In vitro stability studies on gold nanoparticles with different stabilizing agents

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Abstract
Gold nanoparticles are widely used for clinical developments in cell labeling, contrast in biological imaging, drug design and time bound delivery at the specific location of the disease by conjugating gold nanoparticles with proteins or biomolecules that impart high affinity to target and at various disease pathologies. In biomedical applications, preventing aggregation of gold nanoparticle is a very challenging task. The media in which the cell grows, the cytoplasm of the cell and the blood stream are the complex aqueous mixtures of nutrients, proteins, electrolytes etc. Application of gold nanoparticles in biomedicine will require the introduction of particles into living cells. When nanoparticles are introduced into the biological systems, nanoparticles interact and precede the steps like distribution, metabolism and elimination. So the stability of nanoparticle is one of the main concern in biomedical application. In the present research work, gold nanoparticles stabilized with citrate with different sizes, starch and gum arabic have been synthesized and characterized. The AuNps were studied for its in vitro stability. The stability of the gold nanoparticles over a period of time of 240 hrs at room temperature, the addition of different concentration of electrolyte and the change of pH were monitored using absorption spectroscopy and Zeta potentiometer. The in vitro stability studies show that, the gold nanoparticles were highly stable under the experimental conditions adopted. In this stability studies, it was found that, the gold nanoparticles are highly stable also the starch and gum arabic stabilized gold nanoparticles were more stable than citrate capped gold nanoparticles.

Keywords: gold nanoparticles; stability; zeta potential; UV-Vis spectroscopy; electrolyte

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Introduction
Nanotechnology is defined as the understanding, manipulating and controlling things at nanoscale, where the unique properties of materials arises that enable novel applications. Gold is a noble of all metals due to its resistance to surface oxidation because of this, it is known as the king of the metals. Contrary to the nobility of bulk gold, exciting properties can be attributed to gold at the nanoscale. Gold nanoparticles also possess a large surface to volume ratio, which renders them suitable for attachment with biomolecules. When nanoparticles are introduced in biological systems, it is very important to understand the physical and chemical interactions of both nanoparticles and biological media to predict the subsequent process involved. When various components of living system interact with the nanoparticles, this will affect the physiochemical properties such as size, aggregation, surface charge and surface chemistry.

The high ionic strength of the biological media and the presence of electrolyte can result in nanoparticle aggregation. Different plasma proteins adsorb on nanoparticle surface and the surface chemistry of the
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A nanoparticle in a growth medium like plasma is not the same as the originally synthesized nanoparticles. Instead, the nanoparticles adopt the physiochemical properties of the adsorbed protein (Cedervall et al., 2007). The sodium chloride present in the biological systems form Cl\(^{-}\) and Na\(^{+}\) ions that can serve different purposes. The cell can change the concentration of certain other compounds using the help of Cl\(^{-}\) and Na\(^{+}\) ions by affecting the direction in which water diffuses to form macromolecules that needed in the human body. In a similar way, when we use nanoparticles for in vivo or in vitro biomedical applications, the presence of electrolyte in the living cells agglomerates the nanoparticles and thus the stability of the gold nanoparticles will be distorted. So the stability is a main concern for nanoparticles in biomedical applications. Also there is a possibility of pH changes when the gold nanoparticles interact with the body fluids and blood cells which lead to the change of its stability. In this work different sizes of citrate, starch and gum Arabic stabilized gold nanoparticles were synthesized and characterized. A number of methods have been previously reported for the synthesis of metal nanoparticles in aqueous solution using stabilizing agents such as citrate (Faraday, 1857; Turkevich et al., 1951; Turkevich and Kim, 1970), starch (Zhang et al., 2004; Raveendran et al., 2006; Lirdprapamongkol et al., 2010) and gum arabic (Nishi and Jayakrishnan, 2005; Kattumuri 2006; Park et al., 2008). The stability of gold nanoparticles were analyzed by the addition of different concentrations of electrolyte (NaCl), change of pH of the gold solution and time of storage at room temperature. The stabilities were monitored with the help of UV-Visible spectroscopy and measuring Zeta potential of the gold nanoparticle solution.

**Materials and Methods**

The chemicals used in the synthesis of gold nanoparticles (AuNPs) were procured from the standard vendors. Gum arabic, starch, HAuCl\(_4\), Tri sodium citrate and polyethylene glycol (PEG) were purchased from Sigma. Tri hydroxyl phosphino alanine (THPAL) was generated in the laboratory according to the standard literature procedures (Neves et al., 2002; Raghuraman et al., 2003). Double-distilled water was used in all the experiments. Sodium chloride (NaCl), nitric acid, buffers of pH\(_{4}\), pH\(_{5}\), pH\(_{6}\), pH\(_{7}\), pH\(_{8}\) and pH\(_{9}\) (Fisher).

*Preparation of Gold Nanoparticles (AuNps): Synthesis of Citrate (CAuNps) Stabilized Gold Nanoparticles*

Conventional techniques for aqueous synthesis of gold nanoparticles involve reduction of Au(III)Cl\(_3\) with trisodium citrate, a process pioneered by Turkevitch and later refined by Frens (Frens, 1972; Aika et al., 1976; Kimling et al., 2006). In this reduction process 250 mL of 0.25 mM HAuCl\(_4\) was heated to boiling, then 2.75 mL of aqueous solution of 1% trisodium citrate was added to the beaker containing HAuCl\(_4\) solution under vigorous stirring and the boiling was continued for 15 min until it turns deep red color. The citrate ions serve as both reducing as well as capping or stabilizing agent thus preventing agglomeration of gold nanoparticles. In this process gold nanoparticles of average size of 30 nm was prepared. This synthesis procedure was used to fabricate gold nanoparticles with different diameter by separately adding 8, 7, 6.5, 5.75, 5.4, 3.9 and 1.2 mL of 1% trisodium citrate to the 250 mL of HAuCl\(_4\) solution taken in a separate beaker. The prepared samples were labeled as C\(_1\), C\(_2\), C\(_3\), C\(_4\), C\(_5\), C\(_6\), C\(_7\) and C\(_8\).

*Synthesis of Starch Stabilized Gold Nanoparticles (SAuNps): About 0.0225 g of starch is dissolved in 6 mL of*
doubly ionized (DI) water by heating the solution to 90-100°C with continuous stirring. To this hot starch solution 0.1 mL of 0.1 M NaAuCl₄ solution is added, followed by the addition of 0.02 mL of 0.1 M THP AL solution with continuous stirring. The change in the color of the solution to pinkish purple confirms the formation of gold nanoparticles. The stirring is continued for a minute without heating (Kattumuri et al., 2006) and the sample is lebeled as SAuNp.

**Synthesis of Gum Arabic Stabilized Gold Nanoparticles**

About 0.012 g of gum arabic (GA) is dissolved in 6 mL of doubly ionized (DI) water by heating the solution to 90-100°C with continuous stirring. To this hot gum arabic solution 0.1 mL of 0.1 M NaAuCl₄ solution is added followed by the addition of 0.02 mL of 0.1 M THP AL solution with continuous stirring. When the color of the solution changes reddish purple, stirring is continued for a minute without heating and the sample is lebeled as SAuNp. The schematic presentation of preparation of gold nanoparticles stabilized with citrate, starch and gum arabic is shown in Figure 1(A).

**Stability studies on gold nanoparticles**

**Stability of Gold Nanoparticles as a Function of pH:** The zeta potential of a colloidal solution is an indicator of its stability. The greater the zeta potential the higher is the repulsion between the particles. As an empirical rule (Everett, 1989) colloidal suspensions exhibiting zeta potentials lying in the interval between -30mV and +30mV are generally unstable. Therefore colloidal suspensions are considered stable when their zeta potentials are more positive than +30mV or more negative than -30mV. The pH of the gold nanoparticle solution was changed without significantly altering the concentration of the nanoparticle solution. A very small amount of an NaOH, HCl or pH buffer pH-4, pH-5, pH-6, pH-7, pH-8 and pH-9 in aqueous solution of optimum concentration were added to the nanoparticle solution to achieve the desired pH value. Zeta potential measurements were performed using a Malvern Instruments Zeta Sizer 1000Hs operating with a variable power, He-Ne laser (5-50 mW) at 633nm.

**Fig. 1.** (A) The schematic illustration of formation of citrate, starch and gum arabic stabilized gold nanoparticles after reduction with citrate and THPAL, (B) The plasmon width can be calculated by subtracting the linear background from the plasmon peak and (C) The surface plasmon resonance wavelength observed for gold nanoparticles stabilized with citrate (3-42 nm), starch (22 nm-Starch) and (21 nm-Gum arabic).

**Stability of Gold Nanoparticles as a Function of Time and Addition of Sodium Chloride**

Stability of the gold nanoparticles over time (0, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hr) was monitored using absorption spectroscopy at room temperature. The analysis of the characteristic absorption peak $\lambda_{max}$ and $\Delta\lambda$ over a 10 day period were checked for the precipitation of gold nanoparticles. The stability studies were performed with sodium chloride (NaCl) as a function of concentration along with AuNp (Aryal et al., 2006; Ghosh and Pal, 2007). The stability of gold nanoparticles was also measured in the presence of different...
concentrations (0.125, 0.5 and 1.0 M) of sodium chloride (NaCl) solutions with UV-Visible absorption spectroscopy. About 0.5 mL of 30% NaCl was added to 4mL of gold nanoparticles solution and incubated for 45 mins before taking the absorption measurements. The UV-Visible absorption spectroscopy was used to study the peak absorption at different concentration of NaCl after 24 hr.

**Table 1.** Summary of the physical and optical characterization of gold nanoparticles stabilized with citrate, starch and gum arabic

<table>
<thead>
<tr>
<th>Sample code</th>
<th>SPR Absorption</th>
<th>Particle size</th>
<th>Plasmon width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>516nm</td>
<td>3nm</td>
<td>81</td>
</tr>
<tr>
<td>C2</td>
<td>518nm</td>
<td>5nm</td>
<td>77</td>
</tr>
<tr>
<td>C3</td>
<td>519nm</td>
<td>6nm</td>
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<td>C4</td>
<td>520nm</td>
<td>8nm</td>
<td>72</td>
</tr>
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<td>C5</td>
<td>522nm</td>
<td>10nm</td>
<td>68</td>
</tr>
<tr>
<td>C6</td>
<td>523nm</td>
<td>17nm</td>
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<tr>
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<td>42nm</td>
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</tr>
<tr>
<td>SAuNP</td>
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<td>22nm</td>
<td>71</td>
</tr>
<tr>
<td>GAuNP</td>
<td>524nm</td>
<td>21nm</td>
<td>76</td>
</tr>
</tbody>
</table>

**Characterization of gold nanoparticles**

Gold nanoparticle solution stabilized with citrate, starch and gum arabic were characterized by UV-Visible absorption spectroscopy using a UV-1700 series spectrophotometer (PSG Institute of Advanced Studies, Coimbatore, India) to study the peak absorption band. The plasmon wavelength, $\lambda_{max}$ is the maximum absorption wavelength of the incident photons on the gold nanoparticles and corresponds to the mean size of the nanoparticles. The plasmon width, $\Delta \lambda$ corresponds to the size distribution of nanoparticles and is estimated from the full width at half maximum (FWHM) of plasmon peak after a linear background was subtracted from the absorption spectrum as shown in Figure 1(B). Zeta potential measurements were performed using a Malvern Instruments Zeta Sizer 1000 Hs operating with a variable power, He-Ne laser (5-50 mW) at 633 nm (Indian Institute of Science, Bangalore, India). Measurements were taken at 25°C and repeated eight times. Before and in between measurements the flow through cell was washed with ultra-high pure (UHP) water. TEM images of citrate, starch and gum arabic capped gold nanoparticles were collected on a Phillips CM200 (Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Bombay, India).

The operating voltage range was 20-200 kV with a resolution of 2.4Å. A dilute suspension of sample in water was drop-coated on a FormvarTM and carbon-coated copper grids. This was allowed to dry in the air and then analyzed.

**Results and Discussion**

It is important to note that the citrate acts as a good reductant as well as a kinetic stabilizer. Adding less sodium citrate leads to bigger particles but these results are less reproducible, indicating the importance of the citrate stabilizing the gold nanoparticles. The increase of surface Plasmon wavelength moves towards the longer wavelength as the particle size increases, $C_1$ to $C_8$ but the plasmon wave width increases with decrease of particle size from $C_6$ to $C_1$ region for the intrinsic size region ($< 25$ nm) and beyond that the extrinsic size region ($25$ nm ≤), it increases with increase of particle size, $C_7$ and $C_8$ as reported in Table 1. It is well established that the bandwidth is inversely proportional to the radius ($r$) of the very smaller particles (Kreibig and Genzel, 1985). Since Mie’s theory has found wide applicability and has generally been successful in explaining optical absorption spectra of metallic nanoparticles (Kerker, 1969; Bohren and Huffman, 1983; Mulvaney, 1996). The size dependence of
the Plasmon absorption in the quasi-static regime is introduced by assuming a size dependent material dielectric function $\varepsilon(x,R)$, the related changes in the optical absorption spectra are referred as intrinsic size effects (Link and El-Sayed, 1999). It is well known that the position and shape of the SPR band are strongly related to particle size, dispersion and degree of aggregation (Lin and Sandroff, 1985; Shipway et al., 2000; George Thomas and Kamat, 2000; Daniel and Astruc, 2004). The broadening of the plasmon band is then usually ascribed to retardation effects.

**Fig. 2.** (A) The transmission electron micrograph of citrate (a) 3, (b) 5, (c) 6, (d) 8, (e) 10, (f) 17, (g) 30, (h) 42, (i) 22 nm-Starch and (j) 21 nm-Gum arabic stabilized gold nanoparticles and (B) The size histogram for citrate (3-42 nm), starch (22 nm-SAuNps) and gum arabic (21 nm-GAuNps) stabilized gold nanoparticles.

Figure 1(C) shows the absorption spectra of different sizes of gold nanoparticles with diameters of 3, 5, 6, 8, 10, 17, 30 and 42 nm citrate stabilized gold nanoparticles (CAuNps), 22 nm starch stabilized gold nanoparticles (SAuNps) and 21 nm gum arabic stabilized gold nanoparticles (GAuNps). They were prepared by chemical reduction of gold ions with trisodium citrate (Handley, 1998), THPAL-Starch and THPAL-Gum arabic in an aqueous solution. Starch is a complex carbohydrate. It is well known that simple carbohydrates like glucose and fructose are reducing sugars that are capable of reducing AuCl$_4^-$ ions to form AuNPs (De La Fuente and Penades, 1760). Starch acts as a
stabilizer and keeps the nanoparticles segregated for longer time without really participating in the reduction of gold. The hydroxyl groups of the gum arabic network hold the nanoparticles through hydrogen bonding helping them stay apart and thus providing stability to nanoparticles.

Several particles with diameter greater than the above sizes are also present but it is suspected that these particles arise from overlap lapping of two or more small particles (Wang et al., 2008). They also show the spacing between the gold nanoparticles. The individual particles allow the citrate layer surrounding the particle to be seen. This is strong confirmation that particles have been attached to the surface of the gold nanoparticles. Although the exact chemical nature of the material absorbed on the surface of citrate stabilized gold nanoparticles is not definitively known (Chow and Zukoski, 1994), an attempt can be made to rationalize the observed trend. There is some evidence showing that citrate ions, chloride, gold chloride (AuCl$_4^-$ and AuCl$_2$) produced by incomplete reduction of AuCl$_4^-$ and hydroxide anions are present on the citrate-stabilized gold nanoparticle surface (Weitz and Lin, 1985; Grabar et al., 1996; Li and Liu, 2003).

Starch is a complex carbohydrate. It is well known that simple carbohydrates like glucose and fructose are reducing sugars that are capable of reducing AuCl$_4^-$ ions to form AuNPs. The hydrophilic sites in the starch network hold the nanoparticles, keeping them away from one another and thus providing stability to nanoparticles. Gum arabic has been used to coat and protect nanoparticles from agglomeration (Effiong et al., 2004). The TEM micrograph of gold nanoparticles are shown in Figure 2 and the particle size histogram is shown in Figure 2. The position and shape of the surface plasmon band did not exhibit any dependence from the pH of the nanoparticle solution indicating good stability of citrate stabilized gold nanoparticles. It was also examined as a function of pH using zeta potential measurements as shown in figure 3. The zeta potential of a nanoparticle solution is an indicator of its stability. The greater the zeta potential, the higher is the repulsion between the particles. Colloidal suspensions are considered stable when their zeta potentials are more positive than +30 mV or more negative than -30 mV. The gold nanoparticles are negatively charged and the potential measurements indicate good stability of colloidal solutions with respect to pH changes. As a consequence the surface of the colloidal gold becomes less negatively charged. For statistical reasons, eight streaming Zeta potentials were measured at each pH value. The mean value of these data was used to calculate the mean potential per pH function. In all the experiments, the Zeta potential for the nanoparticles ranging from 3 to 42 nm were more negative than -30 mV. So the nanoparticles are highly stable with the change of pH.

Stability of the gold nanoparticles over time (0, 24, 48, 72, 96, 120, 144, 192, 216 and 240 hr) was monitored using absorption spectroscopy by measuring the maximum plasmon resonance wavelength as shown in Figure 4(A) and plasmon width as shown in Figure 4(B). The analyses were carried over a period of ten days and the precipitations of gold nanoparticles were monitored. It is important that the use of lower concentrations of AuNPs in biomedical applications and should be stable over a period of time and also it should preserve their chemical and photophysical properties under in vitro and in vivo environments. It should be noted that red shift in $\lambda_{max}$ is associated with either an increase in the mean size of the particles or modification in the surrounding media. Correspondingly any change in $\Delta \lambda$ indicates either
agglomeration of nanoparticles or modification in the surrounding media. A change of around 1nm per day in \( \lambda_{\text{max}} \) and \( \Delta \lambda \) at room temperature was deemed adequate to consider AuNPs stable over that period of time in a particular medium. The electrolyte (NaCl) has many roles in living organisms one of them is to raise the specific heat of water in living organisms. It does this by lowering the freezing point of water in the organism's body and raising its boiling point. Another function of NaCl is that after enters the body the Cl\(^-\) and Na\(^+\) ions can serve different purposes and the cell use them for changing the concentration of certain other compounds using the help of Cl\(^-\) and Na\(^+\) ions, by affecting in the direction in which water diffuses to form macromolecules in the human body.

It was observed that there is some deviation of observed plasmon absorption peak value from its original absorption peak (NaCl = 0 mM) as shown in Figure 4(C). There is also change in surface plasmon width, when the concentration of NaCl increases as shown in Figure 4(D). But the shift in \( \lambda_{\text{max}} \) and \( \Delta \lambda \) were very small. So the prepared citrate stabilized gold nanoparticles are stable over the range of NaCl concentrations. The position and shape of the surface plasmon band did not exhibit any dependence from the pH of the nanoparticle solution indicating good stability of citrate, starch and gum arabic stabilized gold nanoparticles. As the surface plasmon resonance position is very sensitive to the aggregation the minimal change in its position under the above experimental conditions indicates the extra stability of gum arabic capped AuNPs. In comparison with other AuNPs systems like citrate and starch stabilized gold nanoparticles, the gum arabic stabilized gold nanoparticles show less aggregation as pH changes and in the electrolyte environment.

**Conclusion**

In conclusion, we have prepared gold nanoparticles stabilized with citrate, starch and gum arabic using the modified Turkevich method. The aqueous solution of gold nanoparticles was characterized by UV-Visible spectroscopy and Transmission electron microscopy. In addition, the *in vitro* stabilities over the period of time through which, it is stored at room temperature, change of pH and the addition of different concentration of electrolyte (NaCl) were studied using UV-visible spectroscopy and by measuring its zeta potential. These particles were found to be easily soluble in water and stable across the various experimental methods that were adopted to analyze its stabilities moreover the starch and

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**Fig. 3.** Zeta potential of gold nanoparticles stabilized with citrate (3-42 nm), starch (22 nm-Starch) and (21 nm-gum arabic)
gum arabic stabilized gold nanoparticles were found to be more stable compare to the citrate stabilized gold nanoparticles. The findings of the study lead to the conclusion that AuNps can be used effectively in developing catalysts, sensors, nanomedicine and in biomarker applications.

**Fig. 4.** (A) Surface plasmon resonance spectrum of gold nanoparticles stabilized with citrate (CAuNps) (C₁ to C₈), Starch (SAuNps) and Gum arabic (GAuNps) as a function of time (0, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hr), (B) Surface plasmon width of gold nanoparticles as a function of time (0, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hr), (C) Surface plasmon resonance spectrum of gold nanoparticles as a function addition of different concentration of electrolyte (NaCl) and (D) Surface plasmon width of gold nanoparticles as a function of addition of different concentration of electrolyte (NaCl)

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**References**


