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Synthesis of a stable gold hydrosol by the reduction of chloroaurate ions by the amino acid, aspartic acid

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Abstract. Development of reliable protocols for the synthesis of nanoparticles of well-defined sizes and good monodispersity is an important aspect of nanotechnology. In this paper, we present details of the synthesis of gold nanoparticles of good monodispersity by the reduction of aqueous chloroaurate ions by the amino acid, aspartic acid. The colloidal gold solution thus formed is extremely stable in time, indicating electrostatic stabilization via nanoparticle surface-bound amino acid molecules. This observation has been used to modulate the size of the gold nanoparticles in solution by varying the molar ratio of chloroaurate ions to aspartic acid in the reaction medium. Characterization of the aspartic acid-reduced gold nanoparticles was carried out by UV-visible spectroscopy, thermogravimetric analysis and transmission electron microscopy. The use of amino acids in the synthesis and stabilization of gold nanoparticle in water has important implications in the development of new protocols for generation of bioconjugate materials.

Keywords. Gold nanoparticles; reduction by amino acids; surface modification; bioconjugates.

1. Introduction

Development of synthesis protocols for nanomaterials over a range of chemical compositions, sizes and indeed shapes constitutes a steadily evolving branch of nanotechnology [1]. Much of the interest in this direction is fuelled by the unusual physical and chemical properties exhibited by nanomaterials which are quite different from those of their bulk counterparts [2–5]. The synthesis of gold nanoparticles has, in particular, received considerable attention, and potential applications in catalysis, single electron tunneling [6], nonlinear optical devices [7] and DNA sequencing [8] have been demonstrated. There are a number of synthesis procedures for obtaining gold nanoparticles over a range of sizes [9] and shapes [10] that may be broadly classified into two sections depending on whether the nanoparticles are grown in a non-polar organic medium or a polar medium such as water. Most reports on the synthesis of gold nanoparticles in non-polar organic solvents have followed the Brust protocol [11] wherein aqueous chloroaurate ions are transferred into the organic solvent using phase-transfer molecules (tetraalkylammonium salts). The chloroaurate ions in the organic phase are then reduced and capped with

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alkanethiol [11]/alkylamine [12] molecules resulting in stable gold nanoparticles. Some of the advantages of this approach include the ability to assemble the gold nanoparticles into a hexagonal close-packed configuration by simple solvent evaporation [13] and the use of suitably functionalized gold nanoparticles as novel chemical reagents [14]. On the other hand, gold nanoparticles grown in water would contribute immensely to the development of biological applications such as drug-delivery, gene transfer and recognition, biological markers etc. [15] and, in the current context, this is an extremely exciting area of research in nanotechnology.

In this laboratory, we have been interested in the surface modification of gold nanoparticles with amine derivatives as an alternative to the more popular alkanethiols and have shown that aqueous gold nanoparticles may be phase-transferred into non-polar organic solvents by complexation with alkylamine molecules at the liquid-liquid interface [16]. A detailed investigation into the nature of complexation of octadecylamine and laurylamine molecules with gold nanoparticles has recently been completed [17]. As part of this ongoing study, we have recently looked at surface modification of gold nanoparticles with amino acids where binding with the gold nanoparticle surface may be accomplished through the amine functionality. In this paper, we report our interesting finding that the amino acid, aspartic acid, reduces aqueous chloroaurate ions under boiling condition leading to the formation of extremely stable gold nanoparticles. The amino acid caps the gold nanoparticles after the reduction process thereby stabilizing the nanoparticles electrostatically. The ability of aspartic acid to bind to the nanoparticle surface has been used to modulate the size of the gold nanoparticles by simple variation in the chloroaurate ion: aspartic acid molar ratio in the reaction medium. The aspartic acid protected gold clusters have been characterized by UV-Vis spectroscopy, thermogravimetry analysis (TGA) and transmission electron microscopy (TEM). Presented below are details of the study.

2. Experimental

2.1 *Chemicals*

Chloroauric acid ($HAuCl_4$) and aspartic acid were obtained from Aldrich Chemicals and used as-received.

2.2 *Synthesis of aqueous aspartic acid-reduced gold nanoparticles*

90 ml of 10^{-4} M aqueous solution of chloroauric acid ($HAuCl_4$) was reduced by 10 ml of 10^{-2} M aqueous solution of aspartic acid under boiling condition to yield colloidal gold particles (**1**). In an attempt to vary the size of gold nanoparticles, we repeated the experiment by taking mixtures of 90 ml of 10^{-4} M aqueous solution of chloroauric acid ($HAuCl_4$) and 10 ml each of 10^{-3} M (**2**) and 5×10^{-4} M (**3**) aqueous solutions of aspartic acid under boiling conditions. The reduction of the metal ions to yield gold nanoparticles was evident in the appearance of a dark ruby-red to blue colour in the different colloidal solutions. The gold colloidal solution **2** was subjected to ultracentrifugation and the resulting pellet was washed with deionized water to remove uncoordinated aspartic acid molecules. The pellet was then dried and subjected to TGA analysis.

In order to demonstrate the reducing capability of aspartic acid, control experiments were performed as described above using the amino acids valine and lysine. More

specifically, a 10^{-4} M aqueous solution of $HAuCl_4$ was boiled with 10^{-3} M aqueous solutions of valine and lysine. Even after prolonged boiling, no reduction of the chloroaurate ions was observed, clearly underlining the role of aspartic acid in reducing $AuCl_4^-$ ions. In yet another control experiment, 10^{-4} M aqueous $HAuCl_4$ solution was heated to boiling and, in this case as well, formation of gold nanoparticles was not observed.

2.3 *UV-Vis spectroscopic studies*

The optical properties of aspartic acid-reduced gold colloidal solutions **1**, **2** and **3** were monitored as a function of time of reaction under boiling condition on a Hewlett–Packard diode array spectrophotometer (model HP-8452) operated at a resolution of 2 nm.

2.4 *Thermal stability measurements*

TGA profiles of carefully weighed quantities of purified powders of aspartic acid-reduced gold nanoparticles from **2** were recorded on a Seiko Instruments model TG/DTA 32 instrument at a heating rate of 10°C/min.

2.5 *Transmission electron microscopy measurements*

TEM measurements were made on a JEOL model 1200EX instrument operated at an accelerating voltage of 120 kV. Samples for TEM studies were prepared by placing a drop of gold colloidal solutions **1**, **2** and **3** on carbon-coated TEM grids. The films on the TEM grids were allowed to dry for 2 min following which the extra solution was removed using blotting paper.

3. Results and discussion

Figure 1A shows the UV-Vis spectra recorded from the colloidal gold solutions reduced by different concentrations of aspartic acid. Curves 1–3 in the figure correspond to colloidal gold solutions **1**, **2** and **3** respectively. The spectra have been shifted vertically for clarity. In all the spectra a strong absorption at ≈530 nm is observed, this resonance corresponding to excitation of surface plasmon vibrations in the gold nanoparticles [18]. The presence of this resonance in the visible region of the electromagnetic spectrum is responsible for the lovely pink-blue colours observed in gold colloidal solutions [18]. While the absorption band from gold colloidal solution **2** is sharp (figure 1A, curve 2), the spectra from solutions **1** and **3** are much broader. This result indicates some degree of aggregation of the gold nanoparticles in solution in samples **1** and **3**. We would like to mention here that in spite of indications of aggregation of the gold nanoparticles, the three different gold colloidal solutions were stable for months with little evidence of further aggregation. The variation in the optical properties of the three gold nanoparticle solutions is graphically illustrated in the inset of figure 1A which shows a picture of test-tubes containing solutions **1** to **3**. The gold solutions in test-tubes 1 and 3 are blue in colour while the solution in test-tube 2 exhibits a reddish orange colour. The change in colour of colloidal gold solutions from red to blue consequent to aggregation is a well-understood phenomenon [19] and has been used to study molecular recognition processes such as the biotin-avidin interaction [20] and DNA hybridization using gold nanoparticles in

solution [8]. Figure 1B shows the UV–visible spectra recorded from the gold colloidal solution **2** as a function of time of reaction of aspartic acid with chloroaurate ions under boiling conditions. A monotonic increase in the surface plasmon resonance intensity with time is observed, the reaction terminating after ≈10–12 min of reaction. The inset of figure 1B is a plot of the absorption intensity at 530 nm as a function of time and clearly shows how the reaction proceeds in experiment **2**. This measurement thus establishes the optimum conditions for the preparation of gold colloids for further studies.

The long-term stability of the colloidal gold solutions mentioned earlier indicates stabilization of the nanoparticles, possibly through binding of aspartic acid molecules to the nanoparticle surface. There are very few reports in the literature on surface modification of gold nanoparticles with amino acid molecules and therefore, it would be important to estimate the strength of binding of aspartic acid to the gold surface. Consequently, the gold nanoparticles capped with aspartic acid from solution **2** were obtained in the form of a powder and subjected to thermogravimetric analysis. Figure 2 shows a plot of the TGA data recorded from this sample. Two weight losses are seen to occur at ≈150°C (2% weight loss) and 350°C (1·2% weight loss). These weight losses are attributed to desorption of surface-bound aspartic acid molecules and indicates that there are two modes of binding of the amino acid to the gold nanoparticle surface. We have observed a similar behaviour from alkylamine capped gold nanoparticle at roughly the same temperatures [17]. Preliminary measurements by us [17] indicate that the two modes of binding of the amino acid could be via electrostatic interaction of the protonated amine

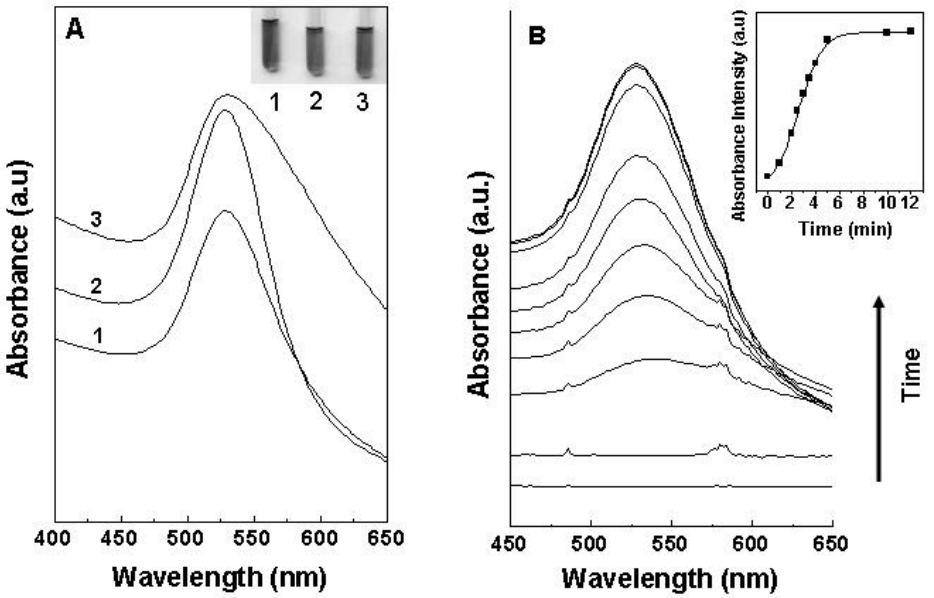

Figure 1. (**A**) UV-Vis spectra of colloidal gold solutions **1** (curve 1), **2** (curve 2) and **3** (curve 3, text for details). The inset shows pictures of the gold colloidal solutions **1**, **2** and **3** with identical labeling. (**B**) UV-Vis spectra recorded as a function of time of reaction in experiment **2** (text for details). The inset is a plot of the absorbance at 530 nm as a function of time and corresponds to the spectra shown in the main part of this figure.

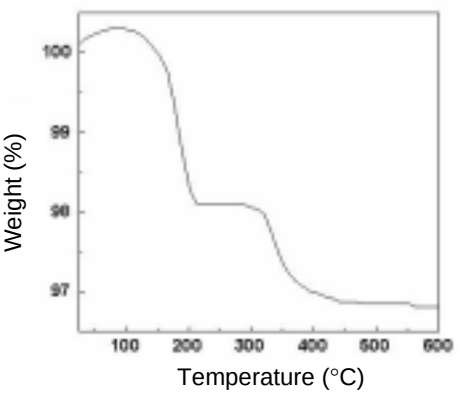

Figure 2. TGA data recorded from carefully weighed powder of aspartic acid-capped gold nanoparticles obtained from the gold hydrosol **2** (text for details).

groups with surface-bound negatively charged $AuCl_4^-$ ions and the other involving direct binding of the amine group to the gold surface. The increase in mass at ≈100°C is not understood at the moment and would require detailed chemical analysis.

Even though effective capping of the gold nanoparticles is indicated by the long-term stability of the gold colloidal solutions and the TGA measurements presented above, one source of disappointment was that the aspartic acid-capped gold nanoparticles after separation as a powder from the three colloidal solutions could not be redispersed in water. This is clearly not a consequence of oxidation of the aspartic acid molecules during formation of the gold nanoparticles since capping of nanoparticles separately synthesised by borohydride reduction of the gold salt also did not yield water-soluble gold colloids. The search for capping functionalities that would render the metal nanoparticles redispersible in water is a fertile area of research [21] having potential benefits akin to those enjoyed by non-polar solvent dispersible hydrophobized gold nanoparticles [14]. We are currently investigating other natural amino acids both from the gold nanoparticle synthesis point of view and for obtaining amino acid-protected gold nanoparticles that would be stable as a powder as well as readily water-dispersible.

Figure 3A shows a representative TEM image recorded from the gold nanoparticle solution **2**. It is seen that the gold nanoparticles are extremely uniform in size. Figure 3B shows the plot of the particle size distribution histogram for particles in the image of figure 3A. The solid line is a Gaussian fit to the histogram and yields an average particle size of 24 ± 3 nm. The gold nanoparticles are thus fairly monodisperse and adequately protected by the amino-acid monolayer. The monodispersity of the gold nanoparticles synthesized using aspartic acid as the reducing agent is comparable to other water-based synthesis procedures that yield standard deviations of typically 10–15% [9]. A closer examination of the micrograph reveals that even though a small fraction of the gold nanoparticles appear to have sintered, the particles are predominantly not in direct physical contact. A careful analysis of the micrograph yielded an average separation of 2·9 nm. This value is a little larger than that expected purely from dimensionality considerations of the aspartic acid monolayer on the gold nanoparticle surface.

Figures 4A and 4B show representative TEM images recorded from gold hydrosols **1** and **3** respectively. While the nanoparticles are roughly spherical in colloidal solution **1** and appear to be reasonably monodisperse (figure 4A), a very large variation in both size and shape of the gold nanoparticles in solution **3** is observed (figure 4B). In the latter case, the particles are also highly aggregated. The TEM result from figure 4B is in good agreement with the UV-Vis spectrum of this solution that indicated aggregation of the particles (figure 1A, curve 3). For the above reasons, a fairly accurate estimate of the nanoparticle size was possible for hydrosol **1** ($29 \pm 3{\cdot}4$ nm, figure 4A) and only a rough estimate of the particle size could be made for hydrosol **3** (42 nm, figure 4B).

The TEM results of figures 3 and 4 indicate the following. The gold nanoparticles synthesised using high aspartic acid concentrations [10^{-3} M (**1**) and 10^{-4} M (**2**)] yield

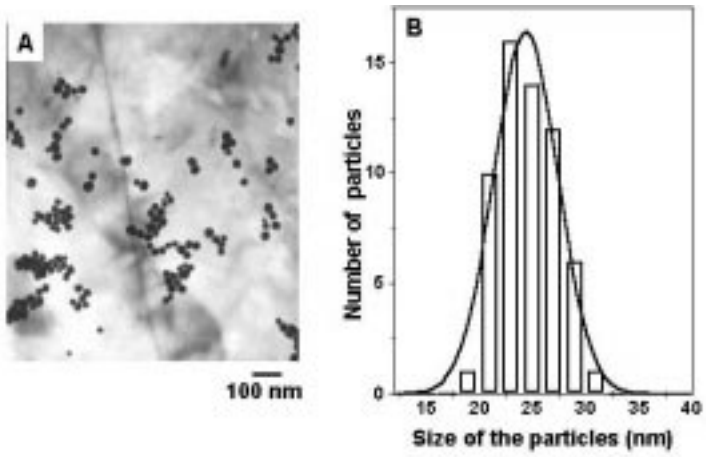

Figure 3. (**A**) Representative TEM micrograph showing a number of gold nanoparticles from the gold solution **2** (text for details). (**B**) Particle size distribution histogram measured from the image shown in A.

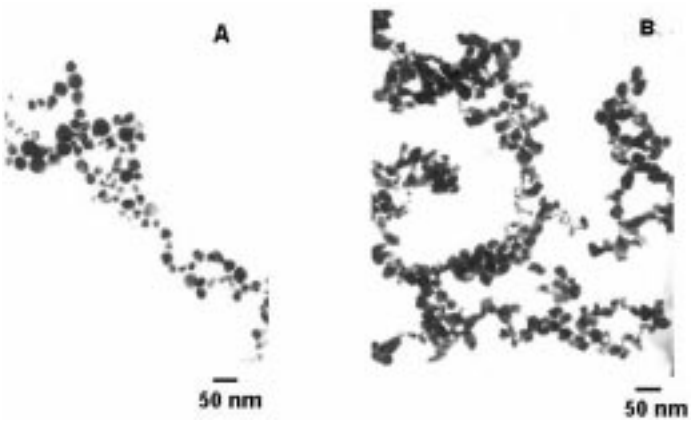

Figure 4. **A** and **B** – representative TEM micrographs of gold nanoparticles obtained from the gold nanoparticle solutions **1** and **3** respectively (text for details).

nanoparticles whose size increases with increasing amino acid content in the reaction medium. The monodispersity in both these solutions is also extremely good. At lower concentrations of the amino acid [5×10^{-5} M (**3**)], the particles are large and highly irregular in shape. The irregularity in shape and the much larger size of the gold nanoparticles in **3** indicates considerable sintering of the nanoparticles due to incomplete coverage of the nanoparticle surface by the stabilizing aspartic acid molecules. The above results are consistent with a strong interaction of the amino acid molecules with the gold nanoparticle surface.

4. Conclusion

In conclusion, the synthesis of gold nanoparticles by the reduction of chloroaurate ions by the amino acid, aspartic acid, has been described. Under certain preparation conditions, highly monodisperse gold nanoparticles stabilized by a monolayer of the amino acid are obtained. While some size control can be exercised by altering the gold ion: aspartic acid molar ratio, the widths of the particle size distributions are large and clearly requires further study for optimisation. We are currently investigating the use of other amino acids based on their hydrophobicity, acidity/basicity in the synthesis and capping of gold nanoparticles, and possibly their use as water-dispersible powders. Another aspect that will be addressed in future communications will be the changes occurring in the amino acids consequent to reduction of the metal ions and formation of nanoparticles. The use of a bio-friendly synthesis process and the stabilization of the gold nanoparticles by the amino acid is interesting from the point of view of forming bioconjugates of proteins and DNA with the nanoparticles. Another exciting possibility is covalent cross-linking of amino acid-capped nanoparticles by formation of amide bonds across nanoparticles and is being pursued.

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