Investigation of Size Evolution of Silver Nanoparticle and Its Use in Medical Field

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Abstract Metallic nanoparticle (NP) is one the most important nanostructures used in medical purposes. However, for successful use of NPs, the size, shape, and growth kinetics should be known. In this work, we have demonstrated how silver nanoparticle (AgNP) size and growth depend on silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) concentrations. The morphology of clusters is simulated by validating the plasmon spectra, generated using DDSCAT (based on discrete dipole approximation) simulation, with the experimental UV–Visible (UV–Vis) spectra. We find that at the concentration [$AgNO_3$] to [$NaBH_4$] ratio of 10:1, the nanoparticle cluster size is smallest and spherical, while for the ratio 3.3:1 this is deformed and large. For the latter, we observe quadrupole plasmon resonance. The AgNP cluster size and growth are also investigated with elapsed time and the stability of the cluster is demonstrated with entropy estimation. Thus, this work could enlighten us to select the AgNP cluster size required for medical purposes by correctly choosing the concentrations of the chemical reagents. Finally, important sectors for future studies of AgNP using γ-radiation have also been discussed, which could be useful in cancer treatment.

Keywords AgNP · DDSCAT · UV–Visible spectra · Nanomedicine · Plasmon

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M. Mukherjee et al. (eds.), *Advances in Medical Physics and Healthcare Engineering*,
Lecture Notes in Bioengineering, https://doi.org/10.1007/978-981-33-6915-3_13

1 Introduction

Nanoparticles are materials having all three dimensions in the nanoscale i.e. within 1–100 nm (Kreibig and Vollmer 1995). However, the property that makes the miniscule nanoparticles (NP) so special in science and technology is the size that is much smaller than their bulk counterparts. The large surface area to volume ratio and the emergence of quantum effects owing to such minute size provide the NP altogether different optical, chemical, electrical, etc. properties over the bulk materials, which gives the former a cutting edge in different scientific fields. The nanomaterials and related science have been well-known in different fields such as Chemistry, Physics, Biology, etc. since the past age. However, only within the last decade, the nanomaterial application rate has been increased multifold. The medical science has, off late, got a new dimension piggybacked by nanomaterial applications (Riehemann et al. 2009; Whitesides 2005; Mauricio et al. 2018).

Nanomaterials and NP are used in medicine for imaging, targeted drug delivery, sensing, artificial implantation, etc. The NP can easily move freely within the human body in comparison to bigger particles. Therefore, by encapsulating therapeutic drugs, one can use NPs to deliver drugs in targeted area, especially for sustained or controlled release. As an example, the use of nanosized liposome and micelles as chemotherapy drug carrier has been known since long time. However, certain disadvantages are always associated with liposome as carrier such as aggregation and lack of stability. Moreover, there could be high production cost to reshape the liposome potentially effective for drug delivery. Recently, the advent of nanocrystals (size within 2–10 nm) or 'quantum dots' has revolutionalized biomedicine due to small size and high molar extinction coefficient (Panahi et al. 2016). Metal and oxides as NP have also several applications in the nanomedicinal field. Metals, such as gold, silver, etc., being biocompatible and having unique electronic and optical properties, may be used for ligand (e.g. protein, antibodies, etc.) conjugation. Besides, metals showing localized surface plasmon resonances (LSPR) can be applied in bioimaging for diagnostic purposes. However, toxicity to the living cells is an important issue and gold is less toxic than any other metals (Mauricio et al. 2018; Patra et al. 2010; El-Sayed et al. 2005).

The too many potential advantages of NP may have eclipsed its disadvantages, still there is dire need to study those to use the NP effectively in medicines. The major problems are aggregation or clusterization and toxicity. Table 1 highlights the size-dependent effectiveness of NP as medicinal purposes. The optimization of size (20–100 nm) is highly useful to achieve stability and less aggregation without losing much in the surface to volume ratio (Longmire et al. 2008). The major problem lies in the fact that the topic of NP stability and aggregation is still not well-understood. It is found that the issue of NP stability depends on different sectors. The selection of base fluid, reducing agent, difference between dielectric constant of nanoparticle and the base fluid, the addition of surfactants, ambient temperature, etc. all could endow NPs stability. But the concerned research studies are very rare in the existing literature (Grassian 2008; Sun and Xia 2176). Grassian (2008) in his recent paper

Table 1 Size dependence of NP in medical physics

NP size	Potential use	Disadvantages
20–100 nm	Applications within the human body. Large surface area useful for drug supply	Aggregation problem
>1 μm	Used as drug carrier for larger size	Opsonized and accumulated within the organs. High rate of aggregation
<20 nm	Good for use in cases where rapid clearance needed	Rapid clearance from blood reduces effectiveness as drug carrier

has showed the size dependent properties and surface chemistry of metallic nanoparticles in gas and liquid phase environments. Sun and Xia (2002) have demonstrated that the presence of poly vinyl pyrrolidone (PVP) in the reduction of silver nitrate with ethylene glycol determines the shape and size of silver nanoparticles (AgNP). By changing the molar ratio of PVP and $AgNO_3$ from 1.5 to 3.3, the aggregation increases. Xia et al. (2011) opined that formation of nanocrystals with specific shape could be difficult and exact knowledge of size and shape evolution of NP is still unassailable. Another work (Xiong et al. 2006) emphasizes that the production of well-defined silver nanostructures is not a trivial job. In view of these, this paper tries to explore how the shape change of NP is manifested by the LSPR. Since the prospect of nanomedicine depends entirely on the NP growth kinetics, stability, and the nature of aggregation, the lack of knowledge in this field has been a major impediment.

In the present work, therefore, we have focused on the growth kinetics of AgNP with water as base fluid. Our aim is to study the dynamics of the AgNP morphology and to understand the size correlation with stability. We have investigated the AgNP size and aggregation with the concentration of reducing agent sodium borohydride ($NaBH_4$) and metallic silver supplier $AgNO_3$. We have also used thermodynamical entropy as a probe to understand the shape and cluster size.

2 Materials and Methods

Nano silver colloid is prepared by chemical reduction, in which the $AgNO_3$ or silver nitrate solution is reduced by ice-cold reducing agent sodium borohydride ($NaBH_4$). The synthesis of the nanoparticle occurs via the following chemical reaction:

$$AgNO_3 + NaBH_4 \rightarrow Ag + 1/2H_2 + 1/2B_2H_6 + NaNO_3$$

The requirement of ice-cold temperature (T) is due to the reaction of $NaBH_4$ at higher temperature (T) producing hydrogen gas and boranes impeding the reduction of AgNP (Piñero et al. 2017; Amendola et al. 2006). In addition, to slow down the reaction rate at lower T is another aim. The $AgNO_3$ and $NaBH_4$ powders, in 99.8 and 95.8% purity, have been procured from Central Drug House, India. At first, we

prepare 100 mL of 0.002 M $NaBH_4$ sample and a part of it (30 mL) is added in an Erlenmeyer flask.

The liquid is initially stirred and cooled for 20–30 min and then 3 mL of $AgNO_3$ is added dropwise into the $NaBH_4$ solution, which is stirred continuously. Emergence of AgNP can be understood when the color of the solution turns light yellow (Islam et al. 2018). We study the UV–Visible (UV–Vis) spectra of AgNP solution by (i) varying the concentration of $AgNO_3$ solution from 0.20 to 3.33 mM/cc, keeping $NaBH_4$ solution constant, (ii) varying the concentration of $NaBH_4$ ($[NaBH_4]$) from 0.03 to 0.10 mM/cc keeping $[AgNO_3]$ fixed at 0.33 mM/cc, (iii) the growth kinetics with number of days elapsed after preparation.

The identification of size and shapes of NP is popularly done by using the techniques such as transmission electron microscopy (TEM), X-ray diffraction (XRD), electron energy loss spectroscopy (ELSS), etc. However, the performance of the techniques could be slow and may provide incomplete information about the particle size, shape, and agglomeration. Moreover, the cost of operation could also be high. One of the simplest measurement techniques is the experimental UV–Vis spectroscopy of NP and theoretical interpretation of the experimental plasmonic data using Discrete Dipole Approximation (DDA). This technique is cost-effective, less time-consuming as well as can emulate the NP and cluster morphology without disturbing the sample solution. This has been used rarely in the existing literature (Amendola et al. 2006; Islam et al. 2018). In this work we have used DDSCAT (Drain and Flatau 2013), a FORTRAN code based on DDA formalism.

3 Results and Discussions

The concentration of $AgNO_3$ solution ($[AgNO_3]$) is varied from 0.20 to 3.33 mM/cc, keeping the $NaBH_4$ solution constant at 0.07 mM/cc. The corresponding extinction spectra are shown in Fig. 1 and the full width at half maxima (FWHM) is depicted at the inset panel. The FWHM reduces with $[AgNO_3]$ and thereafter starts to increase from a minima position. The position 2 denotes the minima, while 1 and 2 are positions with higher values of FWHM. This can be explained heuristically as follows. Position 1 has lowest number of metal particles than that of 2 and 3. The higher number of electrons than the metal particles reduces the effective potential between them, causing agglomeration and higher FWHM. At 2, the number of metal particles increases with $AgNO_3$ concentration to become comparable with the number of electrons. Therefore, at position 2 for the ratio $[AgNO_3]$:$[NaBH_4] = 10{:}1$, the particle size and agglomeration become smallest. At position 3, the number of metal particles increases in comparison to the existing number of electrons. These results increase in FWHM owing to non-uniformity in the amount of electrons between two metal particles. The morphology of the AgNP cluster is shown in the inset of Fig. 1. Similar dynamics of FWHM is found for another set of $[NaBH_4]$ shown by red curve in the inset corroborating our heuristic argument.

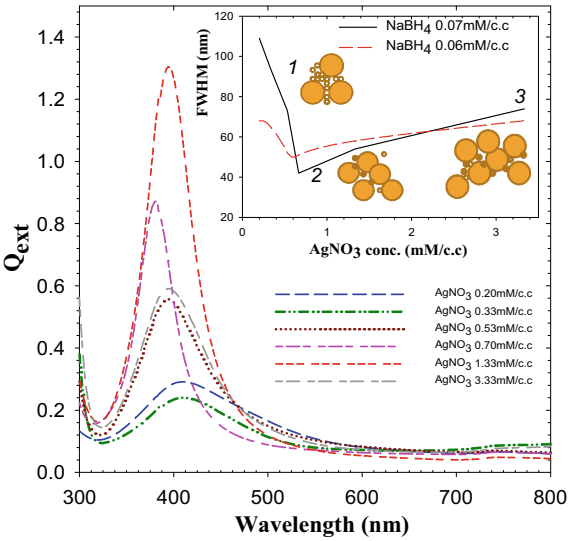

Fig. 1 (Colour online) The variation of experimental extinction spectra with the concentration of $AgNO_3$. Inset depicts FWHM as a function of [$AgNO_3$] and possible cluster structures

In Fig. 2 the variation of extinction spectra with the concentration of $NaBH_4$ is shown, keeping [$AgNO_3$] constant. With the increase in [$NaBH_4$], number of electrons increases, and enhanced probability of agglomeration produces large deformed clusters. This is evident in Fig. 2, where the extinction spectra shown by blue dashed line depicts thicker FWHM along with a spike. The spike is the manifestation of quadrupole plasmon resonance owing to the deformed-shaped cluster. The corresponding ratio [$AgNO_3$]:[$NaBH_4$] is found as 3.3:1. On the contrary, the dotted curve, for lowest [$NaBH_4$], shows thinnest FWHM due to lower electron concentration. The corresponding cluster structures are also shown in the same figure. Interestingly, the smallest AgNP cluster is found for the ratio [$AgNO_3$]:[$NaBH_4$] = 10:1, which is

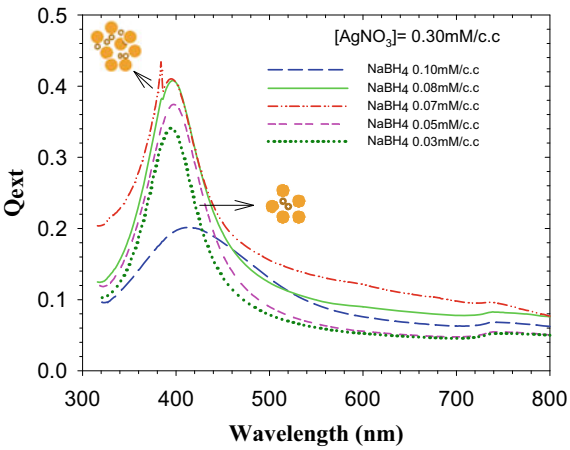

Fig. 2 (Color online) The variation of experimental extinction spectra with the concentration of $NaBH_4$. The corresponding cluster structures are also shown

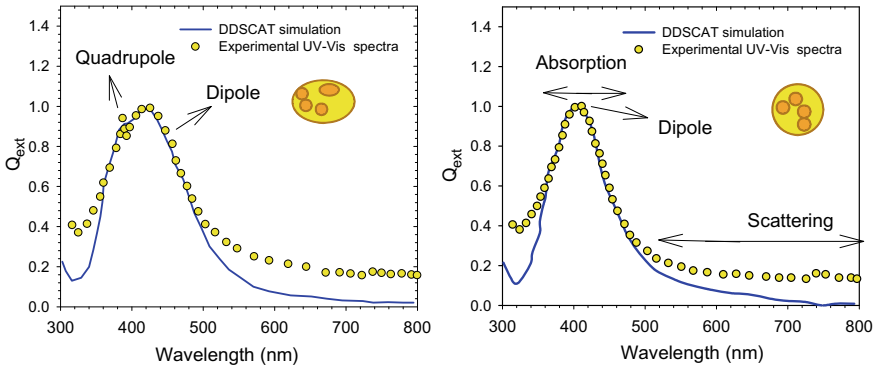

Fig. 3 (Color online) The agreement between DDSCAT simulation and experimental UV–Vis spectra. The absorption and scattering regions are also depicted. Left panel show quadrupole peak along with the dipole absorption, whereas right panel shows only dipole peak. Corresponding cluster morphology is also described

similar to the earlier case of [$AgNO_3$] variation. The cluster morphology is corroborated via DDSCAT simulation. The nice agreement between DDSCAT and experimental UV–Vis spectra in the absorption region is shown in Fig. 3 for quadrupole (left panel) as well as dipole spectra. The mismatch in the scattering region is due to the presence of different scatteres in the experimental solution, which do not affect the result. From the simulation, quadrupole mode generating cluster is found to be deformed in shape with aspect ratio 1.1:1 and size of 58 nm, while the dipole mode generating cluster is found spherical in shape with dimension of 50 nm.

To understand the stability of NP, we observe the growth dynamics of AgNP water solution in the lab with the elapsed time after preparation. For the 1st day, the manifestation of spherical morphology of AgNP can be explained by dipole UV–Vis peak with thin FWHM. In 6th Day, the solution shows quadrupole peak due to deformed cluster shape. But in the 48th day the solution shows only dipole spectra with very broad FWHM. The entropy of the AgNP water system is measured using the van't Hoff equation as per the formalism shown in van Rijjsel et al. (2011). The nanoparticle chain length is simulated using DDSCAT and corresponding absorption spectra is matched with experimental spectra. The entropy is found to increase with number of days elapsed. Actually, on day 1 of AgNP preparation, the cluster size and entropy are found smallest and the tendency to grow by aggregation is highest. Thereafter further aggregation invokes deformation and quadrupole mode of plasmon vibration is achieved with higher value of entropy. Finally, when the cluster is very large at the 48th day, the entropy is highest. Since the aggregation rate becomes almost zero at that point, entropy stabilizes and stable shape is achieved.

Thus, our work elucidates the cluster shape stability and the aggregation dynamics of AgNP in water as base fluid. The concentration ratio of $AgNO_3$ to $NaBH_4$, given by 10:1, provides us the smallest sized NP (within the diameter of 50 nm) having

the highest tendency of clusterization. The smallest sized AgNP generate agglomeration which may induce Caveolae-mediated endocytosis. For the AgNPs with higher dimensions (may be understood easily from the yellow colored solution), such as AgNPs kept for 48 days, or the [$AgNO_3$] to [$NaBH_4$] ratio 3.3:1 (or less), the cluster size is achieved between 58 and 100 nm of radius. Here the utilization may be possible in macropinocytosis that involve processes instead of endocytosis (Kenzaoui et al. 2017). The deformed cluster shapes may also be used in medicinal purposes where reaction rate needs to be higher. This paper, therefore, explains how to select AgNP size based on the medicinal purposes.

Our future aim is to prepare AgNP in a greener way, which will be easier to implement in nanomedicines. Our endeavor is to generate AgNP from $AgNO_3$ solution using γ-ray radiating sources. However, we need to investigate how the rate of preparation of AgNP can be increased using small dose rate, important in human body. External radiotherapy and brachytherapy are the important processes for the exposure of human body to γ-radiation. Whether these therapies can be utilized for nanoparticle generation within the human body is a big question that needs to be answered in the near future.

Acknowledgements The authors gracefully acknowledge the financial support from Department of Science and Technology (India) to procure the UV-Visible spectrophotometer under the FIST scheme at the Department of Physics, Barasat Government College.

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