Environmentally benign synthesis of positively charged, ultra-low sized colloidal gold in universal solvent

**Ajeet Kumar**¹, **Arnab De**², **Amit Saxena**¹ and **Subho Mozumdar**¹

¹Department of Chemistry, University of Delhi, Delhi-110007, India
²Department of Microbiology and Immunology, Columbia University, USA

E-mail: subhoscom@yahoo.co.in

Received 29 July 2013
Accepted for publication 14 April 2014
Published 13 May 2014

**Abstract**

A simple, single-step, one pot method was developed for the synthesis of monodispersed, ultralow sized, water-dispersible, stable, positively charged gold nanoparticles using the branched polyethlyneimine (PEI) in an aqueous media. Sizes of the gold nanoparticles have been tuned by adjusting the concentration of PEI and the gold salt. Formation of gold nanoparticles has been evidenced using various characterization techniques such as quasi-elastic light scattering (QELS), transmission electron microscopy (TEM), x-ray diffraction (XRD), energy dispersive x-ray (EDX) and spectroscopy and UV-visible spectrophotometer.

Keywords: gold nanoparticles, polyethlyneimine, colloidal, reducing agent

**Classification numbers**: 4.02

1. Introduction

Gold nanoparticles are of increasing interest in materials chemistry due to the versatility in their physical and chemical properties. The research related to gold nanoparticles and its applications is becoming an inevitable area of interdisciplinary research. It has been established that these particles are highly water dispersible and remain suspended in water with no alteration of chemicals or physical properties over extended periods of time. At the nanoscale level, gold nanoparticles exhibit distinct colored appearance and they are reported to provide many potential applications in nanoelectronics, nanodevices, chemical separations, bio and chemo sensing, molecular marker, catalysis, coating decorative, optical materials, biosciences and drug delivery etc [1–7].

There are various methods for synthesizing gold nanoparticles, such as arc discharge [8], two phase synthesis [9], one phase synthesis in organic solvent [10], photochemistry [11, 12], reverse micelles [13–15], radiolysis [16], electrochemical methods [17–20], and sonochemical methods [21, 22]. A relatively recent development has been the use of the reducing agent which also acts as the ‘nanoparticle capping species’, and this technique has been demonstrated using amino acids [23–26]. Nevertheless, these above methods required sophisticated apparatus, which severely limits large scale production. Additionally, previous work used expensive surfactants (i.e. benzyl mercaptan, cetyl trimethyl ammonium bromide (C-TAB), sodium bis(2-ethylhexyl) sulfonate (AOT) as stabilizers for preventing aggregation of synthesized gold nanoparticles. The aforementioned methods also needed further purification steps [27–30].

In addition, gold nanoparticles were synthesized using different reducing agents (ascorbic acid, oxalic acid, or hydrazine) [31]. A simple aqueous reduction method has been shown in the past to synthesize negatively charged gold nanoparticles. The process is environmentally friendly and uses a simple apparatus for preparing gold nanoparticles and its derivatives [32, 33].

In contrast, we disclose a simple green approach to synthesize positively charged gold nanoparticles. We report a one-pot methodology to synthesize highly stable, charge/size tunable gold nanoparticles in aqueous solutions using polyethyleneimine (PEI) as a reducing agent (and a particle stabilizer) in this work. The branched nature, high electrical conductivity, reducing as well as stabilizing ability make it an excellent choice for the synthesis of gold nanoparticles for various biomedical and biochemical applications. In this work...
different parameters such as concentration of gold salt and PEI in the water varied to synthesize gold nanoparticles of different shapes, size distribution and stability. These results are anticipated to be of use in the synthesis of biocompatible and sensing materials for biomedical and chemical catalysis applications. We have demonstrated the use of gold nanoparticles as a vehicle for liver-specific drug delivery in the past [34], and have used nanoparticles for catalyzing diverse organic reactions [35–48].

2. Experimental

The methodology for the preparation of the gold nanoparticles using polyethylenimine has been developed, where the PEI acts as stabilizing as well as reducing agent. The method is robust, reproducible and simple. Using the developed methodology one can synthesize gold nanoparticles either at room temperature in a stipulated time period or instantly by mild heating (scheme 1). In a typical procedure gold nanoparticles have been synthesized by mixing a known molar ratio of PEI and gold salt (generally 4:1) in an aqueous solvent and kept for a stipulated time period (6–12 h) to obtain brilliant red colored gold nanoparticles. The same result could be obtained virtually instantly by heating the solution at 60 °C for 5–10 min. Various parameters have been optimized to achieve a method to synthesize size controlled, monodispersed gold nanoparticles with enhanced shelf life.

The absorption spectrum of gold nanoparticles was recorded with a Hitachi AU-2700 spectrophotometer. The synthesized gold nanoparticles were dispersed in double-distilled water (1 ml), out of this sample solution (10 μl) was put on a formvar (polyvinyl formal) coated copper grid and then left overnight for air-drying. TEM pictures were taken with a model electron microscope (FEI Technai Ultra Twin 300 kV). Prior to visualization of samples, a blank grid without sample was also scanned. The average particle diameter of the prepared nanoparticles was analyzed by dynamic light scattering Instrument (Photocor FC, USA). Data analysis was performed with Alango dynal V 2.0 software.

Wide angle x-ray diffraction (XRD) patterns were obtained for gold nanoparticles by using Philips PW 1830 x-ray VB equipped with a 2θ compensating slit, Cu-Kα radiation (1.54 Å) at 40 kV, 40 mA passing through Ni filter with a wavelength of 0.154 nm at 20 mA and 35 kV. Data collection was made in a continuous scan mode with a step size of 0.01° and step time of 1 s over a of 2θ range of 10° to 80°. Data analysis was performed with PC-APD diffraction software.

3. Results and discussion

PEI is a highly branched, aliphatic, water soluble polyamine whose amine groups exist in primary, secondary and tertiary forms (scheme 2). PEI has a high cationic charge density which in turns leads to the reduction of gold salt and protects the gold nanoparticles thus formed by making a charge complex.
It is well known that PEI can easily protonate in acid, even in neutral aqueous solution. PEI reduces $\text{AuCl}_4^-$, causing it to nucleate and gradually leading to the formation of gold nanoparticles in the reaction

$$\text{HAuCl}_3 + 3\text{NR}_3 \rightarrow \text{Au}^0 + 3\text{NR}_3^+ + \text{H}^+ + 4\text{Cl}^-.$$  \hspace{1cm} (1)

During the mixing of oxidative HAuCl₄ solution with PEI, both protonate amine and neighboring methylene of PEI dehydrogenated to form a labile double bond (–C=N–) intermediate, which became amide at an elevated temperature. To gain an insight into the formation kinetics of gold nanoparticles, an \textit{in situ} UV–Vis experiment was performed at a constant temperature of 60 °C (figure 1). In less than 30 s, the onset of formation of nanoparticles was confirmed with the appearance of a surface band centered at 515 nm. Evidently, the intensity of the absorption peak increases significantly in the following minute and then the rate of increase of the peak intensity decreases with elapsing time, possibly because of the increase of the concentration of reduced gold. It is also evidenced that the absorption band shifts continuously to longer wavelength at longer reaction time and ceases at the absorption band centered at 532 nm. This change occurs within an hour which may probably be explained on the basis of particle growth and Ostwald ripening. After this, no change in the absorption spectroscopy is noticed. The reduction of HAuCl₄ occurs due to transfer of electrons from the amine to the metal ion, resulting in the formation of Au⁰ according to the reaction (1). The resulting metallic gold then undergoes nucleation and growth to form gold nanoparticles. The reducing potentials of the system indicates that the reduction is thermodynamically favorable and thus PEI reduces gold salt efficiently to form gold nanoparticles.

Results of TEM analysis (figure 2) and the quasi-elastic light scattering (QELS) revealed that there was no sign of agglomeration and the particles were spherical in shape and well dispersed. A magnified view of a gold nanoparticle is presented in figure 2(c), a typical high-resolution TEM micrography. Figure 2(c) displays fringe patterns, indicating the crystalline nature of these gold nanoparticles.

### 3.1. X-ray diffraction analysis

Gold crystallite sizes were obtained as an average of the three different crystallite size-values estimated from the line width...
of the (111), (200), (220) diffraction lines by using the Scherrer equation. XRD patterns of the gold nanoparticles prepared are depicted in figure 3 with 2θ values between 2° and 90°. The XRD pattern of gold nanoparticles in figure 3 shows four characteristic peaks for 2θ at 38.00°, 44.48°, 64.4° and 77.6° marked indices of (111), (200), (220) and (311) planes, respectively. A significant (111) diffraction peak suggests a prominent growth of network structure along (111) planes compared to (200). Additionally, the other prominent diffraction peak (220) points to the anisotropic (network) nature of the nanoparticles.

3.2. Electron diffraction analysis

Figure 4 shows the electron diffraction patterns of the gold nanoparticles. The diffraction pattern was obtained by aligning the electron beam perpendicular to the facet of the nanoplate. The Debye–Scherrer rings originating from the gold FCC structure are clearly observed because of the sloping background and the overlapping of peak tails for the adjacent (111) and (200) peaks and (311) and (222) peaks.

3.3. Energy dispersive x-ray analysis

Energy dispersive x-ray (EDX) was performed for synthesized gold nanoparticles. The EDX spectrum given in figure 5 shows gold as the only elementary component.

3.4. Zeta potential of gold nanoparticles

Zeta potential of the nanoparticles was measured using the Delsa™ Nano C (size and zeta potential analysis with a duo laser light). The software calculated zeta potential using phase analysis light scattering. The zeta potential of a nanoparticle refers to the electrostatic potential created as a result of the accumulation of electrons at its surface. The software program calculates zeta potential using the electrophoretic mobility of the sample. The charge and intensity of the electrophoretic mobility and zeta potential are reflective of the charge and intensity of the outermost layer of the nanoparticle. Figures 6 and 7 showed the electro-osmosis (EOS) plot and mobility distribution plot for the gold nanoparticles. It shows that the PEI stabilized gold nanoparticles are positively charged with Zeta potential of +23.45 mV with a mobility value of 1.829e-004 cm² Vs⁻¹. The conductivity measurement of the particles comes out to be 0.5968 mS cm⁻¹. The high cationic charge density synthesized gold nanoparticles kept each nanoparticle apart which resulted in the formation of stable colloids.
3.5. Effect of concentration of PEI on size of gold nanoparticles

Concentration of PEI plays a major role in deciding the size of the nanoparticles. On varying the PEI concentration, it was observed that the size of the particles decreased with the increase in the concentration of the PEI (table 1 and figure 8). However, when the concentration of PEI was increased beyond 4 wt%, no further decrease in the size of the particles occurred.

When the concentration ratio of PEI to gold was large enough, the reduction rate of gold chloride was much faster than that of the nucleation rate and almost all the gold ions were reduced to atoms before the formation of proper nuclei. Low concentration of added PEI causes particle aggregation because of the electrostatic attraction of partially coated and uncoated colloids.

3.6. Effect of concentration of gold salt on size of gold nanoparticles

The concentration of the gold salt also plays a crucial role in controlling the diameter of colloidal gold in universal solvent, namely water. On varying the concentration of gold salt keeping the PEI concentration constant, it was observed that at very low concentration of the aqueous gold salt concentration (0.001% w/v), the size of the nanoparticles appeared to be large which finally resulted in the complete precipitation of the nanoparticles (table 2). But, with a gradual increase in the salt concentration, the size of the particles decreased. The minimum size of the nanoparticles could be achieved at 1% concentration of the salt (table 2 and figure 9). Further increase in the salt concentration resulted in a sudden increase in the particle size.

The above observations may be explained by stating that at low concentration of the salt there will be much less interaction between salt and polymer. Therefore, most of the reaction occurred in the bulk phase and the few particles that formed were not stabilized by the system, resulting in the complete precipitation. However, when the concentration of the salt was increased the interaction between polymer and

| Sample No. | PEI (% w/v) | HAuCl₄ (% w/v) | Size (nm) | Polydispersity | Remarks               |
|------------|-------------|----------------|-----------|----------------|----------------------|
| a          | 4.000       | 1              | 05.10     | 0.616          | Stable              |
| b          | 2.000       | 1              | 17.10     | 0.579          | Stable              |
| c          | 1.000       | 1              | 29.15     | 0.300          | Stable              |
| d          | 0.500       | 1              | 54.16     | 0.412          | Stable              |
| e          | 0.250       | 1              | 61.65     | 0.313          | Stable              |
| f          | 0.100       | 1              | 87.54     | 0.506          | Precipitated after 12 h |
| g          | 0.050       | 1              | 129.9     | 0.282          | Precipitated after 6 h  |
| h          | 0.001       | 1              | 226.0     | 0.293          | Precipitated after 2 h  |

Conditions: Stock = 3 ml dd-H₂O; PEI = 100 μl; HAuCl₄ = 100 μl.
4. Conclusion

We report the first environmentally benign synthesis of positively charged gold nanoparticles. This is a simple poly-ethylenimine based synthesis and stabilizing approach for the facile formation of well dispersed, stable, pure, positively charged gold nanoparticles in aqueous solution. The results have implications for the synthesis of biocompatible and sensing materials for biomedical and chemical catalysis applications.

Table 2. Variation of concentration of HAuCl₄.

| Sample No. | PEI (% w/v) | HAuCl₄ (% w/v) | Size (nm) | Polydispersity | Remarks |
|------------|-------------|----------------|----------|----------------|---------|
| a          | 4           | 0.001          | 102.300  | 0.310          | Precipitated in 5 h |
| b          | 4           | 0.050          | 085.040  | 0.504          | Precipitated in 5 h |
| c          | 4           | 0.100          | 051.880  | 0.110          | Stable    |
| d          | 4           | 0.250          | 028.510  | 0.851          | Stable    |
| e          | 4           | 0.500          | 011.060  | 0.011          | Stable    |
| f          | 4           | 1.000          | 004.700  | 0.414          | Stable    |
| g          | 4           | 2.000          | 009.786  | 0.142          | Stable    |
| h          | 4           | 4.000          | 013.892  | 0.350          | Stable    |

Conditions: Stock = 3 ml dd-H₂O, PEI = 100 μl, HAuCl₄ = 100 μl.

References

[1] Maye M M, Luo J, Han L, Kariuki N N and Zhong C J 2003 Gold Bull. 36 75
[2] Haruta M and Date M 2001 Appl. Catal. A: General 222 427
[3] Gittins D I, Bethell D, Schiffin D J and Nichols R J 2000 Nature 408 67
[4] Shirai M, Haraguchi K, Hiruma K and Katsuyama T 1999 Gold Bull. 32 80
[5] Maxwell D J, Taylor J R and Nie S 2002 J. Am. Chem. Soc. 124 9606
[6] Taton T A, Mirkin C A and Letsinger R L 2000 Science 289 1757
[7] Park S J, Taton T A and Mirkin C A 2002 Science 295 1503
[8] Lung J K et al 2000 Nature 408 67
[9] Shirai M, Haraguchi K, Hiruma K and Katsuyama T 1999 Gold Bull. 32 80
[10] Rowe M P, Plass K E, Kim K, Kurdak C, Zellers E T and Matzger A 2004 J. Chem. Mater. 16 3513
[11] Dong S A and Zhou S P 2007 Mater. Sci. Eng.: B 140 153
[12] Marin M L, McGilvray K L and Scaiano J C 2008 J. Am. Chem. Soc. 130 16572
[13] Spirin M G, Brichkin S B and Razumov V F 2005 Colloid J. 67 485
[14] Bulavchenko A I, Arymbaeva A T, Bulavchenko O A, Tatarchuk V V and Petrova N I 2000 Russ. J. Phys. Chem. A 80 1980
[15] Herrera A P, Resto O, Briano J G and Rinaldi C 2005 Nanotechnology 16 618
[16] Henglein A 1999 Langmuir 15 6738
[17] Yu Y Y, Chang S S, Lee C L and RC Wang C 1997 J. Phys. Chem. B 101 6661
[18] Wei G T, Liu F K and Wang C R C 1999 Anal. Chem. 71 2085
[19] Qi H, Zu T and Liu Z F 2000 Acta Phys. Chim. Sin. 16 956
[20] Bianca M, vander Z I, Böhmer M R, Fokkink L G J and Schönenberger C 2000 Langmuir 16 451
[21] Mizukoshi Y, Okitsu K, Maeda Y, Yamamoto T A, Oshima R and Nagata Y 1997 J. Phys. Chem. B 101 7033
[22] Okitsu K, Yue A, Tanabe S, Matsumoto H and Yobiko Y 2001 Langmuir 17 7717
[23] Polavarapu L and Xu Q H 2008 Nanotechnology 19 5601
[24] Kashburi J and Rajendiran N 2009 Colloids Surf. B Biointerfaces 73 387
[25] Wang gao N, Bhasin K K, Mehtab S K and Suri C R 2008 J. Coll. Interf. Sci. 323 247
[26] Petean I, Tomoaia G H, Horovitz O, Mocanu A and Tomoaia-Cotisel M 2008 J. Optoelectron. Adv. Mater. 10 2289
[27] Vitale F, Vitaliano R, Battocchio C, Fratoddi I, Piscoppiello E, Tapfer L and Russo M V 2008 J. Organomet. Chem. 693 1043
Adv. Nat. Sci.: Nanosci. Nanotechnol. 5 (2014) 025017

Kumar A, Aerry S, Saxena A, De A and Mozumdar S 2012
Green Chem. 14 1298

Kumar A, Dewan M, Saxena A, De A and Mozumdar S 2012
PLos One 7 e29131

Dewan M, Kumar A, Saxena A, De A and Mozumdar S 2010
Tet. Lett. 51 6108

Dewan M, Kumar A, Saxena A, De A and Mozumdar S 2012
PLos One 7 e43078

Dewan M, Kumar A, Saxena A, De A and Mozumdar S 2011
Tet. Lett. 52 4835

Kumar A, Singh P, Saxena A, De A and Mozumdar S 2008
Catal. Commun. 10 17

Kumar A, Dewan M, Saxena A, De A and Mozumdar S 2013
RSC Adv. 3 603

Aerry S, De A, Kumar A, Saxena A, Majumdar D K and Mozumdar S 2013
J. Biomed. Mater. Res. Part A 101 2015

Aerry S, Kumar A, Saxena A, De A and Mozumdar S 2013
Green Chem. Lett. Rev. 6 183

Kumar A, Saxena A, De A, Shankar R and Mozumdar S 2013
RSC Adv. 15 5015

Kumar A, Saxena A, De A, Shankar R and Mozumdar S 2013
Adv. Nat. Sci.: Nanosc. Nanotechnol. 4 025009