Synthesis of Gold Nanoparticles: Steps Towards Paper based Aptamer Conjugated Gold Nanoparticle Bioassay

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Abstract—Gold nanoparticles were synthesized by three different reducing agents: Sodium borohydride, Tannic Acid and Ascorbic acid to study the synthesis efficiency. They were characterized using different parameters and protocols like UV spectroscopy, DLS, SEM. Further, it was studied that it could be patterned on DNA and the aggregates could be used in bioassay, pathogen detection and drug delivery.

Keywords—Gold nanoparticle, Reducing agents, UV spectrometry, SEM, DLS, Gold nanoparticle aggregate, Bioassay.

I. INTRODUCTION
Nanoparticles are particles which are defined as small objects that behave as a whole unit with respect to its transport and properties. Their size range is between 1-100 nm. Three different ways are reported to synthesize gold nanoparticles using three different reducing agents followed by their characterization using different techniques like UV (Ultra Violet) Spectroscopy, DLS (Dynamic Light Scattering) technique and SEM (Scanning Electron Microscopy) technique. Gold nanoparticles have specific characteristics as discussed below which is why they are synthesized more often than nanoparticles of other elements. They have high specific area, very good biocompatibility, and easy surface modification. Moreover, gold nanoparticles can be used in various applications such as in electronics as conductors from printable inks to electronic chips; photodynamic therapy; therapeutics agent delivery; as sensors; probes; diagnostics; catalysis etc.[2] A very important property of nanoparticles is that, using different concentrations of the growth solutions reagents, nanoparticles of different shapes and sizes could be synthesized.[1]The future work involves conjugating these nanoparticles with DNA and the gold nanoparticle –DNA conjugates could be used for paper based bioassay of the aptamer conjugated gold nanoparticle. Bioassay is a procedure to determine the concentration or purity of a substance such as a vitamin, hormone etc by measuring the effect on a receptor. Aptamers are single stranded DNA or RNA molecules that can bind to pre- selected targets including proteins and peptides with high affinity and specificity. Aptamers are nucleic acids that selectively bind to low molecular weight organic or inorganic substrates. The affinity constant of an aptamer towards their substrates lies in the micro molar to nanomolar range, comparable to the binding constants of antibodies to antigens[3]. All the chemicals used in the experiments were taken from Sigma-Aldrich.

II. SYNTHESIS OF GOLD NANOPARTICLES AND ITS ANALYSIS
A. SYNTHESIS OF GOLD nanoparticle SEED USING SODIUM BOROHYDRIDE AS REDUCING AGENT

Table 1: Tabulated compound, molecular formula and the concentration used

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>FORMULA</th>
<th>CONCENTRATION (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroauric acid trihydrate</td>
<td>HAuCl₃.3H₂O</td>
<td>0.098</td>
</tr>
<tr>
<td>Cetyl trimethyl ammonium bromide(CTAB)</td>
<td>C₁₉H₄₂BrN</td>
<td>27.32</td>
</tr>
<tr>
<td>Sodium Borohydride</td>
<td>NaBH₄</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Chloroauric acid bears the gold ions to be reduced and CTAB is a surfactant to increase the surface area of reaction. The details are given in Table 1.

A 50 mL beaker was placed inside an ice cold tray and 0.098g/L of Chloroauric acid trihydrate was added to it. Onto it, 27.32g/L of CTAB was added. Freshly prepared ice cold Sodium Borohydride (0.027g/L) was added to the 50mL beaker in the ice cold tray for reduction of gold ions (+3). Constant stirring was done during the whole process to ensure complete reduction of gold ions by sodium borohydride. For preparation of 10mL of the gold seeds 3.33mL each of the reagents were taken maintaining the said concentration i.e.

0.327mg in 3.33mL water: Chloroauric acid trihydrate.
91.02mg in 3.33mL water: Cetyl trimethyl ammonium bromide (CTAB).
0.090mg in 3.33mL water: Sodium Borohydride. [4]

In another experiment for synthesis using the same reducing agent the concentration of the seed was changed to 10 times its earlier concentration. Thus for preparation of 1mL of the gold seeds in this case (a concentration of 10 times of the earlier one) 0.333mL each of the reagents with the same quantity of the reagents (with constant stirring...
and maintaining the ice cold framework as given in the procedure above) was taken i.e.

0.327mg in 0.333mL water: Chloroauric acid trihydrate.
91.02mg in 0.333mL water: Cetyl trimethyl ammonium bromide (CTAB).
0.090mg in 0.333mL water: Sodium Borohydride.

RESULTS AND DISCUSSION

The colour of both the prepared seeds was found to be pink. It was then characterized by using UV spectroscopy as according to Figure 1. Gold ions absorb at a wavelength of 520 nm. From the 1st curve i.e. the blue curve (relatively dilute seed) there is a hump at 500nm, but also, there are multiple humps in the same curve which is suggestive of the non uniformity in the particle size distribution of the gold nanoparticles and thus it gives multiple peaks. To counteract this problem, the concentrated seeds were synthesized and in the UV spectra of the concentrated seeds, unlike the first one, the peak was quite prominent at 520 nm and also did not have any multiple peaks. [6], [9]

The concentrated seeds were processed through a growth cycle which included the following:

Table 2: Tabulated compound, molecular formula and the concentration used

<table>
<thead>
<tr>
<th>COMPOUND USED</th>
<th>MOLECULAR FORMULA</th>
<th>CONCENTRATION USED (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroauric acid trihydrate</td>
<td>HAuCl₃·3H₂O</td>
<td>0.00444</td>
</tr>
<tr>
<td>Cetyl trimethyl ammonium bromide (CTAB)</td>
<td>C₁₉H₄₂BrN</td>
<td>0.1</td>
</tr>
<tr>
<td>Silver Nitrate</td>
<td>AgNO₃</td>
<td>0.01</td>
</tr>
<tr>
<td>L-ascorbic acid</td>
<td>C₆H₈O₆</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Chloroauric acid bears the gold ions to be reduced and CTAB is a surfactant to increase the surface area of reaction. Silver nitrate was used as a capping agent to provide for mechanical stability. The details are given in Table 2.

0.011mL of 0.00444 M Chloroauric acid trihydrate was taken in a 50mL beaker. Onto that, 5mL (0.1 M) of CTAB; 5mL (0.01 M) Silver nitrate and 5mL (0.1 M) L-ascorbic acid were added. Constant stirring was ensured during the whole mixing process and room temperature was maintained. The specifications of reactions were:

0.011mL of 0.00444 M; Chloroauric acid trihydrate. 178.9mg in 5mL water: CTAB.
8.35mg in 5mL water: AgNO₃
87.5mg in 5mL water: L-ascorbic acid.

In this mixture 0.01mL of the seed solution (10 times concentrated one) was added and mixed completely to give the growth solution.

RESULTS AND DISCUSSION

The colour of the prepared seeds was found to be ochre yellow. It was characterized by using UV spectroscopy:

Figure 2: UV Spectroscopy of the growth seeds

From the absorbance peak at around 425 nm Figure 2, it was inferred that the silver ions have capped all the gold ions in the solution and thus light is not able to absorb gold ions and give an absorbance peak at 520 nm. This discussion can be taken into consideration and can be proved to be correct as the core-shell analysis-TEM images supplement the above discussion and show a capping layer over a core molecule.
B. SYNTHESIS OF GOLD NANOPARTICLE SEED USING TANNIC ACID AS REDUCING AGENT

Table 3: Tabulated compound, molecular formula and the concentration used

<table>
<thead>
<tr>
<th>COMPOUND USED</th>
<th>MOLECULAR FORMULA</th>
<th>CONCENTRATION USED (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroauric acid trihydrate</td>
<td>HAuCl₄·3H₂O</td>
<td>10</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>C₇₆H₅₂O₄₆</td>
<td>10</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>Na₃C₆H₅O₇</td>
<td>10</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>K₂CO₃</td>
<td>3.45</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H₂O₂</td>
<td>300</td>
</tr>
</tbody>
</table>

Chloroauric acid bears the gold ions to be reduced; potassium carbonate was the buffer used and sodium citrate was the capping agent. The details are given in Table 3.

79mL of DI water was taken into an Erlenmeyer flask and heated to 60°C and that temperature was maintained. 1mL of 10g/L Chloroauric acid trihydrate solution was added to this. In a 50mL glass beaker, 3mL of 10g/L tannic acid and 4mL of 10g/L sodium citrate solution was added. 10mL DI was added to this. 0.3mL of 3.45g/L solution of potassium carbonate was added to this to maintain the pH at 7.5 to 8. The solution was made up to 20mL by adding DI water. This solution was also brought to 60°C. Both the solutions are mixed and the temperature of the mixture was maintained at 60°C for 15 minutes. Constant stirring was continued during the experiment till the end. The colour of the mixture changed within 2 seconds from pale yellow to wine red. After 15 minutes the solution was heated to boiling and boiled for 10 minutes. 1mL of 300g/L hydrogen peroxide solution was added to this and again boiled for 10 minutes. Then it was cooled with ice cold water. [4], [5]

RESULTS AND DISCUSSION

For the characterisation of these nanoparticles 3 different techniques were:

The first one was UV-spectroscopy of 50 times diluted(1mL nanoparticle solution+49mL DI water) and 500 times diluted(1mL nanoparticle solution+499mL DI water) as depicted below:

Both the curves give us a peak at around 520-530 nm as in Figure 3. Gold ions absorb at a wavelength of 520 nm. The absorbance peak signifies the absorption of light by the gold nanoparticles.

Evidently, the solution which is more dilute i.e. 500 times (red line) has a lower absorption than the 50 times one because of the Beer’s law which says that the amount of absorption is directly proportional to the concentration of the solution.

From Beer Lamberts law:

\[ A = ebC \]

\[ b = \text{PATH LENGTH (cm)} \]

\[ e = \text{EXTINCTION COEFFICIENT (M}^{-1}\text{cm}^{-1}) \]

\[ C = \text{CONCENTRATION (M)} \]

50 TIMES DILUTED SEED:

ABSORBANCE \( A \) = 0.14 absorbance units

PATH LENGTH \( b \) = LENGTH OF CUVETTE = 4.5 cm

EXTINCTION COEFFICIENT \( e \) = 8.56 *10^6 M^{-1} cm^{-1}

C = 0.00363 micro molar.

500 TIMES DILUTED SEED:

ABSORBANCE \( A \) = 0.01 absorbance units

PATH LENGTH \( b \) = LENGTH OF CUVETTE = 4.5 cm

EXTINCTION COEFFICIENT \( e \) = 8.56 *10^6 M^{-1} cm^{-1}

C= 0.000259 micro molar. [8], [10]

The second method that we used is the DLS (Dynamic Light Scattering) technique to find out the effective size of the nanoparticle:

![Figure 4: DLS plot of no. of gold ions vs. diameter of the prepared nanoparticle.](image-url)
The histogram as in Figure 4. of the range of values of the particle size diameter has been depicted in the DLS result as shown above. This analysis was run for 5 minutes and the range in which all the particles were found was from 24.4nm to 26.2 nm which is an even distribution of particles.

The effective diameter was calculated to be 25.42 nm.

The third method that was used was SEM (Scanning Electron Microscopy) technique.

![SEM image of the prepared nanoparticle.](image)

From the SEM images as in Figure 5, the gold nanoparticles have agglomerated. The reason of agglomeration has been discussed below:

The gold nanoparticles are stabilized by the electronic charge repulsion. The buffer we used in this case was 0.3mL of 25mM potassium carbonate. The cause of agglomeration is that, this amount of buffer used is very high. The salt effect and the ionic charge i.e. K⁺ is fairly high enough to disrupt the electron charge over the gold nanoparticle surface (this surface negative charge is neutralised by the high concentration of K⁺ ions) leading to a non stabilisation of the gold nanoparticles in suspension. So to avoid this lesser amount of buffer of lesser concentration should be used.

![UV Spectroscopy of the seeds synthesized: Dilute and concentrated](image)

Though agglomerated, the smaller particles are observed to be monodisperesed.

C. SYNTHESIS OF GOLD NANOPARTICLE SEED USING ASCORBIC ACID AS REDUCING AGENT

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<td>Chloroauric acid trihydrate</td>
<td>HAuCl₃·3H₂O</td>
<td>0.098</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>C₆H₈O₆</td>
<td>0.07</td>
</tr>
<tr>
<td>Tri sodium citrate</td>
<td>Na₃C₆H₅O₇</td>
<td>0.064</td>
</tr>
</tbody>
</table>

Chloroauric acid bears the gold ions to be reduced and citrate molecule was the capping agent. The details are given in Table 4.

A 50mL beaker was taken and 0.098g/L of Chloroauric acid trihydrate was added to it. Onto that we added 0.064g/L of tri sodium citrate which is a capping agent. Ascorbic acid (0.07g/L) was added to this mixture for reduction of gold ions (+3). Constant stirring was done during the whole process to ensure complete reduction of gold ions by ascorbic acid. The temperature was maintained at room temperature.

For preparation of 10mL of the gold seeds 3.33mL each of the reagents were taken maintaining the said concentration i.e.

0.327mg in 3.33mL water: Chloroauric acid trihydrate.
0.234mg in 3.33mL water: tri sodium citrate.
0.214mg in 3.33mL water: Ascorbic acid. [4]

In another experiment for synthesis using the same reducing agent the concentration of the seed was changed to 10 times its earlier concentration.

For preparation of 1mL of the gold seeds in this case (a concentration of 10 times of the earlier one) 0.333mL each of the reagents was taken with the same quantity of the reagents (with constant stirring and maintaining the room temperature framework as given in the procedure above) i.e.

0.327mg in 0.333mL water: Chloroauric acid trihydrate.
0.234mg in 0.333mL water: Tri sodium citrate.
0.214mg in 0.333mL water: Ascorbic acid.

RESULTS AND DISCUSSION

The colour of the 1st prepared seed was found to be brown and that of the concentrated one was found to be pink. It was characterized by using UV spectroscopy.

![UV Spectroscopy of the seeds synthesized: Dilute and concentrated](image)
In the UV spectra of the seeds prepared from the 1st experiment (blue line) there is a broad peak at 500nm (gold ions absorb light at a wavelength of 520 nm) as in Figure 6. There are multiple absorbance peaks that we see on the blue curve which is a result of particle size distribution of the gold nanoparticles in the solution which gives rise to two distinct absorbance peaks at various wavelengths.

In the concentrated seeds’ UV spectra an absorbance peak at 530 nm was found and there were no multiple humps (or multiple absorbance peaks) unlike that of the earlier seed.

III. FUTURE WORK

A. COUPLING OF DNA WITH GOLD NANO Particles TO FORM GOLD NANO Particle-DNA CONJUGATE

Figure 7: Coupling of DNA with gold nanoparticles. [7]

2 ways of coupling the gold nanoparticles with DNA are shown in Figure 7. In the 1st case i.e. (a): Thiol modified (-SH group) DNA is coupled with gold nanoparticle to give the gold nanoparticle-DNA conjugate. The blue colour strand is the DNA molecule and the gold nanoparticle (red ball) attaches to the sulphur atom.

On the other hand i.e. in case (b), at first lipoic acid is made to react with amine modified DNA strand in presence of coupling agents, DCC (N,N’-Dicyclohexylcarbodiimide) or NHS (N-Hydroxysuccinimide) which couples the two molecules and gives us lipoic acid-amine modified DNA molecule which interacts with the gold nanoparticle in the second step of part (b) to give gold nanoparticle-DNA aggregates in which the gold nanoparticle attaches with the sulphur molecule of the reactant originating from lipoic acid.[7]

Once the gold nanoparticle-DNA molecule was achieved, the biosensing platform using the adenosine aptamer was studied. These are the two ways which were studied and could be adopted in future work.

B. PAPER BASED BIOASSAY USING ADENOSINE APTAMER

The colour of the gold nanoparticle-DNA aggregates is studied to be purple/black. When the gold nanoparticles would come closer i.e. aggregate the individual surface plasmon resonance of the nanoparticles couple to each other and the colour would change from red (discrete gold nanoparticle) to purple (aggregated gold nanoparticles).

Figure 8: Paper based bioassay using adenosine aptamer. [2]

A. It is the schematic illustration of an adenosine colorimetric sensor using gold nanoparticle aggregates. Adenosine binding DNA aptamer is studied to be used as a crosslinker to bridge gold nanoparticles attached with DNA strands.

B. The sequence of the DNA molecule studied to be used. The addition of adenosine which binds to and dissociates the aptamer molecule from the DNA strand leads to the redispersion of gold nanoparticle aggregates.

C. Adenosine sensing bioassay studied to be carried out on hydrophobic paper.

D. Adenosine sensing bioassay studied to be carried out on hydrophilic paper. [2]

IV. CONCLUSION

Three protocols of synthesizing gold nanoparticles using three different reducing agents were discussed. It was characterized by various techniques like UV (Ultra Violet) spectroscopy, DLS (Dynamic Light Scattering) technique and SEM (Scanning Electron Microscopy) technique.

For the experiment with sodium borohydride as reducing agent it was inferred that the absorbance peak got broader; closer to 520 nm and it was observed that a single absorbance peak was found using a 10 times concentrated seed than the relatively diluted seed which gave multiple absorbance peaks at different wavelengths due to particle size distribution. For the growth solution UV-analysis of the concentrated seeds it was inferred that the silver ions have capped all the gold ions in the solution and thus light was not able to absorb gold ions and give an absorbance peak at 520 nm but gives around 425 nm.
Using tannic acid as the reducing agent the absorbance peaks at around 520 nm for both was observed, 50 times and 500 times diluted nanoparticle solution. On DLS technique characterisation it was found the effective diameter of the seeds to be 25.42 nm. Moreover, the SEM images of the sample showed some agglomeration (instability) while the smaller particles were still monodisperesed.

Lastly, using ascorbic acid as the reducing agent it was inferred that the absorbance peak was closer to 520 nm and a single absorbance peak was observed using a 10 times concentrated seed than the relatively diluted seed which gave multiple absorbance peaks at different wavelengths due to particle size distribution.

It is planned to use these nanoparticles coupled with DNA strands to form complexes which in turn could be used for bioassay platform using adenosine aptamer.

V. ACKNOWLEDGEMENTS

I would like to thank the Chemical Engineering Department of Indian Institute of Science, Bangalore, for selecting me in their outreach programme for summer internship of 40 days to conduct the whole research and perform the experiments without any problems. I would like to thank Prof. Banani Chakraborty, for guiding me through all literature analysis, experimental work, result analysis and all other project related issues throughout my work and giving me this opportunity to work under this project. I would like to also thank Mr. Sushant Kumar and Mr. Pushkaraj Joshi (PhD students) for helping me out throughout the project from the beginning till the end of it especially in the lab work performed. I would lastly thank my Department at BMS College of Engineering for helping me through all my concepts throughout my B.E. course.

VI. REFERENCES

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