

Applications, Synthesis and Characterization of Gold Nano Particles

A Thesis

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DECLARATION

We hereby declare that thesis work titled “Applications, Synthesis and Characterization of Gold Nano Particles” is our own work. The work hasnot been presented elsewhere for assessment. Where materials used from other sources have been properly acknowledged and referred.

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ABSTRACT

In the Nano scale, gold exists in different shapes and sizes and behaves very differently from its bulk. From 1-100nm in size the gold nanoparticles exhibit an array of different size and shape related properties. These properties offer many uses for gold nanoparticles in the field of electronics, photonics, medicine, chemistry and other fields. Research and applications of gold nanoparticles has been growing rapidly over the last decade and is one of the most emerging and sought after nanoparticle. This thesis covers the Synthesis of Gold Nanoparticles using the Turkevich/citrate reduction method using HAuCl_4 (Hydrogen tetrachloroaurate(III)) as precursor and trisodiumcitrate as reducing agent as well as stabilizing agent. Reaction parameters are changed to see the effect on the produced nanoparticles. Citrate amount, citrate concentrations and initial gold salt concentration are the parameters which were changed. After formation of these Gold Nanoparticles, characterization techniques like UV-Vis Spectroscopy, microscopy and some other detection methods were used to characterize and analyse the changes in sizes and shapes. By changing the synthesis parameters the size can be tuned to our preference and be used for further application. Some practical applications of GNPs are also discussed in this paper.

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ABBREVIATIONS

GNPs- Gold Nanoparticles

SPR- Surface Plasmon Resonance

TEM- Transmission Electron Microscope

SEM- Scanning Electron Microscope

AFM- Atomic force microscope

UV-Vis- Ultraviolet-Visible light

FWHM- Full Width Half Maximum

CHAPTER 1

INTRODUCTION

1.1 Nanoparticle

Nanoparticles are particles between 1-100nm in size. In nanotechnology a nanoparticle is defined as a small object that behaves like a whole unit with respect to its transport & properties. [1]

Nanoparticles can exhibit unique size & shape related properties, significantly different from those of large particles/bulk materials. That's why its application is growing rapidly and it is one of the most emerging topics and so researches based on nanoparticles has increased widely over the last decade [2]. Undoubtedly we can say that nanotechnology is preparing to play a significant and commercial role in our future society.

1.2 Gold Nanoparticles

Gold, a precious metal widely used in the form of jewellery, coinage and as monetary exchange in the past. In its purest form gold is an inert element which means it does not react to other chemicals/elements easily it is considered a 'boring' element in terms of chemistry [3]. In the Nano scale, gold exists in different shapes and sizes and behaves very differently from its bulk. From 1-100nm in size the gold nanoparticles exhibit an array of different size and shape related properties [4]. These properties offer many uses for gold nanoparticles in the field of electronic, photonics, medicine, chemistry, etc. It has not been a long time until recent time after extensive research that scientists knew about gold nanoparticles, its properties and its uses but the reality is that gold nanoparticles were used back in the 4th Century by the Romans, it was discovered that the Lycurgus Cup, a glass cup was made from gold and silver nanoparticles, what made it so special was that the colour of the glass changed depending on the passage of light. This changing of colour is just one of the few features of gold nanoparticles which we would not see Gold atoms exhibit. Gold nanoparticles (GNPs) have been used since ancient times to make stained-glass, but it was long assumed that the colour of the gold suspension was a result of the chemicals used to prepare it. In 1857

Michael Faraday produced the first pure sample of gold colloid and discovered that its colour is due to the size of the gold particles [5].

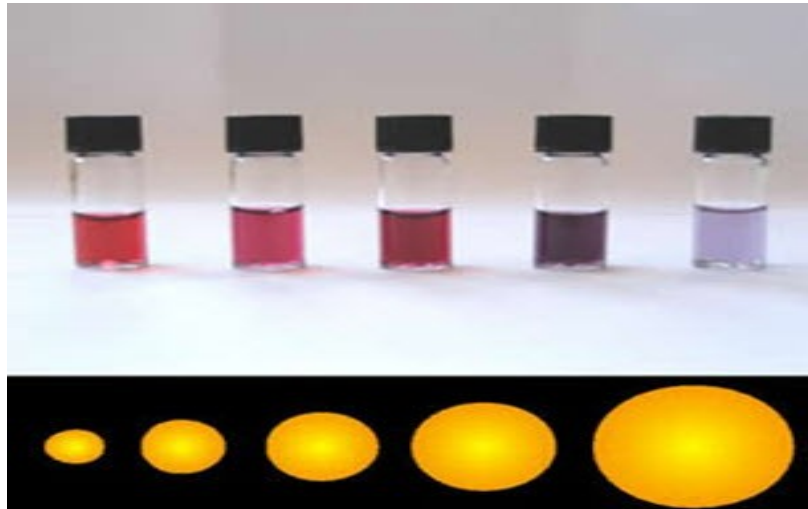


Fig 1. different color of solutions for different size of GNP's [48]

1.2.1 Properties of Gold Nanoparticles:

SPR :

Gold Nanoparticles cooperation with light is actively directed by their environment, size and physical amplitude. Oscillating electric fields of a light beam propagating close to a colloidal nanoparticle interact with free electrons resulting in collaborative oscillation of electron charge that resonates with visible light's frequency. This phenomenon of oscillating resonance of electrons is known as Surface Plasmon Resonance. It is this phenomenon that influences absorption and scattering of light. Because of SPR the interaction of light with electrons on the gold nanoparticle surface is possible. And due to collective oscillation of electrons in the conduction band, different shape varies in color like gold nanospheres are usually red whereas gold nanorods have changed colors. More about this phenomenon is described later. [41] [44]

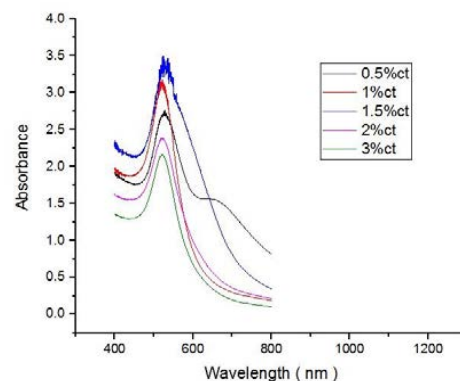


Fig 2.SPR of GNP

Shape and Crystallinity :

Variety of shapes and sizes of Gold nano-particles can be formed depending on the fabrication technique. Generally, anisotropic shapes are produced in the presence of stabilizing polymer that specially ties to one crystal face causing one crystal direction growing faster than others. Basically, fabrication technique determines the size of the crystalline domains in nano-particles. [42]

Stability:

Gold, in general, is inert and does not react with oxygen in the atmosphere. Generally, gold is more stable than silver. But same is not the case when viewed in nano-scale. It is, generally, the most stable nano-particle but at nano scale a certain size (approximately 5 nm) Gold nano-particles are highly reactive and are used as catalysts and reducing agents in many chemical reactions. Nano-particle stability can be precisely evaluated using UV-visible spectroscopy or Dark Field microscopy, also Dynamic light scattering. [44] [42]

Physical property data:

Some data of physical properties of gold nano-particles are shown in table below [43]

Properties	Metric	Imperial
Density	19.30 g/cm ³	0.697 lb/in ³
Molar mass	196.97 g/mol	

Table 1 Physical Properties of GNP

Thermal property data :

Some data of thermal properties of gold nano-particles are shown in table below [43]

Properties	Metric	Imperial
Melting point	1064.43°C	1947.9741°F
Boiling point	2807°C	5084.6°F

Table 2 Thermal Properties of GNP

Biocompatible and Non-Cytotoxic:

Gold is also biocompatible and non-cytotoxic in nature. Which means it does not affect living cells and tissues. This useful property of GNPs can be used in various biomedical applications of GNPs. For example, gold nano-particles are used by researchers in

investigating endocytosis in macrophages, tumour cells. This property also helps in targeted drug delivery. It can be used in applications which at some can be used in dealing with living tissues, as it does not react or harm them in any way and eventually gets the required work done.

1.3 Scope of this Thesis

There are so many other nanoparticles like Gold such as Silver (Ag), Platinum (Pt), Copper (Cu) but Gold is more preferred because of its special properties. As mentioned earlier Gold is a chemically boring element and also it is one of the most stable nanoparticles but at Nano scale a certain size (approximately 5 nm) GNPs are highly reactive and are used as catalysts [6]. It also exhibits a very special and rare property which is Surface Plasmon Resonance (SPR). Though Silver and Platinum both exhibit this property but silver is not stable like gold and platinum is more expensive than gold. Gold is also biocompatible and non-cytotoxic as it does not affect living cells. These properties make GNPs very interesting and is one of the most researched nano particle.

GNP's can be synthesized in a range of different shapes and sizes. The size ranges from 5-400nm, they be manufactured in the form of spheres, rods, cubes, wires, urchins, etc. Knowing the size and shape is important because of the electronic, optical, magnetic, biocompatible and non-cytotoxic and catalytic properties depend on it.

In Bangladesh there is almost no research has been done on GNP's. It is also quite hard to produce gold nanoparticle industrially. In this research an effort has been done to characterize Gold nanoparticle solution using UV-VIS spectroscopy as other characterization techniques such as SEM and AFM are expensive and TEM is not available in Bangladesh. Above mentioned factors have worked as our motivations to work on this thesis.

1.4 Summary of the Following Chapters

In the second chapter of the paper, the applications of Gold NanoParticle are described. This chapter shows some advance applications of Gold nanoParticle which shows why it is one of the most researched nanoparticle. the steps of the experiment of synthesizing gold Nanoparticle are studied in the next chapter. Some easy detection methods to ensure the presence of colloidal Golds are also discussed here. The fourth chapter describes the parameters we changed while doing experiment. It also includes the calculations of the changed parameters. In chapter five, advanced characterization techniques are covered. this chapter also covers a brief description of the instruments and the procedures to carry out the tests.

Chapter six particularly portrays all the results after a successful experiment. All the pictures of the solutions after synthesizing GNP are added in this chapter. Results of different characterizations techniques are also added here and the comparisons with usual values were also showed here. Finally, in chapter seven, the paper was concluded highlighting the results of the synthesis and some limitations with the scope of future work of this thesis.

CHAPTER 2

Applications of Gold Nanoparticles

2.1 Introduction

Recent studies show a wide variety of applications of gold nanoparticles. Range of applications in the areas of medicine, biology and electronics has attracted a lot of attention over the last decade. Biocompatible, non-cytotoxic, optical, electrical and magnetic properties allow many uses for gold nanoparticles.

2.1.1 APPLICATIONS IN MEDICINE & BIOLOGY

Biomedical applications of gold nanoparticles include diagnostics, genomics, biosensors, phototherapy of cancer cells or tumours, targeted delivery of drugs, bio imaging, etc.[8].

Cancer/tumour treatment

Treatment of Cancer, generally, is extremely challenging. Even with vast improvements in medicinal surgery, micro tumours (micro cancer cells), still remain a serious threat. Even when severe cancers get treated through a complete tumour treatment, some residual tumour cells get left behind. These residual tumour cells, if untreated, might gradually experience growth, and eventually reintroduce cancer. Occasionally these tumour cells are difficult to remove as they strongly bind to the critical organs of the body. Throughout the years researches have been going on in order to find a method to detect and eliminate these residual cancer cells. Some are showing good results. One such innovative method describes engulfing of numerous nanoparticles by cancer cells, which eventually helps to destroy these residual cancer cells, without causing significant harm to the cells of the organs the tumours bind with. This method uses the concept of Plasmonic Nano Bubble nanosurgery often known as PNB to detect and eliminate these residual cancer cells. Studies show that, this surgical method prevented reintroduction of cancer and came across with 100% tumor-free survival. This Nano surgery was deemed more effective than standard surgical methods.

Plasmonic Nano bubbles are basically temporal Nano sized bubbles in gaseous state. The nano bubbles are developed around clusters of Gold Nanoparticles via short laser pulses. When short Laser pulses hit the gold nanoparticles cluster, they absorb the laser pulses and convert the energy absorbed into heat energy via non-stationary plasmonic mechanism. There are fluids that are located around these cells. The converted heat energy then evaporates the fluid into small nano sized bubbles that expands within itself and eventually collapses in nanoseconds. These large clusters of gold nanoparticles are formed around the cancer cells through a process known as endocytosis. Basically, this is a method of transportation of materials in and out of cells. Clinically certified gold colloids are attached to the antibodies against the receptors of the cancer cells. This surgical method improves the surgical results and reduces errors, and also allows selective targeting of the cancer cells without causing harm to nearby healthy cells. This selective targeting of cancer cells is not possible in methods such as chemotherapy and radiation therapy. So, patients trying to avoid the two latter treatments can opt to gold nanoparticles surgery. Moving onto another aspect, determining a single residual cancer cell may be very difficult but clusters of cancer cells can be easily detected by gold nanoparticles method. Hence, it can be said that detection of cancer cells and their elimination both are possible through this method [31].

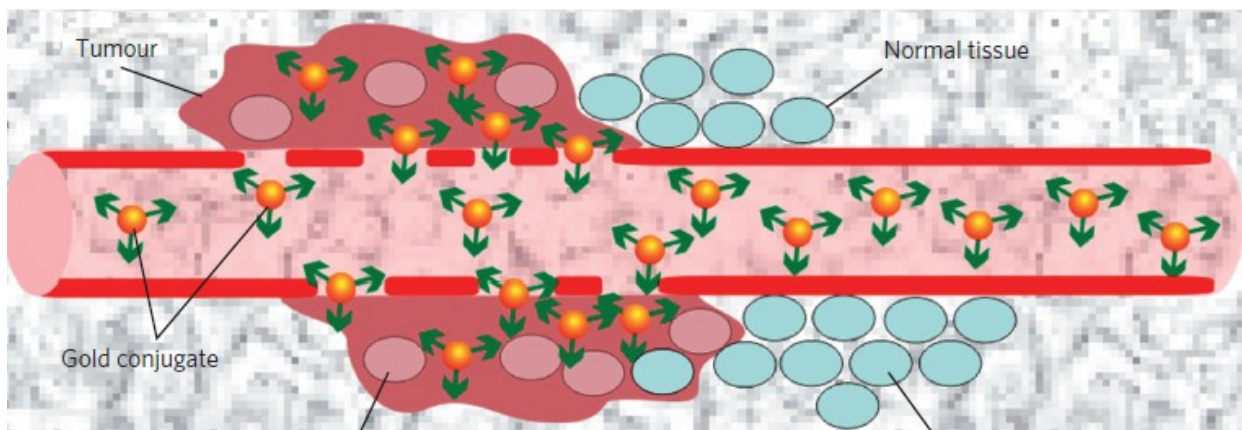


Fig3. systematic Delivery of gold nanoparticles to the tumour cells via leaky blood vessels^[31]

Solidified tumour cells often tend to have leaky blood vessels. Via systematic delivery, as clusters of gold colloids are inserted in the patient's blood they leak out of the leaky blood vessels of cancer cells to bind around the tumour cells. They generally, tend to avoid normal

healthy tissues and target only the tumour tissues. This is how the healthy cells remain unaffected by this surgical method. The cancer cells then envelop the gold nanoparticles. While they are inside the tumour cells and as soon as they get hit by short near infra-red laser pulses, the fluid surrounding the cells gets heated to ultimately destroy the cancer cells. These short laser pulses are penetrable across centimetres of tissues without harming them. There are still some shortcomings of this method. If this was a 100% effective method to get rid of cancer permanently, we would have already acquired the cure for cancer!. One of the shortcomings is that, some normal healthy tissues end up acquiring some of these gold nanoparticles. This can be a problem as while short laser pulses are shot, some of these normal cells containing gold nanoparticles might get destroyed in the process. Another shortcoming is that there is a possibility of infrared light from the laser pulse transmitting onto normal cells surrounding the cancer tissues. This whole experiment has been conducted only on mice and not humans, for the time being. Researchers and clinical experts are setting up working on the procedures so that they can be carried out on humans instead of mice. This will take a few more years. If it shows positive results on humans, a drastic decrease in the number of cancer patients can be seen. In cases where practically removing an entire tumour is impractical, this method could come in very handy. Removing tumour entirely is very essential as the unresolved remaining tumours can grow further into another tumour. To counter the problem of infrared light from laser pulse transmitting into normal tissues and damaging them, researchers came up with the technique of ‘zapping’ nanoparticles. The researching team includes Dmitri Lapotko, a physicist formerly with Rice and now head of laser science at Masimo Corporation, a medical nanotechnology company in Irvine, California. They, first, inserted human squamous cell carcinoma, cancer cells common in human head and neck tumours, into mice. These are normally very challenging to deal with when using standardized treatments. After insertion into mice, they then, coated Gold Nanoparticles with immune protein antibodies. These antibodies attach with receptors on the surface of squamous cells. Hence concentrated particles form clusters inside the cancer cells and also surrounding the cancer cells. This time they shot ultra-short infrared pulses rather than shooting continuous laser beams. Fortunately, they succeeded in limiting the heat to cancer cells only, and causing no heat to reach the normal tissues. But this method had a higher impact on another part. The heat caused temperatures to increase in regions containing large clusters of gold nanoparticles. The surrounding water molecules then gets vaporized into small bubbles that promptly expand and eventually burst, in the meantime destroying the cancer cells. The key, Lapotko says, is that “nanoparticle clusters produce nanobubbles in

cancer cells and not normal tissue.” Laptko and his researchers has said that due to the mini explosions sounds were detected and hence located. As few as three cancer cells could be detected by this method and destroyed as well. Laptko reported that in the vigorous experiments performed, all the animals survived and no residual cancer cells did. The survival rate increased to double, where tumours were surgically and partially removed. “This is very, very interesting,” says Mien-Chie Hung of the University of Texas MD Anderson Cancer Centre in Houston, who is exploring treating tumours with nanoparticles. The new technique, he says, acts like microscopic surgery to target those residual cells. He also depicted that most oncology surgeries that are performed in animals and are successful in animals are not as much successful in humans. But if this surgery does work, it could create wonders in the field of biological science, in detecting and destroying residual cancer cells left behind after surgery [32].

Targeted drug delivery

Targeted drug delivery is one of the most promising applications of gold nanoparticles. Gold nanoparticles works as a non-toxic carrier of drugs [33,34].The developing field of nanotechnology shows development of extremely sensitive organ (tumour) targeted diagnostics and therapies. Without doubt, the mixing of material science and tumour biology is prompting the advancement of inventive vectors with the capability of accomplishing the long sought-after objective of tumour-targeted drug delivery, where we can get the drug-delivery agent exactly where it is required, which is at the solid tumour.Yet for the objective to be fully effective some natural biological barriers must be overcome. Fortunately, bionanotechnologists have built effective methods to conquer this barrier related problems. In recent times, GNPs have been accumulated into scaffolds and used in biosensors and used in deoxyribonucleic acid (DNA) diagnostics. Some historical data, along with some data from Good Laboratory Practices (GLP) toxicology study in rabbits, shows that gold nanoparticles are comparatively inert and biologically suitable carriers. [35]

Gold nanoparticles have the ability to deliver large biomolecules, without themselves having to act as carriers for just small molecular drugs. Their unique functionality along with their tuneable size makes them an appropriate scaffold for efficient recognition and delivery of large biomolecules. They have demonstrated fruit in delivery of peptides, proteins, or

nucleic acids like DNA or RNA. Gold Nanoparticles also provide themselves as appealing contenders in Gene Delivery [36].

Cells themselves, naturally, do a good work in safeguarding their valuable contents, and thus, it is exceptionally hard to infiltrate their membrane walls in order to deliver drugs, nutrients or biosensors without harming or pulverizing the cell. A viable method for doing so, is using colloidal gold nano-particles being covered with a thin layer of a special polymer. In the process, researchers first coated the gold nano-particles with the thin layer of the polymer and combine them with lipids - a category of natural fats, waxes and vitamins that gives the cell wall its shape. Researchers additionally exhibited an upper limit on the sized particles that can enter the cell wall. This limit is determined by the particles' coating composition.

This coating comprises of a blend of hydrophobic and hydrophilic segments forming a monolayer, a one molecule thick layer on the surface of the particle.

"Cells tend to engulf things on the surface," says Alexander-Katz, an associate professor of materials science and engineering at MIT, but it's "very unusual" for materials to cross that membrane into the cell's interior without causing major damage. Irvine and Stellacci exhibited in 2008 that monolayer-coated gold nanoparticles could be able to do so.

As the nanoparticles themselves are fully coated, direct effect that they are made of gold is clearly observed, with the exception that gold nano-particles are an effectively arranged model framework. Nonetheless, there is some proof that the gold nano-particles have some therapeutic properties, which could act as an additional advantage.

Capturing X-ray beams is also a useful side of Gold nano-particles. If they could be made to enter cancer cells, and if heat was applied to them by X-ray beams, they could annihilate those cells from the inside. "So the fact that it's gold may be useful," says Irvine, a professor of materials science and engineering and biological engineering and member of the Koch Institute for Integrative Cancer Research. The system that allows nano-particles to enter through the membranes, seems also to enclose the opening as the particles enter.

A potential application of this research could be in inserting and attaching biosensing molecules on or into specific cells. Along these lines, researchers could detect or screen particular biochemical markers, for example, proteins that show the starting or diminishing of a disease or metabolic process.

Roughly, attachment to the surface coating of Nano-particles could provide a key to the insides of the cells for the molecules that usually are able to enter the cells [37].

2.1.2 ELECTRONICS APPLICATIONS

Stretchable/flexible electronics

Flexible electronics has drawn a lot of research in recent years. This research is allowing manufacturers to dream about making flexible devices like solar panels to even smart phones. The requirement of a conductor which maintains its conductivity even when it is stretched has seemed to end. Researchers at the University of Michigan have invented a material made of spherical gold nanoparticles embedded in flexible polyurethane, which stays electrically conductive when it is stretched. Previous nano materials used for the same cause proved to be less effective as they could not maintain their conductivity after being stretched, but gold nanoparticles when embedded in a polymer maintains its conductivity very well even after it has been stretched up to twice its original length. This happens because the nanoparticle polymer composite when stretched aligns itself into chainlike form which allows conduction and at relaxed state it restores its original shape. This research has opened a lot of potential applications of gold nanoparticles in flexible electronics. Professor Nicholas Kotov team leader at UMich for this project also quoted “potential applications for this technology, such as less harmful electrode brain-implants for treating Parkinson's, epilepsy, and other diseases” [38, 39].

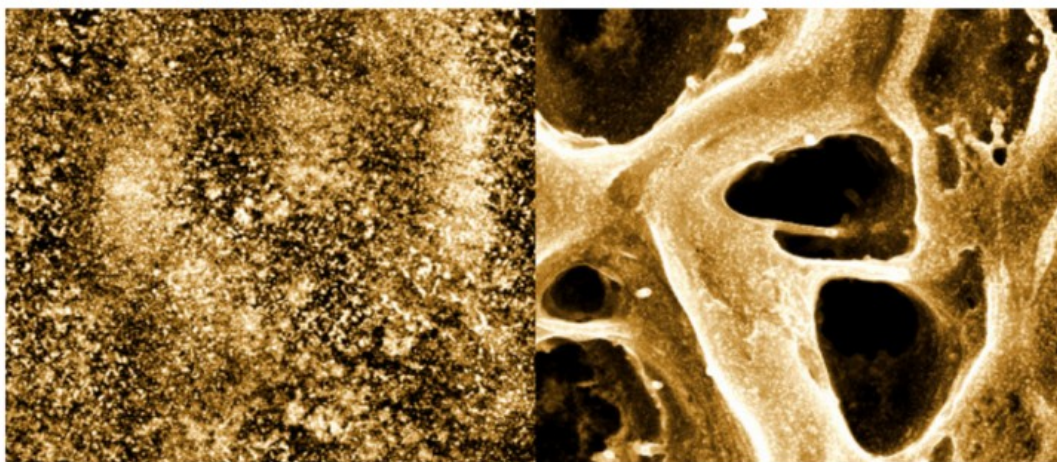


Fig4. Electron microscope images of the gold nanoparticles embedded in polyurethane [38]

The team at University of Michigan has used two methods in making this nanoparticle polymer composite. The layer-by-layer method in which a glass slide is dipped into solutions of gold nanoparticles and polyurethane alternatively and the Vacuum assisted filtration in which a mixture of gold nanoparticle solution and polyurethane solution was filtered using a filtration assembly. In both processes they have used spherical gold nanoparticles suspended in a colloid which was synthesised using the citrate reduction method just like ours [40]

Chapter 3

Synthesis of Gold Nanoparticle

3.1 Introduction

There are many different methods of synthesis of gold nanoparticles; these methods are suitable for different types of gold nanoparticles. Although many advanced techniques exist, like photochemical UV irradiation [7], Seed-mediated growth, Sonoelectrochemical [8], Sonochemical [9], and the chemical reduction method [10, 11]. The chemical reduction method which is the simplest method comprises of a gold salt solution which is reduced by a reducing agent that yields colloidal gold nanoparticles in a solution which is basically spherical gold nanoparticles suspended in a fluid. The size and shape of the nanoparticle is not only dependent on the synthesis procedure but also its parameters. Parameters like rate of reaction, temperature, amounts of reagents used and even pH are a major factor in determining the size and shape of nanoparticles [12].

3.1.1 Common terminologies& Concepts

Before we begin to discuss about the synthesis procedure we must be familiar with a few terminologies and concepts essential for better understanding of the synthesis process [47].

1. **Nucleation-** Nucleation is the first step in the formation of either a new thermodynamic phase or a new structure via self-assembly or self-organization. Nucleation is typically defined to be the process that determines how long an observer has to wait before the new phase or self-organized structure appears. It is basically the arrangement of small number of molecules in a pattern.
2. **Growth-Crystal growth** is a major stage of a crystallization process, and consists in the addition of new atoms, ions, or polymer strings into the characteristic arrangement of a crystalline Bravais lattice. The growth typically follows an initial stage of either homogeneous or heterogeneous (surface catalyzed) nucleation, unless a "seed" crystal, purposely added to start the growth, was already present.

3. **Aggregation** - The number of potentially relevant materials resulting from the aggregation of elementary units with a finite functionality continues to increase. The growth of branched clusters and networks may proceed through the formation of reversible (physical) or irreversible (chemical) bonds.
4. **Agglomeration**-According to the IUPAC definition, flocculation is "a process of contact and adhesion whereby the particles of a dispersion form larger-size clusters." Flocculation is synonymous with agglomeration and coagulation / coalescence. Molecules are joined loosely in solid state but can be broken down.
5. **Super saturation**- There is a point when a solution contains more solute than the solvent can dissolve. This solution is known as a supersaturated solution. You normally cannot see the solute in a solution because it is fully dissolved. You are able to see the solute in a supersaturated solution because all of the solute doesn't fully dissolve into the solvent.
6. **Precursor**- a precursor is a compound that participates in a chemical reaction that produces another compound. In biochemistry, the term "precursor" often refers more specifically to a chemical compound preceding another in a metabolic pathway, such as a protein precursor.
7. **ElectrostaticStabilisation**- Electrostatic stabilization of Colloids is the mechanism in which the attraction van der Waals forces are counterbalanced by the repulsive Coulomb forces acting between the negatively charged colloidal particles.
8. **Stabilizing agent** - In chemistry a stabilizer is a chemical which tends to inhibit the reaction between two or more other chemicals. [citation needed] It can be thought of as the antonym to a catalyst. The term can also refer to a chemical that inhibits separation of suspensions, emulsions, and foams.It prevents aggregation of particles.
9. **Capping agent**- A capping agent is a strongly absorbed monolayer of usually organic molecules used to aid stabilization of nanoparticles.Particles can be functionalized using capping agents to impart useful properties. A capping agent is used to protect

the surface of materials, commonly nanoparticles. It prevents degradation and can help preserve different properties of the material.

3.2 Synthesis Procedure

This paper will cover the citrate reduction method which was introduced by John Turkevich[13] in 1951 and was later developed by G.Frens in the 1970's [10]. The citrate reduction method uses Trisodium Citrate as the reducing agent and also acts as a stabilizing agent and Chloroauric acid is the precursor. In this method Trisodium Citrate is added to heated solution of Chloroauric acid while being rapidly stirred which results in the production of spherical gold nanoparticles and change the colour of gold solution from yellow to ruby red.

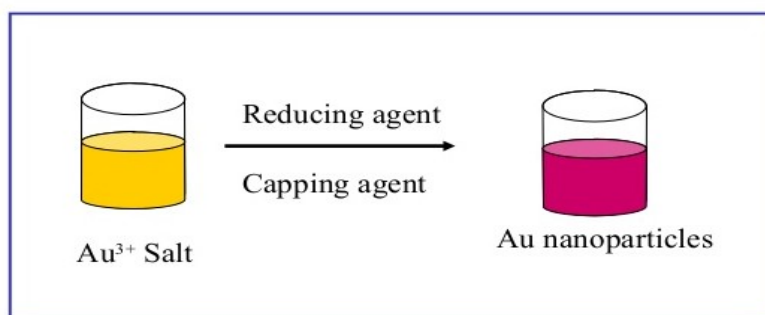


Fig 5. change of solution color [48]

3.2.1 Apparatus & Chemicals

Chemicals:

1. Hydrogen tetrachloroaurate(III)/Chloroauric acid - HAuCl_4 (1.0mM)
2. 1% of Trisodium Citrate - $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$
3. Distilled Water

Apparatus:

1. Beaker
2. Test Tube
3. Conical Flask
4. Pipette
5. Measuring Cylinder
6. Glass funnel
7. Dark glass bottle with stopper
8. Erlenmeyer Flask
9. Magnetic Stirrer and hotplate combination



Fig 6. apparatus used for preparing Gold Nano Particle

3.2.2 Experiment

- Dissolved 1.0g of HAuCl_4 in 250ml of distilled water to make 10.0mM stock solution
- Diluted 25ml of stock solution to 250ml distilled water to make 1.0mM of HAuCl_4 solution
- Dissolved 0.5 g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ (sodium citrate) in 50 mL distilled water to produce 1% trisodium citrate.
- We added 20ml of 1.0mM of HAuCl_4 to a 50ml Beaker placed on a magnetic stirrer and hotplate combination and brought the solution to boil.
- To the boiling and rapidly stirring solution we added 2 mL of a 1% solution of trisodium citrate dihydrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$

Here the citrate ions reduce the Au^{3+} ions to neutral Au atoms, the citrate ions acts as the reducing agent, stabilizing agent and capping agent. The solution becomes supersaturated due to vigorous stirring, gold atoms collide to form a stable nucleus, growth starts and leads to the formation of monodisperse spherical gold nanoparticles after about ten minutes. The colour of the chloroauric acid turns from transparent light yellow to dark black to finally a characteristic ruby red colour which confirms the presence of gold nanoparticles.[14]

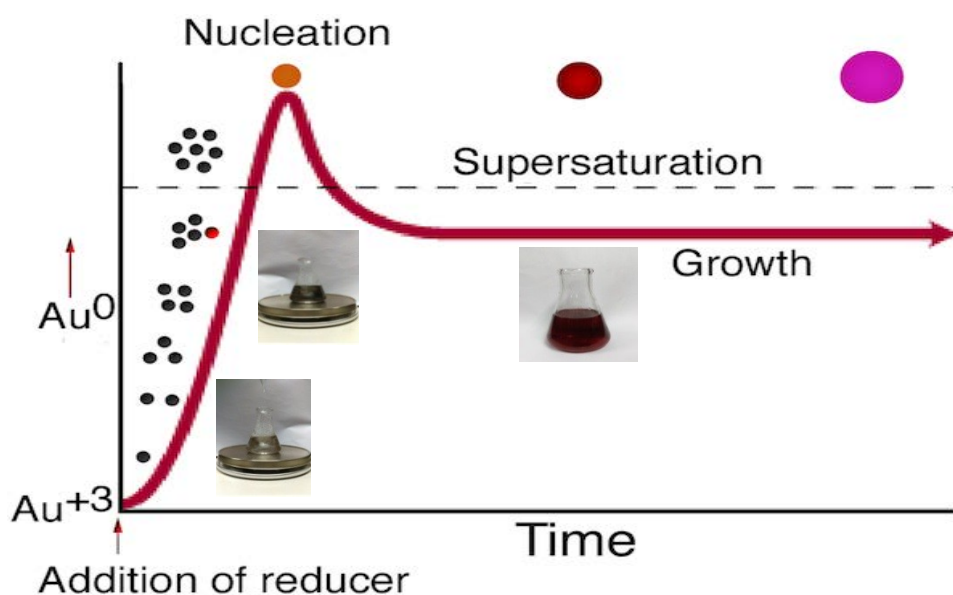
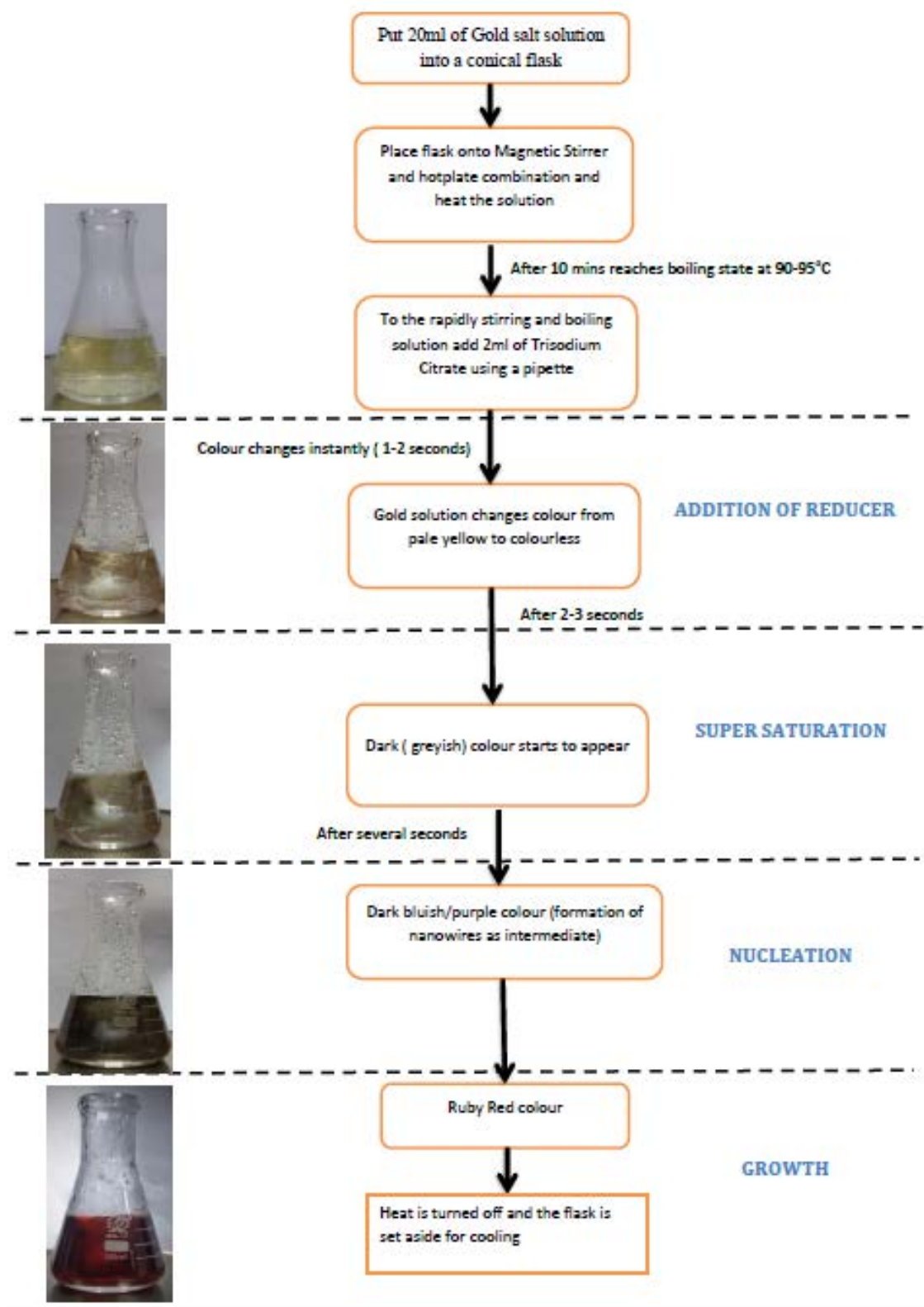


Fig 7. processes of gnp formation [14]

Fig8.flow chart for our synthesis process



Observations



Fig 9.different phases of reaction

This picture shows the different phases of gold solution which is placed over the magnetic hotplate and stirrer combination. Starting from the left the first picture shows the boiling solution of the gold solution which is pale yellow in colour. The next picture shows the moment when the trisodium citrate is added to the boiling and rapidly stirring solution, the colour changes from pale yellow to a clear solution almost instantly. This clear phase lasts for about a few seconds after which a light greyish colour starts to appear. This light colour darkens into a dark blue-grey colour (third picture) after several seconds and continues to change its colour to a purple and finally a ruby red colour as shown in the last picture. The gold solution takes almost 10 minutes to bring to a boil after which citrate is added and almost after 5 minutes of adding citrate the red colour can be seen and the heat is turned off and the ruby red solution is allowed to cool. The whole process takes about 20-25 minutes.

3.2.3 Tests and observations

After the synthesis of ruby red gold nanoparticle solution other than the characteristic colour there are no ways to ensure that it is in fact gold nanoparticles in the solution which we made. To further clarify this we did some more experiments/ test on the final solution.

Laser Pointer

The simplest approach to confirm the presence of gold nanoparticles is shining a laser pointer into the ruby red gold solution stored in an Erlenmeyer flask or beaker, if the path of the laser is not visible through the solution then gold nanoparticles are not present. If the path of the laser pointer is visible through the solution it means gold nanoparticles are present. This happens because the suspended gold nanoparticles are reflecting and scattering the light this is known as the Tyndall effect [15].

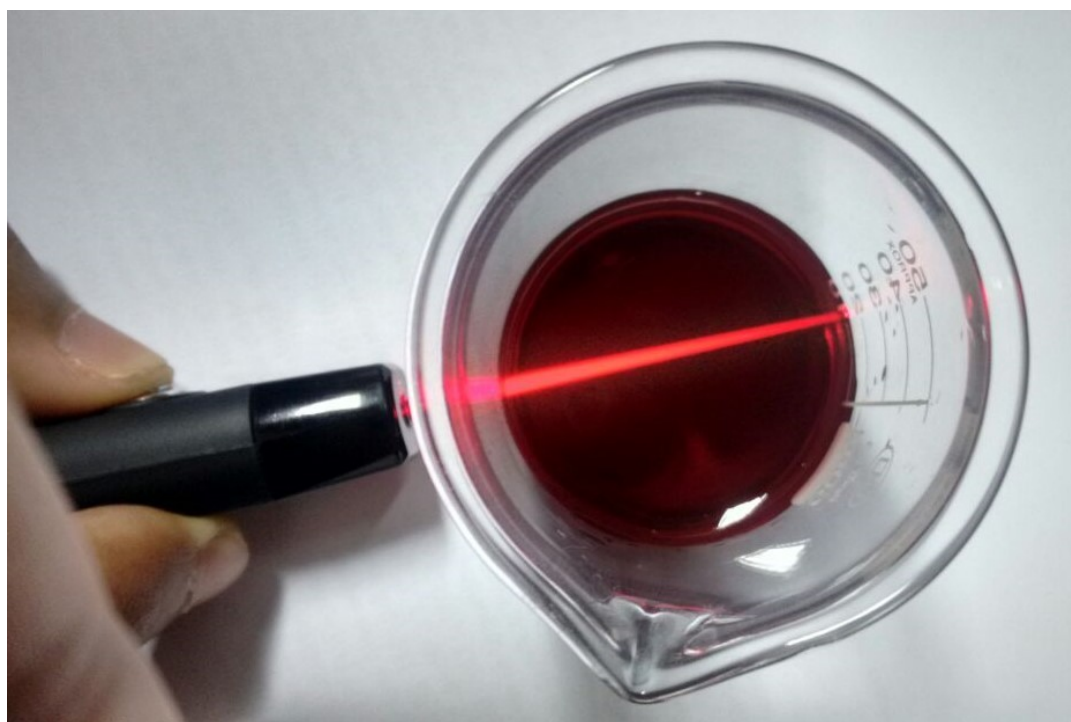


Fig 10.laser test on GNPs solution

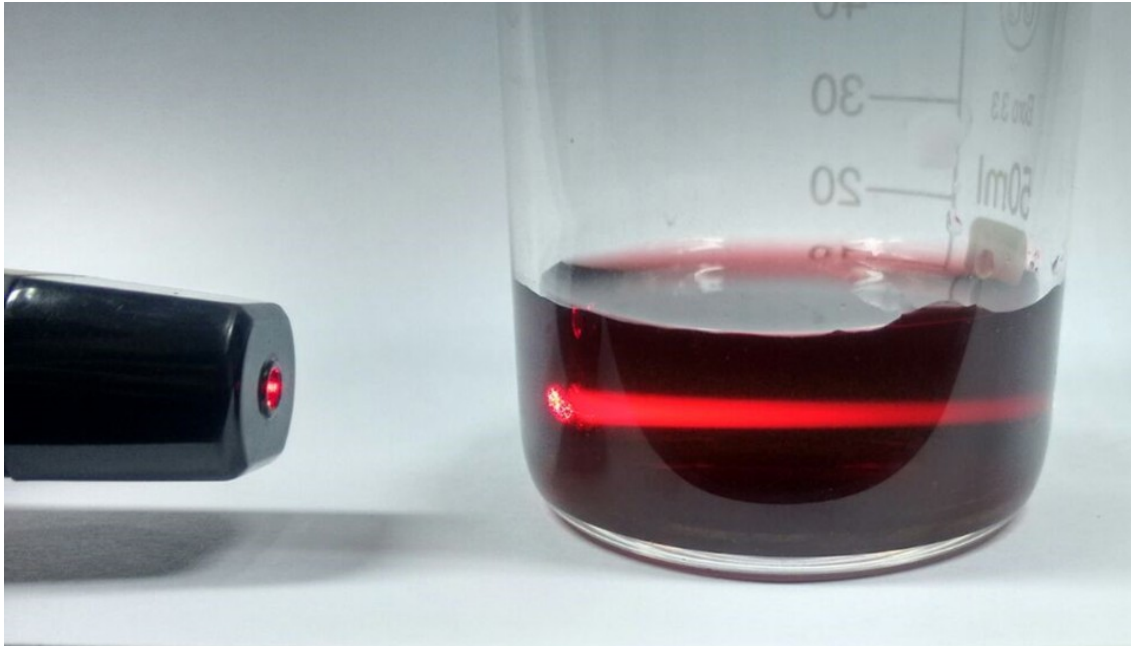


Fig 11. laser test on GNPs solution

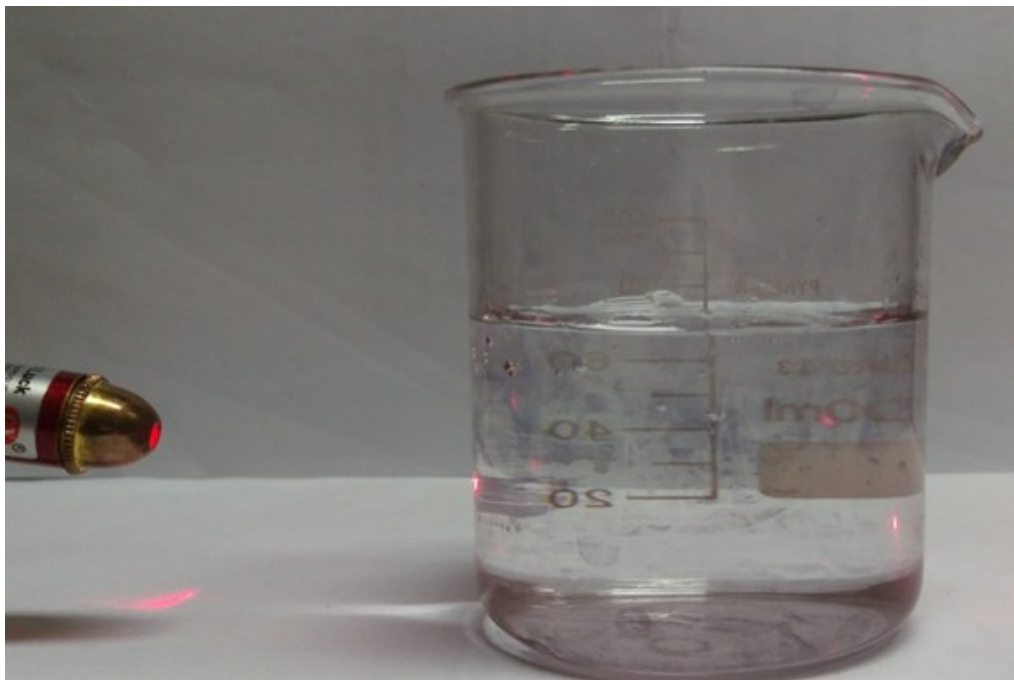


Fig 12.laser test on Water

Tyndall Effect

The Tyndall effect, which is also known as Willis-Tyndall scattering, is light scattering by particles in a colloid or else particles in a very fine suspension. It is named after the 19th-century physicist John Tyndall. It is similar to Rayleigh scattering, in that the intensity of the scattered light depends on the fourth power of the frequency. It is particularly applicable to colloidal mixtures and fine suspensions. The Tyndall effect is used in nephelometers to determine the size and density of particles in aerosols and other colloidal matter.

For Tyndall effect, the longer-wavelength light is more transmitted while the shorter-wavelength light is more reflected via scattering. The Tyndall effect is seen when light-scattering particulate-matter is dispersed in an otherwise-light-transmitting medium, when the cross-section of an individual particulate is the range of roughly between 40 and 900 nanometers, i.e., somewhat below or near the wavelength of visible light (400–750 nanometers).

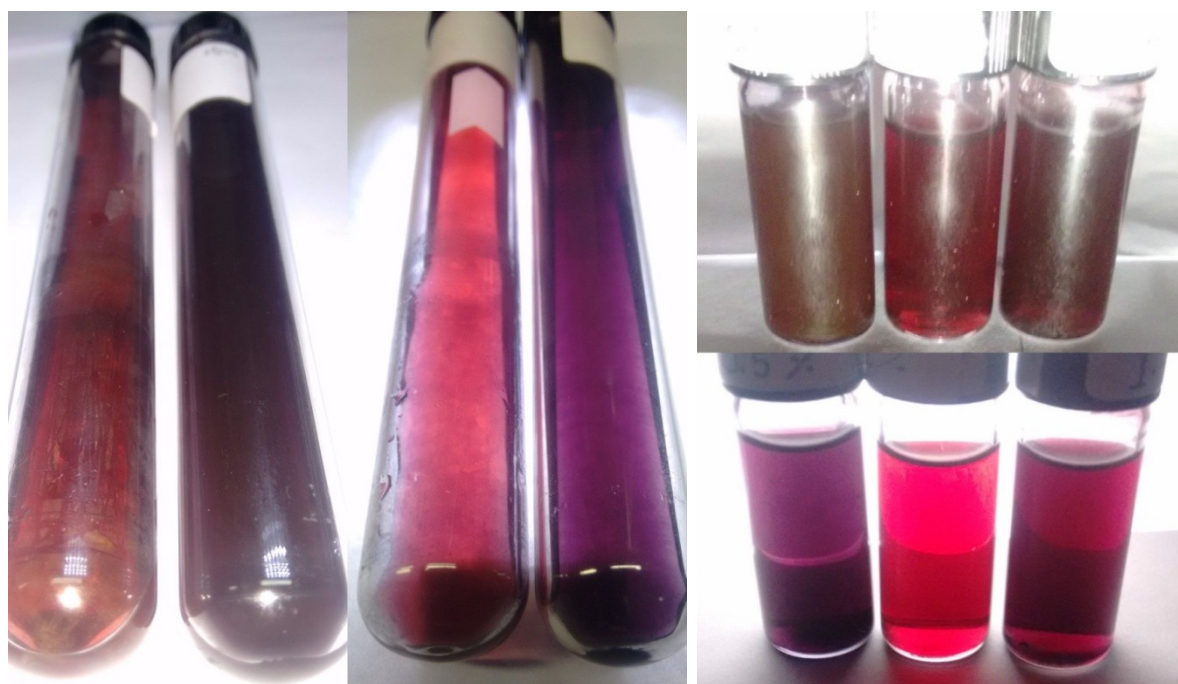


Fig 13. Tyndall Effect of Gold nanoparticle

Salt & Sugar Test

This test was done with the ruby red gold nanoparticle solution after the synthesis process. This is a rather inexpensive way of determining the presence of gold nanoparticles just like the laser pointer test. Here we took three test tubes of the ruby red gold nanoparticle solution and added few drops Sodium Chloride solution in one test tube, added few drops of sugar solution and another test tube was kept as a colour reference

Salt test

0.5 g of NaCl or simply table salt was dissolved in 10 ml of water to make the salt solution. 5-10 drops of this solution was added to a test tube containing gold nanoparticle solution and was shaken well. The nanoparticle solution contains gold atoms which are capped by negatively charged citrate ions this allows the gold atoms to be dispersed in the solution and not bind together. After the addition of an electrolyte such as NaCl the Na^+ ions neutralize the anion layers that shield the gold atoms. The nanoparticles then starts to agglomerate and the colour of the solutions turns deep blue as the particles now absorb longer wavelength (green, yellow, orange and red: 550-650 nm) but not the shorter ones (blue and purple 400-500 nm). Therefore the colloid now looks bluish whereas previously it looked red as it absorbed shorter wavelength: blue and green (475-550 nm) instead of orange and red (600-700 nm) [16]. After adding more drops of NaCl the solution turns clear as no particles remain suspended in the solution it aggregates and precipitates form at the bottom so no light is absorbed by the solution so it appears clear [17].

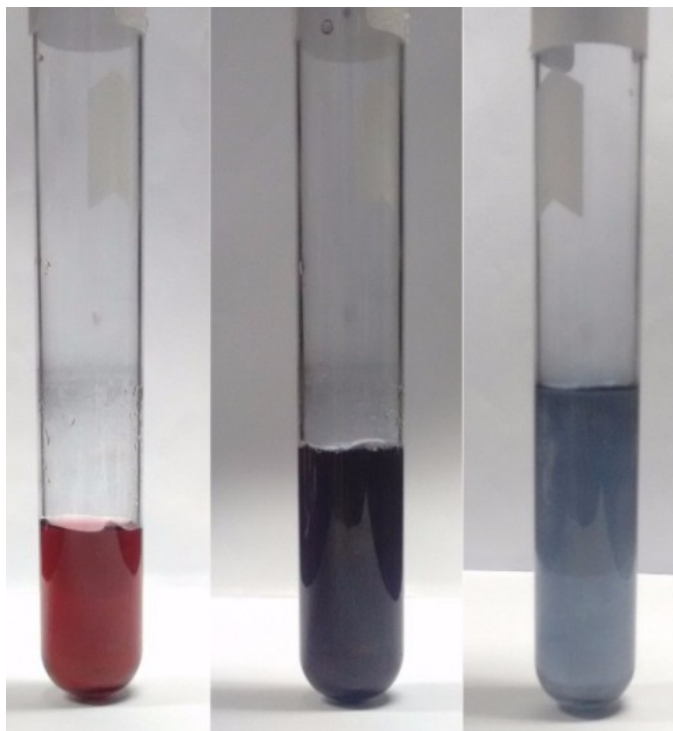


Fig 14. different steps of salt test

Sugar test

2g of sugar is dissolved with 10 ml of distilled water to make the sugar solution. 3-4 drops of the solution was added to the red gold nanoparticle solution and was shaken well. There was no significant change in colour. If a weak or non-electrolyte such as sugar is added to the nanoparticle solution the anion layer of citrate ions is not disrupted so this does not result in any change of colour. There might be a slight change from ruby red to slight pinkish red which results from very small agglomeration. [18]

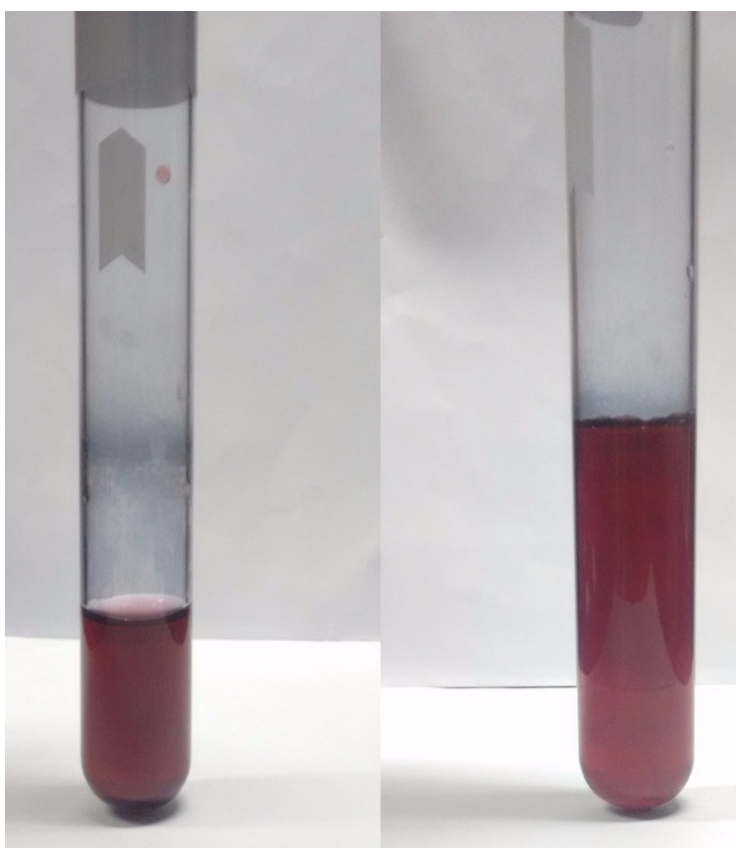


Fig15. Sugar test

Chapter 4

Experiments with changed Parameters

4.1 Introduction

The synthesis of gold nanoparticles via the turkevich or citrate reduction method gives spherical gold nanoparticles of certain size this size and size distribution can be altered by adjusting the synthesis process. Many properties of colloids and suspensions depend on the particle size [10]. By changing certain parameters we get the desired particle size which we can apply.

4.2 Parameters

The turkevich method or citrate reduction method is a very sensitive method in which slight changes in experiment parameters will cause significant changes in the result of the experiment. Parameters like Temperature, PH, mixing rate, initial gold concentration, citrate concentration and amount of citrate all are responsible for changes in the final result. In 1951 turkevich reported the basic experimental approach and the effect of temperature and reagent concentration on size and size distribution and in 1973 Frens reported the effect of changing the concentration of sodium citrate [19]. In our work we have changed the amount of trisodium citrate added, Initial gold concentration and the concentration of trisodium citrate and analysed its effects on nanoparticle size and size distribution.

Amount of Trisodium Citrate added

In this experiment we only changed the amount of trisodium citrate added other parameters like temperature, gold concentration, concentration of trisodium citrate, mixing rate, etc. were kept constant throughout. Gold salt concentration was 1mM and 1% trisodium citrate was used. In the basic approach only 2ml of citrate was added to a rapidly mixing heated solution of gold salt. Amount of trisodium citrate added was 0.5ml, 1.5ml, 2ml, 3ml and 4ml.

Trisodium Citrate Concentration

In this experiment only the concentration of trisodium citrate was changed other parameters were kept constant. The gold salt concentration was 1mM, 20ml of gold salt was reduced by adding 2ml of trisodium citrate with different concentration/percentage. In our basic approach 1% of trisodium citrate was added, we used 0.5%, 1%, 1.5%, 2% and 3%.

Calculations

Weight / Volume percentage concentration is used to measure the concentration of a solution.

To calculate W/V % concentration:

$$\text{W/V \%} = [\text{mass of solute (g)} / \text{Volume of solution (ml)}] \times 100\%$$

So in case of $\text{Na}_3\text{C}_6\text{H}_{507.2}\text{H}_{20}$. To obtain 0.5% of $\text{Na}_3\text{C}_6\text{H}_{507.2}\text{H}_{20}$.

$$0.25 \text{ g} / 50\text{ml} \times 100\% = 0.5\%$$

Therefore we need to dissolve 0.25g of $\text{Na}_3\text{C}_6\text{H}_{507.2}\text{H}_{20}$ in 50ml distilled water to get 0.5% of $\text{Na}_3\text{C}_6\text{H}_{507.2}\text{H}_{20}$

In the same way we calculated for 1%, 1.5%, 2% and 3%.

For 1% ----- 0.5g was dissolved in 50 ml water

1.5%----- 0.75g was dissolved in 50 ml water

2% ----- 1g was dissolved in 50 ml water

3% ----- 1.5g was dissolved in 50 ml water



Fig 16. measuring mass in Weighing Balance



Fig 17. different concentration (%) of Sodium Citrate

Initial gold salt concentration

In this experiment the initial gold salt concentration was changed and all other parameters were kept constant. In our standard approach we used 1mM of AuCl_3 solution we changed the concentration to 0.5mM, 1mM, 5mM, 10mM and 20mM. Amount used were same as before we used 20ml of gold salt and 2ml of citrate. The concentration of citrate used was 1%.

Calculations

For 10mM & 1mM

Molar Mass, $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O} = [1 + 197 + 4(35.5) + 3(18)] = 394 \text{ g/mol}$

Mass = 1 g

Volume of Distilled water = 250ml

No. of Moles = Mass/Molar Mass = $1\text{g} / 394\text{g/mol} = 0.0025 \text{ mol}$

Concentration (molarity) = No. of moles / Volume = $0.0025 \text{ moles} / 250\text{ml} = 0.01\text{Mol/litre}$
 $= 10\text{mM}.$

Hence by dissolving 1g of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ in 250 ml water we get a solution of concentration 10mM.

By serial dilution method we can easily dilute 10mM solution with water and get 1mM solution. With Number of Moles remaining same:

$$X / 25\text{ml} = 10\text{mM}$$

$$X / 250\text{ml} = 1\text{mM}$$

Therefore we dilute 25ml of 10mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ with distilled water to get a solution of 250ml of 1mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$.

For 0.5mM, 5mM & 20mM

As previously we made stock solutions of 1mM and 10mM the solutions of 0.5, 5 and 20mM were made later. The calculations are as follows.

Molar Mass, $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O} = 394 \text{ g/mol}$

No. of Moles = Mass / Molar Mass = $1.97 / 394 = 5 \times 10^{-3}$ moles

Concentration (Molarity) = No. of Moles / Volume = $0.005 / 250 \text{ ml} = 0.02 \text{ Mol/litre} = 20\text{mM}$

By using the serial dilution method we made the solutions for 0.5 mM and 5mM from the 20mM solution.



Fig 18.measuring mass of Gold Salt



Fig 19.dilution of Gold salt solution

Let number of moles be x and with the number of moles remaining same.

For 5mM

$X / 62.5 = 20\text{mM}$, divide by 4

$X / 250 \text{ ml} = 5\text{mM}$

For 0.5mM

$X / 25 \text{ ml} = 5\text{mM}$, divide by 10

$X / 250 \text{ ml} = 0.5 \text{ mM}$

Therefore, we dilute 62.5 ml and 25ml of 20mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ with distilled water to get 250 ml solutions of 5mM and 0.5mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ respectively.



Fig 20. 1mM , 10mM & 20mM HAuCl_4

Chapter 5

Characterization

5.1 Introduction

There are different methods for characterizing gold nanoparticles after synthesis like Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), atomic force microscopy, UV-Vis Spectroscopy, Dynamic Light Scattering (DLS), X-ray Diffraction, gel electrophoresis and others. Almost all of these methods are able to deduce the size, size distribution and the shape of the nanoparticles. TEM and SEM give the most accurate measure of particle size and shape [20].

5.2 UV-Vis Spectroscopy

Another very useful characterization technique is UV-Vis spectroscopy, it is the measure of degree at which light is absorbed by a medium in different wavelengths like Ultraviolet, Visible light, Infrared, etc. using a spectrophotometer. This gives a spectrum which is a graph of intensity of absorbed or emitted radiation by sample versus frequency or wavelength. Usually gold nanospheres give a single absorption peak between 510-550nm depending on the size (diameter) of the particle [2].

The shapes of the absorption peak vary in different sizes and shapes of gold nanoparticles. For example, nanorods have two peaks in the absorbance curve this is due to transverse and longitudinal surface plasmon resonance whereas nanospheres have a single peak. With increase in particle size absorption peak shifts to longer wavelength. By observing these trends and by collecting data about their absorption the average size of the nanoparticles can be estimated using mathematical approximations or by matching it with given or standard values [27]. UV-Vis can also be used to determine the concentration of the solution based on the absorbance [21]. In UV-Vis Spectroscopy the solution which is to be tested is put into a cuvette, which is a clear plastic, glass or sometimes quartz (for UV-light) test tube specially made for optical analysis.

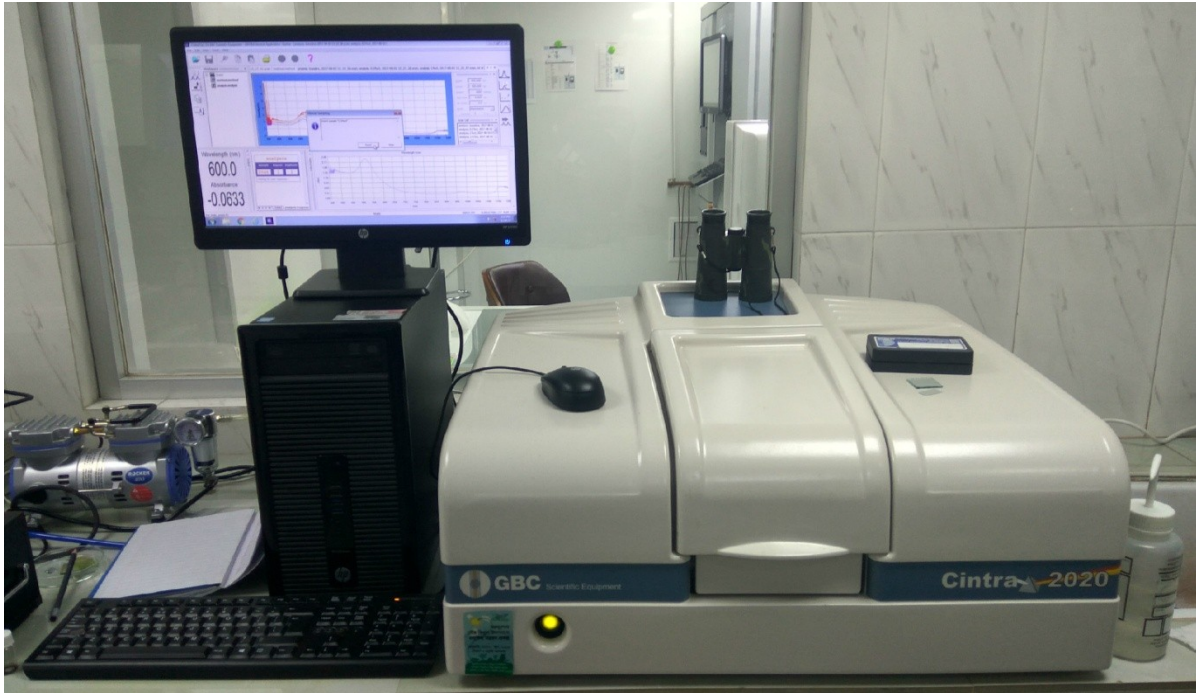


Fig 21. The instruments used for UV-VIS

In UV-Vis, a beam with a wavelength usually varying between 180 and 1100 nm passes through the solution in a cuvette. The sample in the cuvette absorbs this UV or visible radiation [22].

I_0 is the radiation/Intensity coming in,

I the radiation/Intensity coming out

Transmittance = I/I_0 (indicates concentration)

Absorbance, $A = -\log_{10} T = -\log_{10} (I/I_0)$

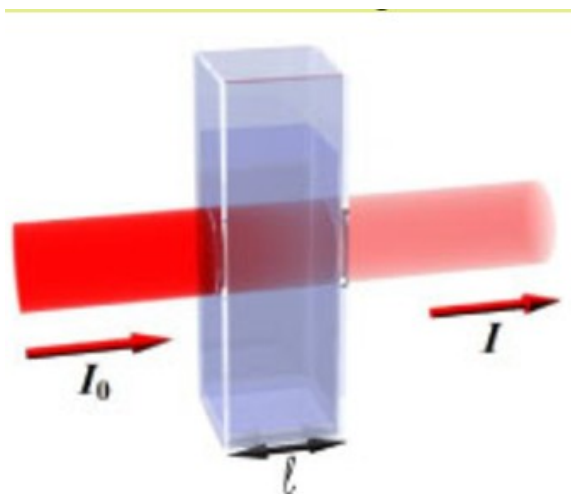


Fig 22. showing incident beam I_0 and transmitted beam I [22]

We mostly require absorption peak which is the graph of absorbance VS wavelength. The absorption peak gives information about size shape and also the size distribution of the nanoparticles.

The wavelength at which absorbance/extinction is highest is the wavelength at which SPR occurs. The peak gives information about the size of the nanoparticle, the peak shifts to the right if the particle size increases. Smaller nanospheres primarily absorb light and have peaks near 520 nm, while larger spheres exhibit increased scattering and have peaks that broaden significantly and shift towards longer wavelengths (known as red-shifting). Larger spheres scatter more light both because they have larger optical cross sections [23]. If the particles are mono-dispersed (uniform in size) the peak is narrow but broader size distribution is indicated by a broader peak which means a broader range of light is absorbed due to the presence of different sized nanoparticles.

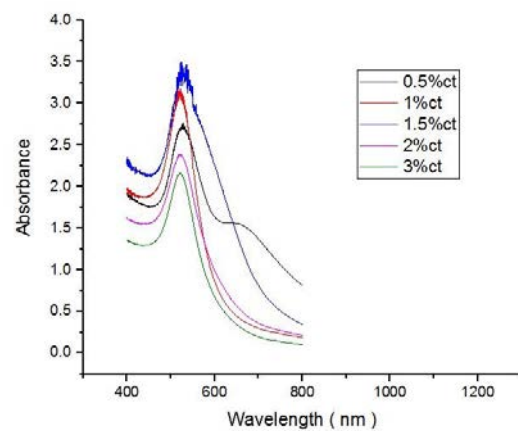


Fig 23. absorption spectra of different sized GNP's

5.3 Transmission Electron Microscopy(TEM)

The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. What you can see with a light microscope is limited by the wavelength of light. TEMs use electrons as light source and their much lower wavelength makes it possible to get a resolution a thousand times better than with a light microscope. These high resolution images allows quantitative analysis of gold nanoparticle size distribution with the help of a software average diameter of gold nanoparticles can be measured [24]. To sum up, although an expensive method TEM gives a very magnified high resolution image from which we can interpret the size(diameter) and exact shape of the nanoparticle.

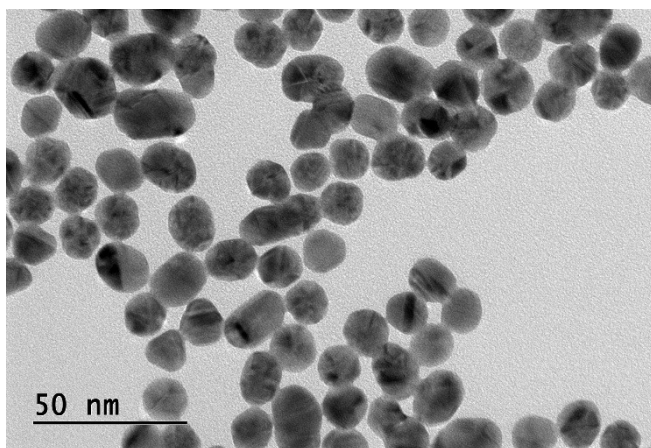


Fig 24. TEM image of colloidal gold nanoparticles [24]

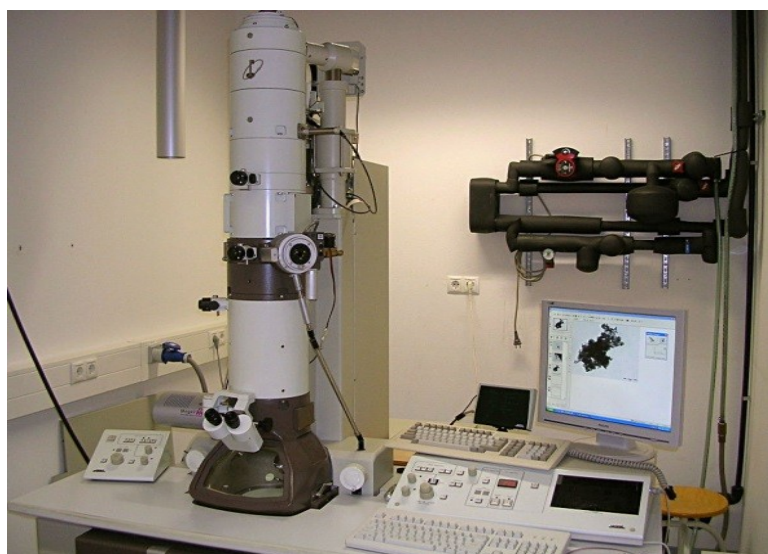


Fig 25. Transmission Electron Microscope [24]

5.4 Atomic Force microscopy

The Atomic force microscopy involves a closed loop laser beam deflection system where a laser beam is deflected off of a cantilever or AFM lever on to a position detection sensor. The cantilever has a sharp tip made of a piezo electric element which acts as a mechanical probe to scan the sample the AFM operates by measuring the force between the probe and the sample. This interaction of forces causes the cantilever to bend thus the reflected beam gives a change in position which is measured by a photo detector. The movement of the laser spot on the photo detector gives a precise measurement of the movement of the probe. This gives accurate information of the topography of the sample and thus very high resolution images. There are different modes in AFM used to measure different nanoparticle physical properties such as magnetic fields, mechanical properties, electrical properties, and thermal conductivity [25,26].



Fig26. Atomic force Microscopy

5.5 Scanning Electron Microscopy (SEM)

Along with Transmission Electron Microscopy, Scanning Electron Microscopy is also considered as the standard for nanoparticle detection or characterization [28]. This method gives high resolution images of nanoparticles from which many important data like size, shape, size distribution, surface structure etc. can be determined. An SEM works in a similar

way as the TEM, an electron gun transmits an electron beam which is focused on the specimen/sample with the help of condenser lenses which are basically electromagnets. The focused beam hits the specimen and gives off electrons of its own which are ultimately registered by a detector and gives a high resolution microscopic image. After the electron beam hits the specimen there are mainly two types of electrons which come off from the specimen. The initial electron beam is known as the primary electrons, secondary electrons are the electrons which are emitted by the specimen itself after it has absorbed the primary electron beam and the back scatter electrons are actually the same electrons from the electron gun which have been reflected off the surface of the specimen. There are two types of detectors which are positively charged for the two types of electrons. The back scatter electrons are useful for getting the surface features of the specimen which is one of the main reasons for using a scanning electron microscope [29].



Fig 27. Scanning Electron microscope

Although TEM and SEM are considered to be very similar methods they have some features which make them different. An SEM gives a reliable 3D image and TEM produces a 2D image, however both the images are made two dimensional but the TEM can give a higher resolution. Where particle surface characteristics are of interest SEM is more suitable. TEM usually takes a longer time to process so particle aggregation should be taken into consideration as it can cause changes in size distribution. Larger amount of the sample can be measured at one time using SEM [30]. Both SEM and TEM are expensive methods of characterizing nanoparticles.

CHAPTER 6

RESULTS AND DISCUSSION

6.1 Introduction

The solutions of gold nanoparticles which were made by changing the parameters were characterised by UV-Vis Spectroscopy, optical microscope imaging and Scanning Electron Microscopy. Characterisations were done at The Institute of Fuel Research and Development (IFRD), Bangladesh Council of Scientific and Industrial Research (BCSIR).

6.2 Parameter changed: Amount of Citrate

The amount of Trisodium Citrate was changed from 0.5ml to 4ml and other parameters were kept constant.

Gold Solution(mM)	Amount of citrate (mL)	Amount of Gold Solution(mL)	Percentage of citrate	GNP solution Color	Sediments
1	0.5	20	1%	Brownish Purple	No
1	1.5	20	1%	Red	No
1	2	20	1%	Red	No
1	3	20	1%	Dark Red	No
1	4	20	1%	Dark Red	No

Table 3 Observations for Amount of Citrate changed

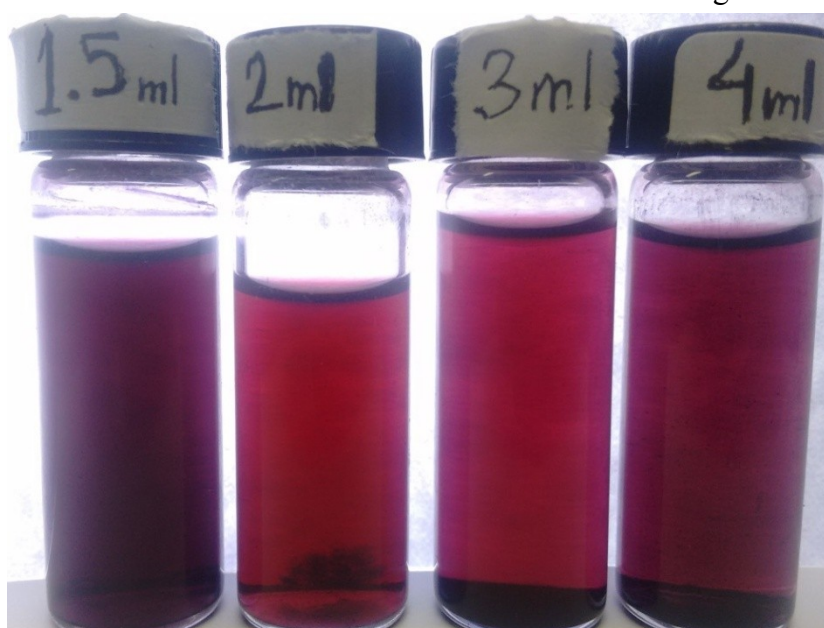


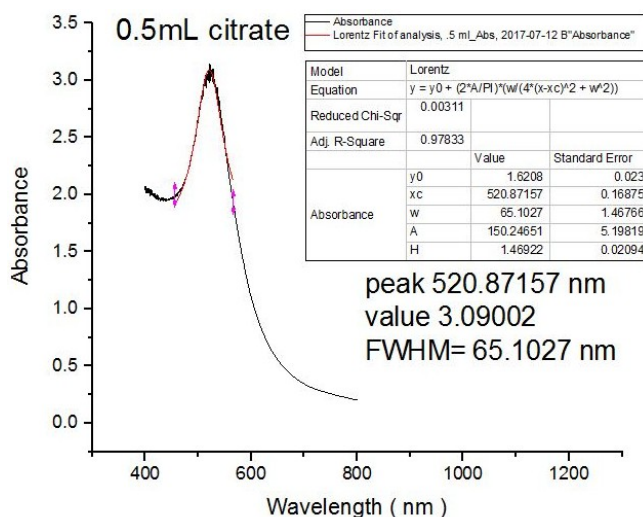
Fig 28. GNP solutions made with different amounts of citrate

The colours of the GNPs produced are seen to be varying from red to purple. Purple colour indicates larger particles and red colour indicates smaller GNPs to get a better knowledge about the size and size distribution we need to analyse the UV-Vis spectroscopy data.

UV-Vis Analysis



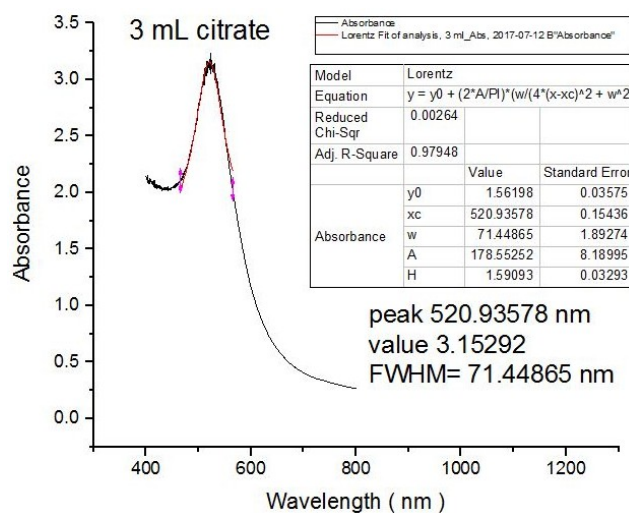
GNP solution with 0.5ml citrate



Absorbance peak for 0.5 ml of citrate added



GNP solution with 3ml Citrate



Absorbance graph for 3ml citrate

UV-Vis test was done for two samples 0.5ml and 3ml. The data from UV-Vis absorbance tests were used to plot the absorbance peaks with Origin Software. The GNP solutions by getting a proper fit (Lorentz) we got the SPR peaks and the FWHM. For both 0.5 ml citrate and 3ml citrate we got similar SPR peaks at 520.87nm and 520.93nm respectively. Considering errors the difference is negligible. The difference in FWHM is visible, for 3ml citrate we got a higher value than for 0.5ml which is 71.44nm and 65.10nm respectively. Therefore we can say that the sizes of GNPs are identical as the SPR peaks were also identical but the GNP solution with 0.5ml citrate is slightly more monodisperse than 3ml citrate considering FWHM values. Thus we can conclude that the amount of citrate added does not have an effect on the size of the GNP but monodispersity is slightly affected. The more we increase the amount of citrate the lesser the monodispersity.

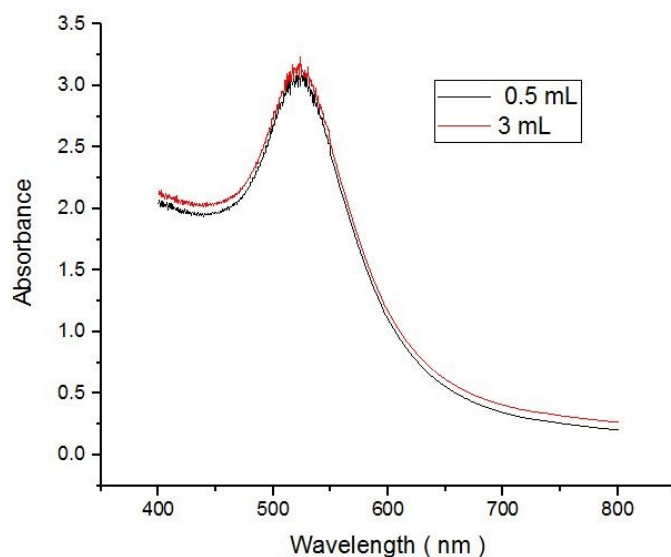


Fig 29. absorbance peak in a same graph for 0.5ml and 3ml

6.3 Parameter changed: Initial gold salt concentration

The initial gold salt concentration was changed from 1mM to 10mM and 20mM; all other parameters were kept constant throughout.

Gold Solution(mM)	Amount of Gold solution (ml)	Amount of Citrate(ml)	Percentage of Citrate	Colour of GNP solution	Sediment
10	20	2	1%	Bright Yellow	Yes
20	20	2	1%	Bright Yellow	Yes

Table 4 Observations for change in initial gold salt concentration



Fig 30. reduced GNP solution with precipitate



Fig 31. Gold precipitate after sedimentation

During this experiment we observed that the pale yellow solution became more yellowish and we could see gold precipitate forming at the bottom of the beaker and settle there. We found that the amount of precipitate increased with the increase in gold concentration from 10 to 20mM. Therefore we can say the particles aggregated and clustered into larger particles thus forming precipitate. As the concentration of citrate was much lower (1%) compared to the concentration of gold solution, the gold solution was not perfectly reduced to make nanoparticles. To get nano sized particles in the solution for high gold solution we must increase the concentration of trisodium citrate added.

6.4 Parameter changed: Concentration of Trisodium Citrate(BATCH 1)

The concentration of Trisodium Citrate was changed from 0.5% to 3% and all other parameters were kept constant.

Gold Solution(mM)	Amount of Gold Solution(mL)	Amount of citrate (mL)	Percentage of citrate	GNP solution Color	Sediments
1	20	2	0.5%	Dark Purple	No
1	20	2	1%	Dark Pink	No
1	20	2	1.5%	Purple	No
1	20	2	2%	Light Red	No
1	20	2	3%	Dark Red	No

Table 5 Observations for change in percentage of citrate (BATCH 1)

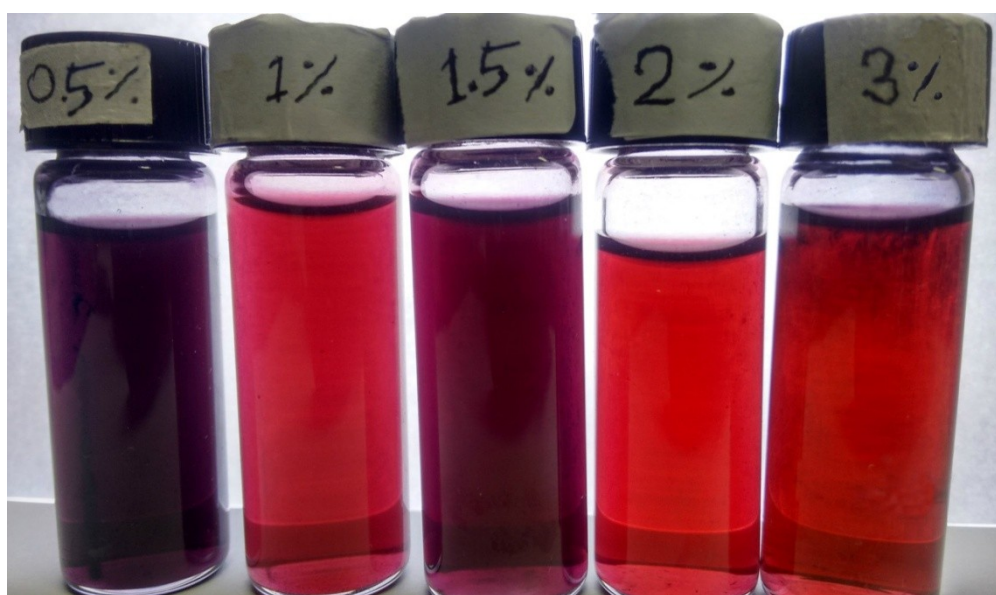


Fig 32. GNP solutions made using different percentage of citrate (BATCH 1)

We observed changes in colour of the produced GNP solutions. Solutions made with 0.5% and 1.5% citrate had a purplish colour whereas solutions made with 1%, 2% and 3% were reddish in colour with different shades of red. All five solutions were taken to BCSIR for UV-Vis Analysis.

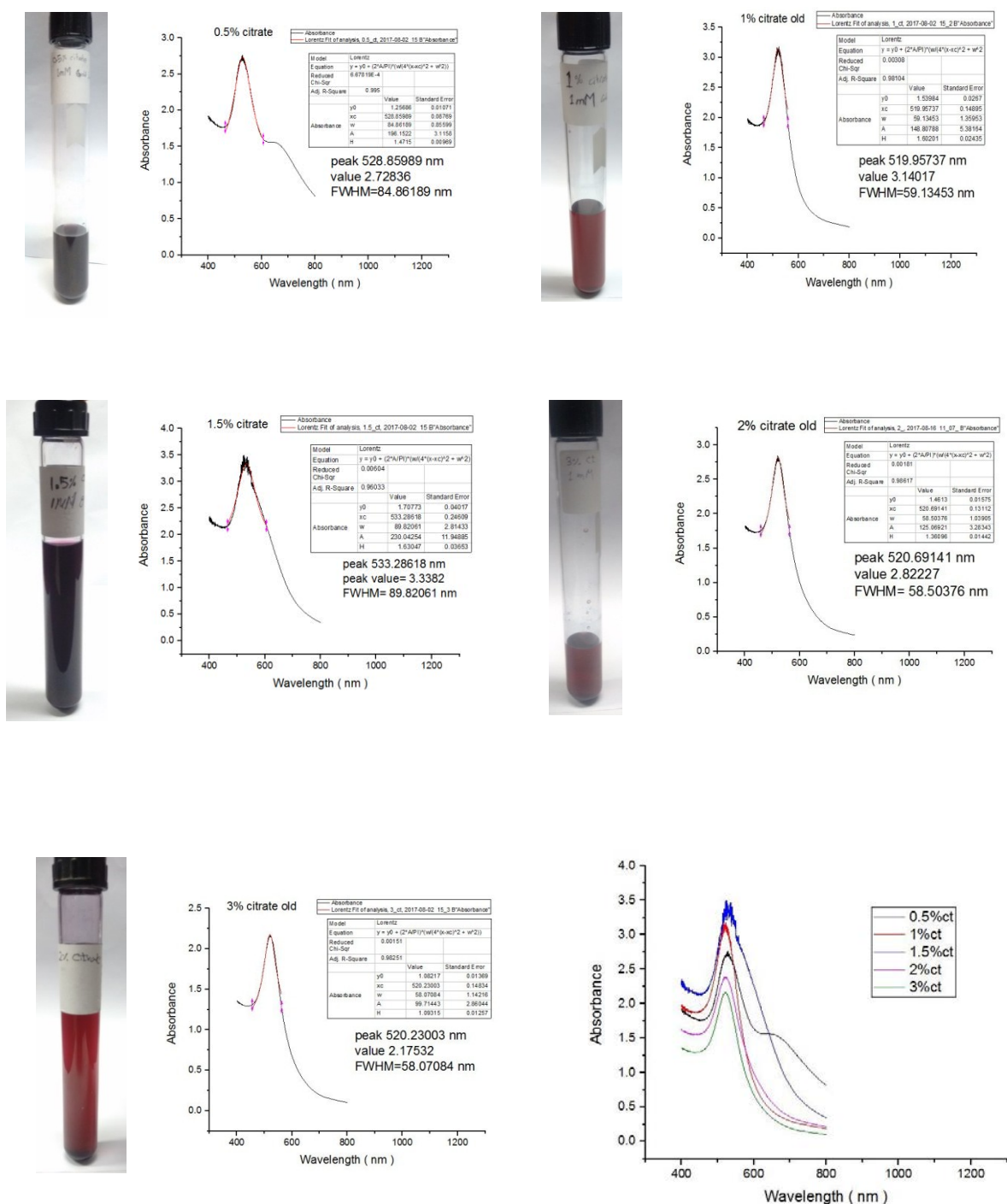


Fig 33. absorbance for different percentage of citrate

The absorbance peaks were curve fitted and the SPR peak and FWHM was found. We noticed some significant changes in both SPR peak and FWHM value. The solutions made with 0.5% and 1.5% had SPR peaks at higher wavelengths and had higher values for FWHM which indicates that the GNPs are bigger in size and are not monodisperse, they absorb a much larger wavelength of light that is why they have a purplish colour whereas the red GNP solutions which were made from 1%, 2% and 3% had significantly different wavelength for SPR peak and also for FWHM value. For 0.5% the SPR peak occurred at 528.85nm and FWHM was 84.86nm. For 3% the SPR peak occurred at 520.23nm and the FWHM was 58.7nm. For lower percentage of citrate we get larger particles with low monodispersity with higher percentage of citrates 1%, 1.5%, 2% and 3% we get smaller particles with 1.5% being an anomaly but we found that the percentage of citrate was a very sensitive parameter as small changes in percentage of citrate caused large change in particle size and monodispersity. To further investigate this parameter we took more values of citrate concentration and go further results in the next section. By matching with **reported** values of sigma Aldrich listed in table in the Appendix, we get particles less than 40nm in size for 0.5%, less than 50nm for 1.5% and for the rest of the percentages we get particle size of 15nm.

Parameter changed: Concentration of Trisodium Citrate (BATCH 2)

The concentration of Trisodium Citrate was changed from 1% to 10% and all other parameters were kept constant.

Concentration of Gold Solution used (mM)	Amount of Gold Solution(mL)	Amount of citrate (mL)	Percentage of citrate	GNP solution Colour	Precipitate
1	20	2	1%	Dark Red	None
1	20	2	2%	Red	None
1	20	2	3%	Purplish Red	None
1	20	2	4%	Ruby Red	None
1	20	2	5%	Light pink	None
1	20	2	6%	Colour less with slight pink shade	Yes
1	20	2	7%	Colour less	Yes
1	20	2	8%	Colourless	Yes
1	20	2	9%	Colourless	Yes
1	20	2	10%	Colourless	Yes

Table 6 Observations for change in citrate percentage (BATCH 2)

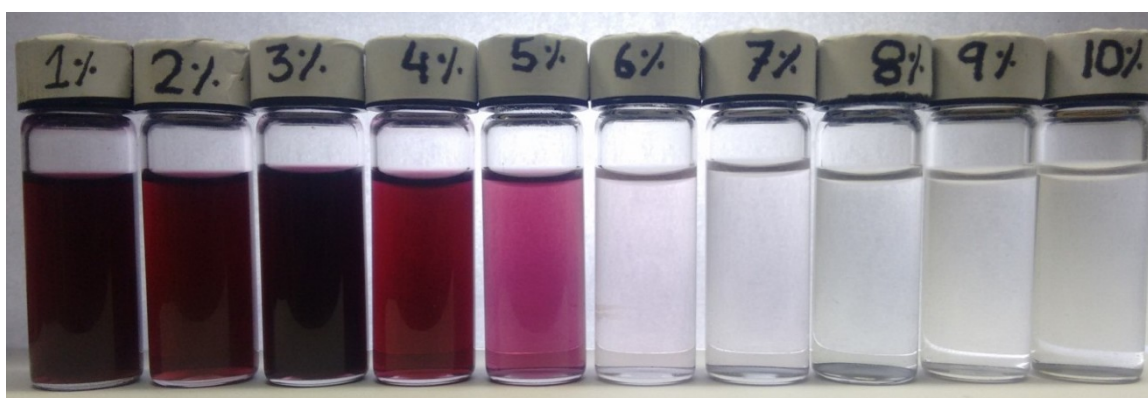
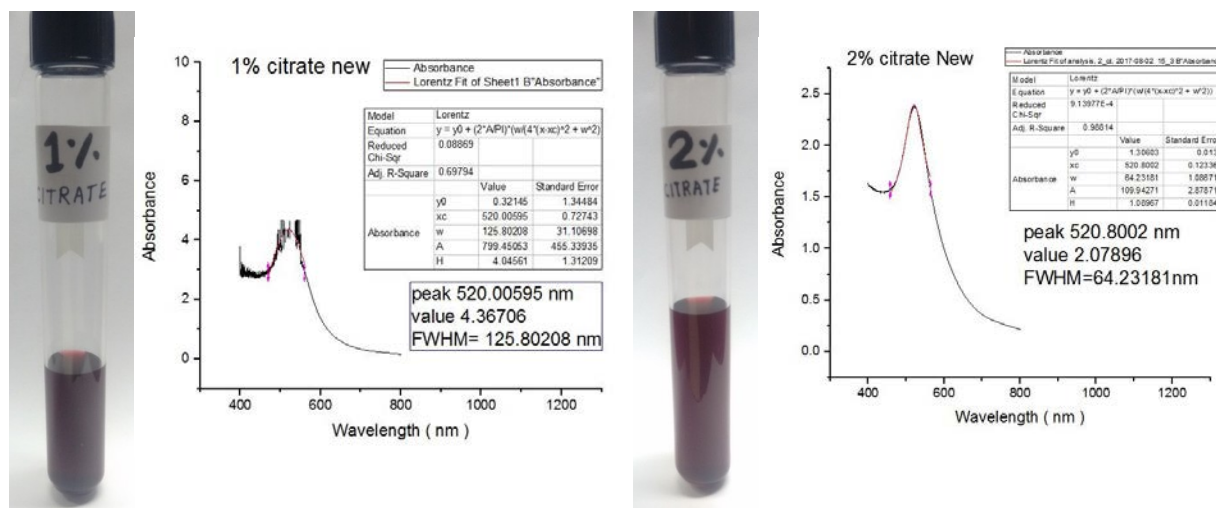
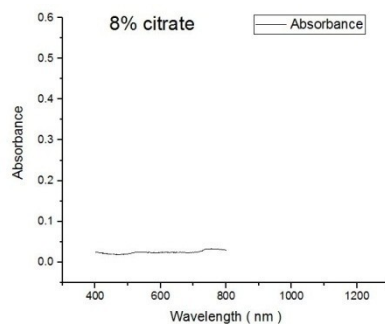
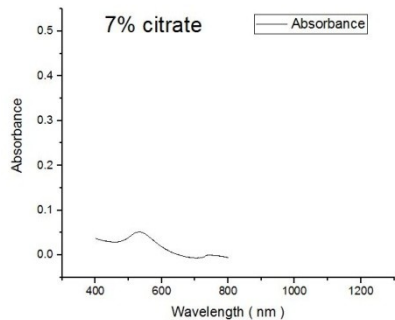
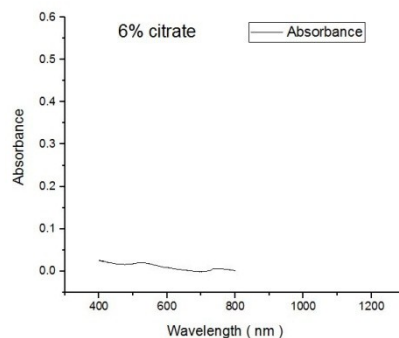
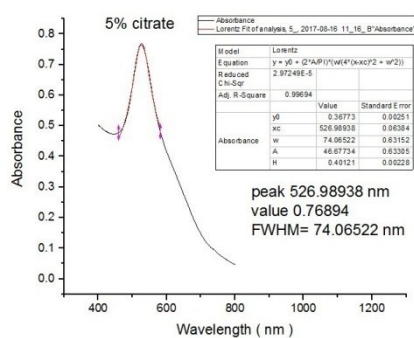
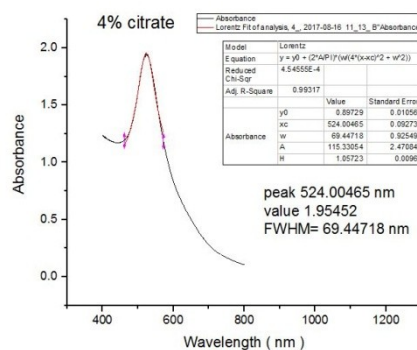
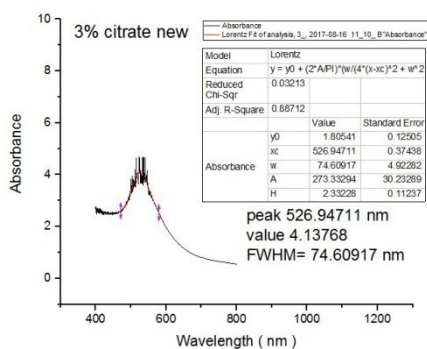


Fig 34. GNP solutions made with different percentage of citrate (BATCH 2)

We observed different shades of red when the Gold solution was reduced with 1%, 2%, 3% and 4% with the red colour getting lighter from a dark red (except for 3% which was purplish) but while reducing the solution with 5% of trisodium citrate we noticed a light pink colour and a very tiny amount of blackish precipitate at the bottom of the conical flask. With 6% of citrate we got almost a clear solution with a slight shade of pink this solution also had a small amount of brownish precipitate at the bottom. The solution made with 7% also was a clear solution with a light shade of pink lesser than that of the solution made with 6%. The solutions made with 8%, 9% and 10 % of citrate were almost identical, each one was a clear and colourless solution with some precipitate forming at the bottom. We saw a trend in the colour of the GNP solution. The colour of the solutions turned from a dark red to a lighter red to a light pink to almost clear solution with a shade of pink to an absolutely clear colourless solution. As the concentration of citrate was increased the colour of the GNP solutions were turning out to be lighter. Which means the size of the nanoparticles present in the solution were getting larger in size and aggregating as citrate concentration was increased. The presence of black/ brownish precipitate for higher percentages of citrate (6%, 7%, 8%, 9% and 10%) also supports this claim. All ten solutions were taken to BCSIR for UV-Vis spectroscopy.

UV-Vis Analysis





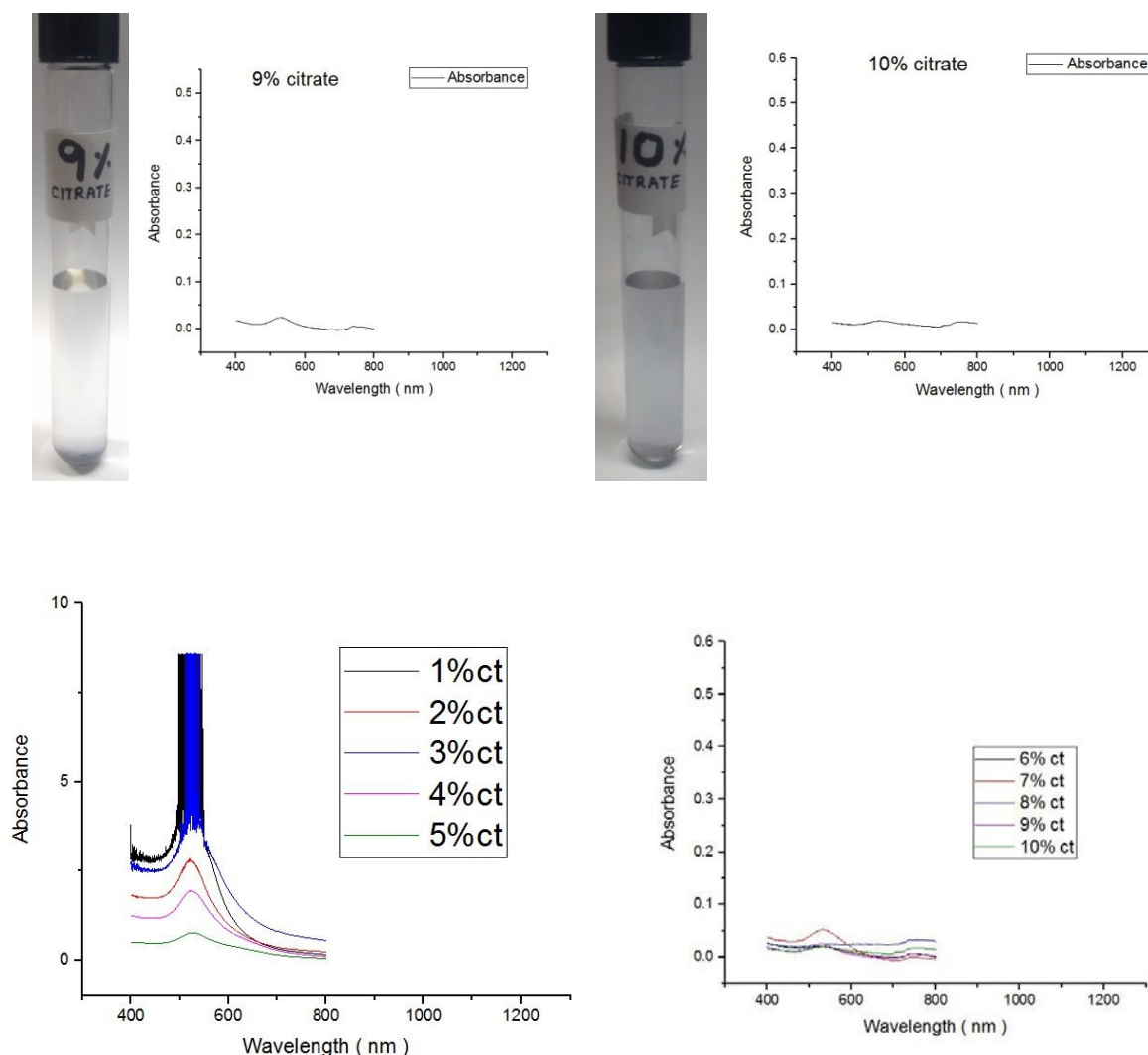


Fig 35. absorbance graph for different percentage of citrate (BATCH 2)

All ten solutions of GNPs underwent UV-Vis test and absorbance peaks were plotted using origin just like previous samples. The trend in colour change strongly supports the trend in absorbance data. Starting from 1% citrate the values for SPR peaks and FWHM increases with the unit increase in % of citrate. SPR peaks shifts to the right indicating an increase in size of the GNPs, FWHM also increases which indicates that monodispersity is also decreasing. The absorbance also decreases gradually from around 4 to 0.7 for 1% and 5% respectively. From 6% we found that the colour starts disappearing and the solutions become colourless. This significantly effects the absorption peaks as we see that the solutions are heavily aggregated and do not have an SPR peak and absorbance is close to 0. From 6% to 10% we get similar data as they all are aggregated and precipitate was formed during

synthesis. Therefore, we can conclude that the increase in citrate concentration increases the sizes of GNPs and for high concentrations greater than 5% the GNP solutions get heavily aggregated and is damaged. By matching the values with sigma Aldrich reported values in table 2 of Appendix, we get GNPs of size 15nm for 1% and 2% of citrate, 30nm and 20nm for 3% and 4% and size for 5% is greater than 30nm.

Powder form and Optical Microscopy

Few solutions of GNPs which were red in colour were chosen to make it into powder form. The process requires the solution to be centrifuged at high speed for a long time and heated overnight. The Centrifuge process was a bit tedious as we had to select the right speed and the right amount of time to be centrifuged as the speed and time strongly depends on the nanoparticle size we matched the value with reported values listed in table 1 of Appendix. After a few failed attempts the speed chosen was 12,000rpm and was centrifuged for about 45 minutes. The test tube then was removed from the machine and the supernatant was disposed and the rest was stored in a petri dish and heated overnight in an oven.



Fig 36. Centrifuge Machine at BCSIR



Fig 37. GNP solutions inside the centrifuge machine

The next day the petri dish contained a purplish film of particle layer which we then viewed through an optical microscope.

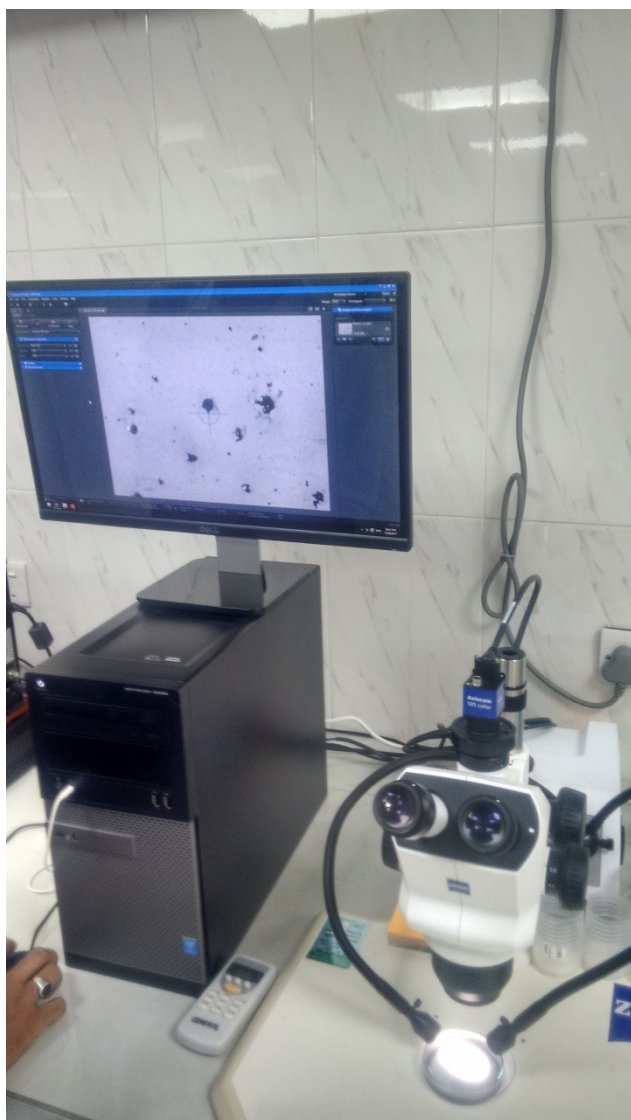


Fig 38. Optical Microscope setup at BCSIR

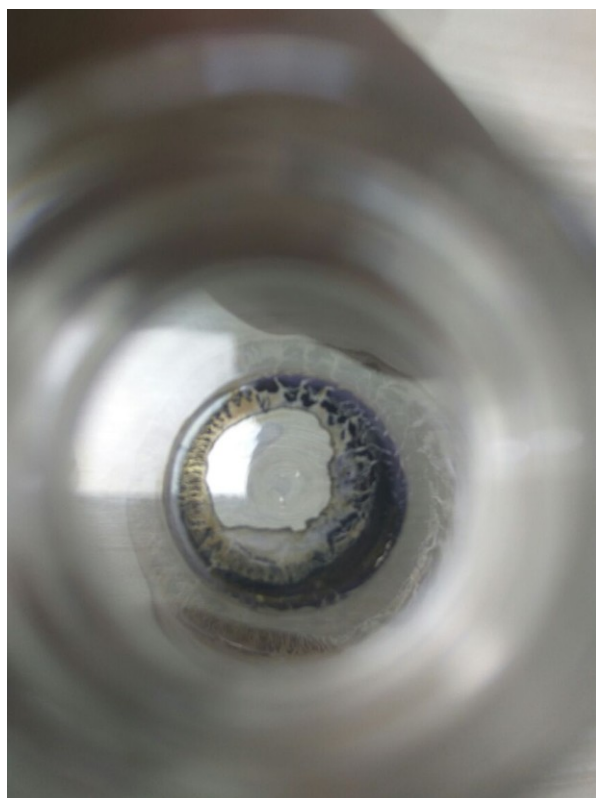


Fig 39. Test tube after centrifuge containing purplish film of gold

Through the microscope we saw some large clusters and irregular shapes. This was a result of aggregation during the centrifuge process. Although not all the particles were aggregated most cannot be seen with this microscope as it is not able to view nano sized particles. The white parts of the image contain nanoparticles whereas the black clusters are of gold nanoparticles which have been aggregated into larger particles and irregular shapes. A golden shine can also be seen.

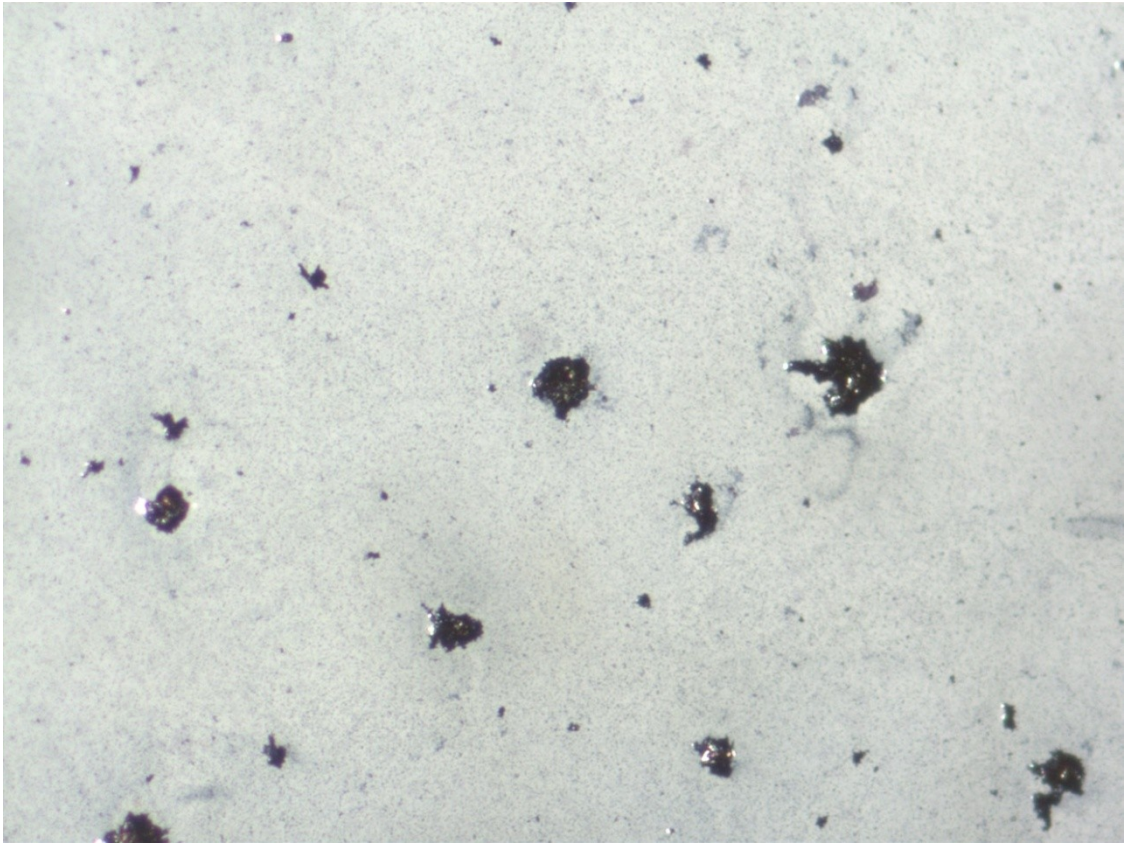


Fig 40. Aggregated gold particles

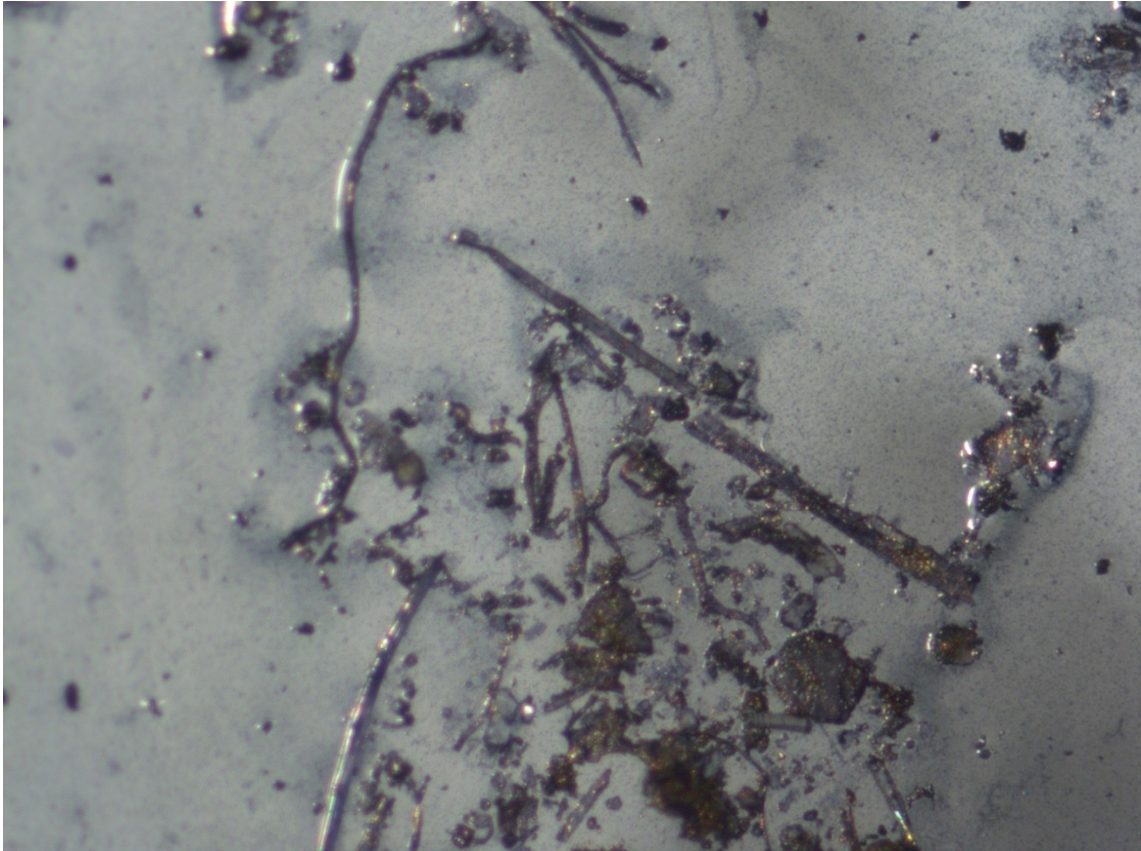


Fig 41. random shapes of aggregated particles

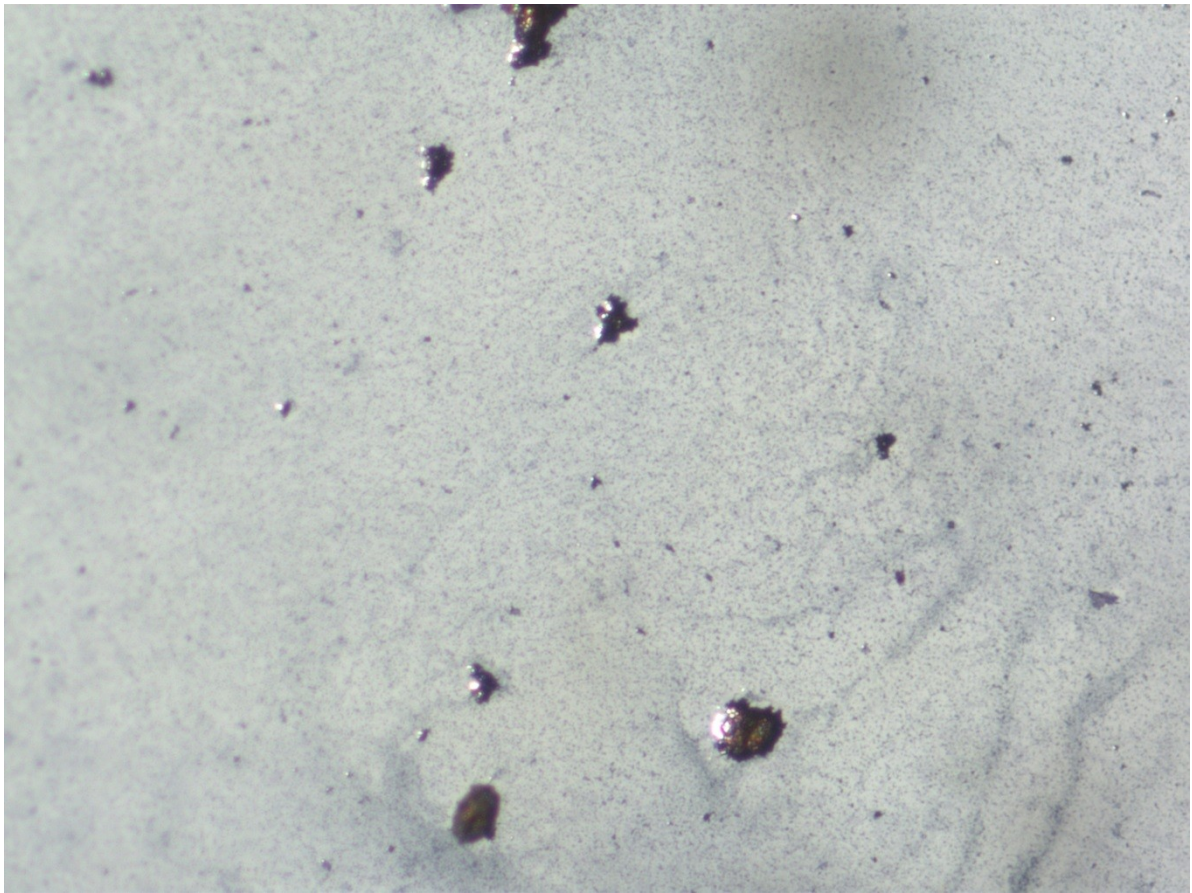


Fig 42. few clusters of aggregated gold particles

CHAPTER 7

CONCLUSION

7.1 Summary

After doing our thesis we can conclude that the synthesis parameters play a vital role in shaping and sizing the final GNPs. Although some are more significant than others like the amount of citrate added did not have a significant change in size although there was a small change in monodispersity. The change in concentration of gold solution did not follow through as large particles with gold precipitate formed instead of GNPs. The most valuable data which we got was that of the concentration of citrate as a small change in concentration caused a significant change in particle size and size distribution. It can be said that as concentration increases the size of particles increases but for concentrations higher than 5% we got heavily aggregated solutions with no SPR peaks. By tuning the synthesis parameters we can effectively tune the properties of the GNPs which then can be applied to perform its specific task in various fields of science and technology.

7.2 Limitations and Challenges faced :

Availability of Characterization techniques:

As discussed before some of the equipments and machines were not readily available for us to work on. We could not get some of the characterization tests perform due to this difficulty. Neither the Transmission Electron microscope nor the Scanning electron microscope could be used for our tests. One of them, Transmission Electron microscope, being not at all available. These are very rare, and are not available in our country for us to use it. Scanning electron microscope is available for us to use. However, we were unable to use it for its high price range. Same goes with Atomic Force microscope. Price range for usage of these equipments is very high. It exceeds our budget price by a long way. The reason, they charge such high price for using the equipments for even once, is they only have few of these equipments in the country, and it is generally authorised for usage by the Government agencies and firms. Since their unreasonable charge for using these equipments exceeds our budget capability, we were, unwillingly, unable to get these characterisation tests

done. But there was a characterisation technique, that we succeeded in performing - The UV-VIS spectroscopy. We got a lot of results from these characterization method. However, we had to face difficulties here as well. The UV-VIS spectrometer is not readily available in our Institution, hence, we had to head far to a place where it could be performed. Every time we had to perform spectroscopy on one of our results we had to travel back and forth to that place. There we had to follow certain standard protocols and get into the long queue of other awaiting products to be scanned by the spectrometer. The long queue is due to the UV-VIS Spectrometer not being readily available in most institutions. Also, the transportation is a major issue in our country. It takes a lot of time to get from one place to another due to the traffic. So, getting the UV-VIS Spectrometry test not only proved to be a big hassle for us but also consumed a lot of our time. Getting one batch of UV-VIS Spectroscopy fully performed, would sometimes take us a whole week, as the results and graphs would get received by us after several days.

Residues sticking to interior walls:

We had to use several test tubes, flasks, beakers, vials for our experiments. Since, we conducted our experiments myriad number of times, we had to use these instruments over and over again. A proper wash of the flasks, test-tubes, beakers, vials had to be ensured. Usually, after chemical experiments, cleaning these instruments properly with distilled water is enough to carry on with the next batch of experiments. In our case, this was not enough as we faced something different in our experiments. After performing different types of synthesis, we at times had nanoparticles of gold colloids sticking onto the interior surface of the flasks, test-tubes and vials. It remained in the test-tubes and flasks like stains and would not get off easily. Neither distilled water nor tap water could get those off. It was a difficulty as, if we performed our next experiment on that same flask containing residues from the previous experiment of different sort, there would be a high chance of those stain-like remaining residues reacting with the chemicals of the next experiment and would eventually get us incorrect results. So, it was very important to wash the instruments and equipments thoroughly before each set of experiments. To get the stains off, proper cleaning of each test-tubes and flasks had to be done with soap water. They had to be rubbed and cleaned thoroughly with test-tube brushes soaked in soap water. Then before use, they were cleaned with distilled water again. This ensured the stains to be completely removed. This was the

solution for the conical flasks, test-tubes and other containers but we faced another such difficulty with the vials. Vials are small cylindrical containers made of glass that are used mainly to hold medicinal liquids. These are relatively small and narrow. Gold Nano-particles stains in the interior walls of the vials could not be removed using those test-tube brushes as it is very hard to get these brushes to reach fully inside the narrow vials. Small narrow test-tube brushes had to be purchased to overcome this problem. Small test-tube brushes that could reach inside the small vials easily and could get the stains completely removed after thorough rubbing and washing. Washing every single container, vial, flasks and test-tubes after each experiments consumed a lot of time. Hence, conducting several experiments in one single day was not that much of a possibility.

Not Long Lasting :

As mentioned before, getting the UV-VIS results was a very hectic task. Hence, we could not go for UV-VIS spectroscopy right after finishing up our experiments and getting our resulting solutions. Moreover, UV-VIS Spectroscopy was not accessible after evening and was inaccessible during weekends. So, we could not perform UV-VIS Spectroscopy whenever we pleased. After getting our solutions we were not able to preserve it perfectly for 2-3 days. After around 3 days the Gold Nanoparticle solution would show signs of losing colour. They would show signs of transparency. This is because with time, the nanoparticles start aggregating. And this aggregation results into the slight change in colour.

Availability and Cost of Gold Salt :

Gold Salt, in our case Tetra Chloro-aurate, was not available in our country's chemical industry. Since, this chemical was not used in large scale, no production of this salt occurred in here. The gold salt had to be imported from abroad, in our case China, and this caused a lot of hassle. Price was also an issue. We received just 10gm of Gold Salt and it took the most from our budget. Thus, these 10gm had to be used with great caution so as to not waste even 1gm as importing again would cost a lot and a lot of time would be needed in shipment.

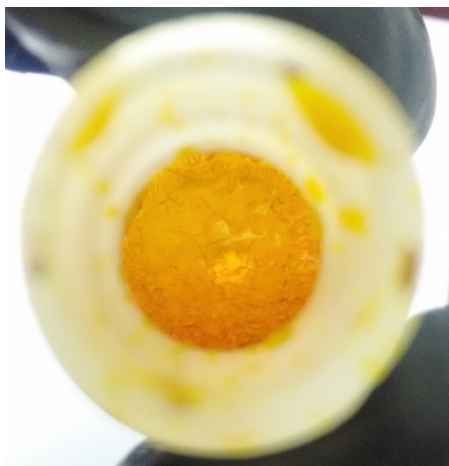


Fig 43. Gold Salt (Tetra Chloro-Aurate)

Storage :

Storing both the Gold Salt and Gold nanoparticle solution proved to be a challenge. Proper storage would require the gold nanoparticle solution to be kept completely away from sunlight and stored in a temperature controlled environment. To prevent it from sunlight we used dark-brown, amber-tinted containers that does not let sunlight into the container. Furthermore, wrapped that container tightly with Aluminium foil for further preservation. Temperature controlled environment was inaccessible to us. Any sort of refrigerator could not be used in this case as the temperature had to be fixed around 4 degree Celsius. This was a big limitation. Proper storage of the gold salt had to be done as well. Gold salt shows sensitivity to light and also tends to absorb moisture from air. To tackle this problem we had to seal the already sealed-in air-tight container containing Gold nanoparticles in an air-tight plastic container. This outer container was, in turn, stored in a dark-brown amber tinted container to keep away from sunlight. This was also enclosed with Aluminium foil for reducing chance of any light entering the bottle. After this much of layering, we were able to preserve the Gold salt for months.



Fig 44. Air-tight plastic container bag



Fig 45. Amber-tinted brown bottle



Fig 46. Brown bottle wrapped in Aluminium foil

Careful Handling and Precautions:

Gold salt, in our case Tetra chloro-aurate, is somewhat toxic and can cause a lot of skin and lung problems. Hence, careful handling is required for safety. It comes with a lot of safety precautions. When coming into contact with skin, it can cause severe skin burns and also can cause allergic skin reaction. When comes into contact with eye, causes eye damage. It is also corrosive in nature and ingestion is strictly prohibited. To avoid these, safety eye goggles and chemical gloves had to be worn in all times during the experiment. On the other hand, fumes of gold nanoparticle solution emitted while solution is being heated during the experiments, can be very harmful if inhaled. If huge amount of gold nano-particle fumes gets inhaled, it might destroy the mucous membrane tissues and upper respiratory tract. To tackle this, inhaling fumes must be avoided and in some cases using chemical face masks would solve the problem.

7.3 Future Scope of GNPs

The field of nanotechnology is an highly developing field because of its boundless applications in various territories of science and technology. The synthesis of gold nanoparticles has received considerable attention and has been a very influential and main focus of research due to their high chemical and thermal stability, fascinating optical, electronic properties, and promising applications such as nano-electronics, biomedicine, sensing, and catalysis. Biosynthesis of nano-particles is an energizing recent area to the vast collection of several methods of nano-particles synthesis and now, nano-particles have entered a commercial exploration period. Outlining and advancement of novel and affordable techniques for scale-up production of nano-materials have not only given a fascinating area of study but in near future will also address the growing human necessities including health safety and environmental issues etc. In the industries, the application of nano-particles is increasing extensively by the day, and they will soon in the future be able to replace the toxic chemicals currently used as antimicrobial agents. Application of nano-particles and their nano-composites will help provide us with a sound and moderately more secure and safer alternative and will thus, introduce new potential for development of antimicrobials. Another development, being worked on is that of, building of computer memory using GNPs. Focusing on an organic non-volatile bi-stable memory, which is a mixture of plastic and gold. Technologies based on GNPs are currently being developed for the environmental

applications for controlling pollution and purifying water using gold nano-particles. [45] It has been researched that bimetallic gold–palladium nano-particles provide an active catalyst which can be used to fix trichloroethene (TCE) into a non-toxic form. This TCE being responsible of causing pollution to ground water. GNPs integrated in a water purification device can adequately catch and remove halocarbon-based pesticides from drinking water and can also elevate the oxidation of mercury generated from coal power plants. [46] In cancer treatment using gold nano-particles, where cancer cells are destroyed by zapping nano-bubbles method, future work is in progress. This has already proved to be successful on mice. Research is going on for these surgeries to be performed on humans. If proven successful on cancer patients, it can effectively help reduce number of cancer patients. Potential applications for this technology is also being developed in flexible electronics area, where research is going on in producing less harmful electrode brain-implants using gold-nanoparticles for treating Parkinson's, epilepsy, and other diseases. Gold nano-particle electrons being bio-compatible and non-cytotoxic will not react or harm brain tissues and is thus a very good material for these electrodes in brain implants.

Future work is still undergoing on GNPs applications as this might turn out to be very useful in a lot of industries including biomedical engineering, nanobiotechnology and also electronic engineering. If GNPs are handled and maneuvered efficiently, it can be applied to many different applications across the field of biology and medicine, environment, and technology. [45]

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APPENDIX

Sigma-Aldrich Reported Values:

Diameter (nm)	SPR Peak Wavelength (nm)	Extinction Coefficient ($M^{-1}cm^{-1}$)
5	515-520	1.10×10^7
10	515-520	1.01×10^8
15	520	3.67×10^8
20	524	9.21×10^8
30	526	3.36×10^9
40	530	8.42×10^9
50	535	1.72×10^{10}
60	540	3.07×10^{10}
80	553	7.70×10^{10}
100	572	1.57×10^{11}

Table 2

SGNP Size (nm)	Speed (rpm)	Notes
1.8-5	150000	Ultracentrifuge
7	20000	
10	15000	
15	12000	
20	10000	
30	6000	
40	5000	
50	4000	
60	3500	
70	3000	
80	2500	
90	2000	
100	1500	

Table 1 Recommended centrifuge speeds vs. spherical gold nanoparticle size