CHAPTER 4

CONTROLLED SYNTHESIS OF NANOSTRUCTURES VIA CHEMICAL AND BENIGN PROCESSES

Overview of the Chapter

In this chapter, optimization of monodispersed nanostructures synthesis has been reported. Spherical and anisotropic gold and silver nanostructures are synthesized via conventional and eco-friendly routes. Furthermore, effect of various parameters like concentration of precursor, reducing agent and rate of addition of reducing agent/precursor has also been studied. This chapter is structured as:

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4.1 Introduction

Nanoscaled materials have received considerable attention with the recognition of their dramatically altered material properties as compared to their bulk counterparts [112-114]. The size and shape of nanostructures are the key parameters controlling diversified properties like electronic, optical, thermal and magnetic properties of materials at nano length scale. Gold, silver, magnetite and copper are some of the most frequently studied metals at nano length scales. Among these metal nanoparticles, gold nanostructures display prodigiously high chemical reactivity and catalytic properties [115-117]. Furthermore, their ease towards surface modification reactions has made them a potential candidate for varied applications in electronics [118, 119], catalysis [120, 121], chemical sensing [122, 123], biomedicines [124-126], etc.

Since the pioneering work of Turkevich et.al. [18], various synthesis methods of gold nanoparticles based on the reduction of chloroauric acid in the presence of a stabilizer have been reported [127-130]. Most of these methods results in isotropic nanostructures, however, it has been observed that the anisotropic nanostructure exhibits interesting physical and chemical properties much different from corresponding isotropic nanomaterials. Recent advances in the chemical synthesis of metal nanostructures has led to synthesis of different anisotropic metal nanostructures including prisms, rods, cores, wires, rings, and cubes [131-134]. However, the one-dimensional assembly of gold nanoparticles into useful morphologies and their associated exciting properties has attracted significant interest due to its potential applications ranging from electronics to chemical/bio-sensors.

4.2 Experimental

4.2.1 Synthesis of Gold Nanostructures

Gold nanoparticles (AuNPs) were synthesized using HAuCl₄ as a precursor following the renowned Turkevich method (trisodium citrate dihydrate - Na₃C₆H₅O₇.2H₂O as reducing agent) and an ecofriendly route using amino acids as reducing and capping agent. Briefly, 0.5 mM reducing agent (trisodium citrate dihydrate/amino acid) was added to boiling aqueous solution of HAuCl₄ (0.2mM) at a constant flow rate (0.25 ml/min). Preheating of the solution not only decreases the reaction time but also helps in controlling the distribution of nanoparticles. The resulting solution turned from yellow to light yellow and then to colorless.
in 5 min. The change in color of the HAuCl₄ solution is attributed to the rapid reduction of Au³⁺ to Au⁺ by electrons donated by the reducing agent (trisodium citrate/amino acid). After few minutes, the color of the yellow solution turned to pale red indicating the commencement of colloidal gold formation. Solution was continuously stirred while the heating was on till it turned bright red and then growth of nanoparticles was arrested by sudden change in temperature using an ice bath. Quenching of the solution was followed by sonication for 15 minutes in an ultrasonicator water bath. The mixture was then thermalized to room temperature and the colloidal red solution of gold nanospheres was centrifuged at 12,000 rpm for 20 minutes. The synthesized gold nanoparticles were stored at room temperature till further processing. Simplified schematic representation of the underlying reaction mechanism of gold nanoparticles synthesis for the above discussed methods are:

**Conventional Method:**

**Eco-Friendly Route:**

![Figure 4.1](image-url)

*Figure 4.1: Schematic representation of mechanism of synthesis of gold nanoparticles via a) conventional method and b) Eco-friendly method.*
4.2.1.1 Conventional Method: Parameter Optimization for Morphological Control

Effect of variation in concentration of precursor and reducing agent was investigated to optimize the synthesis of gold nanostructures of different morphologies. Ratio of trisodium citrate dihydrate/HAuCl₄ concentration (R) was varied as a) R<1; b) R>1, keeping flow rate constant during synthesis experiments.

Another important parameter, effect of rate of addition of reducing agent to the precursor solution during the synthesis of nanostructures, was studied by varying it from 0.1 ml/min to 0.5 ml/min keeping other parameters constant (0.25 mM HAuCl₄ and 0.5mM trisodium citrate dihydrate).

4.2.1.2 Ecofriendly Route : Morphological Control through pH

Synthesis of gold nanostructures of different morphologies was further achieved using benign chemicals (natural amino acids). Effect of variation of pH and choice of amino acids on morphology of synthesized nanomaterial has been studied.

*Effect of variation of side chains of amino acids*

Amino acid with hydrophobic R group (phenylalanine), basic R group (lysine), and acidic R group (glutamic acid) were used as reducing and capping agent to investigate their effect on morphology and size of the synthesized nanostructures.

*Effect of pH*

Effect of variation of pH on morphology of nanostructures was studied by constituting the amino acid in buffers of acidic, basic and neutral pH keeping in mind pKₐ₁, pKₐ₂ and pI of amino acids used.

4.2.2 Synthesis of Silver Nanostructures

In this study, chemical reduction method is followed to synthesize highly monodispersed silver nanoparticles (AgNPs) using silver nitrate (AgNO₃) as a precursor and trisodium citrate dihydrate was used as a reducing agent and stabilizing agent providing sufficient steric hindrance to keep the colloidal suspension stable over a long period of time. A schematic representation of the synthesis process is given below in Figure 4.2,
Effect of reducing agent/precursor concentration AgNO$_3$ (R), on nanostructure synthesis was studied by varying the R value as a) R<1 and b) R>1. Other parameter like rate of addition of reducing agent was kept constant throughout the study.

Effect of rate of addition of reducing agent to the precursor solution during the synthesis of nanostructures was also studied by varying it from 0.1 ml/min to 0.3 ml/min keeping other parameters constant.

### 4.2.3 Synthesis of magnetite nanoparticles

Magnetite nanoparticles (Fe$_3$O$_4$NPs) were prepared by method of co-precipitation of ferrous and ferric salts under alkaline environment proposed by Zhu et al. Briefly, the NaOH (5N) solution and mixture of ferric (5M)/ferrous(2.5M) chloride solution were added at a constant flow rate (0.2 ml/min) to a continuously stirred preheated aqueous solution at 80°C. Prior to synthesis the mixture of the ferric and ferrous salts, was sparged with Argon gas to prevent oxidation. Initial appearance of black precipitates in the boiling solution, marked the onset of Fe$_3$O$_4$NPs synthesis. The colloidal solution was further stirred for 2 hours at 80°C followed by centrifugation at 12,000 rpm for 20 minutes. Fe$_3$O$_4$NPs pellet was stored at room temperature till further processing. The reaction mechanism of Fe$_3$O$_4$NPs synthesis is:

\[
\begin{align*}
Fe^{3+} + H_2O & \xrightarrow{\text{deprotonation}} Fe(OH)_{3-x}^3- \\
Fe^{2+} + H_2O & \xrightarrow{\text{deprotonation}} Fe(OH)_{2-y}^{2-y} \\
Fe(OH)_{3-x}^{3-} + Fe(OH)_{2-y}^{2-} & \xrightarrow{\text{oxidation/dehydration}} Fe_3O_4
\end{align*}
\]
4.3 Results and Discussions

Synthesized nanoparticles of gold, silver and magnetite were characterized using physical and chemical methods as discussed in chapter 3. The unique properties of metallic nanoparticles like exhibition of intense color in colloidal solution which is absent in their bulk counterpart as well as at atomic scale has been exploited as an effective characterization tool to confirm the presence of nanoscale material. The relationship between the color and size of particles is investigated to characterize the nanostructures. Transmission electron microscopy (JEOL 2100) was employed for studying morphology and estimation of size of the nanomaterials.

Chemical characterization of synthesized nanomaterials was done by elemental analysis using energy dispersive X-ray (EDX) spectra (JEOL 2100), surface charge and stability analysis by studying zeta potential of synthesized nanomaterials (zetasizer from Malvern technologies) and assessing the optical properties of nanomaterials using UV-visible absorption spectra (ND 1000 UV–Vis spectrophotometer from Nanodrop technologies). The reaction kinetics of nanoparticle synthesis was also studied by analyzing the absorption spectra of sample extracted from reaction mixture after specific time interval.

4.3.1 Conventional Method of Nanostructure Synthesis

4.3.1.1 Synthesis of Gold Nanostructures

The onset of nanoparticle synthesis in reaction mixture can be correlated with the variation in the color of the solution. Figure 4.3 shows the color changes during the synthesis of gold nanoparticles. Appearance of bright red color indicates synthesis of monodispersed spherical AuNPs while appearance of purple or blue color indicates larger size of the NPs or agglomerated NPs.

![Figure 4.3: Color variation during the synthesis process of gold nanoparticles.](image-url)
UV visible absorption spectra of synthesized AuNPs showed the characteristic absorption peak of AuNPs at 526 nm and a broad hump from 580 nm to 750 nm (see Figure 4.4A). The absence of a sharp absorption peak or in other words the peak broadening represents the polydispersity of the synthesized nanoparticles or mixture of nanostructures. Figure 4.4B shows TEM micrograph of solution centrifuged at 12000 rpm showing a mixture of nanochains of different lengths and spherical nanoparticles.

![Figure 4.4: A) UV – Vis absorption spectra and B) TEM micrograph of synthesized gold nanostructures](image)

Spherical nanoparticles of gold showed an average diameter of 20 nm, whereas the average length and diameter of synthesized gold nanochains was 224 nm, and 20 nm, respectively. However, the usage of nanomaterials in any application or any industry is highly dependent on synthesis of monodispersed particles of a particular morphology. This can be achieved by two approaches – a) separation of particles of different size and shapes from the synthesized mixture of NPs or b) optimized synthesis of NPs of desired size and morphology.

**Separation of NPs Based on Centrifugal Forces**

Gold nanostructures prepared using the above protocols were separated on the basis of effective centrifugal force acting on different nanostructures. The morphological analysis of the solution separated at different centrifugal speeds is done using TEM. The nanoparticles and nanochains were separated based on their hydrodynamic velocity or the effective centrifugal force experienced by them at high centrifugation speed. Nanochains being heavier in mass have larger terminal settling velocity and hence settle down faster along the wall of the centrifugation tubes while comparatively lighter spherical particles tend to take longer
time and settle at the bottom of the centrifugation tube. The red colored pellet of nanoparticles at the bottom of the tube when characterized using UV-Vis spectroscopy showed sharp single absorption peak at 526 nm (curve a; Figure 4.5A) whereas the purplish black pellet at the wall of centrifuge tube shows two distinct peaks at 532 and 668 nm (curve b; Figure 4.5A), confirming the anisotropic character of the synthesized nanochains. The morphological analysis of the pellets was further done using TEM which confirmed the separation of two different shape nanostructures using centrifugation (Figure 4.5(B-C)).

\[ \text{Figure 4.5: A) UV–vis absorption spectra and TEM micrograph of B) spherical Au NPs and C) Au nanochains after centrifugal separation.} \]

4.3.1.2 Morphological Control Mechanism

Understanding the mechanism of synthesis of nanoparticles is a prerequisite for specific identification of crucial parameters controlling the synthesis. The reduction of chlorauric acid by trisodium citrate can be explained by the multistep reaction mechanism mentioned below,

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{(OCCH}_2\text{)}_2\text{C(OH)}\text{CO}^- & \quad \text{(OCCH}_2\text{)}_2\text{C=O} \\
\text{AuCl}_3 + 2e^- & \quad \text{AuCl} + 2\text{Cl}^- \\
\text{AuCl} & \quad 2\text{Au}^0 + \text{AuCl}_3
\end{align*}
\]

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{(OCCH}_2\text{)}_2\text{C(OH)}\text{CO}^- & \quad \text{(OCCH}_2\text{)}_2\text{C=O} + \text{CO}_2 + \text{H}^+ + 2e^- \\
\text{AuCl}_3 + 2e^- & \quad \text{AuCl} + 2\text{Cl}^- \\
\text{AuCl} & \quad 2\text{Au}^0 + \text{AuCl}_3
\end{align*}
\]

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In this study, the molar ratio of reducing agent/precursor is found to be the crucial parameter for controlling the monodispersed gold nanoparticles or nanochains. All other parameters except molar ratio were kept constant throughout the synthesis experiment. Broadly, it can be explained in terms of two cases:

a) Case 1: Low molar ratio (R<1.0)

It was observed that the low molar ratio (R<1.0) facilitates formation of gold nanochains (AuNCs) (see Figure 4.6A). Figure 4.6A shows the surface plasmon absorption spectra of AuNCs with well defined two bands: one of the band is in blue- green region around 530 nm due to the transverse dipole resonance, while the other band is in -near infrared region corresponding to longitudinal dipole resonance at 635 nm (curve a). With increase in concentration of reducing agent (molar ratio: 0.5) the absorption spectra (curve b) shows transverse band at 532 nm and longitudinal band as a hump at 630 nm depicting smaller gold nanochains. The longitudinal electronic oscillation lies between 600–1300 nm depending on the chain length of AuNCs. TEM micrographs shows that the decreasing concentration of reducing agent induces anisotropic character in gold nanoparticles resulting in longer chains.

b) Case 2: High molar ratio (R>1.0)

When the reducing agent to precursor molar ratio is high (R>1.0), highly monodispersed spherical gold nanoparticles (AuNPs) were synthesized (see Figure 4.6B). Nanostructures of gold are known to display strong surface plasmon resonance (SPR) absorption, which is sensitive to the particles size and shape. Figure 4.6B shows the UV–Vis absorption spectra of spherical AuNPs characterized by a single SPR band at 522 nm. However, if molar ratio, R >>1 the synthesized nanoparticles are no more spherical in shape (see Figure 4.6C) rather a mixture of different shapes like triangle, trapezoidal, cubic, hexagonal, rods etc are formed. The SPR peak of such a mixture is observed as a broad band with absorption maxima around 543 nm representing the polydispersity of the mixture. The effect of variation of molar ratio of trisodium citrate/HAuCl₄ on of colour of colloidal solution is summarized in Table 4.1.

Table 4.1: Effect of molar ratio on color of colloidal solution

<table>
<thead>
<tr>
<th>Molar ratio (R) of trisodium citrate/HAuCl₄</th>
<th>Color of colloidal solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;1</td>
<td>Purple</td>
</tr>
<tr>
<td>R&gt;1</td>
<td>Ruby red</td>
</tr>
<tr>
<td>R&gt;&gt;1</td>
<td>Reddish Purple</td>
</tr>
</tbody>
</table>
TEM micrograph and corresponding UV-Visible spectras of the synthesized nanoparticles is shown below.

**Figure 4.6:** UV–Vis absorption spectra and corresponding TEM micrograph of AuNPs synthesized at a) R<1, b) R>1 and c) R>>1.

**Proposed Mechanism**

The reaction mechanism of formation of spherical gold nanoparticles (AuNPs) and gold nanochains (AuNCs) as shown above shows that the electrons released during the oxidation of citrate to form dicarboxy acetone, facilitates the reduction of auric salt to aurous salt. This is followed by disproportionation of aurous species to form gold atoms. These small size gold
nuclei act as nucleation sites for further growth of nanoparticles. The dual behavior of citrate ions present in the growth solution as reducing as well as capping agent is a well studied fact. When the molar ratio is high (R>1.0), sufficient amount of citrate ions are present in solution to cover the surface of nanoparticles and hence stabilizing the high surface energy NPs. The negatively charged citrate ion capping on NPs surface leads to electrostatic repulsion among the charged NPs and act as a barrier to the process of fusion of NPs. If the molar ratio is less than 1.0, insufficient concentration of citrate ions in growth solution limits the function of citrate ions as capping agent and thus the Van der Waals attractive force act as driving force for integration of gold nanoparticles resulting in formation of gold nanochains (AuNCs).

However, at molar ratio much greater than one, the reducing agent is present in excess and reduces almost all the precursor instantaneously leading to nucleation. Since almost all the nuclei are formed together, the size of the nanoparticles formed is small and reducing agent concentration being in excess should cap the NPs and hence stable monodispersed spherical AuNPs must be synthesized. However, the results are not in agreement with theoretical expectation. This implies there is probably some other factor which is also contributing to morphological control of NPs. We explored the rate of addition of reducing agent as the third factor.

Faster rate of addition of reducing agent gears up the nucleation process thus leading to a high concentration of nuclei in the solution. Under such circumstances size of NPs is regulated by the molar ratio of reducing/precursor concentration (R), i.e, R>1 efficient capping and stabilization leads to monodispersed spherical NPs, while R<1 inefficient capping and high concentration of nuclei leads to agglomeration of the particles.

It was observed that decreasing the rate of addition of reducing agent results in interesting nanostructures since the synthesis is not just thermodynamically controlled rather kinetically governed as well.

c) Effect of rate of addition of reducing agent

The influence of rate of addition of reducing agent on particle size distribution and morphology was investigated by varying the value of flow rate from 0.1ml/min to 0.5 ml/min. All other parameters like HAuCl₄ concentration (0.25mM) and trisodium citrate dihydrate (0.5mM) concentrations were kept constant. The obtained results are presented in Table 4.2,
The above results showed that the slow addition of reducing agent results in large size NPs with broad size distribution of nanoparticles. High flow rates resulted in nanoparticles with smaller size, but with broad size distribution. The optimum rate of addition in order to synthesize nanoparticles of the size ranging between 10-16 nm was found to be between 0.2-0.3 ml/min.

The stability of monodispersed citrate stabilized AuNPs was assessed through zeta potential analyser. A high zeta potential of -41.7 mV indicates highly stable AuNPs (see figure 4.7).

**Table 4.2:** Effect of rate of addition of reducing agent on synthesis of AuNPs.

<table>
<thead>
<tr>
<th>Rate of addition (ml/min)</th>
<th>Color of Colloidal Solution</th>
<th>Dispersity of the Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Blue</td>
<td>Highly Polydisperse</td>
</tr>
<tr>
<td>0.2</td>
<td>Red</td>
<td>Monodisperse</td>
</tr>
<tr>
<td>0.3</td>
<td>Ruby red</td>
<td>Highly Monodisperse</td>
</tr>
<tr>
<td>0.4</td>
<td>Dark pink</td>
<td>Polydisperse</td>
</tr>
<tr>
<td>0.5</td>
<td>Purple</td>
<td>Polydisperse</td>
</tr>
</tbody>
</table>

**Figure 4.7:** Zeta potential distribution of citrate stabilized spherical AuNPs.
Zeta deviation or width of the peak (7.76mV) indicates difference in surface charge that indirectly reflects the polydispersity in terms of size of the particles.

### 4.3.1.3 Controlled Synthesis of Silver Nanostructures

The controlled synthesis mechanism proposed on the basis of AuNPs synthesis was validated through silver nanoparticles (AgNPs) synthesis. AgNPs synthesis was taken as model system for validating the hypothesis, since it is a major challenge to synthesize stable monodispersed silver nanostructures via chemical synthesis route. Silver nitrate (AgNO₃) was used as precursor, whereas trisodium citrate dihydrate not only behaved as reducing agent but also helps in stabilizing the high surface energy particles from agglomeration. Synthesis was performed by taking precursor and reducing agent concentrations as reported in literature [135]. The color of the reaction mixture changed from colorless to yellow and finally to green depicting the presence of different facet nanostructures (uncontrolled synthesis). Moreover, the change in color after 40 minutes shows unstability of the synthesized nanostructures (see Figure 4.8).

![Figure 4.8: Variation in color of the reaction mixture during synthesis and afterwards representing the polydispersed unstable silver nanostructures.](image)

The synthesized colloidal solution was further analyzed using UV-Vis spectroscopy. A broad peak from 400 to 600 nm was observed in the absorption spectra confirming highly polydisperse or broad size distribution of the synthesized nanoparticles (see Figure 4.9). Further, the morphology of nanostructures was confirmed by performing TEM analysis. TEM
micrographs show a mixture of nanostructures of different morphologies like spherical, triangular, tetragonal, hexagonal, rod shaped etc.

Achieving monodisperse AgNPs of a particular morphology at nanometer scale in solution phase is a real challenge. Our understanding of the mechanism of nanostructures synthesis, i.e., controlled flow rate of reducing agent, in addition to molar ration of precursor to reducing agent concentration (R>1 or R<1), was exploited to achieve a control over morphological variations of AgNPs. Using the above control, synthesized nanostructures of different morphologies in colloidal solution are shown below in Figure 4.10.

Figure 4.9: UV-Vis spectra of silver nanostructures with its corresponding electron micrograph

Figure 4.10: Colors of colloidal solution containing different shape and size silver nanostructures.
Effect of flow rate and molar ratio of reducing agent to precursor concentration (R) on morphology of synthesized silver nanoparticles.

a) Case 1: Low molar ratio (R<1.0)

At low molar ratio i.e. R<1.0, reducing agent, trisodium citrate dihydrate is available in low amounts and hence it only facilitates reduction and no capping or partial capping of the synthesized nanoparticles. These high surface energy nanoparticles undergo process of agglomeration for their stabilization. Figure 4.11 shows TEM micrographs and corresponding UV-Visible absorption spectra of the synthesized NPs. The peak at 300 nm in graph represents the unreacted AgNO₃ in the sample whereas the peak at 400 nm confirms the presence of silver structure in nanometer scale and a broad hump near 525 nm representing agglomerated NPs.

b) Case 2: High molar ratio (R>1.0) with variable flow rates

At high molar ratio of reducing agent to precursor concentration (R>1.0), synthesis of highly monodispersed spherical silver nanoparticles (AgNPs) is expected. However, based on above mentioned probable mechanism, by modulating the flow rate of addition of reducing agent,
different morphological nanostructures i.e., monodispersed spherical AgNPs, 1-D nanochains of AgNPs, 2-D/3-D networked nanochains of AgNPs could be synthesized.

**R>1 and Flow rate is 0.1 ml/min**

Figure 4.12A shows the absorption spectra of silver nanostructures showing one of the peak around 400 nm due to the transverse dipole resonance, while the presence of other peak corresponding to longitudinal dipole resonance at 635 nm confirms the presence of anisotropic nanostructure of silver in the sample. The corresponding TEM micrograph is in accordance with the absorption spectra showing ribbon like structure. The fusion of synthesized nanoparticle is a consequence of slow rate of addition of reducing agent i.e. despite R being greater than one, at any instance of time point the available concentration of reducing agent is insufficient for effective capping, resulting in fusion of NPs forming 2-D/3-D nanochains.

**R>1, flow rate is 0.2 ml/min**

With increased rate of addition of reducing agent, more trisodium citrate dihydrate is available for capping the synthesized nanoparticles leading to 1-D nanochains due to slow addition of reducing agent. The TEM micrograph in Figure 4.12B shows 1-D nanochains along with spherical nanoparticles. The synthesized spherical uncapped AgNPs when collide with each other tend to form nanochains. Hence at lower AgNPs concentration they would be predominantly be spherical, however, at higher concentrations the probability of collision increases and so is the chances of 1-D nanochains formation. Corresponding absorption spectra is in accordance with the observed TEM micrograph images, showing a sharp peak near 400nm and a decline in the second peak at 550 nm (as compared to Figure 4.12A).

**R>1, flow rate is 0.3 ml/min**

At high molar ratio and high flow rate of reducing agent i.e., R>1 and flow rate of 0.3 ml/min sufficient amount of reducing agent is available for effective capping of synthesized NPs at a given instant of time leading to highly monodispersed spherical silver nanoparticles as shown in TEM micrograph (Figure 4.12C). Nanoparticles in the range of 2-4 nm were synthesized and further characterized by a single sharp absorption peak at approximately at 400 nm shown in corresponding UV-Visible spectra.
The high concentration of reducing agent and optimum rate of addition of reducing agent results in faster reduction of precursor. Thus resulting in rapid nucleation rate and further large number of nuclei are synthesized spontaneously.

Figure 4.12: UV-Visible spectra and corresponding TEM micrographs of silver nanoparticles at flow rates A) 0.1 ml/min; B) 0.2 ml/min; C) 0.3 ml/min.
Dynamics of nanoparticle synthesis was studied in terms of nucleation, growth and termination under optimized condition for monodispersed spherical AgNPs (see Figure 4.13).

The synthesized AgNPs are characterized by absorption peak at 400 nm. Increase in amplitude of absorption peak at 400nm which follows a sigmoidal shape curve representing different reaction stages as shown in Figure 4.13. The low absorbance value during nucleation period is reflective of the fact that during initiation phase less reduction and hence low nuclei concentration is present. While the second stage - growth phase is marked by rapid increase in absorbance with time and finally the absorbance reaches a constant value after which there is no increase in the intensity showing the termination of the synthesis process. After reaction kinetic studies the elemental analysis of synthesized nanoparticles was performed using energy dispersive X-ray spectroscopy (EDX). Figure 4.14, shows the results of elemental mapping of the synthesized AgNPs. The red colored dots depicting 41% of silver were confirmed by characteristic X-ray absorption peaks of silver at 1.25 keV and 3-3.25 keV.

The stability of the synthesized AgNPs was monitored intermittently over a period of two years through UV-Vis absorption spectroscopy. There was no change in absorption maxima upto one year while a marginal shift (4nm) was observed at the end of two years representing highly stable AgNPs (see Figure 4.15)
Figure 4.14: Elemental analysis of synthesized silver nanoparticles using EDX spectroscopy.

<table>
<thead>
<tr>
<th>Element Series</th>
<th>Cu</th>
<th>Ag</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic %</td>
<td>58.83</td>
<td>41.17</td>
<td>5.1</td>
</tr>
<tr>
<td>Weight %</td>
<td>70.83</td>
<td>29.19</td>
<td>5.1</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.15: UV-Vis spectra confirming the stability of synthesized silver nanoparticles.
The stability was further evaluated by calculating zeta potential using zeta potential analyzer. The observed zeta potential value of -48.9 mV for the synthesized AgNPs further validated the stability of the synthesized AgNPs (see figure 4.16).

**Figure 4.16**: Zeta potential distribution curve of AgNPs.

### 4.3.2 Ecofriendly Route

The reaction kinetics of NPs synthesis via ecofriendly route was studied by observing the change in color of the precursor solution from yellow to red as well as by recording the alterations in the absorption peak corresponding to formation of elemental gold. Synthesis of AuNPs at pH 10.5 (Figure 4.17A) results in gradual disappearance of choloauric acid (~300 nm) with time and formation of a fairly constant absorption peak for nanoparticles of gold (~523 nm) that increased in intensity with time. However, at pH 3.0 (see Figure 4.17B) there is broadening of the absorption peak at 523 nm with time indicating polydispersity and eventually a small hump at 651 nm indicative of chain formation is observed. Figure 4.18 shows nucleation, growth and saturation or stable nanoparticles formation. As seen from the graph the onset of nucleation is slightly delayed in case of lower pH (curve a, pH 3) as
compared to higher pH (curve b, pH 10.5) because of better reducing capabilities of amino acid at higher pH. The stable nuclei formation is marked by saturation in peak height in case of spherical nanoparticles (curve a). However, curve b shows slower growth and a much delayed saturation. After the growth process was arrested the nanoparticle solution was centrifuged at 9000 rpm. NPs synthesis at pH 10.5 showed the same single peak at 523 nm while NPs solution (pH 3) showed two absorption peaks corresponding to both transverse (523 nm) and longitudinal absorption (660 nm) from the gold nanochains. On centrifugation, the particles come nearer and the effect of attractive forces becomes more pronounced leading to chain like Au NPs. The TEM micrographs of the two synthesis confirmed the monodispersed spherical AuNPs and chain like AuNPs formation at pH 10.5 and 3 respectively (see Figure 4.19(A-B))

**Figure 4.17** Kinetics of (A) spherical AuNPs and (B) Au nanochains synthesis.
Figure 4.18: Reaction kinetics of Au amino NPs synthesis marking induction period for nucleation, growth and termination periods at (A) pH 10.5 and (B) pH 3.

Figure 4.19: TEM micrographs of (A) spherical NPs (B) 1-D nanochains, (C) branched chains and (D) agglomerated Au amino NPs

4.3.2.1 Mechanism of pH Controlled Synthesis

Controlled synthesis of gold nanostructures (nanospheres, 1-D nanochains, branched nanochains) and random fusion of nanoparticles (agglomerated NPs) using amino acids, as reducing and capping agent, can be achieved by modulating the pH of the solution (see Fig4.19(A-D)). The present study illustrates that pH is not just influencing the nucleation and growth of NPs, rather it also alters the morphology of the synthesized particles. Amino acids, in general, contain a carboxylic group (–COOH, weak acid) and an amino group (–NH₂, weak base) that undergo dissociation and ionization in solution. As a result, they behave as a cation [RCH(NH₃⁺)COOH] at pH<<pI, as an anion [RCH(NH₂)COO⁻] at pH>pI or electrically neutral molecule (zwitterion – [RCH(NH₃⁺)COO⁻]) at pH=pI, as represented in Figure 4.20. In recent past, the ratio of concentration of the reducing agent (amino acid) and the precursor (HAuCl₄⁻) was used as a control parameter to explain variation in morphologies of
nanostructures – nanoplates, nanospheres, nanochains and nanowires. However, we propose a pH controlled selective synthesis of monodispersed nanospheres, directed assembly of 1-D nanochains and random coupling of amide linked branched nanochains.

![Figure 4.20: Schematic representation of different ionization states of amino acids at different pH](image)

We have exploited the pH dependence of amino acids for morphological control over the synthesized amino acid decorated nanostructures. The ionization state/surface charge of these amino capped nanoparticles results in electrostatic interaction among these spherical nanoparticles leading to morphological variation. Dissociation and ionization constants of most of the amino acid is in the range of 1.73-2.63 (α-COOH pK\(_{a1}\)) and 8.95-10.78 (α-Amino pK\(_{a2}\)). In addition to pK\(_{a1}\) and pK\(_{a2}\), some amino acids contain an acidic or basic R-group giving rise to third ionization/dissociation constant (pK\(_{R}\)). Table 4.1 shows pI and pK values for different amino acids. At neutral pH, the carboxylic group of most of the amino acids dissociates and the released electron reduces AuCl\(_4^-\) ions forming Au nanoparticles. The growth of nuclei eventually results in isotropic growth (spherical particles) or polycrystalline nanoparticles having different facets of varying surface energy. The low concentration of amino acids results in partial coverage of the NPs surface resulting in ineffective electrostatic stabilization and hence the fusion of NPs along lowest energy plane driven by high surface energy leading to formation of 1-D nanochains (see Figure 4.19B). However, at high
concentration of amino acids the surface charge is the sole determinant of the overall interaction among the NPs.

<table>
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<tr>
<th>Name</th>
<th>pK</th>
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<th>pK</th>
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Amino acid with hydrophobic R group (phenylalanine), basic R group (lysine), and acidic R group (glutamic acid) are used as reducing and capping agent at high molar ratio of amino acid to precursor to study the effect of pH on surface charge and hence morphologies of nanostructures.

pH<pK\_α1 : Highly acidic pH conditions favor carboxylic group of amino acid in protonated state (COOH) and amine groups as (NH\_3\(^+\)) and hence cannot facilitate the reduction of the precursor.

a) Case I: For Amino acids with Basic R group (Lysine)

L-Lysine titration curve showing different ionization states as a function of the pH of solution is shown in Figure 4.21.
a) $pK_{a1} < pH < pK_{a2}$: The pH of solution results in amino acid with 2 NH$_3^+$ and 1 COO$^-$ group. The amino acid reduces HAuCl$_4$ to Au and gets oxidized itself. This leads to amino acid capping of the AuNPs with COO$^-$ and 1NH$_3^+$ group on the surface hence facilitating the formation of amide bond between groups present on surface of adjacent nanoparticles. Thus, resulting in formation of peptide coupled highly branched gold nanochains as shown in TEM micrograph shown in Figure 4.22A.

b) $pK_{a1} < pK_{a2} < pH < pK_R$: These conditions favor neutral $\alpha$-amino group while side chain R amino group remains in ionized state (NH$_3^+$). As a result the surface of nanoparticle is decorated with 1 NH$_3^+$ and 1 COO$^-$ thus the presence of both negative as well as positive charge on the surface NPs (dipolar behavior) promotes their multipoint coupling leading to amide linked branched nanochains. Electron micrographs show the branched network of amide linked Au NPs (see Figure 4.22B).

c) $pK_{a1} < pK_{a2} < pK_R < pH$: Under highly basic conditions surface of the nanoparticles consist of 1 neutral amino group (NH$_2$) and 1 carboxylate group (COO$^-$). This results in electrostatic repulsion among the carboxylate groups present on the surface of nanoparticles and helps in maintaining the monodispersity among the nanoparticles. TEM micrograph of the nanoparticles synthesised under the above discussed conditions is shown in Figure. 4.22C.
b) Case II: Amino acids with acidic R group (Glutamic acid)

Ionization states of glutamic acid at different pH is shown in Figure 4.23.

Figure 4.23: Schematic representation of ionization states of Glutamic acid as a function of pH of solution.
a) $pK_{a1}<pH<pK_R$: Under such circumstances, AuNPs surface would be functionalized with a carboxylate and an unmodified COOH group. This leads to electrostatic repulsion among the particles because of carboxylate groups. At the same time, H-bond interaction due to the COOH groups on surface of nearby AuNPs. The interplay of two forces – attractive H-bond interaction and repulsive electrostatic interaction results in spherical nanoparticles with the low but significant probability of fusion or chain formation as well since electrostatic. The TEM micrograph in Figure 4.24A shows spherical AuNPs with polydispersity due to agglomeration or chain formation.

b) $pK_{a1}<pK_R<pH<pK_{a2}$: The NH$_2$ group of amino acid binds to AuNPs hence the two carboxylate groups are presented on surface that leads to high electrostatic repulsion and hence highly spherical AuNPs (see Figure 4.24B).

c) $pK_{a1}<pK_R<pK_{a2}<pH$: Similar situation as in previous case is observed, i.e. two COO$^-$ groups on surface hence monodispersed spherical nanoparticles are observed (see Figure 4.24C).

![Figure 4.24: TEM micrographs of synthesized glutamic acid mediated synthesis of AuNPs at (A) $pK_{a1}<pH<pK_R$ (B) $pK_{a1}<pK_R<pH<pK_{a2}$ and (C) $pK_{a1}<pK_R<pK_{a2}<pH$](image-url)
c) Case III: For Amino acids with hydrophobic R group (phenylalanine)

Shown below is the titration curve of phenylalanine showing different ionization states (see Figure 4.25).

![Figure 4.25: Schematic representation of ionization states of Phenylalanine as a function of pH of solution.](image)

Figure 4.25: Schematic representation of ionization states of Phenylalanine as a function of pH of solution.

a) $pK_{a1} < pH < pK_{a2}$: As seen from equation (1) the amino acid exists in a zwitterionic state in solution. The AuNPs have COO$^-$ and a hydrophobic group on the surface. There is interplay of two forces i.e., repulsion among nanoparticles because of negatively charged carboxylate groups and attractive hydrophobic interaction because of phenyl ring. Hydrophobic interaction being stronger it results in a linear chain like arrangement of particles as observed in TEM micrograph (see Figure 4.26A).

b) $pK_{a1} < pK_{a2} < pH$: The surface of the nanoparticles have same groups on the surface as discussed above, i.e. carboxylate and a hydrophobic R group. The hydrophobic attractive forces would result in 1-D nanochains (Figure 4.26B).

Monodispersed spherical AuNPs synthesized through eco-friendly route were further analysed for long term stability using zetasizer. The zeta potential was found to be -28.3mV with a very narrow band width, i.e, zeta deviation of 5mV(see Figure 4.27). Though zeta potential was lesser than that of citrate stabilized AuNPs or AgNPs, however, but it was greater than 20mV that indicates stable amino acid capped AuNPs.
Figure 4.26: TEM micrographs of phenylalanine assisted synthesis of AuNPs at (A) $pK_{a1} <\text{pH} <pK_{a2}$ and (B) $pK_{a1} <\text{pH} <pK_{a2}$.

Figure 4.27: Zeta potential distribution of amino acid capped AuNPs.