

Acknowledgements

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.....Divyashree D.

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Chapter 1

General Introduction

1.1. Nanotechnology

Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale, normally 1 to 100 nanometers, and the fabrication of devices with critical dimensions that lie within that size range.

It is a highly multidisciplinary field, drawing from fields such as applied physics, materials science, interface and colloid science, device physics, supramolecular chemistry (which refers to the area of chemistry that focuses on the noncovalent bonding interactions of molecules), self-replicating machines and robotics, chemical engineering, mechanical engineering, biological engineering, and electrical engineering. Much speculation exists as to what may result from these lines of research. Nanotechnology can be seen as an extension of existing sciences into the nanoscale, or as a recasting of existing sciences using a newer, more modern term.

Two main approaches are used in nanotechnology. In the "bottom-up" approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition. In the "top-down" approach, nano-objects are constructed from larger entities without atomic-level control. The impetus for nanotechnology comes from a renewed interest in Interface and Colloid Science, coupled with a new generation of analytical tools such as the atomic force microscope (AFM), and the scanning tunneling microscope (STM). Combined with refined processes such as electron beam lithography and molecular beam epitaxy, these instruments allow the deliberate manipulation of nanostructures, and led to the observation of novel phenomena.

Examples of nanotechnology in modern use are the manufacture of polymers based on molecular structure, and the design of computer chip layouts based on surface science. Despite the great promise of numerous nanotechnologies such as quantum dots and nanotubes, real commercial applications have mainly used the advantages of

colloidal nanoparticles in bulk form, such as suntan lotion, cosmetics, protective coatings, drug delivery and stain resistant clothing.

A nanoparticle (which historically has included nanopowder, nanocluster, and nanocrystal) is a small particle with at least one dimension less than 100 nm. This definition can be fleshed out further in order to remove ambiguity from future nano nomenclature. A nanoparticle is an amorphous or semi crystalline zero dimensional (0D) nano structure with at least one dimension between 10 and 100nm and relatively large ($\geq 15\%$) size dispersion [1][2][3].

1.2. Metal nanoparticles

Metal nanoparticles, i.e. particles in the approximate size range 1 – 10 nm are used in a variety of applications, the most important of which is catalysis. Industrial processes such as petroleum reforming depend on catalytic metal nanoparticles, as do the catalytic converters used to clean up motor vehicle exhausts. Metal nanoparticles are also used in a range of other applications, including the labeling of biological samples and hydrogen storage. One of the challenges in this field is to make the particles as small as possible, with as narrow as possible a range of diameters. Dynamic light scattering (DLS) is an essential tool for this research. .

The extremely small size of silver nanoparticles means they exhibit enhanced or different properties when compared with the bulk material. The extremely small size of nanoparticles results in the particles having a large surface area relative to their volume. In the case of silver nanoparticles this allows them to easily interact with other particles and increases their antibacterial efficiency. This effect can be so great that one gram of silver nanoparticles is all that is required to give antibacterial properties to hundred of square metres of substrate material [4].

All bacteria use an enzyme as a form of ‘chemical lung’ in order to metabolise oxygen. Silver ions cripple the enzyme and stop the take up of oxygen. This effectively suffocates any bacteria, killing it within 6 minutes and leaving surrounding tissue or material unaffected. Viruses grow by taking over another living cell and reprogramming the nucleus to replicate the virus rather than the healthy cell. As part of this process, the

cell reverts to a more primitive form that relies upon a primitive form of oxygen metabolizing enzyme as a chemical lung. Again the silver ions stop oxygen being brought into the virus-producing cell and it dies by suffocation. A fungus is composed of a series of single cells. Each cell survives by means of a 'chemical lung' much like that seen in bacteria. Just like bacteria, the presence of silver ions disables the chemical lung and the fungus dies. Antibiotic drugs can be used to kill the pathogens attacked by silver nanoparticles but bacteria and viruses are becoming increasingly resistant to drug therapies. Silver nanoparticles kill all types of fungal infections, bacteria and viruses, including antibiotic resistant strains. No drug based antibiotic is effective on all types of bacteria. Additionally, research to date has shown that bacteria have been unable to develop any immunity to silver. Elemental silver occurs naturally. It is considered non-toxic, non-allergic, is not cumulative and is not known to harm either wildlife or the environment [1][2][3].

1.3. Applications of silver

Silver metal in its pure state has a brilliant white metallic luster. It is a little harder than gold and is very ductile and malleable. It is classified with the precious metals. Silver is stable in pure air and water, but tarnishes when exposed to ozone, hydrogen sulphide, or air containing sulphur because of the formation of a silver sulphide.

The most important silver inorganic compound is, undoubtedly, the nitrate, because it is used in almost all the processes of production other compounds. Silver nitrate has a wide application in painting, xerography, chemical electroplating, in components for electric batteries and in Medicine as catalyst. Silver chloride is another important compound, due to its ductility and malleability. The organic compounds of the element are used in the coating of several metals and in dynamite or other explosive bars. The most important alloy of this element is silver-copper, traditionally used in producing coins. Nowadays this alloy was replaced by a cheaper alloy, copper-nickel. There are other silver alloys used in producing radiators for the automobile industry, and in producing musical instruments.

The chemical industry uses metallic silver as catalyst of several oxidation reactions such as those of ethanol and other alcohols. The oil industry also uses silver nitrate as catalyst. For many years, the mirrors were made by deposition of a small silver coat on a glass surface. Nowadays aluminum is used for this purpose.

Silver as sterling silver is used mostly as a precious metal and as alloys for jewelry, silverware, coinage, in electrical contacts in electronics and radio equipment for more corrosion resistance and conductivity. Silver is used in the making of solder and brazing alloys and electrical contacts of high capacity silver-zinc and silver-cadmium batteries. Silver halogenated compounds are of the utmost importance in photography (where approximately 30% of the U.S. Industrial consumption goes into this application). Silver's catalytic properties make it ideal for use as a catalyst in oxidation reactions, in organic and inorganic synthesis, e.g. in converting alcohols into aldehydes and acids or ethylene oxide.

Silver's sterilizing, antisepticising effects are commonly known: preparations have antibacterial, anastaltic and catheteris properties which are connected to their ability to disturb germs' enzyme systems and to precipitate proteins. Silver nitrate solution was a standard of care but was largely replaced by silver sulfadiazine cream. Other options such as silver coated dressings (activated silver dressings) are used in addition to SSD cream, and may present advantages such as pain reduction and capacity for treatment at home.

1.3.1 Food industry

In a world where there is increasing concern over the safety of food and how it is processed, packaged and stored, silver-based biocides are being evaluated for many different purposes. Sales of silver for such purposes are still small, less than 10,000 ounces world wide, and mostly in Japan and in Europe, but major growth is expected.

Silver nanoparticles, because of its antibacterial qualities, offers greater hygiene, control of odor, and prevention of discoloration and structural damage in processing areas. Use of silver-based biocides also offers reduced downtime because there is less need to take processing equipment offline for cleaning. Additional uses are in specialty packaging, occupational clothing worn by food processing workers, prevention of pathogen build-up in climate control systems, and on floors, walls and ceilings of food

processing and storage facilities. Hygiene needs are greatest for meat and poultry processing because of the danger of potentially deadly microbes such as Salmonella and Listeria. Dairy and bakery products can also be a large potential market for control of pathogens in processing areas. Ice making and beverage preparation also make use of silver biocide coated parts in processing machinery. So far, there has not been any use or need for the processing and packaging of fresh fruits and vegetables as the pathogen danger is lower. Silver-based biocides in packaging can also help keep the foods inside fresh for longer periods of time, but are generally too expensive to be used on throw-away items. For cost-effective adoption, a minimum of a 1,000 fold decrease in pathogen count is necessary and often a 100,000 fold decrease is necessary to really excite food processors.

Lower loadings are acceptable against gram positive and gram negative bacteria than are needed for control of usually less prevalent and less dangerous molds and fungi. Meat and poultry processors are taking increasingly more steps to minimize any possible build up of particularly virulent pathogens such as E. Coli H157:O7 and Listeria. Current steps include welding of metals and other components of machinery so that no biofilms suitable for supporting pathogens can form in small or narrow spaces. Stainless steel surfaces, especially for meat and poultry, are widely used and have traditionally not had problems. Meat and poultry processors also use other well established processes such as washing and bleaching, high-energy irradiation, and organic antimicrobial treated parts and surfaces. Different government and non-government entities are involved in keeping foods safe. The agency is starting to look more carefully at health effects of both one-time and cumulative biocide intake resulting from the use of these chemicals. It must be remembered that although food and beverages can come into contact with biocide treated surfaces, these biocides must not become food or beverage ingredients. Consequently, it must be demonstrated that all biocides remain bound to the surface and do not leach into the food. Most food processors in North America are just beginning to be acquainted with products containing silver-based biocides.

1.3.2. Textile industry

Also this could be used to get rid of dust mites on fabrics. It was noticed by National Aeronautics and Space Administration (NASA) crewmembers that when they wore silver-based clothing it cut down on bacteria and odor. It was tested as how to protect crewmembers from bacterial growth in their clothing and linens while on space missions in which laundry and bathing facilities like those at home are not available.

The NEEMO 6 crew used clothing, towels and bedding made with Fosshield. The closed-system Aquarius test capsule provided with an ideal opportunity to evaluate the performance of the silver ion fabric in a space station situation.. The crew tested silver-based materials as well as regular clothing, and the silver-based materials remained noticeably fresher and more comfortable throughout the mission, NASA reported.. Other silver-based fiber applications includes mattress components, pillows, hospital scrubs, wound care products, air and water filters, automotive upholstery, wall coverings, athletic and intimate apparel, shoe insoles and cleaning supplies.

1.3.3. Biosensors

Silver nanoparticles have unique optical properties, especially enhanced sensitivity to changes in their environment, and show promise as sensors used in applications ranging from laboratory testing equipment to biohazard alarms.

When light is reflected off a substance, it scatters into many different wavelengths, depending upon the material's unique chemical make-up. This was used to built a silver nanoprobe based on these light scattering techniques. The probe can detect and analyze chemicals, explosives, drugs, and other substances of close to a single molecule size. This ability makes it possible to detect minute amounts of substances despite the environment. The probe is a 100 nanometer optical fiber honed to a point with an extremely thin coating of silver nanoparticles that are sensitive to light. When a laser is pointed at the sample, light is reflected and scattered according to a pattern unique to that substance. This pattern is captured by the probe and fed into a computer for analysis.. The silver ink was introduced that was used in electronics applications such as smart cards, radio frequency identification (RFID) tags, flexible circuits and antennas for handheld devices.

The silver inks essentially act as flat wires that are placed on surfaces and used to connect electrical components. A major use is in printed circuit boards. This drives demand from industries such as health care, inventory control and logistics, access/security badges, communications, transportation and defense. The silver inks are placed on a variety of materials including thermoset and thermoplastic materials, paper and polyvinyl chloride (PVC). The materials deliver electrical conductivity that is an order of magnitude greater than other products in the market, along with an ability to maintain low resistivity even during long-term environmental stress [5].

1.4 References

1. Akhlesh Lakhtakia , ‘Nanometer structures’, Prentice Hall of India private limited, 2007.
2. Sulabha Kulkarni, ‘Nanotechnology: Principles and practices’, Capital publishing company, 2007.
3. Richard Booker, ‘Nanotechnology’, Wiley publishers, Delhi, 2005.
4. Chen et al., ‘Nanosilver: A nano product in medical application’, *Toxicology Letters*, 2008, 176, 1-12
5. www.wikipedia.org/silver accessed on 15/02/2008

Chapter 2

Characterization techniques

The general characterization techniques used to analyze nanoparticles are Dynamic Light Scattering (DLS), Atomic force Microscopes (AFM), and Transmission electron microscope (TEM) and UV-Vis spectroscopy. DLS is used to analyze the size of nanoparticles where monochromatic light is passed through the laser beam and gets scattered depending on Brownian motion. In the present chapter we will be discussing the characterization of DLS and UV-Vis spectroscopy only.

2.1. Dynamic light scattering

It is also called as photon correlation spectroscopy or quasi elastic light scattering. Its objective is to measure size of the particles in sub micron range.

2.1.1. Principle:

A monochromatic laser beam such as laser is focused onto a sample containing particles that are in Brownian motion. Because of this Brownian motion of the particles, the interference pattern formed at the detector and the scattering intensity measured by the detector changes with time. Since the incoming light is a laser, which has high intensity and optical stability, the fluctuations in the intensity of the scattered light can be measured. The translational diffusion co-efficient of the particle is measured based on the auto correlation function, which represents the correlation between the two scattered light signals different in time domain. Using this translational diffusion co-efficient, hydrodynamic dynamic diameter of the particle will be measured from Stokes Einstein equation,

$$D_t = \frac{K_B T}{3\pi\eta d_H}$$

where

K_B = Boltzmanns constant

η = Viscosity of sample at temperature T

Dt = Diffusion coefficient

d_H = hydrodynamic diameter

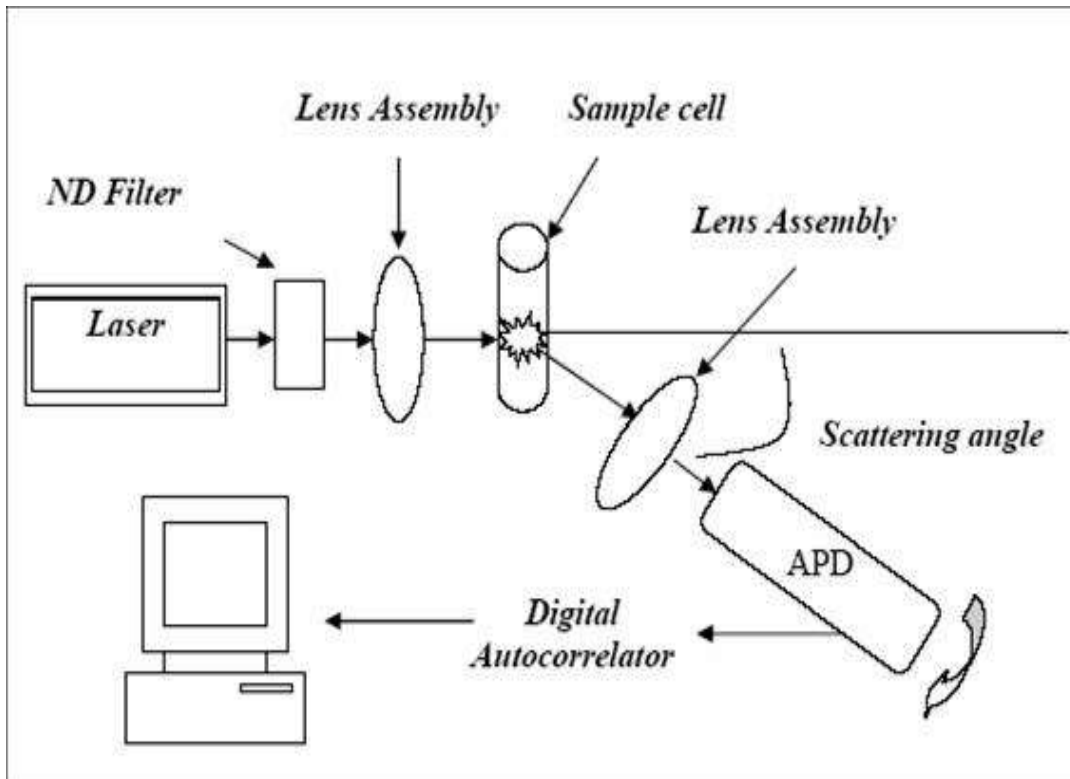


Figure 2.1: Block diagram of Dynamic Light Scattering [1].

2.1.2 Experimental set up of DLS:

DLS comprises of four sections namely source, sample cell assembly, detector optics and digital correlator.

Source:

This section comprises of a laser (source) and an assembly which focuses the beam to the sample cell assembly. Here, He-Ne laser operated at 632.8nm. A neutral density (ND) filter with an optical density of 3 is used for varying the power of the beam. Steering lens assembly is used for focusing the beam to the sample cell.

Sample cell assembly:

Samples are transferred in to the sample cell and kept inside the assembly. The sample cell is surrounded by index matching liquid, decahydronaphthalene (decalin) to reduce the stray light. The index matching liquid is kept inside the vat, cylindrical vessel.

Detector optics:

The front portion of the detector optic assembly consists of an eye piece which focuses light onto a slit. Behind the slit, a two position mirror is located. When the mirror adjustment is rotated counter clockwise, light can be seen on the eye piece else it passes through the variable pinhole and then through a filter into the photomultiplier tube. The pinhole wheel is used to change the aperture size. The filter wheel is used to allow the beam of desired wavelength. The filtered beam then passes through an Avalanche photodiode (APD) which detects the temporal fluctuations of the scattered light intensity from the sample. These fluctuations are fed to the correlator. The detector assembly is usually placed at an angle of 90°, where the effects of flare, dust and misalignment are insignificant.

Digital correlator:

BI-9001AT correlator is used. It has an adjustable delay range capable of producing continuous correlation function starting from 100ns. The mean particle size and polydispersity is estimated by the method of cumulants, software packages like non-negatively constrained least squares (NNLS) and CONTIN.

2.1.3. Sample preparation

In DLS, the intensity varies as D^6 . So, the intensity of scattering due to a micron size particle will be huge when compared with the sub micron size particle. Care has to be taken in the following steps to prevent scattering due to dust particles. It involves 3 steps:-

1. Preparing the sample(chemicals, glass wares, water used)
2. Sample cell cleaning
3. Filtration of index matching liquid

During preparation of the sample, all glass wares must be cleaned with Aquaregia, deionised water. Dust must be avoided from all perspective. The index matching liquid can be filtered with the help of an externally connected filtration system.



Figure 2.2: Photograph of DLS setup

2.1.4. Precautions to be taken during measurement

The fluctuations in the average count rate (ACR) must be minimized. Intermediate jumps in the ACR represent the presence of dirt in the sample or decalin. With dilution of the sample with solvent, ACR should decrease. Increase of ACR with dilution indicates multiple scattering. Then, the temporal fluctuations of the intensity are not random. To make the motion random, sample must be free of multiple scattering which can be avoided by sample dilution. Too much dilution can make ACR very low. Recommended value of ACR is 10kcps to 200kcps. This can be obtained by adjusting either ND filter or pinhole wheel or filter wheel. A plot of ACR with time should not have any slope. Slope indicates the presence of interparticle aggregation. This can be avoided by increasing either the concentration or increasing the measurement time.

In the correlator window baseline difference is reported. Baseline difference refers to the difference the measured baseline and the calculated baseline. Calculated baseline depends on the Seigart relationship while the measured baseline depends on the sample. The baseline difference must be positive and less than 1%. Negative baseline difference can be avoided by increasing the sample measurement time. High baseline difference indicates the presence of bigger size particles probably dirt. This can be avoided only by removing dirt from the scattered volume.

Hydrodynamic diameter is based on Stokes-Einstein equation which depends on the properties like temperature, viscosity of the sample. Hence, these properties can affect the result but their affect is not as significant as dirt [2][3].

2.2 UV-Visible spectrophotometer

In order to obtain an absorption spectrum, it is necessary to measure the absorbance of a substance at a known series of wavelengths. The instruments that are used to study the absorption or emission of electromagnetic radiation as a function of wavelength are called spectrometers or spectrophotometers. More or less similar optical

principles are employed in these instruments. There are, however, some important differences in the specific components used in the regions of spectrum.

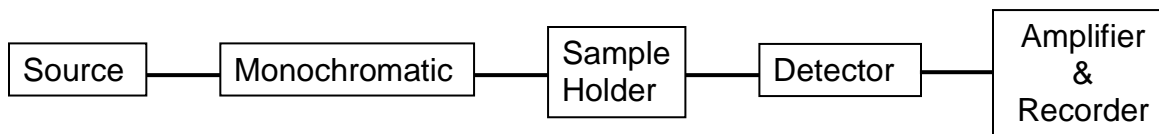


Figure 2.3: Scheme of UV visible spectrum

2.2.1. Radiant Energy Sources:

Materials which can be excited to high energy states by a high voltage electric discharge or by electrical heating serve as excellent radiant energy sources. Most commonly used sources of ultraviolet radiation are the hydrogen lamp and the deuterium lamp. Both the systems consist of a pair of electrodes in a glass tube provided with a quartz window.

The most commonly used source of visible radiation is tungsten filament. It is inexpensive emits continuous radiation in the region between 350 and 2500nm.

2.2.2. Wavelength selectors

Filters operate by absorbing light in all other regions except one which they reflect. Gelatin filters are made up of a layer of gelatin, colored with organic dyes and sealed between glass plates. Most modern filter instruments, however, use tinted- glass filter. Filters resolve polychromatic light into a relatively wide bandwidth of about 49nm and are used only in colorimeters. One of the disadvantages of glass filters is their low transmittance (5-20%).

2.2.3. Sample containers

Samples to be studied in the ultraviolet or visible region are usually gases or solutions. Mostly, the spectrophotometer studies are made in solutions. The solutions are dispensed in cells known as cuvettes. Cuvettes meant for the visible region are made up of quartz. Since glass absorbs in the ultraviolet region, quartz or fused silica cells are used in this region. Standard pathlengths of these cuvettes is usually 1 cm. The surfaces

of the cuvettes must be kept scrupulously clean; fingerprint smudges and traces of previous samples may cause serious errors in quantitative measurements. Rinsing with water should normally clean quartz cuvettes. If however, the dirt is abnormally tenacious, sulfonic detergents or nitric acid may be used. The use of rectangular cuvettes in spectrophotometers effectively curtails the chances of dirt being transferred during handling. The two sides of such cells through which the light passes are precision ground and polished to be optically flat. The other two sides are rough ground glass and the cell may be handled by these sides.

2.2.4. Detector devices

These near infrared detectors are usually photoconductive cells which detect infrared radiation in the range $0.8\text{--}3.0\mu$. The sensing element is a semiconductor (germanium, lead sulphide, or lead telluride). Upon illumination with radiation of appropriate wavelength, the electrons of the semiconductor are raised to conduction bands. This causes a drop in electrical resistance. Consequently, if a small voltage is applied, a large increase in current can be noted. The resistance of the system is such that the current may be amplified and finally indicated on a meter is recorded [4].

2.3 References

1. S. P. R. Daliparthi, 'Synthesis and characterization of gold nanoparticles', Master of engineering report, Indian Institute of Science, 2006.
2. 'Instruction manual for BI 200SM Goniometer Ver. 2.0' Brookhaven Instrument Corporation.
3. Van de Hulst, 'Light Scattering by small particles', Dover Publication, 1957
4. Upadhaya and Chakravarthy, 'Bio Physical chemistry', Tata McGraw Hill, 2004.

Chapter 3

Synthesis of Silver Nanoparticles

3.1. Introduction

Silver nanoparticles exhibit unusual optical, electronic and chemical properties, depending on their size and shape thus opening many possibilities with respect to technological applications [1]. They are produced by various methods including the chemical reduction of silver ion with or without stabilizing agents [2], laser ablation [3], sonochemical [4], electrochemical [5] and reverse micelles [6]. We get spherical, octahedral, tetrahedral, hexagonal, cubic, wire, coaxial. Cable, triangular prism, disc, triangular mark, belt, shell shapes have been manufactured.

Several methods have been reported for the preparation of organic inorganic hybrid materials. Most of the methods that can be used to incorporate metals into polymeric matrices include some form of chemical reactions such as sputtering, plasma deposition, and layer- by layer deposition. Most of these methods add cost, multi-step synthesis along with complexity to the processes for fabrication of metal doped materials. Hence, the preparation of nanoparticles without using external reagents in a single step (in situ) by excluding extra purification processes or transfer protocols will have advantages over the present methods.

Also, silver nanoparticles have known to exhibit antibacterial properties and this antibacterial activity mediated by silver nanoparticles have been investigated by many researchers [7]. As the size of silver particles decrease to nanoscale regime their antibacterial efficacy increases to their larger total surface area per unit volume. This has made is applications possible in various industries as that in food packaging, .medicine, textiles.

3.2. Tannic acid

Tannic acids are oligomeric compounds with multiple structure units with free phenolic groups. Its molecular weight ranges from 500-20000kDa. They are soluble in water with exception of some high molecular weight structures [8].

3.2.1. Sources

Tannic acid are abundant in many different plant species, in particular oak (*Quercus* spp.), chestnut (*Castanea* spp.), stag horn sumac (*Rhus typhina*), and fringe cups (*Tellima grandiflora*). They can be present in the leaves, bark, and fruits, and are thought to protect the plant against infection and herbivores [2].

3.2.2. Classification

Tannins can be classified in three groups: condensed tannins, hydrolysable tannins, and complex tannins (Khanbabaee and Van Ree, 2001). These groups can then be further divided as shown in figure1.

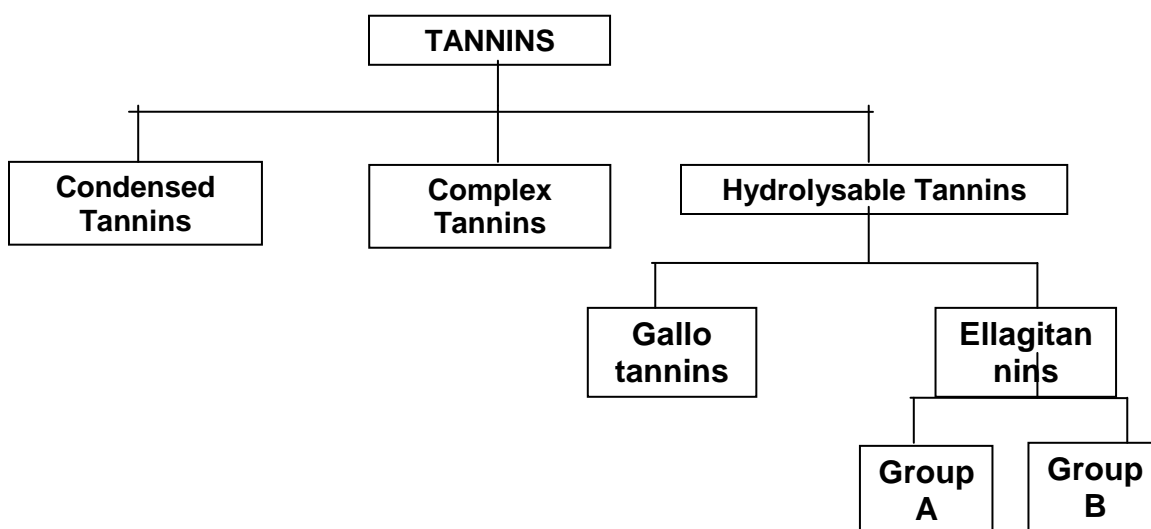


Figure 3.1: Classification of tannins

3.2.2.1. Condensed tannins

Condensed tannins are also referred to as proanthocyanidins. They are oligomeric or polymeric flavonoids consisting of flavan-3-ol (catechin) units. Hydrolysis under harsh conditions, such as heating in acid, yields anthocyanidins. Polymers are formed through the action of acids or enzymes. Polymers made up of between two and ten residues are called flavolans. Polymers made up of more than 50 catechin units have been identified (Khanbabaee and Van Ree, 2001). The degree of polymerization affects the ability to

precipitate proteins. This is of importance in wine making, where a high level of condensed tannins, especially in red wines, can result in the dry feeling on the inside of the mouth.

3.2.2.2. Gallotannins

Gallotannins are hydrolysable tannins with a polyol core (referring to a compound with multiple hydroxyl groups) substituted with 10-12 gallic acid residues. The most commonly found polyol is D-glucose, although some gallotannins contain catechin and triterpenoid units as the core polyol. Gallotannins with a D-glucose core are synthesized from 1, 2, 3, 4, and 6- pentgalloylglucose.

3.2.2.3. Ellagitannins

Ellagitannins are also hydrolysable tannins derived from pentagalloylglucose, but unlike gallotannins they contain additional C-C bonds between adjacent galloyl moieties in the pentagalloylglucose molecule. This C-C linkage is formed through oxidative coupling between the two adjacent galloyl residues, and results in the formation of a hexahydroxydiphenoyl unit.

3.2.2.4. Complex tannins

Complex tannins are defined as tannins in which a catechin unit is bound glycosidically to either a gallotannin or an ellagitannins unit. As the name implies, the structure of these compounds can be very complex. An example is Acutissimin A. This is a flavogallonyl unit bound glucosidically to C1, with an additional three hydrolysable ester bonds to a D- glucose derived open- chain polyol. This complex tannin is formed during the aging process of red wine, whereby the catechin unit originates from the grapes, and the ellagitannin, in this case vescalagin, originates from the oak barrels [8].

3.2.3. Structure of Tannic Acid

In nature there is abundance of mono and di-galloyl esters of glucose (Molecular weight-900). But they are not considered as tannins. At least, 3 hydroxyl groups of the glucose must be esterified to exhibit a sufficiently strong binding capacity to be classified as tannins.

Tannic acid is glucose ester of digallic acid. It contains 25 hydroxyl groups among which 20 hydroxyl groups are available for the reaction. This is a schematic representation showing the structure of tannic acid [9]

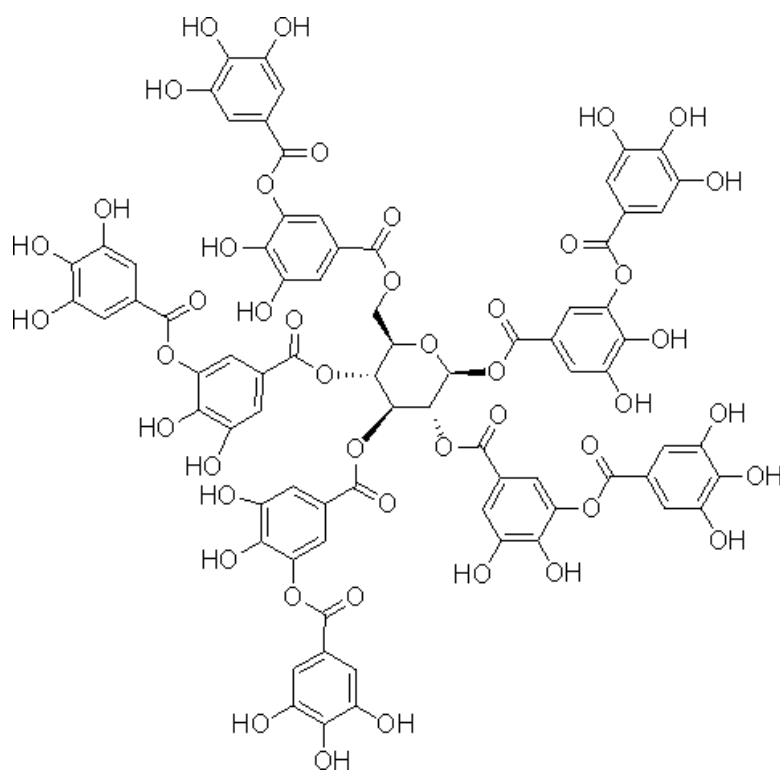
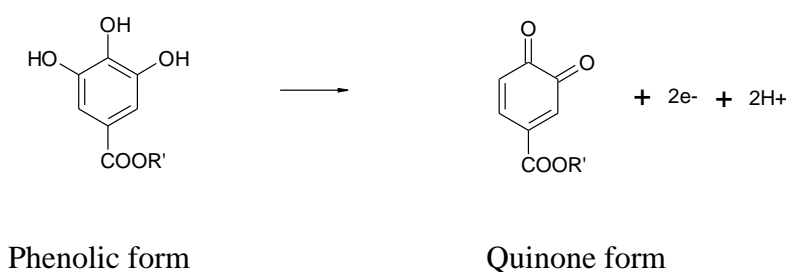
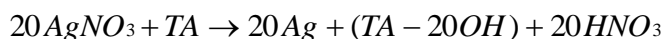


Figure 3.2: Structure of tannic acid

3.3. Synthesis of silver nanoparticles by tannic acid reduction

In tannic acid there are 25 hydroxyl groups among which only 20 are available for reaction. The alcoholic group gets converted to ketone [10]. The chemical reaction taking place is shown here.



2.3 References

1. Zhao et al. , 'A convenient phase transfer routr for Ag nanoparticles', *Physica E*, 2004, 23, 92-96.
2. Kim et al., 'Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection', *Nanotechnology*, 17, 2006, 4019-4024.
3. Mafune et al. 'Structure and stability of silver nanoparticles in aqueous solution produced by laser ablation' *J. Phy. Chem B*, 2000, 104, 8333-8337
4. Sanchez et al., 'Electrochemical synthesis of silver nanoparticles', *J. Phys. Chem. B.*, 2000, 104, 9683-9688.
5. Salkar et al., 'The sonochemical preparation of amorphous silver nanoparticles', *J. Mater. Chem.*, 1999, 9, 1333-1335.
6. Zhang et al., 'Preparation of silver nanoparticles in water in oil AOT reverse micelles', *J. Colloid and Inter. Science*, 2006, 302, 370-373.

7. Chen et al., 'Nanosilver: A nano product in medical application', *Toxicology Letters*, 2008, 176, 1-12.
8. <http://www.users.muohio.edu/hagermae/tannin.pdf> accessed on 14/02/2008
9. <http://www.sigmaaldrich.com/catalog/search/SpecificationSheetPage/SIAL/T3437> accessed on 15/02/2008.
10. Yoosuf et al, 'Insitu synthesis of metal nanoparicles and selective naked eye detection of lead ions in aqueous media', *J. Phys. Chem. C*, 2007,

Chapter 4

Results and discussion

4.1. Introduction

Since our area of interest was to obtain smaller nanoparticles at room temperature with reduced polydispersity, tannic acid reduction of silver nitrate was carried out. The different parameters to check for the size of nanoparticles were known by the following types:

- Mixing reactants
- Reduction mixture pH
- SR on magnetic stirrer
- SR using Ruston turbine baffle system

4.2. Effect of mixing reactants on nanoparticle size

Experiments were conducted to know the effect of mixing reactants on nanoparticle size. The same volume of reagents was added in both the cases. However, the concentration in both cases varied. While AgNO_3 was added as the precursor for synthesis of silver nanoparticles, tannic acid (TA) was added to perform its role as stabilizing and reducing agent. The tannic acid pH was increased by using 1% w/v K_2CO_3 . Also, the amount of K_2CO_3 added in both cases was same. Both the solutions were mixed instantaneously. In the first case, TA was added to AgNO_3 and in the second case, AgNO_3 was added to TA. Both these solutions were mixed on magnetic stirrer for about 30min. DLS was performed for these two samples and were analyzed.

AgNO_3 1% w/v ml	AgNO_3 pH	TA 1% w/v ml	K_2CO_3 1% w/v ml	TA pH	DLS dia nm	Remarks
1	6	5	1.4	9	18.9±1.6	Tannic acid was added to AgNO_3
1	6	5	1.4	7.5	14.4±0.9	Tannic acid was added to AgNO_3

Table 4.1: Effect of mixing reactants on nanoparticle size

When DLS was performed, it was noticed that in the first case, when TA was added to AgNO_3 it resulted in larger nanoparticles. In the second case, when AgNO_3 was added to TA then it resulted in smaller nanoparticles. The reason behind this is unclear. Since our area of interest was to obtain smaller silver nanoparticles all the proceeding experiments were carried out by the addition of AgNO_3 to TA.

4.3. Effect of reducing mixture-pH

The pH of AgNO_3 was found to be 6. The pH of tannic acid was adjusted to 6 by using 1% w/v K_2CO_3 . This solution was stirred for 30 minutes using magnetic stirrer. Similarly, the above experiment was repeated by adjusting the pH of tannic acid to 7.5 and 9 using the required amount of K_2CO_3 . It was then stirred for 30mins as discussed in the above conditions. The nanoparticles formed were characterized by DLS, UV-Vis spectrophotometer.

AgNO_3 1% w/v ml	AgNO_3 pH	TA 1% w/v ml	K_2CO_3 1% w/v ml	TA pH	DLS dia nm	Remarks
1	6	5	0.6	6	$80.6 \pm$	AgNO_3 was added to TA.
1	6	5	1.4	7.5	$14.4 \pm$	AgNO_3 was added to TA.
1	6	5	2.1	9	13.0 ± 1.3	AgNO_3 was added to TA.

Table 4.2: Effect of reducing mixture-pH

A DLS instrument was used to analyze the size of nanoparticles. It was observed that when pH 6 was maintained, silver nanoparticle size were larger than compared to silver nanoparticles solution with pH 7.5 and 9. As pH was increased to higher range, silver nanoparticle size decreased considerably. This inferred that lower pH; there was formation of large silver nanoparticles. As pH increased, the size of silver nanoparticles

decreased. The reason behind this could be possibly the stabilization of tannic acid at higher pH.. Also, the reaction kinetics is faster at higher pH than at pH 4. It is known that phenolic group in tannic acid dissociates at higher pH [1]. This dissociated tannic acid is highly active and this could lead to faster reaction.

4.4. Effect of stoichiometric ratio on nanoparticle size – magnetic stirrer

The other most important parameter to be considered in the synthesis of silver nanoparticles is stoichiometric ratio (SR). It was varied in the range of 1, 5, 10, 15 and 20. Appropriate amounts of tannic acid for each SR to be added were calculated. The amount of AgNO₃ added was same in all cases but the amount of tannic acid was varied depending on SR. The pH of all the tannic acid solutions was changed to 7.5 using K₂CO₃. The solutions were stirred on a magnetic stirrer at room temperature for period of 30 minutes. The DLS was performed on all the samples at different SR of TA.

AgNO ₃ pH	AgNO ₃ 1% w/v ml	TA 1% w/v ml	TA SR	TA pH	K ₂ CO ₃ 1% w/v ml	DLS dia nm	SD nm
6	1	0.5	1	0.7	7.5	9.8	3.1
6	1	2.5	5	0.9	7.5	30.9	11.1
6	1	5	10	1.4	7.5	33.5	6.0
6	1	7.5	15	1.2	7.5	49.5	0.8
6	1	10	20	2	7.5	50.9	12.5

Table 4.3: Effect of stoichiometric ratio on nanoparticle size- magnetic stirrer

It was observed with the help of DLS that at lower SR silver nanoparticle s size was lesser than at higher SR. As SR of TA increased, size of silver nanoparticle also increased. An increasing trend was observed in all these solutions ranging from SR-1 to

SR-20. Generally, a decrease in the size was speculated with the increase of SR [2]. However, to my surprise there was an increase in size with increase in SR.

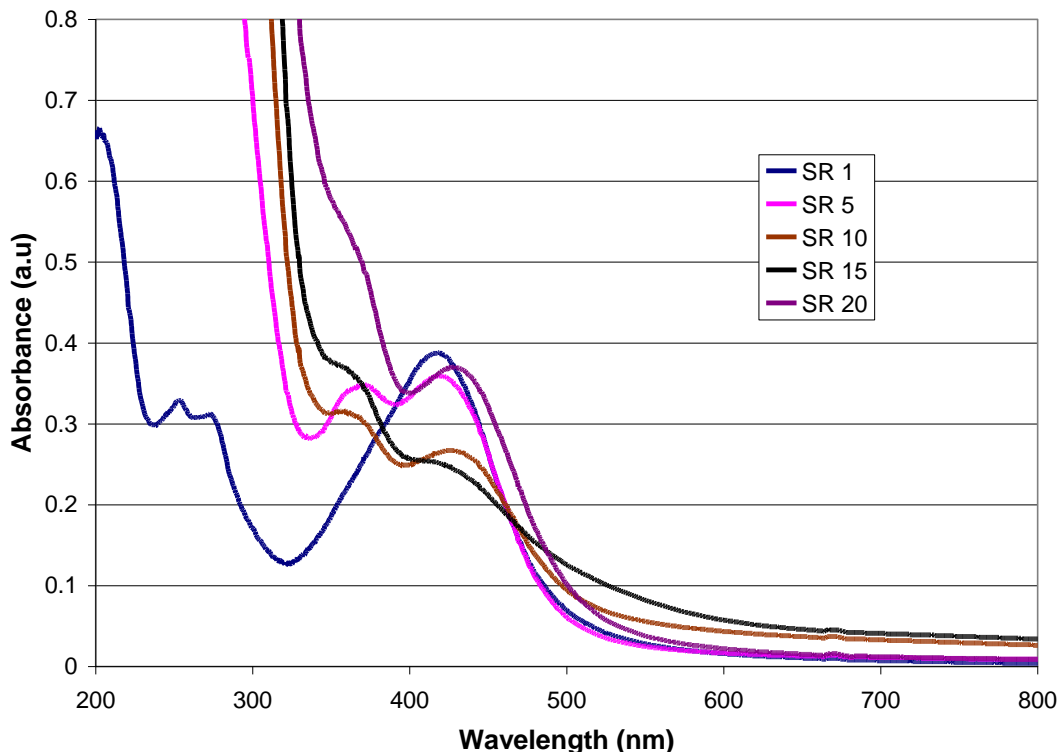


Figure 4.1: UV- Vis of silver nanoparticles synthesized SR 1-20

4.5 Effect of stoichiometric ratio on nanoparticle size – turbine and baffles

The above experiment was repeated with the same quantity of all reagents. The amount of these reagents was scaled down by a factor of 4. The total volume of these solutions was made up to 25ml. AgNO_3 solution made up to 3ml was added to TA which was made up to 22ml (In both the cases, deionised water was added.) To check the effect of SR beaker was placed fitted with baffles. This was connected to a agitator. The schematic representation of the Ruston turbine baffle system is shown here.

AgNO_3 was injected into TA using syringe such that time scale of addition of reactants is in the order of milliseconds. For SR 1 there was instantaneous reaction taking place. However, for SR 20 there was gradual change color of reaction mixture

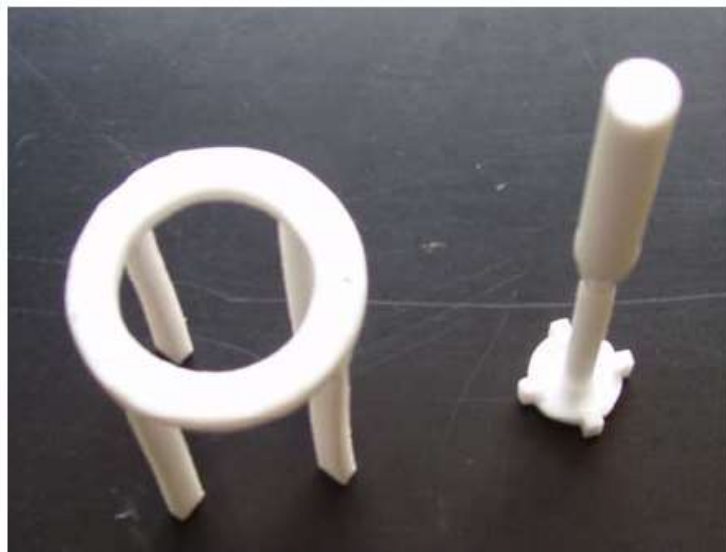


Figure4.2: Photograph of Ruston turbine baffle system.

With the information obtained from DLS it was known that the trend remained the same i.e., increase in nanoparticle size with increase in SR. Due to the effective rate of mixing in Ruston turbine baffle system there was uniform formation of nanoparticle.

4.6. Comparison of nanoparticle size with magnetic stirrer and Ruston turbine baffles system.

The sizes of nanoparticles were larger when performed in magnetic stirrer than when it was performed in Ruston turbine baffle system.

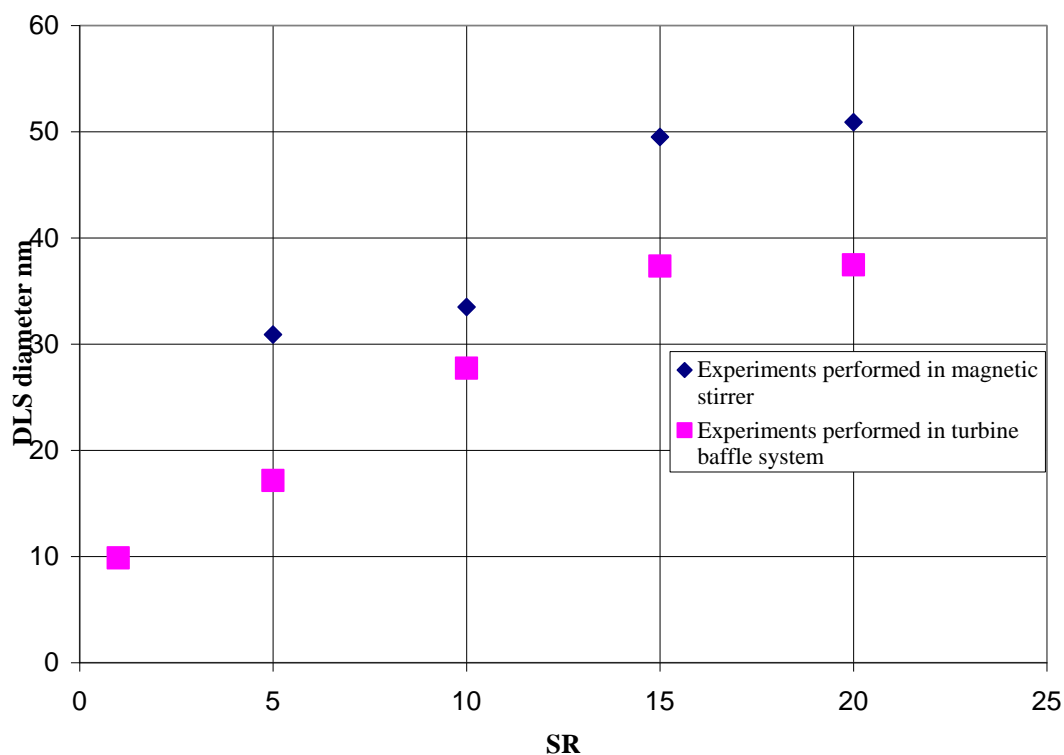


Figure 4.3: Comparison of nanoparticle size obtained with magnetic stirrer and Ruston turbine baffle system

The turbine baffle mixing differs from magnetic mixing in two ways. The mixing is very efficient in turbine system than the magnetic stirrer system. The total volume of the reaction mixture was scaled down to 25 ml in turbine baffle system. So the time taken for addition decreased from 4 s (magnetic stirrer) to 1 s in turbine baffle system.

4.7. Experiments to check for unreacted AgNO_3

Experiments were conducted to check if there was any unreacted silver in solution which may give rise to larger particles. No change in the particle size was observed which confirmed the absence of unreacted silver in the solution. UV-Vis graph showed no change in peak wavelength.

AgNO ₃ 1% w/v ml	AgNO ₃ pH	TA 1% w/v ml	TA SR	TA pH	K ₂ CO ₃ 1% w/v ml	DLS dia nm	DLS dia nm
0.25	6	0.12	1	7.5	0.2	8.7±0.9	11.9±0.8
0.25	6	0.25	6	7.5	0.2	11.9±2.4	12.0±1.5
0.25	6	0.5	11	7.5	0.2	10.0±1.8	11.4±0.9

Table4.4: Experiments to check for unreacted AgNO₃

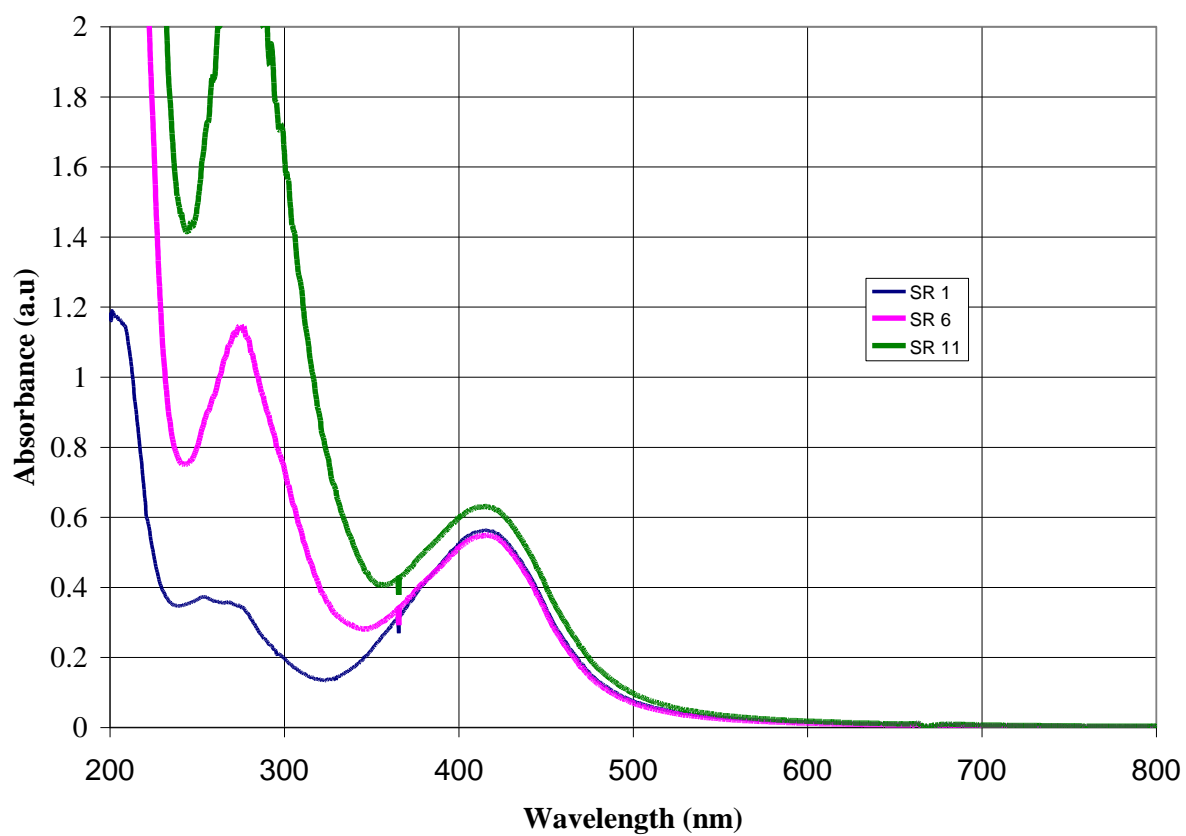


Figure4.4: UV-Vis of silver nanoparticles for unreacted AgNO₃

4.8. References

1. Jong-Hyok et al, 'Adsorption of tannic acid on chitosan-montmorillonite as a function of pH and surface charge properties', *Applied Clay Science*, 2007, 36, 256–264.
2. Frens, G, 'Controlled nucleation for the regulation of the particle size in monodispersed gold suspensions', *Nature Physical Science*, 1973, 241, 20-26.

Chapter 5

Conclusion

The main focus theme behind this study was to obtain smaller size nanoparticles. Synthesis of silver nanoparticles using tannic acid reduction in aqueous phase was achieved. Different parameters were checked as already discussed and the following conclusions were drawn as listed below:

- Silver nanoparticles produced by tannic acid were found to be stable at 15°C.
- At higher reducing mixture pH, the sizes of silver nanoparticles were found to be decreasing.
- The type of mixing also affects the particle size.
- With increase in SR of tannic acid, there was increase in silver nanoparticle size.
- Mixing in Ruston turbine baffle system results in smaller particles than in magnetic stirrer.
- Also, when the experiments were checked for any unreacted silver by the addition of tannic acid nanoparticle size remained the same.

A better uniformity of nanoparticles was obtained when Ruston baffle system was used. This led to less polydispersity than when performed in magnetic stirrer.

Appendices

Appendix-1: Synthesis protocol for silver nanoparticles.

1. Materials:

The materials used for the intended experiment include silver nitrate, tannic acid, potassium carbonate, aquaregia (1:6 HCl and HNO₃) and dodecanethiol. Deionised water from Millipore was used for preparation of aqueous solutions and for rinsing glass wares.

2. Experimental procedure

1. All the glass wares were rinsed with Aquaregia and then washed with tap water followed by deionised water.
2. 1ml of 1% w/v silver nitrate solution and 79ml of deionised water was mixed in a 100ml conical flask. pH was found to be 6.
3. 5ml of 1% w/v tannic acid and 14ml of deionised water was taken in 100ml beaker. Initial pH was found to be 4.
4. The pH of solution was changed to 9 by using 2% w/v potassium carbonate to the tannic acid solution in beaker to raise the pH to desired value.
5. Place the silver nitrate solution on an magnetic stirrer and add tannic acid solution to this as quickly as possible. Observe for any change in the color of the solution.
6. Stir the above mixture for about 30 minutes allowing the reaction to proceed.
7. The average of DLS for 6 times was taken for each sample and their mean was considered.

Appendix-2: Chemicals used and supplier information

1. Silver nitrate, 99.99% from MERCK.
2. Tannic acid (M.W 1701.23) ACS reagent from ACROS ORGANIC.
3. Potassium carbonate from MERCK
4. Deionised water from Millipore