

Synthesis and Functionalization of Gold Nanoparticles by Using of Poly Functional Amino Acids

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Abstract:

Synthesis and characterization of two functionalized gold nanoparticles by using of two poly functional amino acids (L-Arginine and L-Aspartic acid) are reported. The gold nanoparticles were reduced by sodium citrate and functionalized with L-Arginine at the pH of 7 and 11 and L-Aspartic acid at the pH of 7. Transmission electron microscopy, UV-Vis spectroscopy, dynamic light scattering, zeta potential and agarose gel electrophoresis techniques were used for characterization and identification. The transmission electron microscopy image showed well distributed particles with an average size of about 10 nm. At the pH of 7, the results obtained, verified that the interaction between L-Arginine and gold nanoparticles was electrostatic and it was covalent/coordinate in the case of L-Aspartic acid. The functionalized gold nanoparticles possessing free amine and carboxylic groups (poly functional amino acid) could play an important role in conjugating biomolecules such as proteins (e.g. antibodies) and DNA in nanomedicinal and nanobiotechnological applications. In addition, if the pH of the target in vivo environment is constant, functionalized gold nanoparticles bound electrostatically will be preferred. On the other hand, if the pH of the target in vivo environment is variable, then amino acid capped gold nanoparticles bound covalently/coordinate are recommended.

Keywords: L-Arginine, L-Aspartic acid, Functionalization, gold nanoparticles (GNPs)

1. INTRODUCTION

Today, the synthesis and characterization of metal nanoparticles such as Gold [1], Silver [2], and etc [3] is of considerable interest due to their attractive size- and shape-dependent optoelectronic and physicochemical properties [4-5]. Moreover, their applications in catalysis [6-7], imaging [8-9] and data storage [10] have highlighted the current growing interest. Particularly, Gold nanoparticles (GNPs) have been greatly investigated since the Middle Ages, when they were used in glasses, pigments, and applied in both medical treatments and diagnostic procedures [11]. The most usual

method for the production of GNPs dispersions is the reduction of Au (III) by a reducing agent such as sodium borohydride (NaBH₄) in a suitable solvent (e.g. water) [12-13]. Moreover, water-soluble GNPs dispersions potentially possess important biological applications after being functionalized with particular functional groups such as amino acids [14]. These functionalized GNPs with a specific size, dispersion pattern, and shape are categorized as bio-functionalized nanoparticles [15].

The surface modification of GNPs by functional groups would play an important role in many applications such as novel organic reactions [16],

biosensors [17], biodiagnostics [18], DNA /drug delivery [19], and imaging [20]. Although a common choice for GNPs surface modification is thiol-mediated binding of ligands [21], it is increasingly being recognized that amine groups can bind to GNPs quite strongly as well [22-23]. For instance, the strong binding of alkylamines with GNPs led to a successful phase transfer of aqueous GNPs to a non-polar organic solvent as well as the modification of the particles' surface [24]. These alkylamines-modified GNPs have important implications for biomedical applications [25-26]. On the other hand, amino acids as naturally occurring amine groups are preferred over the other amines e.g. alkylamines for in vivo applications [27-28]. Joshi et al. reported the successful production of water-dispersible GNPs by capping them with L-Lysine [29]. Bhargava et al. also produced GNPs by reduction and stabilization with sodium citrate, by using the amino acids L-Tyrosine and L-Tryptophan [30]. However, to the best of our knowledge, there has been no report on the strength and nature of the interactions between GNPs and the other amino acids till now.

The aim of this study was to synthesize GNPs functionalized by L-Arginine (Arg) and L-Aspartic acid (Asp) to obtain well-dispersed, identically-sized and -shaped particles at the pH of 7. The functionalized GNPs were characterized using high resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), UV-Vis spectroscopy, zeta potential and agarose gel electrophoresis (AGE) techniques.

2. EXPERIMENTAL

2.1. Material and methods

Tetrachloroauric (III) acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), L-Arginine and L-Aspartic acid, sodium citrate dihydrate, AGE chemicals (i.e. agarose, Tris-base, boric acid, and ethylenediamine tetraacetic acid (EDTA)) were obtained from Merck (Darmstadt, Germany). The deionized distilled water used was produced by the deionized water section of Pasture Institute of Iran. The UV-Vis spectroscopy measurements of

GNPs were performed on a Shimadzu dual-beam spectrophotometer (model UV-1601). DLS method (Malvern ZetaSizer 3000) was used to measure the size and zeta potential of the particles and the particle images were obtained by using a HR-TEM (JEOL-JEN 2010). Moreover, the preparation efficiency of the functionalized-GNPs at different pH was investigated by AGE.

2.2. Synthesis of functionalized GNPs

The aqueous GNPs were prepared by reducing Au (III) salt in presence of Arg and Asp. To achieve that, a solution of HAuCl_4 (3×10^{-3} M) in deionized distilled water was heated to boiling, and then sodium citrate solution (8×10^{-3} M) was added to the boiling solution (HAuCl_4 to sodium citrate molar ratio of 1:5). The production of GNPs was confirmed by a color change from colorless to ruby-red. After that, water solutions of Arg and Asp. (5×10^{-3} M) was added to the GNPs colloidal solution in a 1:10 molar ratio of gold to Arg or Asp solution at the pH of 3 and 7 for Asp and the pH of 7 and 11 for Arg. Finally, the solutions were incubated in a shaker-incubator at 4°C for 18 hr [29].

2.3. Characterization

The first characterization step in order to confirm the production of GNPs was carried out by using UV-Vis spectroscopy. Afterward, DLS and TEM techniques were used to determine the size and the distribution of the nanoparticles. In order to investigate the interaction between the Arg, Asp and the GNPs, Arg and Asp -capped GNPs were run on a 1% agarose gel at 130 V for 1 hr and also zeta potential analysis were used. Agarose gel was prepared in TBE buffer at pH=7.2 (tris base 0.02 M; boric acid 0.1 M and EDTA 1×10^{-3} M) [30]. Gel electrophoresis has been previously used in nanotechnology to investigate the binding behavior of L-Lysine [29], to separate DNA-capped nanogold [35-36], and protein-capped GNPs [37]. Charged nanoparticles may be separated by AGE based on their mass and surface charge.

3. RESULTS AND DISCUSSION

After producing the GNPs by using citrate as previously mentioned, the characterization of nanoparticles was carried out by TEM, DLS and UV-Vis spectroscopy methods. Figure 1 shows the TEM image and the DLS size distribution histogram, respectively. The results obtained, indicates that the synthesized GNPs are of high quality for the functionalizing experiment due to their acceptable size of about 10nm. This was further confirmed by the TEM images. Figure 2 shows the UV-visible spectrum recorded for the GNPs. A strong absorption corresponding to the excitation of surface plasmon vibrations in the particles is observed at $\lambda_{\max}=516$ nm [31-32]. When the GNPs were functionalized with Arg or Asp, a consequent red-shift accrued and the λ_{\max}

was shifted to 520 and 527nm for functionalized GNPs by Arg at the pH of 7 and 11, respectively (figure 2-a). The λ_{\max} values for functionalized GNPs by Asp were also shifted to 522 nm at the pH of 7, respectively (figure 2-b).

Some studies reported the extremely strong binding of amine groups to GNPs [33-34], which is comparable to that of thiol [33]. However, for in vivo applications, amino acids as a naturally occurring group are preferred [27-28]. L-lysine, L-tyrosine and L-tryptophan have been successfully bound to GNPs [29-30]. In the present study, functionalizing GNPs with poly functional amino acids possessing active free functional groups i.e. Asp (one free carboxylic acid) and Arg (two free amine groups) was accomplished. The only difference observed between the binding behavior of Asp and Arg with GNPs at the pH of 7, was the protonation of Arg's

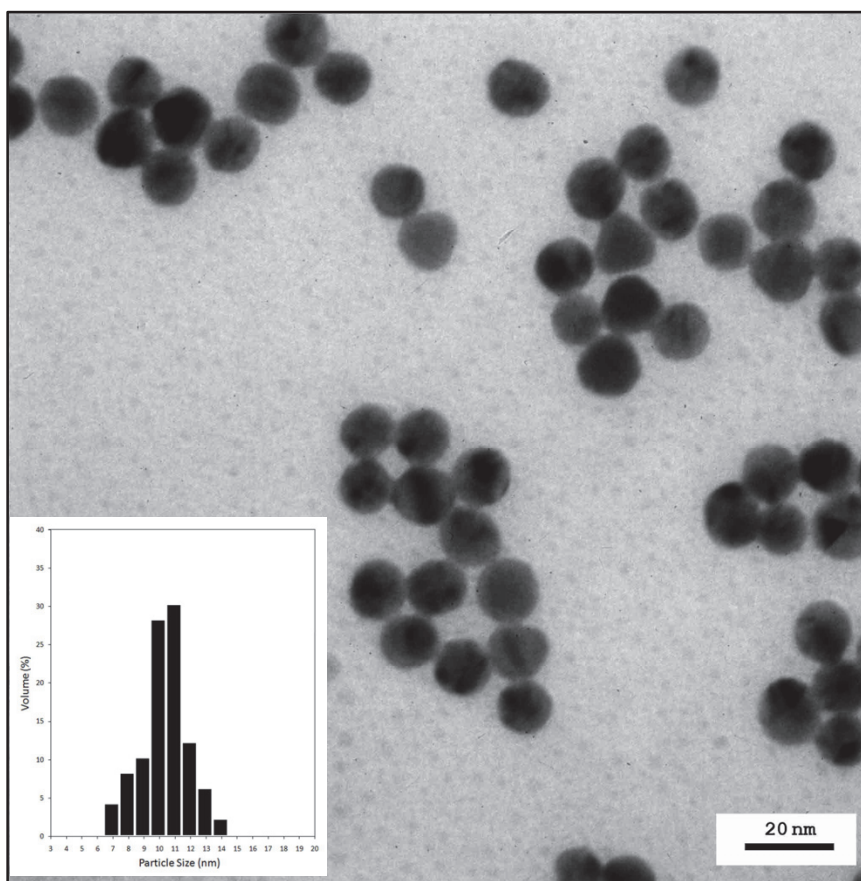


Figure 1: Transmission electron microscopy image and Size distribution of GNPs synthesized by sodium citrate.

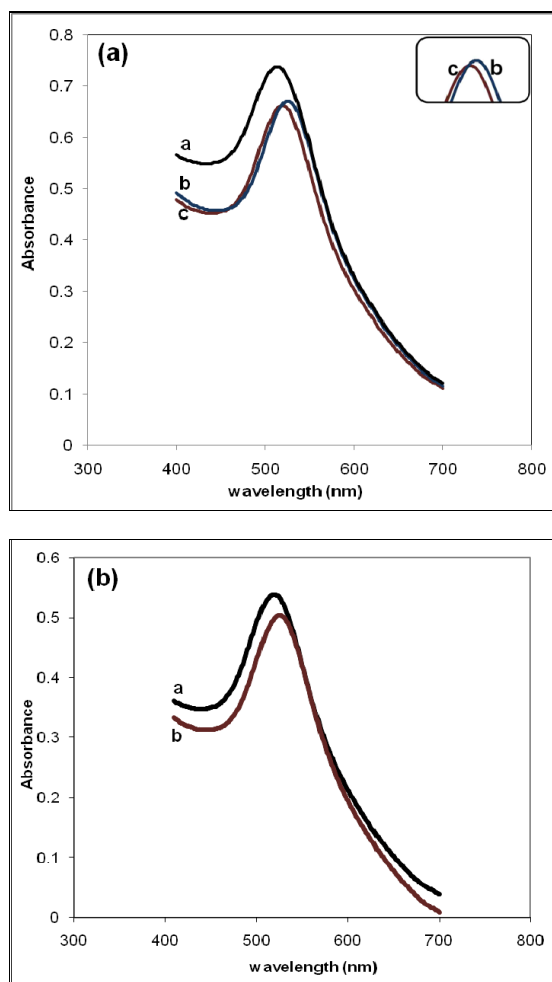


Figure 2: (a) UV-Vis Spectra of GNPs (Curve a) and functionalized GNPs by L-Arginine at the pH of 11 (Curve b) and 7 (Curve c), (b) UV-Vis Spectra of GNPs (Curve a) and functionalized GNPs by L-Aspartic at the pH of 7 (Curve b).

amine groups due to its isoelectric pH (pI) of 10.75 while the Asp's amine groups remained as -NH_2 because of its pI of 2.77. Under these conditions (pH=7), the type of bond between Arg and GNPs is electrostatic because the citrate-reduced GNPs at the physiological pH are negatively charged due to the surface-bound $\text{AuCl}_4^-/\text{AuCl}_2^-$ ions. On the other hand, Arg is positively charged at the same pH and therefore, complexes electrostatically with the gold surface. The low binding energy component of Au would be assigned to electron emission from Au (0),

while the high binding energy component is ascribed to binding of $\text{AuCl}_4^-/\text{AuCl}_2^-$ ions to the surface of the GNPs [33-34]. These ions are a general feature of the aqueous nanogold formulations reduced by borohydride in the +3/+1 oxidation states.

Apparently, the binding of the amine groups to GNPs only occurs in the unprotonated state. At the pH of 11, the Arg amine groups are not protonated due to its pI (10.76) and so, they are accessible for binding to GNPs. It must be noted that it is not obvious what kind of bond is formed between the amine groups and the GNPs, but the non-bonding pair electrons of nitrogen are clearly involved. Moreover, the strength of the interaction between Arg and nanogold is a covalent/coordination-like bond, and so, it may be expected to be directional. The consequent steric constraint on binding Arg to the surface of gold causes limitation on the coverage of Arg to lower packing values. On the other hand, electrostatic interactions are isotropic and allow higher coverage of Arg. This accounts for the variation in the binding strength of this amino acid with GNPs.

Zeta potential and Agarose gel electrophoresis techniques could be used to verify the binding behavior of different amino acids to GNPs at different pH. Gel electrophoresis has been previously used in nanotechnology to investigate the binding behavior of L-Lysine [29], to separate DNA-capped nanogold [35-36], and protein-capped GNPs [37]. Charged nanoparticles may be separated by AGE based on their mass and surface charge. When Arg-capped GNPs, synthesized at the pH of 7 and 11, were run on the gel along with uncapped GNPs, considerably more mobility toward the positive pole (anode) was observed for Au-Arg (pH=11) (Figure 3-a, lane 2) than both the uncapped GNPs (Figure 3-a, lane 1) and Au-Arg (pH=7) (Figure 3-a, lane 3). In fact, little movement of the Au-Arg prepared at the pH of 7 indicated the rather complete neutralization of the negative surface charge of Au by binding to Arg molecules electrostatically. Moreover, the higher mobility of Au-Arg prepared at the pH of 11 (Figure 3-a, lane 2) in comparison with uncapped GNPs (Figure 3-a, lane 1) clearly showed that the negative surface charge of the Au-Arg was higher

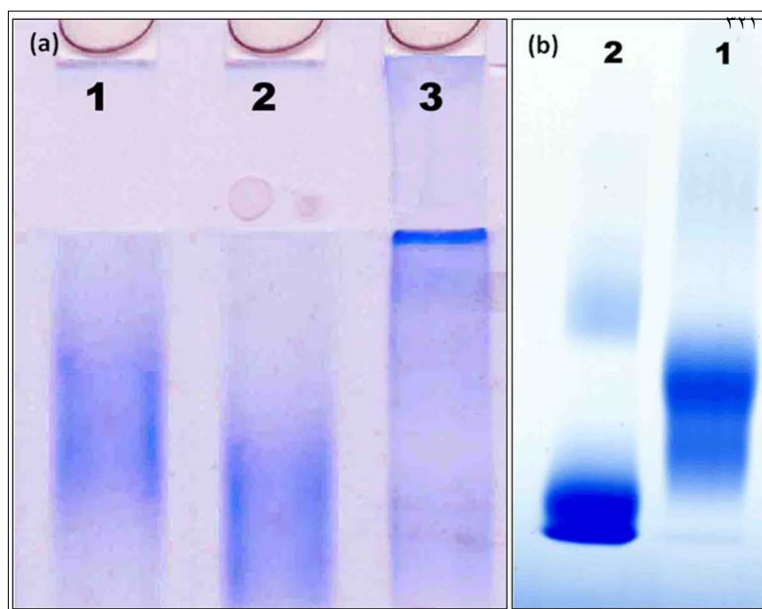


Figure 3: (a) Gel electrophoresis image of functionalized GNPs by L-Arginine. Lane 1) un-functionalized GNPs, Lane 2) Au-Arg at the pH of 11 and, Lane 3) Au-Arg at the pH of 7. (b) Gel electrophoresis image of functionalized GNPs by L-Aspartic acid. Lane 1) un-functionalized GNPs and, Lane 2) Au-Asp at the pH of 7

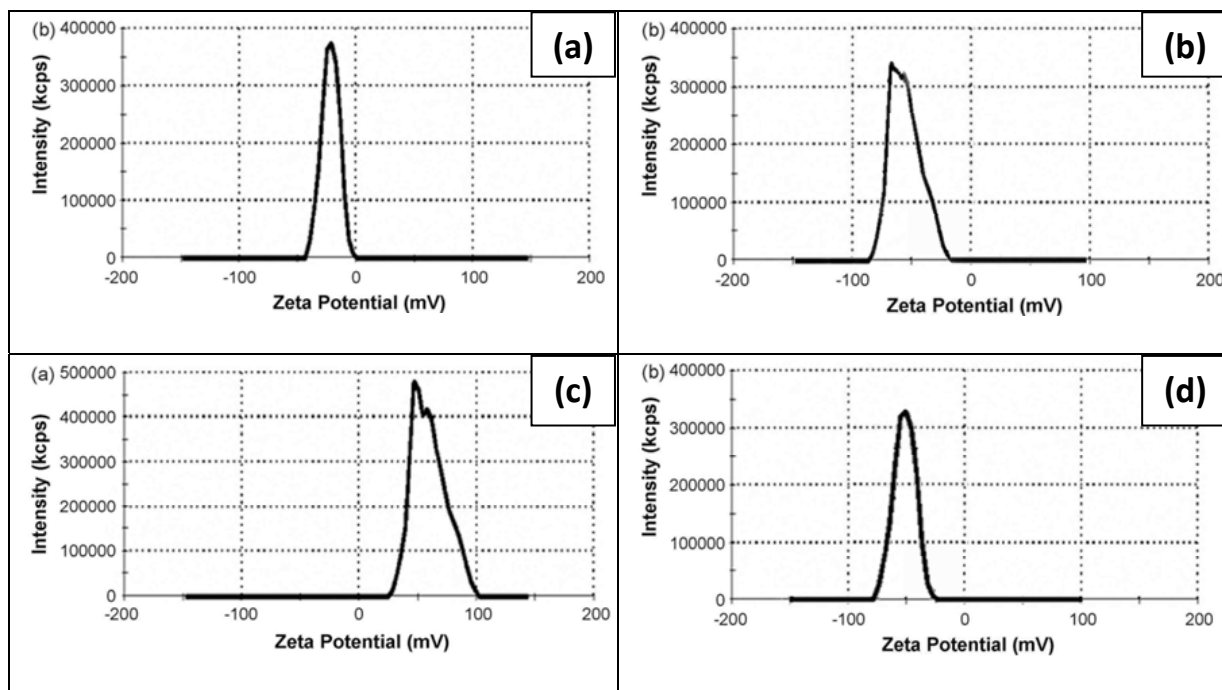


Figure 4: Zeta potential results (a) GNPs (b) Au-Arg the pH of 7 (c) Au-Arg the pH of 11 and, (d) Au-Asp the pH of 7.

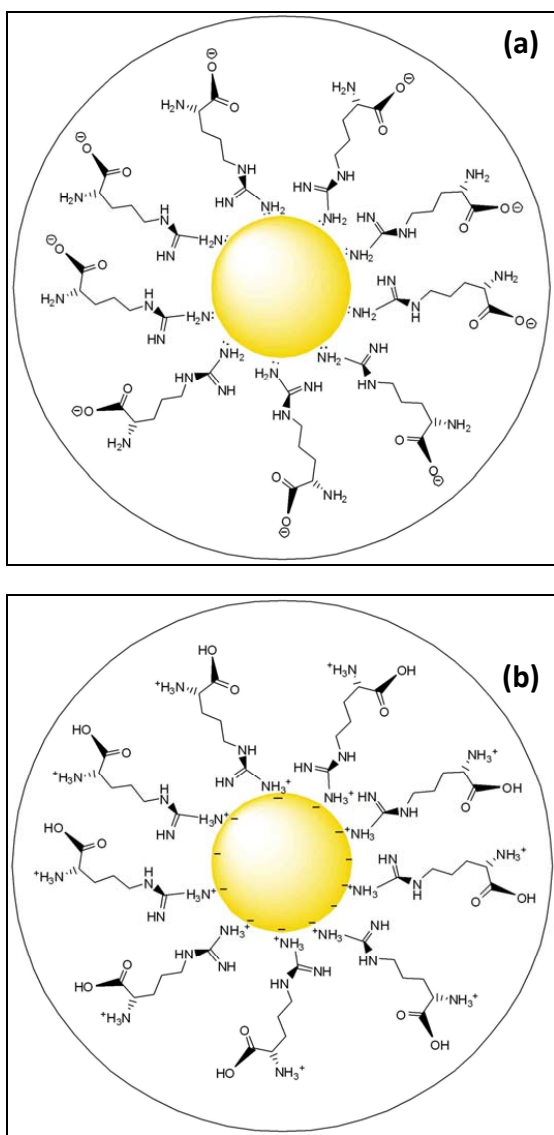


Figure 5: Schematic picture of functionalized by L-Arginine GNPs at the pH of a) 7 and, b) 11.

and attributed to the Arg's carboxylate ions. On the other hand, the smaller negative charge on the uncapped GNPs was only due to the surface bound $\text{AuCl}_4^-/\text{AuCl}_2^-$ ions. Also zeta potential results shown in figure 4a-c confirm the previous data.

Figure 3-b presents the mobility of functionalized Au-Asp synthesized at the pH of 7 on agarose gel (lanes 2), and on lane 1, the mobility of un-functionalized GNPs is shown. The Au-Asp and uncapped GNPs moved to the positive

pole (anode), indicating their negative charge. The results will be adapted by zeta potential data (figure 4-a,d). In addition, in case of Asp synthesized at the pH of 7, there was a stronger covalent/coordinate bond observed between N/Au. This resulted from proton elimination from NH_3 and the consequent formation of NH_2 , which supplied a non-bonding electron pair for making the covalent bond. Furthermore, investigation of the Au-Asp prepared at different pH by using UV-Vis spectroscopy showed a few red-shifts in the spectrums of functionalized GNPs by Arg or Asp due to the strong binding of the amino acid to the gold surface (Figure 2).

4. CONCLUSION

This study revealed the strong binding of Arg and Asp as functionalizing agents for GNPs due to their unprotonated amine groups. Moreover, an electrostatic interaction was observed between GNPs surface and the amine group of Arg at the pH of 7 (Figure 5-a). On the other hand, AGE and zeta potential analysis confirmed the covalent/coordination nature of the interaction which took place at the pH of 11 (Figure 5-b). The same covalent/coordination interaction was verified by AGE for Au-Asp capped nanoparticles synthesized at the pH of 7. In conclusion, if the pH of the target in vivo environment is constant, functionalized GNPs bound electrostatically are preferred because of the coverage of amino acids to higher packing values. On the other hand, if the pH of the target in vivo environment is variable, then Arg and Asp-capped GNPs bound covalently/coordinate are recommended because of the stronger and pH-independent nature of these bonds in comparison with the electrostatic bonds.

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