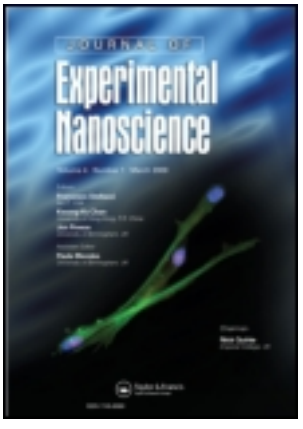

Journal of Experimental Nanoscience
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tjen20

Differential interaction of silver nanoparticles with cysteine

Aswathy Ravindran [a], Sindhu Priya Dhas [a], N. Chandrasekaran [a] & Amitava Mukherjee [a]

[a] Nanobiomedicine Laboratory, School of Bio Sciences and Technology, VIT University, Vellore, India

Published online: 22 May 2012.

To cite this article: Aswathy Ravindran , Sindhu Priya Dhas , N. Chandrasekaran & Amitava Mukherjee (2013) Differential interaction of silver nanoparticles with cysteine, Journal of Experimental Nanoscience, 8:4, 589-595, DOI: 10.1080/17458080.2011.577100

To link to this article: http://dx.doi.org/10.1080/17458080.2011.577100

Journal of Experimental Nanoscience, 2013
Vol. 8, No. 4, 589–595, http://dx.doi.org/10.1080/17458080.2011.577100

Taylor & Francis
Taylor & Francis Group

Differential interaction of silver nanoparticles with cysteine

Aswathy Ravindran, Sindhu Priya Dhas, N. Chandrasekaran and Amitava Mukherjee*

Nanobiomedicine Laboratory, School of Bio Sciences and Technology, VIT University, Vellore, India

(*Received 20 December 2010; final version received 25 March 2011*)

Understanding the characteristics of cysteine on a solid surface is an important issue in protein study and amino acid analysis. Therefore, cysteine was selected as a model biomolecule to study the interaction with plasmonic silver nanoparticles. In this study, we report the differential interaction of cysteine with silver nanoparticles synthesised by Lee and Meisel (using citrate as reductant), and modified Creighton (citrate and borohydride as reductant) methods. In Lee and Meisel's method, the red-shifting of silver plasmon resonance in the UV–vis spectra and the aggregation of the particles occurred owing to a decrease in stability of silver nanoparticles upon interaction with cysteine. In contrast, the other method did not cause any aggregation or significant spectral changes. The differential behaviour may be due to surface chemical changes on cysteine with pH, which plays a major role in the nanoparticle-biomolecule interaction. The synthesis of silver nanoparticles applying two sol gel methods followed by interactions with cysteine induces different functionalities on the nanoparticles, which may find specific applications in bio-sensing and drug delivery.

Keywords: cysteine; surface plasmon resonance; aggregation; silver nanoparticles; functionalisation

Nanoparticles are defined as particles between 10 and 100 nm in size. Metallic nanoparticles (like Ag and Au) have unique optical, electrical and biological properties that have attracted significant attention due to their potential applications, such as catalysis, biosensing, drug delivery and nanodevice fabrication. Among noble-metal nanomaterials, silver nanoparticles have received considerable attention due to their attractive optical and electronic properties [1]. The surface plasmon resonance (SPR) and large effective scattering cross-section of individual silver nanoparticles make them ideal candidates for trace analysis and even for probing single molecules [2–4], where phenomena such as surface enhance Raman scattering can be exploited. The synthesis of particles that bear surface functionality is desirable for the purpose of nanoparticle handling and the construction of functional architectures. This functionalisation of the nanoparticle surface can be accomplished during the nanoparticles synthesis by the addition of a suitable agent or by interaction of the nanoparticle with a suitable

*Corresponding author. Email: amit.mookerjea@gmail.com

biomolecule after the synthesis of the particle. The surface functionalisation also imparts an enhanced stability and thus gives additional control over their size [5]. The interaction of plasmonic nanoparticles like gold and silver with biomolecules has been studied in detail recently [6,7]. A number of peptides have been designed for the stabilisation of gold nanoparticles aiming to interact with biomolecules [8]. This design reflects the need of cysteine as an anchor group in polypeptide to stabilise metallic nanoparticles under different physiological conditions. L-cysteine (Cys) is an amino acid synthesised by the liver, and is involved in a variety of important cellular functions, including protein synthesis, detoxification, and a metabolic process [9,10]. It is a small, zwitterionic molecule, and well used in biochemical and electrochemical research. Understanding the characteristics of Cys on a solid surface is an important issue in protein study as well as in differentiating amino acid molecules [11–13]. Therefore, we have selected cysteine as a model biomolecule for interaction with plasmonic silver nanoparticles. In this communication, we report the differential behaviour of cysteine with silver nanoparticles prepared in an aqueous medium using two different methods – Lee and Meisel [4] and a modified Creighton's method (sodium borohydride method) [14].

L-cysteine was obtained from Sigma–Aldrich, USA (high purity), silver nitrate and trisodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) was purchased from SD fine Chemicals (high purity, above 99.5%), Mumbai. Deionised and distilled water was used in all the experiments. In Lee and Meisel's method [4], 100 mL of 0.001 M silver nitrate solution was heated to boiling. To the boiling solution, 2 mL aqueous solution of 1% trisodium citrate was added drop wise, accompanied with vigorous stirring. The mixed solution was kept for boiling for further 20 min. Finally, a green grey silver colloid was obtained. The pH of the resulting colloidal solution was found to be 6.4. In another method [14], silver nanoparticles were prepared by the reduction of sodium borohydride. In a typical experiment, 100 mL aqueous solution of silver nitrate (0.001 M) was reduced by 0.01 g of $NaBH_4$ at room temperature resulting in the formation of yellow colloidal solution of silver nanoparticles. The pH of the resulting colloidal solution was found to be 8.6. Thereafter, 10^{-3} M of as prepared colloidal solution of silver nanoparticles was interacted with 10^{-2} and 10^{-3} M of cysteine for 10 min in an orbital shaker at 120 rpm.

UV–visible spectra of silver nanoparticles synthesised from the two methods and the Ag/cysteine mixtures having varying concentrations of cysteine were recorded in the UV–visible spectrophotometer (Shimadzu UV-1700, Japan). To determine the size and shape of the particles synthesised using the two sol gel methods, transmission electron microscopy (TEM) was performed using Technai G 10 (Philips 80 kV). The 90Plus Particle Size Analyser (Brookhaven Instruments Corporation Holtsville, NY, USA) was used to analyse the hydrodynamic diameter, and the zeta potential of the silver nanoparticles synthesised by the two different procedures and their mixtures obtained after interaction with cysteine. All the tests were carried out in triplicate, and mean values of the results were reported. The experimental error limit was strictly kept within ±5%.

The silver nanoparticles display intense colours due to the intense absorption in the visible range owing to SPR phenomenon. The resonance essentially arises due to the collective excitations of the electrons in the conduction band; near the surface of the nanoparticles [15]. For silver nanoparticles, the λ_{max} values were reported in the visible range 400–500 nm [16]. It was observed from the UV-visible results that differently prepared silver nanoparticles differed in their mode of interaction and behaviour

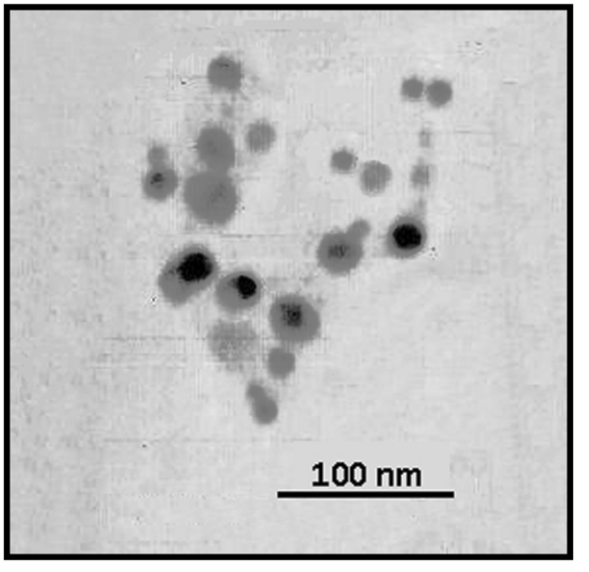

Figure 1. TEM image of particle synthesised using Lee and Miesel's method.

with cysteine. Silver nanoparticles synthesised using Lee Miesel's method gave a λ_{max} of 442 nm. The plasmon peak gives an indication about formation of the silver nanoparticles and the average size of the particles in the colloidal solution. The broadening of the band was observed due to the wide size distribution of particles rather than aggregated particles [17]. The TEM image of the synthesised particles gave an average particle size of 15–30 nm and suggests that the particles were spherical in nature (Figure 1). The interaction of these particles with 10^{-2} and 10^{-3} M cysteine resulted in immediate aggregation of the particles and a λ_{max} value of 460 nm denoting red-shifted plasmon bands (Figure 2). The optical absorption spectrum of metal nanoparticles is dominated by the SPR and an increase of particle size always results in the red-shift of plasmon band to higher wavelength ranges [18]. The red-shift in plasmon band essentially signifies the aggregation of the particles in the presence of the adsorbed molecule. In contrast, the silver nanoparticles synthesised using sodium borohydride method gave a λ_{max} value of 400 nm. The TEM images suggest that the particles were spherical and well-dispersed with an average particle size between 10 and 20 nm (Figure 3). On interaction of these samples with cysteine, no significant spectral changes were observed and cysteine failed to induce any aggregation of the particles (Figure 4).

The hydrodynamic diameter of the particles synthesised in Lee and Miesel's method was found to be 60 nm and those obtained after interaction with cysteine was 122 nm. An increase in the particle size of the Cys treated samples in the first method confirmed the aggregation of the particles (Table 1). The silver nanoparticles synthesised in the sodium borohydride method had a hydrodynamic diameter of 51.6 nm and no significant changes were observed in the cysteine treated samples (Table 1). Hence the UV-visible readings were in complete accordance with the particle size analysis. To determine the stability of colloidal solution, zeta potential experiments were carried out. The silver nanoparticles

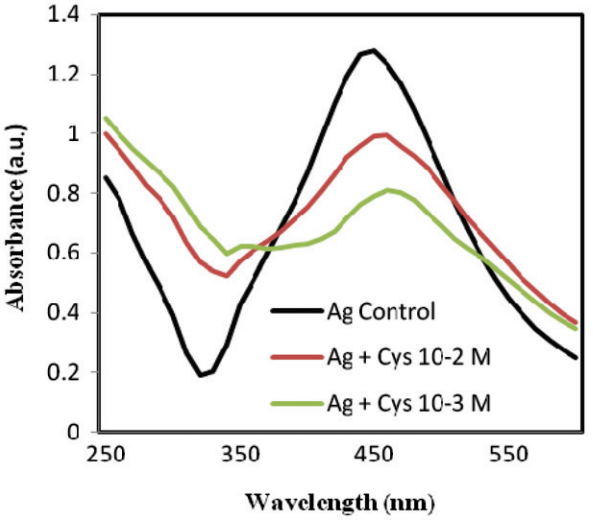

Figure 2. (Colour online) UV–vis spectra of Ag and Ag/Cys mixtures synthesised using Lee and Meisel's method.

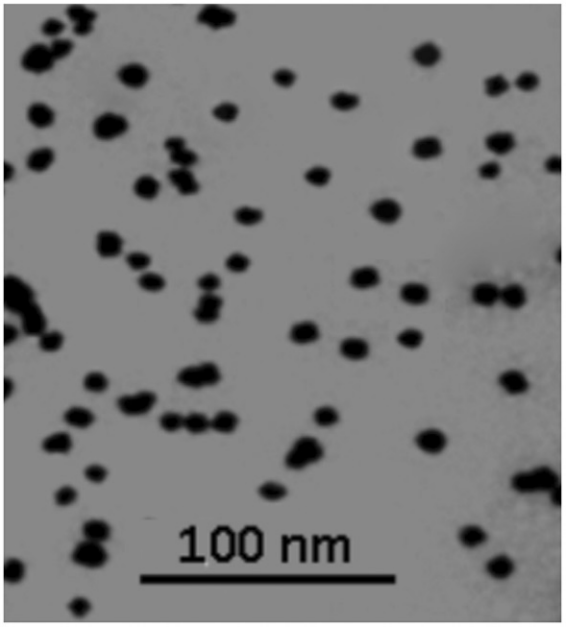

Figure 3. TEM image of particle synthesised using modified Creighton's method.

synthesised by Lee and Meisel's method gave a zeta potential value of −31.12 mV and the Ag/Cys interacted samples resulted in a decrease in zeta potential value (−20.32 mV). The citrate bound silver nanoparticles are negatively charged. The addition of cysteine to the colloidal silver reduced the effective negative charge on the surface of the silver

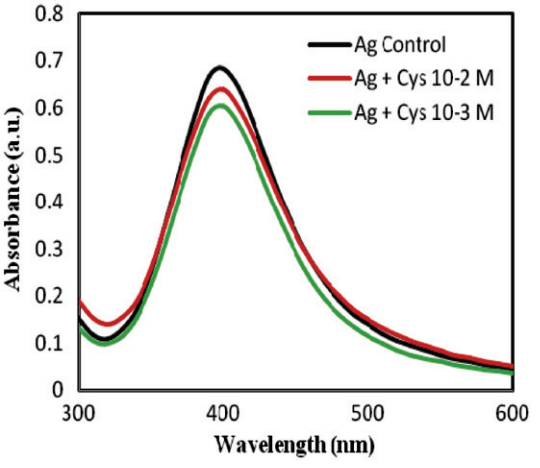

Figure 4. (Colour online) UV–vis spectra of Ag and Ag/Cys mixtures synthesised using Creighton's method.

Table 1. The average hydrodynamic size of the particles synthesised using two processes before and after interaction with cysteine.

Process	Ag nanoparticle (nm)	Ag nanoparticle ± cysteine (nm)
Lee and Miesel's method	60 ± 0.33	122 ± 0.57
Modified Creighton's method	51.6 ± 0.88	53.3 ± 0.66

Note: Values are the mean of 3 (n = mean ± standard error).

nanoparticles thereby, inducing aggregation. Borohydride method synthesised particle gave a zeta potential value of −30.76 mV and the Ag/Cys interacted samples gave similar zeta potential values which confirmed the stability of the colloid even after addition of cysteine. The above results were in substantial agreement with the UV and dynamic light scattering results.

A possible mechanism describing the differential behaviour of cysteine in the two synthesis methods is discussed. Cysteine is a thiol containing amino polar amino acid having pK_a values: 1.96 (for the carboxylic group), 8.18 (for the amine group) and 10.28 (for the thiol group) [19]. The isoelectric pH of cysteine is 5.07. The pH of the colloidal silver solution using Lee method was about 6.4. At this pH, functional groups of cysteine are protonated; thus, the thiol groups of cysteine bind to silver surface through ligand exchange reactions [20] and the amine group of one cysteine-coated nanoparticle forms a bond with carboxyl group of the other cysteine-coated nanoparticle. Hence, the cysteine molecule constitutes a bridge among silver nanoparticles, thus forming large aggregates, which deposit in time. In the method two, the pH of the synthesised colloid was 8.6. At this pH, the carboxyl groups will be negatively charged, the thiol groups will be positively charged and the amino groups behave as zwitter ionic species. Hence in absence of

electrostatic interactions, the amino groups failed to bind with the carboxyl groups to induce aggregation.

Hence, the particles synthesised using the Lee and Miesel's method upon interaction with cysteine caused the aggregation of particles, which could be applied for the colorimetric detection of biomolecules [21]. The silver nanoparticles synthesised using modified Creighton's method was more stable. The further alteration of this process can result in stabilised silver nanoparticles applicable for drug delivery and biomedical applications [22]. The differential functionalisation of silver nanoparticles with the same biomolecules may lead to differential biomedical applications. Additional studies are in process to establish the detailed mechanistic aspects of the process.

Conflict of interest

The authors have declared no conflict of interest.

References

[1] J.K. Daniels and G. Chumanov, *Spectroelectrochemical studies of plasmon coupled silver Nanoparticles*, J. Electroanal. Chem. 575 (2005), pp. 203–209.
[2] N. Shuming and R.E. Steven, *Probing single molecules and single nanoparticles by surface-enhanced Raman scattering*, Science 275 (1997), pp. 1102–1106.
[3] X.M. Dou, Y.M. Jung, Z.Q. Cao, and Y. Ozaki, *Surface-enhanced Raman scattering of biological molecules on metal colloid II: Effects of aggregation of gold colloid and comparison of effects of pH of glycine solutions between gold and silver colloids*, Appl. Spectrosc. 53 (1999), pp. 1313–1482.
[4] P.C. Lee and D.P. Meisel, *Adsorption and surface-enhanced Raman of dyes on silver and gold sols*, J. Phys. Chem. 86 (1982), pp. 3391–3395.
[5] F.Z. Jing, R. John, S. Rossen, and A.B. David, *Functionalized gold nanoparticles: Synthesis, structure and colloid stability*, J. Colloid Interface Sci. 331 (2009), pp. 251–262.
[6] R.M. Bright, D.G. Walter, M.D. Musick, M.A. Jackson, K.J. Allison, and M.J. Natan, *Chemical and electrochemical Ag deposition onto preformed Au colloid monolayers: Approaches to uniformly-sized surface features with Ag-like optical properties*, Langmuir 12 (1996), pp. 810–817.
[7] R. Elghanian, J.J. Storhoff, R.C. Mucic, R.L. Letsinger, and C.A. Mirkin, *Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles*, Science 277 (1997), pp. 1078–1081.
[8] R. Levy, N.T.K. Thanh, R.C. Doty, I. Hussain, R.J. Nichols, D.J. Schiffrin, M. Brust, and D.G. Fernig, *Rational and combinatorial design of peptide capping ligands for gold nanoparticles*, J. Am. Chem. Soc. 126 (2004), pp. 10076–10084.
[9] W. Droge, V. Hack, R. Breitkreutz, E. Holm, G. Shubinski, E. Schmid, and D. Galter, *Role of cysteine and glutathione in signal transduction, immunolpathology and cachexia*, BioFactors 8 (1998), pp. 97–102.
[10] L.P. Osman, S.C. Mitchell, and R.H. Waring, *Cysteine, its metabolism and its toxicity*, Sulfur Rep. 20 (1997), pp. 155–172.
[11] P. Tengvall, M. Lestelius, B. Liedberg, and I. Lundstroem, *Plasma protein and antisera interactions with L-cysteine and 3-mercaptopropionic acid monolayers on gold surfaces*, Langmuir 8 (1992), pp. 1236–1238.

[12] Y.C. Sasaki, K. Yasuda, Y. Suzuki, T. Ishibashi, I. Satoh, and Y. Fujikiet, *Two-dimensional arrangement of a functional protein by cysteine-gold interaction: Enzyme activity and characterization of a protein monolayer on a gold substrate*, Biophys. J. 72 (1997), pp. 1842–1848.
[13] I. Willner, E. Katz, B. Willner, R. Blonder, V. Heleg-Shabtai, and A.F. Buckmann, *Assembly of functionalized monolayers of redox proteins on electrode surfaces: Novel bioelectronic and optobioelectronic systems*, Biosens. Bioelectron. 12 (1997), pp. 337–356.
[14] N.R. Jana, L. Gearheart, and C.J. Murphy, *Wet chemical synthesis of silver nanorods and nanowires of controllable aspect ratio*, Chem. Commun. 7 (2001), pp. 617–618.
[15] M.G. Guzmán, J. Dille, and S. Godet, *Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity*, Proc. World Acad. Sci. Eng. Technol. 43 (2008), pp. 357–364.
[16] N. Mohri, M. lnoue, Y. Arai, and K. Yoshikawa, *Kinetic study on monolayer formation with 4-aminobenzenethiol on a gold surface*, Langmuir 11 (1995), pp. 1612–1616.
[17] M. Cyrankiewicz, T. Wybranowski, and S. Kruszewski, *Study of SERS efficiency of metallic colloidal systems*, J. Phys.: Conf. Ser. 79 (2007), pp. 012013–012019.
[18] A.A. Lazarides and G.C. Schatz, *DNA-linked metal nanosphere materials: Structural basis for the optical properties*, J. Phys. Chem. B 104 (2000), pp. 460–467.
[19] M.J. Betts and R.B. Russell, *'Amino acid properties and consequences of substitutions*, in *Bioinformatics for Geneticists*, M.R. Barnes and I.C. Gray, eds., Wiley, Chichester, 2003, pp. 289–316.
[20] S. Aryal, B.K. Remant, B. Narayan, C.H. Kim, and Y. Kim, *Study of electrolyte induced aggregation of gold nanoparticles capped by amino acids*, J. Colloid Interface Sci. 299 (2001), pp. 191–197.
[21] H. Li, Z. Cui, and C. Han, *Glutathione-stabilized silver nanoparticles as colorimetric sensor for Ni^{2+} ion*, Sens. Actuators B 143 (2009), pp. 87–92.
[22] M.E. Anderson, *Glutathione: An overview of biosynthesis and modulation*, Chem. Biol. Interact. 112 (1998), pp. 1–14.