeping other reaction conditions same throughout the protocol. As shown in the figure, AgNPs formed over the pH range 5-7 were of irregular shape with larger size, as compared to those formed over the pH range 8-9 where, the AgNPs were quasi-spherical in shape and depicted lower aggregation tendency. Interestingly, the pH range 10-11 was also observed to be favourable for synthesizing spherical and monodispersed AgNPs and thereby, a pH of 10.5 was chosen as the optimized pH for synthesizing silver nanoparticles employing the co-reduction approach. Further, increase in pH i.e., 12-13 resulted in formation of undesired morphologies, such as nanorods and AgNP clusters.

**Table-S5** Characterization of different sized silver nanoparticles

| Sample Code[‡] | UV-Vis Absorption | | Particle size using FEG-TEM (nm) | | Crystallite size Using XRD (nm) | Zeta Potential (mV) |
|---|---|---|---|---|---|---|
| | ($\lambda_{max}$) | FWHM | Mean ± SD[!!] | Size Range | | mean ± SD* |
| Ag (5) | 393 | 55 | 5 ± 0.7 | 4 to 7 | 5.1 | -22.8 ± 0.8 |
| Ag (7) | 394 | 57 | 7 ± 1.3 | 4 to 11 | 6.2 | -27.3 ± 1.2 |
| Ag (10) | 398 | 62 | 10 ± 2.0 | 6 to 13 | 9.3 | -30.2 ± 1.2 |
| Ag (15) | 401 | 75 | 15 ± 2.3 | 11 to 19 | 13.9 | -34.0 ± 2.0 |
| Ag (20) | 406 | 85 | 20 ± 2.5 | 14 to 26 | 16.8 | -35.1 ± 0.9 |
| Ag (30) | 411 | 68 | 30 ± 5.1 | 21 to 38 | 14.4 | -33.7 ± 2.0 |
| Ag (50) | 420 | 138 | 50 ± 7.6 | 33 to 70 | 19.7 | -41.8 ± 1.3 |
| Ag (63) | 429 | 167 | 63 ± 7.4 | 45 to 78 | 30.3 | -48.5 ± 0.9 |
| Ag (85) | 449 | 160 | 85 ± 8.2 | 62 to 108 | 52.1 | -52.4 ± 3.4 |
| Ag (100) | 462 | 162 | 100 ± 11.3 | 81 to 131 | 65.9 | -53.1 ± 1.8 |

‡For convenience, the different sized AgNPs are designated as Ag(x), where x is the average particle size in nanometer.

!!The particle size was estimated on counting ≥400 nanoparticles, using multiple TEM micrographs
*Samples were tested in triplicates

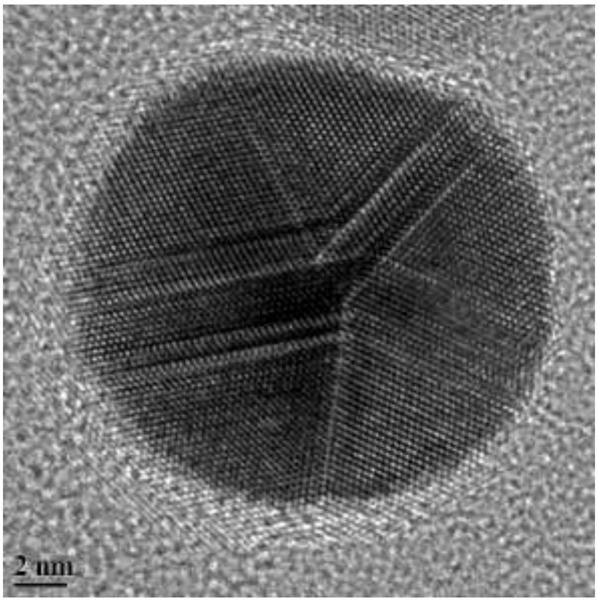


**Fig. S6** High resolution TEM micrograph showing twinned crystal in as synthesized silver nanoparticles

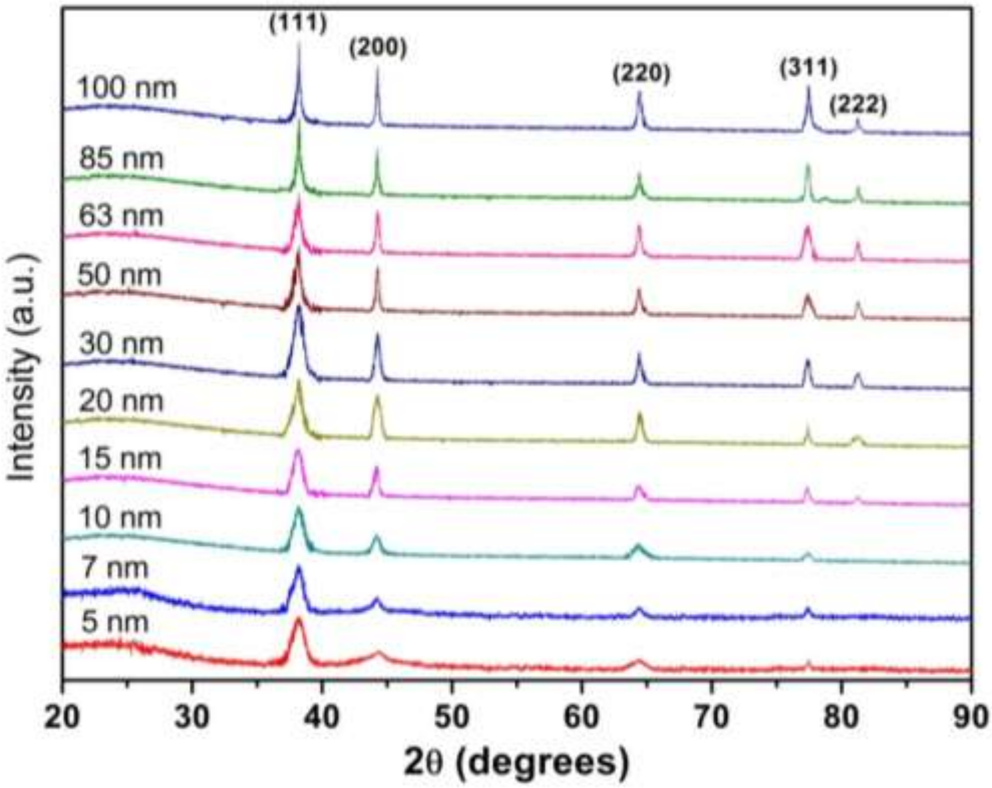


**Fig. S7** XRD spectrum of as synthesized silver nanoparticle

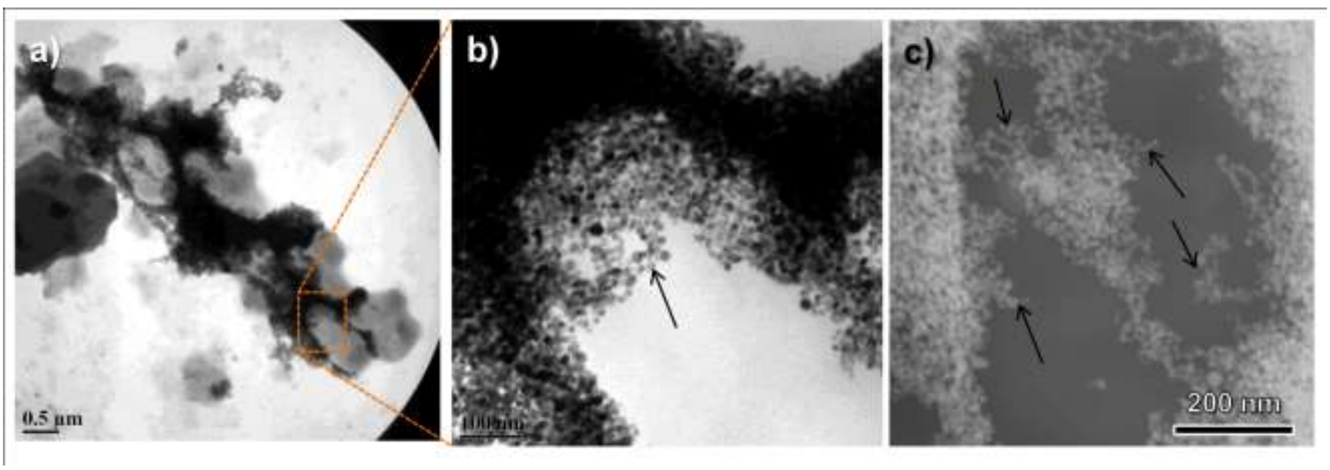


**Fig. S8** FEG-TEM micrograph of *E. coli* cells treated with silver nanoparticles (a) low magnification (b) high magnification (c) dark field STEM micrograph showed internalization of silver nanoparticles within the bacterial cells (indicated by arrows)