Synthesis and surface modification of colloidal silver nanoparticles and their cytotoxic effect on HuH7 cells

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ALL OF MY WORK IS
DEDICATED
TO
MY LOVING PARENTS

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Table of Contents

Abstract

Stable colloidal solutions containing metal nanoparticles are important for biomedical applications. Phase transfer of metal nanoparticles from aqueous solutions to organic solvents plays a vital role in development of stable metal nanoparticles. The transferred metal nanoparticles show superior stability for longer periods of time if stored under proper physiological conditions. Moreover colloidal metal nanoparticles can also be transferred back to aqueous solution via polymer coating and further used in different application.

This work proposed silver nanoparticles synthesized via two phase method where dodecylamine is used as phase transfer agent. Nanoparticle synthesis was confirmed by UV-vis spectroscopy. The presence of surface Plasmon absorption peak corresponding to metal nanoparticles and a clear red shift from the absorption peak of citrate coated silver nanoparticles shows that the agglomeration occurs during phase transfer. Furthermore silver nanoparticles were coated with polymer to suspend it back in aqueous solution and also able to use in further application like drug attachment. Polymer and drug attachment both were confirmed from UV-spectroscopy, gel electrophoresis and FTIR. Silver nanoparticles synthesized were used for studying their antibacterial and cytotoxicity.

Chapter 1

Introduction

1.1. Nanotechnology

The word Nano is derived from Greek word ‘dwarf’ on matric scale$1nm = 10^{-9}m$**.** It is science, engineering and technology applied at the smallest scale such as Nano scale about less than 100 nm. Nano science and Nanotechnology are the fields of study and can be applied in other fields of natural sciences such as physics, biology, chemistry, materials science and engineering. The whole theory or idea about this came under consideration after a talk by Richard Feynman entitled as “there is plenty of room at the bottom” at American physical society meeting CALTECH in December 1959. He discussed a method in which researchers will be capable of using and controlling single atom and molecules. Modern nanotechnology revolution started after the invention in 1981 of scanning tunneling electron microscope with the help of which individual atom or molecule can also be observed. The name nanotechnology was coined by professor Taniguchi.

The study of Nano materials involves the ability to observe and control individual atom and molecules which are impossible to observe with naked eyes and simple microscopes. So more powerful microscopes were invented to observe material at Nano scale almost three decades ago. So we can say that the age of this technology began three decades ago with the invention of STM and AFM.

As far as the use of Nano size material is concerned they are being used since ancient times For example, silver and gold particle stained glass windows were used in medieval churches centuries ago but the people at that time didn’t have an idea about the composition of the material they were working on. Nowadays scientist and engineers are working on different nanomaterial to be benefited from their various properties. Historically NPs root back far beyond their current interactive scientific study. A simple example is the dyeing of glass by the freshly precipitated colloidal solution of gold which was known in Greek roman time [1].

1.2. Nano materials

Nano-materials are the material that has structural component smaller than 1 micro-meter in no less than one spatial measurement. While the molecular and atomic building blocks (~ 0.2 nm) of matter are considered Nano materials, illustration for example, such as bulk crystal with lattice spacing of Nanometer yet microscopic measurement generally speaking are ordinarily prohibited [2].

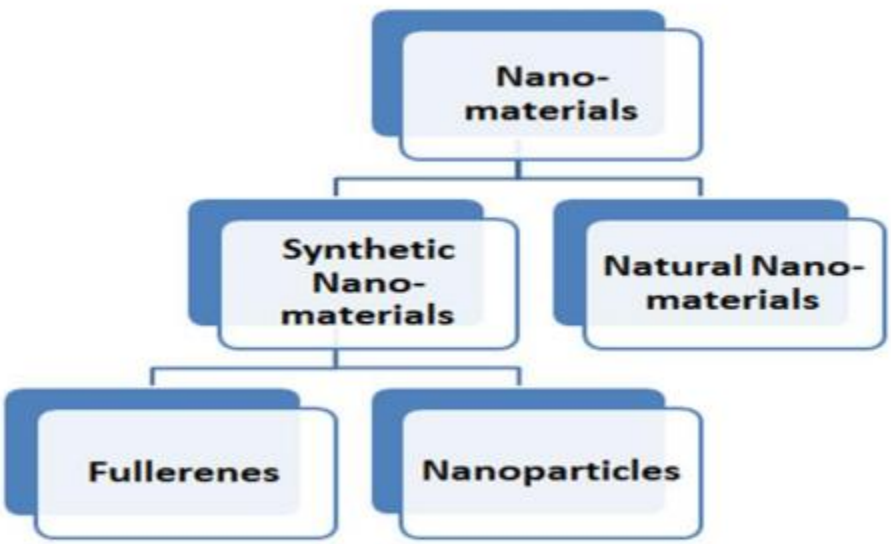

Fig1.1: schematic of nanomaterial’s [2]

1.3. Nanoparticles

Nanoparticles are class of material which has at least one dimension less than 100 nm. Its shape can be 0D, 1D, 2D, 3D. An interesting aspect about nanoparticles is that size and shape can vary it physicochemical properties like optical properties. Nanoparticles may also contain certain species attached on its surface. In general a nanoparticle may be composed of different possible layers i.e. surface, shell and core (put an image here). The surface layer is generally used for functionalization with various molecules which possibly can be number of tiny molecules, ions, surfactant compound. The shell is generally chemically different material from the core, and the core is mainly the central portion of nanoparticles or nanoparticle itself. Due to these various significant characteristics, these materials have attracted the interest of researchers [3].

1.3.1. Classification of nanomaterial's

Based on dimension we can classify the Nanomaterial's into the following types.

a) Zero-Dimensional Nano material

In zero dimensional Nano-material (0D) electron movement is bound in every single spatial measurement which implies that no measurement is more prominent than 100 nm. Quantum dots are the illustration of zero dimensional material. These materials can be amorphous or crystalline.

b) **One-Dimensional Nano material**

In one dimensional material (1D) electron motion is confined in two dimensions and can move only in one direction. 1D Nano material can be crystalline or amorphous structure. Examples of 1D are nanowires and nano-rods.

c) *Two-Dimensional Nano material*

In two dimensional materials (2D) electron are confined in one dimension and can move in two directions. Examples of 2D are thin films, Nano coating and quantum wells.

d) *Three-Dimensional Nano material*

In 3D materials electrons are not confined at all in different dimensions. All the dimensions of material are greater than 100 nm. These Nano-materials are also known as bulk Nano-material.

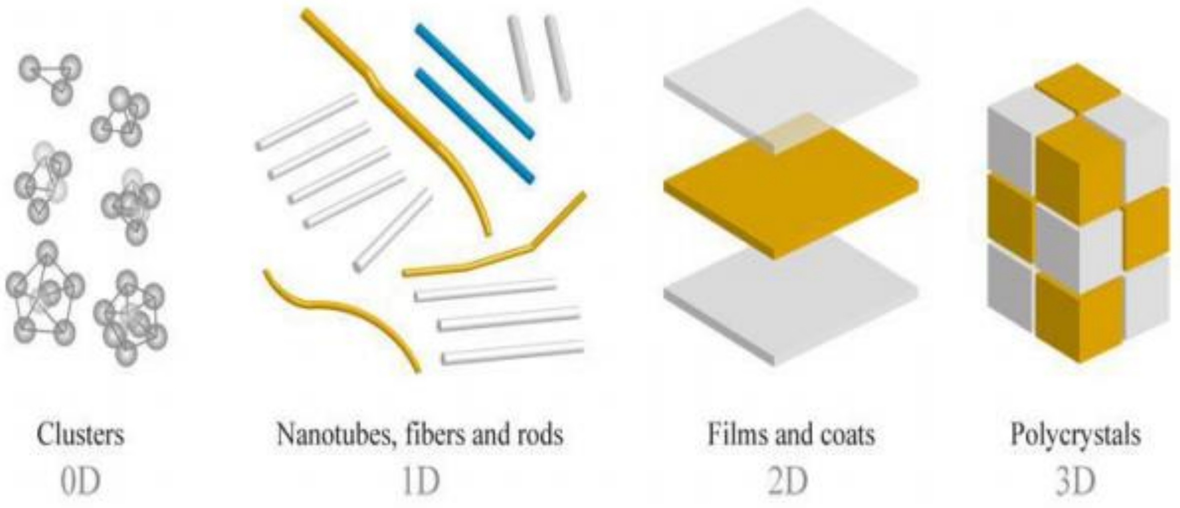

Fig 1.1 different types of nanomaterial's [4].

Nanoparticles can be categorized into different groups on the basis of structures, size and chemical properties. Below mentioned are well-known classes of NPs are based on physical and chemical properties [4, 5].

1.3.2. Classification of nanoparticles

1.3.2.1. Carbon-Based NP's

Carbon based Nps have two major types that are carbon nanotubes and fullerence. Main benefit or quality characteristic of carbon nanotube is along the length they are thermally conductive and insulated along the tube. We can simply say that CNTs are graphene sheets rolled into a tube. Application of CNTs is mainly in structural enforcement as they are strong than steel. They are further divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes MWCNTs. Structure of Fullerene is the combination of number of carbon atoms. The carbon units of these structures are pentagonal and hexagonal arrangements. They have good electrical conductivity, high strength and electron affinity [6].

1.3.2.2. Ceramic NPs

Inorganic solids synthesized of oxides, carbides and phosphates, having high heat resistance and chemical inertness, which can be used or have application in photo catalysis, photo degradation of dyes, drug delivery and imaging. Their applications can be easily improved by controlling or improving some of their characteristics i-e size, surface area, porosity and surface-volume ratio make it a good drug delivery agent. They are effectively used as drug delivery agent in different diseases like bacterial infections, glaucoma, cancer etc. [6].

1.3.2.3. Semiconductor NPs

Semiconductors are in central groups in periodic table as their properties are like those of metals and nonmetals both due to which they have gotten place in various applications. Due to the wide band gap and easily tunable it has shown greater variations in properties for example these materials have greater applicability in photo catalysis, photo optics and electronic devices. According to previous literature it is worth considering that exceptional efficiency during water splitting applications it was also concluded that this is because of their suitable band gap and band-edge positions [6].

1.3.2.4. Polymeric NPs

According to literature, organic-based NPs or polymer nanoparticles are also termed as PNP's. Most of these are Nano sized spheres or Nano sized capsules shaped. Nano sized spheres are usually found in solid form while the other molecules are absorbed at the external layer of the spherical surface. In Nano sized capsule case, the solid mass is enclosed inside the NP wholly. The PNPs are promptly functionalized and hence have vast applications in natural sciences [6].

1.3.2.5. Metal NPs

Metal NPs are synthesized from the metal precursors. There are different methods and techniques to synthesize these nanoparticles i.e. chemical, electrochemical and photochemical method. They are mainly produced by reducing metal ions precursor in solution through reducing agent. Furthermore they have potential to assimilate tiny molecules and possess high surface energy. They have application mainly in drug delivery molecular detection, imaging etc.. Metals nanoparticles have localized surface Plasmon resonance (LSPR) characteristics, due to which their opt electrical properties are unique from other nanoparticles. Alkali and noble metal nanoparticles have a broad absorption band in the visible zone of the electromagnetic solar spectrum. Metal NPs are also significant in present day cutting-edge materials considering that they are synthesized based on controlled size, surface and shape [6].

1.3.3. Properties of Nanoparticles

As we have discussed earlier that the discovery and development of novel materials has played a very vital role in the development and growth of industry and technology. It has opened the door for many innovative and critical approaches. The main breakthrough occurred in this field by exploiting the properties of Nano scale dimension materials. Due to which scientists were able to get vast knowledge about the properties of matter as it changes from bulk to Nano phase, the phase in which particle take a clear turn from the properties in bulk and atomic or molecule form. The change in properties i.e. physical or chemical of these particles is credited to the transformation of phase which effected the arrangement of atoms that leads to energy level quantization. So we will here discuss these properties, their effect and applications [6].

1.3.3.1. Electronic and optical properties

Optical and electronic properties of nanoparticles are interlinked. Since metal nanoparticles optical properties are size dependent and have strong UV-Visible extinction band which are non-existent in bulk. When the oscillation frequency of conduction band electrons in metals and incident photon frequency of electrons is same, it results in excitation band, which is known as Localized Surface Plasmon Resonance (LSPR). It is evident from various studies that LSPR spectrum depends upon shape, size and inter-particle spacing of nanoparticles. It also depends on its own dielectric properties and local environment i.e. substrate, solvent and adsorbents. Moreover, free electrons on the surface of metal nanoparticles are easily transportable through the nanomaterial. The mean free path for silver and gold is 50 nm which is comparable to their size; therefore no scattering is expected in bulk during light interaction. Instead a standing resonance condition takes place, which is responsible for LSPR in nanoparticles. Other aspects and difference of metal nanoparticles which make it unique from others nanoparticles is that it have different electronic band structure. For instance, band edge of metal nanoparticles has more effect of quantum confinement and Fermi level is at the Centre of the band. Spatial confinement has less effect on metal instead of semiconductors and insulators etc., while quantum confinement is more effective for smallest metal nanoparticles (1nm or less). For larger size regime, metal nanostructure shows classical behavior which is in line with most of the experiment [6, 7].

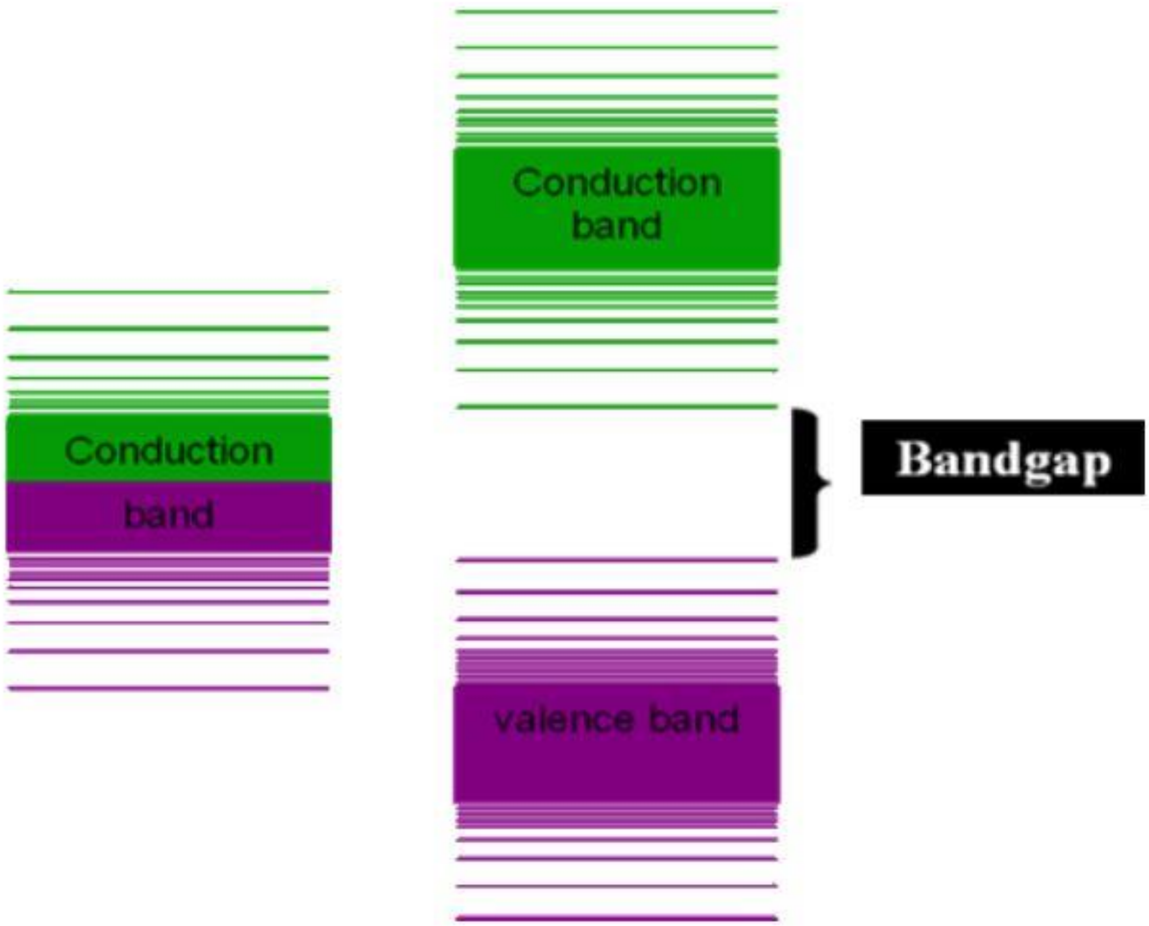

Fig1.2. electronic band structure of metal (left) versus semiconductor (right) nanomaterial. The Fermi level can easily be observed in both structures [7]. As in metal it is more likely in conduction band and in semiconductor in center.

Optical properties of metal nanoparticles are relatively more sensitive to the shape rather than the size as occurs in semiconductors and insulator nanomaterial. This point is more or less directed toward classical behavior of metal nanoparticles if they attain very small size its optical behavior is then dominated by collective behavior of conduction band electrons. As such small size is not much of use here for us so classical dynamics or mechanics can be easily used to study or describe the behavior of metal nanostructure both for electronic and phonon properties. From much of the work it is noted that surface-to-volume ratio is one of the well-known feature that have effect on properties of metal nanostructure such as chemical reactivity, thermodynamic and kinetic stability, interaction with embedding environment and electronic and phonon relaxation dynamics.

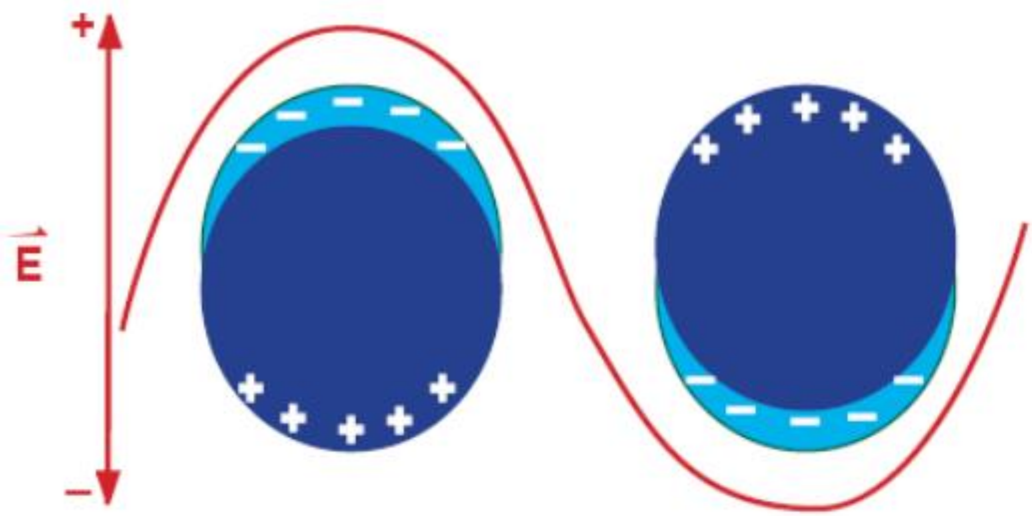

Fig 1.3 Illustration of dipolar S.P.R in spherical nanoparticles [7]

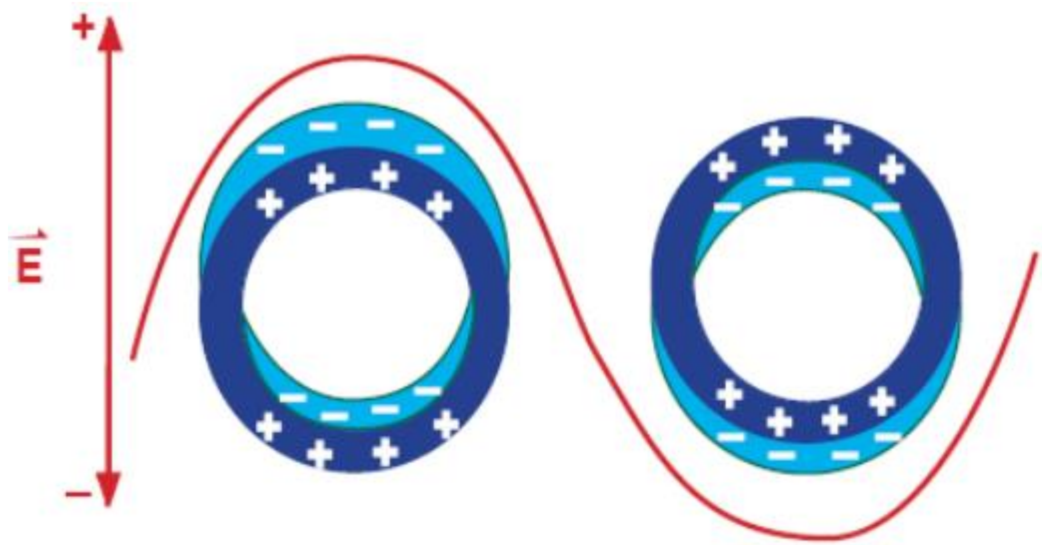

Fig 1.4 illustration of dipolar SPR in core/ shell structure [7]

Fig 1.5 illustration of dipolar SPR in Nano rods [7]

1.3.3.3. Magnetic properties

Magnetic nanoparticles are of great importance and it has created great curiosity for researchers across the field as it includes both homogenous and heterogeneous catalysis, biomedicine, magnetic fluids, data storage, MRI and environmental remediation water decontamination. From different studies it has been found out that nanoparticles performs optimal when the average size is less than critical value i-e 10-20nm. At such small level magnetic properties of nanoparticles prevails which make these particle effective, and workable in various applications**.** The abnormal (uneven) distribution in nanoparticles also leads to effective magnetic property. Platinum (Pt.) nanoparticles of diameter ranging from 2.3-3.8 nm have shown super magnetic behavior in the magnetization process. As it has paramagnetic moment in the bulk state but in nanostructure it has shown enhanced magnetization. Pt nanoparticle has shown increase in magnetic moment with decrease in diameter from 3.8nm and have $5.3\mu_{\beta}$/Pt nanoparticle at 2.3nm diameter. Ferromagnetic polarization in Pt nanoparticles is attributed to spin polarization of surface of these nanoparticles, as predicted by theorists, that Ferromagnetic or antiferromagnetic polarization occur in transition metal with diminish dimension such as clusters or thin film. It is noted that number of atom on surface is comparable to that exist in the core, consequently the effect of surface should be significant in nanoparticles. Polymers which play a significant role in stabilizing nanoparticles also interact with surface of nanoparticles**.** The ligand molecules with larger affinity play a role in diminishing the magnetic moment at the surface of nanoparticles [6].

1.3.3.4. Mechanical properties of nanoparticles

The peculiar behavior and mechanical properties of Nanoparticles have gained the attention of researchers to study its novel application in various fields such as Surface engineering, Tribology, Nanofabrication and Nano manufacturing. Various parameters including stress, strain, friction, elastic modulus and hardness are potential areas to discover the mechanical nature of Nanoparticles. Additionally coagulation, lubrication and surface coating will provide additional support in finding the mechanical properties of Nanoparticles. According to various studies the mechanical properties of Nanoparticles varies with respect to micro particles and their bulk material. Moreover, when there is lubricated contact there is visible contrast in the stiffness among nanoparticles and contacting external surface. Moreover it also control position of nanoparticles, they may either be engraved in the surface of the plan or damaged considering that when there is high pressure at the surface. These findings are of great importance as it provides us with evidence about the behavior of nanoparticle when they are in contact with a surface. When we are able to control the mechanical properties of nanoparticle during its interaction with surface, it provides us opportunity to improve the surface quality and removal of material. These studies also enable us to have thorough understanding mechanical properties of nanoparticles such as elastic modulus, movement law, hardness, adhesion etc. [6].

1.3.3.5. Thermal properties of nanoparticles

It is a well-established fact that metal nanoparticles have thermal conductivities higher than those of fluid in solid form**.** It is already known that copper thermal conductivity at standard room temperature is 700 times is higher than that of water and about 3000 time higher than that of engine oil. Even metal oxide has higher thermal conductivity than that of water. From this it can easily be concluded that the fluid containing solid particles have higher enhanced thermal conductivity compared to conventional heat transfer fluids. It is known that Nano fluids are generally produced by suspending solid particles in to liquid for example water, or oils etc. Nano fluids have much strong properties compare to conventional heat transfer fluids or that in which microscopic size particles are suspended because the heat transfer take place at the surface of nanoparticles, so it is useful to use the particle having large surface area which also helps in increasing the stability in suspension[6].

1.3.4. Application of nanoparticles

On the basis of above discussion about properties of nanoparticles there are variety of applications in which nanoparticles help in improvement and developing. Some of them will be discussed under.

1.3.4.1. Application in drugs and medicine

We have previously discussed that nanoparticles have different nature i-e simple and complex, unique display, interesting physical and chemical properties which enable them to play a vital role in some interesting and important applications like physical, biological, biomedical and pharmaceutical. Nanoparticles are of great interest among the scholars specially in fields of medicine such as in finding optimal level of dosage of drugs. These studies have so far delivered better results considering therapeutic efficiency of drugs and diminishing the side effects. Nanoparticles have also a significant role in biological and cell imagining due to its optical properties. Additionally Mie theory and discrete dipole approximation is a measurement tool for commonly used categories of nanoparticle such as measuring absorption and scattering efficiencies as well as optical resonance wavelengths[6].

The evolution of hydrophilic nanoparticles as drug carrier is declared as the most challenging task of the following years. When given the appropriate surface chemistry Fe_20_3 (Super paramagnetic iron oxide nanoparticle) is used in Vivo application, such as drug delivery, detoxification of biological fluid hypothermia, tissue repair and MRI contrast enhancement etc. All of the above mentioned biomedical application requires a smaller size less than 100nm, high magnetization value and narrow size distribution of Nanoparticles.

Analytes can be identified in tissue by antigen-antibody interaction by using antibodies tagged with fluorescent radioactive compounds or colloidal gold, dyes and enzymes. Liposomes have been usually used for drug carrier because of its advantages to protect drug from degradation, target active sites and minimizes side effects specifically nauseouness. But work on liposomes was restricted due to pre-existing health issues such as squat encapsulation issue, rapid water leakage in the commodity of blood components, inefficient storage and stability. Contrary to these, nanoparticles especially polymer coated nanoparticles have more advantages with respect to liposomes. Nanoparticles increase retention of drug and poses convenient drug release properties. As metallic nanoparticle as well as semiconductors Increases the probability of cancer diagnosis and therapy on the account of surface Plasmon resonance enhanced light scattering and absorption. Silver nanoparticles are successfully used for dressing of wounds, catheters and others house hold activity because of their antimicrobial activity. Antimicrobial agents are very useful in industrial activity such specifically in textile, and pharma, water treatment and food packaging techniques. So inorganic nanoparticles get more importance as compared to organic nanoparticle as the later are relatively more toxic to the biological system. Also some nanoparticle are functionalized with various group to overcome and also boostup its antimicrobial activity [6].

1.3.4.2. Application in manufacturing and materials

As the properties of nanoparticles deviate from bulk due to size dependency so they provide interesting substance for material science. Synthesized nanoparticles shows physiochemical properties which provide distinct electrical, mechanical, optical and imaging properties which are pretty much valuable in various applications in the medical, mechanical, environmental, and ecological sectors. Nanoparticles have been widely used in characterization, designing and engineering of biological as nonbiological structure less than 100nm, which have interesting and unique functional properties. The advantages of nanotechnology have been witnessed in many products which can be produced in bulk specifically in microelectronics, aerospace and pharmaceutical industries. Nanotechnology has been widely used in processed food and its packaging [Food Packaging Husnain Janjua]. Resonant energy transfer system considering of organic dye molecule and noble metal nanoparticles are recently considered for use in bio photonics as well as material science [6].

1.3.4.3. Application in the environment

With the development of nanoparticles increasing use in industrial and house hold application, which are responsible for the production of such material to the surroundings. Threat of these nanoparticles to environment can be assessed on the base of their mobility, reactivity, Eco toxicity and persistency. The engineering material applications are increasing the concentration of nanoparticles in ground water and soil which are the most exposed avenue for environmental risks. Due to the high surface-mass ratio natural nanoparticles absorbed some part of contamination to its surface and their absorption depends upon the size, composition, porosity and other characteristics of nanoparticles.

Environmental applications of nanoparticles fall into three categories.

- Environmental friendly products Indemnification of material effected with hazardous substances Sensors for environmental stages.

The most important elements having environmental effects on natural water are mercury, lead, thallium, cadmium and arsenic which are hazardous both for environment and human health. For this purpose super paramagnetic iron oxide nanoparticles are used as effective absorbent for such hazardous materials. Photo degradation by nanoparticle is also one of the important applications of nanoparticles and practiced commonly [6].

1.3.4.5. Application in electronics

In recent times there is a huge interest in printed electronic due to its potential for low cost, mainly due to its application in flexible displays, sensors and attractiveness of traditional silicon techniques. Printed electronics have various functional inks which contain nanoparticles i.e. organic electronic molecules, CNTs, ceramic nanoparticles and metallic nanoparticles is expected to flow rapidly as production on large scale of new electronic equipment. Given the unique structural, electronic and optical properties of metals and semiconductors, they become key structural block for new generation of electronics, photonic materials and sensors [6].

1.3.4.6. Application in energy harvesting

As in recent studies it has been pointed out the non-renewable nature of fossil fuels shift of research occur toward the renewable sources at low cost. They have reached the conclusion that nanoparticles are the best future candidates due to several reasons. The salient features of nanoparticles are large surface area, which ensues their optical and catalytic behavior. As a result nanoparticles are being widely used in photo electrochemical and electrochemical water splitting for energy generation. Beside water splitting they are also being used in carbon dioxide reduction to fuel precursor, solar cells and piezoelectric generator. Nano generators are the most advanced as they convert mechanical energy to electricity using piezoelectric [6].

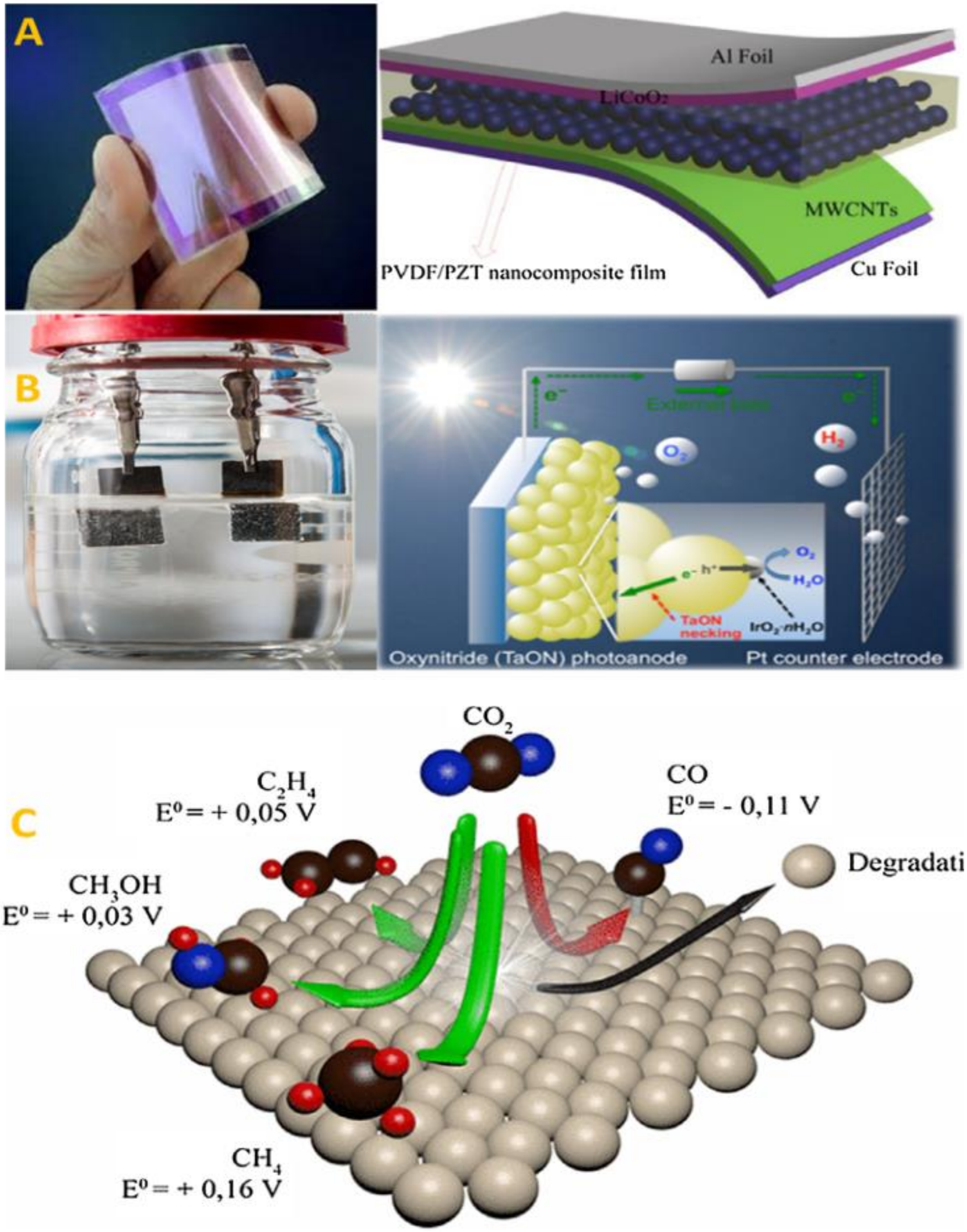

Fig1.6 Energy generation approaches from (A) Piezoelectrics actuators (B) Water splitting (C) CO_2 reduction [6].

1.4. Colloidal silver nanoparticles

In this study we have synthesized silver nanoparticles, characterized them and used them for various applications. Why silver? As it has been already previously mentioned that metal nanoparticles, specifically silver being a relatively economical noble metal, frequently exhibit unique optical characteristics. The most peculiar application of silver is as antimicrobial agent. From a while now silver ions are treated as an effective antimicrobial agent against a wide range of microorganisms at a low concentration having therapeutic activity. Silver is described as oligodynamic because of its exertive ability as a bactericidal at relatively low concentration. Silver nanoparticles can be easily characterized by UV-spectroscopy and other optical technique. Having known their UV response, size of the nanoparticles can be theoretically determined by using Mie theory [8].

There are different routes for synthesis of silver nanoparticles which can be bottom up ortop down approaches. The method we have used here for synthesis of silver nanoparticles is phase transfer method which can be categorized as bottom up approach.

1.4.1. Phase transfer method

Phase transfer method was first introduced in 1965 and has become a prominent method for synthesis. The major issue with this technique is the transfer of precursors from one liquid to another in soluble form. A phase transfer agent is normally used for this purpose. In case of metal nanoparticles it was first introduced in 1995 by Brust et al. [9]. They used tetra butyl amine as a phase transfer agent for gold nanoparticles. However this method cannot be adopted the same way due to the charge on the precursor ions. $AuCl^{4-}$ anions can be easily captured by $[CH_3(CH_2)_7]_4N^+$ due to oppositely charged nature. In the present case $AgNO_3$ has been used as a source for Ag^+ cations. Due to the same charge on the two ions the typical electrostatic interaction is not possible.

The methods we have followed here for synthesis of silver nanoparticles is as follows Citrate capped silver nanoparticles have been prepared in water by Turkevich approach. The synthesized nanoparticles were then transferred to toluene with the help of dodecyl amine and ethanol was used as mediated solvent between water and organic solution. Here dodecyl amine also played the role of capping agent just like thiol in case of Brust method. The benefit of this method is that the particles remained stable in organic phase for longer periods of time. The basic principle for phase transfer is introduced by Reubon and Sjoberg in 1981. It can be possible on the capacity of phase transfer agent to assist the transfer of one reagent from one phase to another wherein the other reagent already exists. It is also necessary that the transferred species remain in active state in the new phase also and can be regenerated during organic reaction.

1.4.2. Polymer coating

A polymer is a substance that contains a molecular structure that mainly contains a large number of small units. Amphiphilc molecules are compounds that have covalently connected hydrophilic and hydrophobic components. Colloidal nanoparticles are dispersed in a solvent which can either be water based or organic solvent for hydrophilic or hydrophobic particles, while amphiphilic nanoparticles can be dispersed in both solvent. Hydrophobic side chain linked hydrophobic nanoparticle and hydrophilic backbone provide water solubility through charged group and it also act as anchoring site for the attachment of biological molecule with bio conjugate chemistry. The coating does not rely upon the material of inorganic nanoparticle core but only depend on hydrophobic interaction. For phase transfer of nanoparticle from organic solvent to aqueous solvent there two approaches mercaptocarboxylic acid based and amphiphilic polymer based.

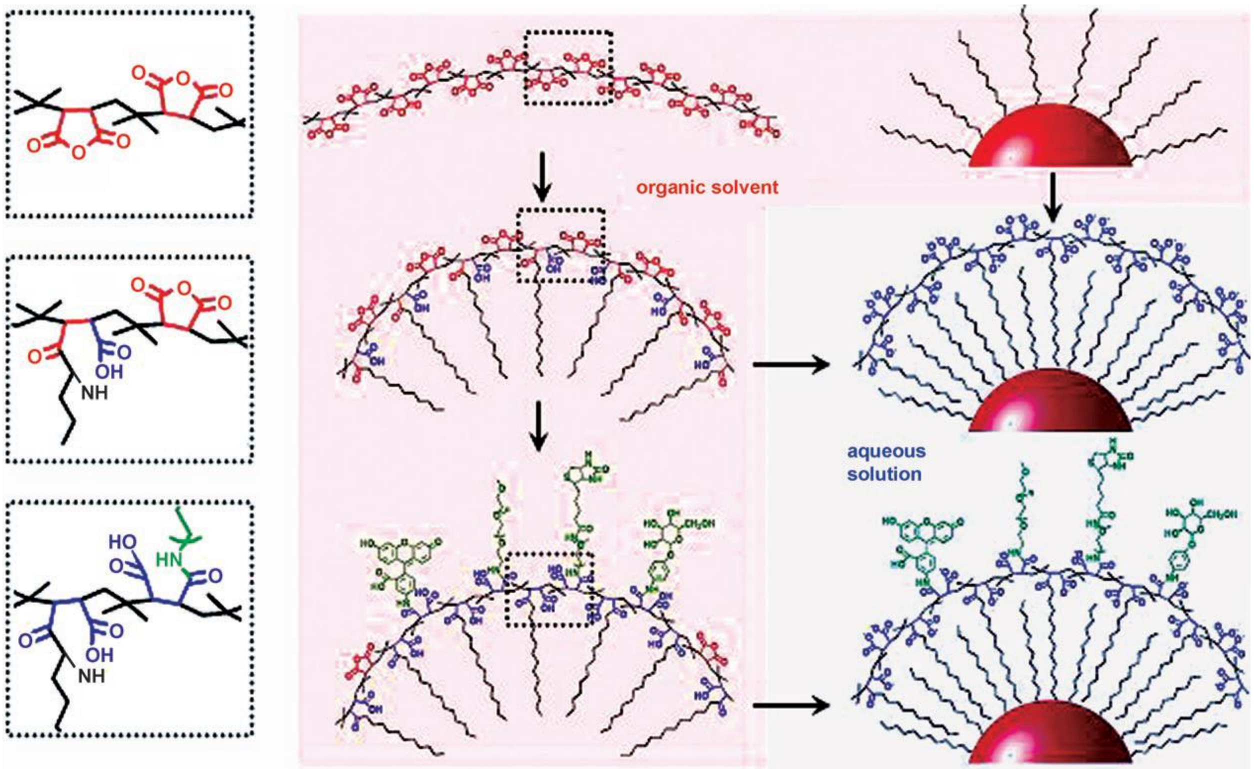

Fig 1.7 schematic of synthesis of amphiphilic polymer and the coating of nanoparticles [27].

1.4.3. Drug attachment to silver nanoparticles

As it is previously discussed that silver nanoparticles exhibit bioactivities so we have attached methotrexate drug with it. Methotrexate formerly known as amethopterin is a chemotherapy agent and immune system suppressant. It is used for treatment of cancer autoimmune diseases. There are different methods for drug attachment for some particles it can be attached directly but in some cases cross linkers are required such as EDC (1-Ethylene-3-(3-dimethylaminopropyl) carbodiimide). Here for polymer coated particles we have followed the same techniques.

1.4.4. Cytotoxicity

Cytotoxicity is the quality of being toxic to the cell. After drug attachment the nanoparticles are applied on the cells to study cytotoxicity. By this we can easily study the effectiveness of drug attached nanoparticles on cells. Cell cytotoxicity shows capability of few chemicals or mediators cells to demolish living cell. For measuring cell cytotoxicity we actually measure cell viability through use of vital dyes, protease biomarkers or by measuring ATP content are few common method used in measuring cytotoxicity. The formazan dyes are chromatic formed by the reduction of tetrazolium salts include INT, MTT, MTS and XTT.

Chapter 2

Literature Review

The development in synthesis and characterization of metal nanoparticle was previously initiated by Faraday in his bakarian lecture to the royal society in London which is also supported by a study that was conducted later (has published a paper "experimental relation of gold and other metals to light"). The purpose of this research was to study the light interaction with metal nanoparticles but most of his work is related to nature, different properties and various aspects of formation of ruby gold which can be declared as starting point of colloidal chemistry. This study has led to the emergence of the Nano science specifically of gold and nanotechnology. Although Faraday has conducted studies on several metals but the ruby-color arose in the solution by fine particles of gold which are very minute in dimension and synthesized via various approaches. One typical example of an aqueous solution of a gold compound was that of sodium aurochlorate which is treated with a reducing solution of phosphorus in carbon disulfide in a two-phase system which changes the yellow color of sodium aurochlorate to deep ruby color of colloidal gold. Faraday [10] study concluded that ruby color in the fluid confirms that gold is dispersed in the liquid in very evenly distributed metallic form. A century later Turkevich [11] used electron microscopic investigation to reveal that Faraday's method synthesizes colloids of a size is in the range of 6???. Further, it was investigated by W.D. Mogermaan, Leslie b. Hunt and John Turkevich. Mogerman explained that Faraday covered many important points in his paper. Mogerman[11] further explained that Faraday was fascinated by the color of gold suspension and different method of its synthesis. Hunt[11] covered historical facts about metal in his article "true story of purple Cassius" published in 1976 explained that from last three centuries literature has given the credit to Andrew Cassius for discovering the purple preparation of colloidal gold and hydroxide which is still described as effective method for producing color ranging from pink to maroon. By that method, ruby glass was produced. The most effective and detailed explanation of Faraday work was given by Turkevich who summarized maximum work done at the end of 19^{th} century and at the beginning of 20^{th} century which contain bridges arc method for synthesis, invention of slit ultra-microscope by Zsigomy[11], theoretical explanation for size and color of gold in colloidal solution by Mie[12]. Many researchers have explained that the ruby color is due to the absorption of light in gold.

A second important development in metal synthesis has been done by Turkevich[11] as is previously discussed that he studied nanoparticle synthesized by Faraday and then work on the synthesis of alternative method during his work, the most remarkable development is the synthesis of nanoparticle via reduction method in 1951. They also contributed to the study of growth process and nucleation of colloidal gold. Furthermore Turkevich also concluded that a hybrid polymer is formed with aurotic ions, which can be identified as reducing agent, prior to the reduction and nucleus formation, proving that law of growth is exponential**.** Consequently, quantity of gold, nucleation process and the law of growth influence the average size, the variation from the mean size and the character of the particle size distribution curve. Turkevich et-al[11] produced various types of sols like breeding sol, Faraday sol, Tenin gold sol, oxalic sol, Hydrooxyl amine sol, Donaw sol, acetylene sol, citric acid gold sol and sodium citrate sol and examined the growth and other properties of all of them. The most important which needs to be discussed here is citrate sol. As by examining all of these they have noted that nucleation in gold sol is a complex phenomenon to examine directly because the nucleus is too small and unstable to examine.

Natalia et al [13] have revised some of the comparative and broadly applied production methods for particular of metallic silver nanoparticles. They have discussed briefly every perspective and different methods of synthesis including nucleation and growth of silver nanoparticles which were previously discussed by John Turkevich et al for gold nanoparticles. They have discussed generally two methods which are widely used top-down approach and Bottom-up approach. In the Top-down approach, the bulk material is used and then reduced into nanoparticles via physical, chemical and other methods, while in Bottom-up approach molecules or atoms are combined to form nanoparticles. They have discussed three different categories of synthetic routes i.e. chemical reduction, light assisted method and electrochemical method.

The chemical reduction method as discussed previously also such as Turkevich method (1951) for gold nanoparticles has been extended by the researchers for silver and other metals also. In 1981 Lee and Meisal have synthesized silver nanoparticles in aqueous solution for Surface-

Enhanced Raman Scattering (SERS) application using Turkevich method [14]. They have not focused on the mechanism or full characterization of nanoparticles. They have investigated negatively charged dyes absorbed on surface metal nanoparticles which revealed surface enhancement of Raman scattering and was observed for carbocyanine dye for both gold and silver sol. Excitation dependence and absorption studies both confirmed the surface-enhanced Raman scattering nature obtained from spectra. From the results obtained it was found out that absorption of dye molecules on the surface of metal nanoparticle is quite an efficient process and it produces strong surface-enhanced Raman spectrum which can be easily observed at sub molar dye concentration. The result obtained from them even in the quantitative matter can reveal much of the structural information including isomerization, orientation and aggregation.

Pillai and Kamat worked on controlling the size and shape of nanoparticles through citrate method [15]. Their finding was that using boiling temperature method at different concentration can produce silver nanoparticles with absorbance at 420 nm. They have also confirmed the use of citrate as a stabilizer by synthezing SiO_2@Ag NPs using sodium borohydride and adding sodium citrate given that the synthesis of SiO_2@Ag-Citrate complex with absorption constant of $220M^{-1}$ among citrate and silver was established.

Henglin and Gerseg has also worked on capping effect of citrate on of silver nanoparticle by radiolytic method and has tallied their UV result with the previous method and concluded that absorption is at 400 nm as in Turkevich method [16].

As sodium borohydride is one of the main reducing agent, van Hyning and Zukoski has done some work on it. They have investigated the reduction process by sodium borohydride both in situ and ex situ through UV-Vis and transmission electron microscopy. They have concluded that nucleation and growth of these nanoparticles do not follow Lamers model which is classical theory of growth [17].

Other notable methods based on chemical reduction method are reduction by gallic acid [18], hydro quinine synthesis of silver nanoparticle in an organic solvent [19], and polyol method and shape-controlled synthesis [20].

Other methods which were developed for synthesis of metal nanoparticles are light assisted methods which have two types namely photo physical method and photochemical method. In the last decade, much of work was done on these methods for synthesis of nanoparticles due to adaptability of these methods to produce in-situ reduction species having high spatial resolution and have no requirement for the change in media around. In the photo physical method, laser ablation method is used in which nanoparticles produced are stabilized by anionic surfactant molecule that depends on surfactant coverage and charge state of nanoparticles as silver nanoparticles aggregate if the coverage degree is less than unity. Electrostatic repulsion forces results in strong stability given that surface of the particle is covered with dual layered surfactant. It is concluded from this that longer the chain of hydrocarbon better is the stability of the particles in solution. They have also studied the impact of Sodium chloride on the stability of silver nanoparticles [21]. The photochemical approach is a bottom-up approach in which reduction of metal cation M^{n+} to M^{0}occurs either by direct or indirect photolysis. This method was introduced by Hada et al in 70s when they investigated silver perchlorate in both aqueous and organic solution radiated with UV-light at 254 nm.

Another alternative method for the production of silver nanoparticles is electrochemical method. This method was first introduced by Rodrigues-Sanchez et al who used a silver sheet as anode and platinum or aluminum as cathode and tetra butyl amine in acetonitrate as an inert solvent [22]. Silver nanoparticles were only obtained when Platinium used as electrode they have concluded that absorption bandwidth of Plasmon band depends on size and shape of nanoparticles and its contact with encircling media and auto-catalytic phase in the synthesis. From the results they concluded that product does not depend on synthetic method largely but it mainly depends upon metal precursor, capping agent and temperature. The most important finding is that nucleation and growth is not always occurring according to the classical model.

J yang and H lin have reviewed different methods for synthesis of metal nanoparticles via to phase method [23].

Briefly describe the two phase method here, its advantages and challenges first. The main method which was first introduced for gold nanoparticles by Burst el al. The burst method is the most cited approach and initial phase transfer approach to synthesize thiol stabilized metal nanoparticles. In this method transfer metal ions are initially transferred through a phase transfer reagent (tetraoctyl ammonium Bromide). After transferring metal ions they are reduced with the help of sodium borohydride in the presence of alkanethiols. Average particle diameter obtained through this method is 20 nm. So it can be assumed that nucleation and growth of gold nanoparticles and attachment of thiol occur in a single step[9]. This method was idealized from the work of Meguro et al in 1988 which was first attempt for synthesis of metal nanoparticles in organic solvent[24]. Brust et al modified the method by replacing Dodecanethiol with Dodecylamine to stabilized gold nanoparticles. By this method, they have produced gold nanoparticles of larger size i.e. up to 20 nm.

In view of above, phase transfer approach can be divided into two methods. The first method which is already previously discussed is a straight forward one-step approach in which already synthesized particles in water are directly transferred from aqueous to organic solvent by ligand exchange. The second approach is two-step approach in which a second solvent is used as a mediator normally ethanol and hence this approach is called ethanol mediated phase-transfer approach. This approach can be further subdivided into two different approaches: one is the transfer of metal nanoparticles in which we first synthesize metal nanoparticles in an aqueous solvent and then transfer it to organic solvent and in the second approach, metal precursors are transferred into an organic solvent and then they are reduced in an organic solvent. Yang et al in 2009 has worked on these methods initially which were then modified and progressed by other people [25]. Metal ions in aqueous solution were mixed with equal volume of ethanol containing dodecyl amine and stirred for two minute. Toluene was then added to the solution and stirred for 1 more minute before transferring the solution into separation funnel. Two mutually immiscible layers of solvents appear in the separation funnel. As the transfer completes one of the layer became completely colorless. As in Brust method we know that phase transfer is due to electrostatic interaction between phase transfer agent and metal ions at the interface of two solvent but in ethanol mediated it did not follow the same procedure rather a milky suspension appears at first which slowly clears out. Phase transfer via ethanol offers good ions intake, high stability in opposition to hydrolysis, selective ion complexation of heavy metals accompanying with no affinity for alkaline and alkali earth ions leads to high concentration than that of oil and water. Also, adequate binding strength can be obtained for metal ions and selection of metal complex derived for organic phase over aqueous phase which would be of particular significance for application in environmental studies.

Other method of ethanol mediated phase is transfer of nanoparticles directly to organic phase via ethanol mediation. In this method ions are first reduced and stabilized in water and then transferred into organic phase. As this is not easy for metal nanoparticles to directly transfer to organic phase from water, therefore, citrate is first replaced with dodecyl amine or dodecanethiol and then through stirring they are transferred to organic solution. The main reason for not directly transferring the particles to the organic phase is the interaction between ligands and nanoparticles at interface which is not much efficient. As ethanol and water both are immiscible so it is easy for both nanoparticle and ligands to interact and have strong interfacial contact as in water and toluene it is difficult. The conclusion of these methods beside the synthesis of stable particles is that citrate ions are replaced from surface of nanoparticles by amine or thiol [26].

Many studies for inorganic colloidal nanoparticles such as quantum dots or gold nanoparticles have been taken place over the time in many fields. In many applications such as optics, electronics and several others have been reported. With regards to biological application applications stability is the key factors. Apart from stability in organic solvents with help of ligands and others amphiphilic polymers have used by several groups for stability of hydrophobic nanoparticles in aqueous solvent. These polymers not only enable nanoparticles phase transfer from organic to aqueous but also provide great platform for chemical modification and bio conjugation of nanoparticles because other biological molecule can be easily covalently linked to the polymer surface. It is due to the point that stability of polymer coating around the nanoparticles depends solely on hydrophobic interaction and have no role of material of inorganic nanoparticle core as happens for ligand exchange protocol. This coating of polymer and nanoparticles is highly stable due to numerous contact points. The amphiphilic polymer under discussion has two parts: hydrophobic which is used for linkage to nanoparticle and hydrophilic backbone which provides water solubility through charged group. Cheng-An J. Lin et al in this work has used a polymer whose hydrophilic part has also capacity to attach other functional molecules [27].

Chapter 3

Synthesis and Characterization of Silver Nanoparticles

For synthesis of Nanoparticles there are two approaches given as follows;

- Top-down approach
- Bottom-up approach

Top-down approach is basically to slice bulk materials by cutting it successively hence to get nano-size particles. Conversely building of material from bottom which goes atom by atom or molecule by molecule is known as Bottom-up approach. Consequently through the atom by atom deposition the self-assembly of atoms/molecules occurs and hence clusters are created. The size of these clusters depends upon a number of thermodynamic factors including, precursor concentration, temperature, pH etc.

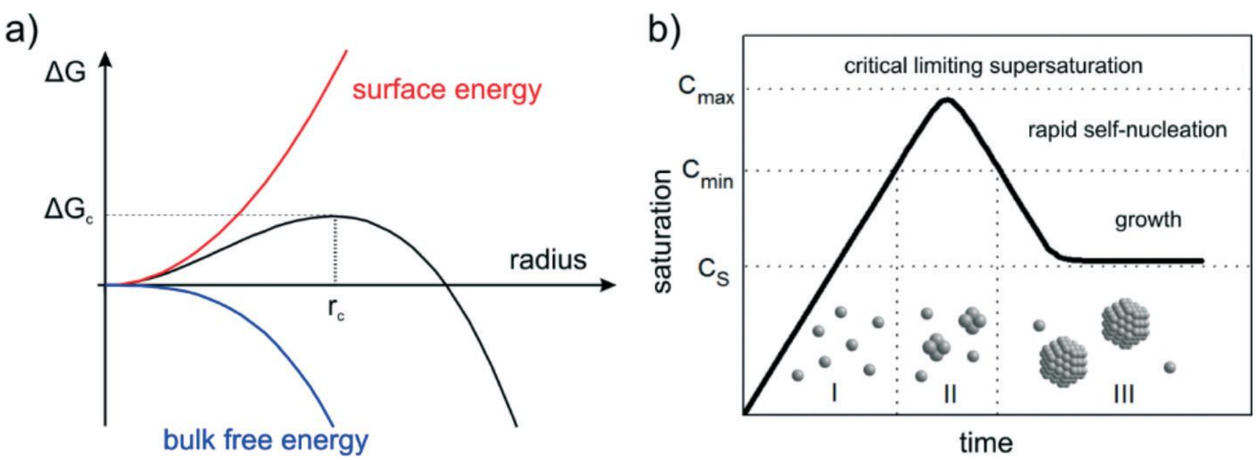

Fig 3.1 (a) The dependence of the cluster free energy, ΔG, on the cluster radius, r according to the classical nucleation theory (CNT). The curve has a maximum free energy ΔG at a critical cluster size, rc, which defines the first stable particles – the nuclei. (b) The principle of NP nucleation due to LaMer's mechanism of (sulfur) nucleation derived from CNT. The (theoretical)

qualitative curve describes the monomer concentration as a function[Fundamental growth principles of colloidal metal nanoparticles – a new perspective by Jörg Polte 2015]

3.1 Description of Apparatus

For synthesis of different materials the following apparatus are used.

- Digital Balance
- Magnetic Stirrer
- Hot Plate
- Separation funnel
- Centrifuge
- Rotary evaporator
- pH paper or pH Meter

3.2 Synthesis Process

Colloidal silver nanoparticles are dispersed in a solvent which can be water based or organic based. Synthesis processes for both were discussed in details in previous chapter. Here our goal is to synthesize silver nanoparticle via phase transfer. There are two potential methods for phase transfer of nanoparticles Brust method and ethanol mediated phase transfer method. The main difference in both methods is that in one method ions are transferred to organic solution and then reduced while in other methods synthesized nanoparticles are transferred to organic solution. The method we used here to synthesized nanoparticles is ethanol mediated phase transfer in which dodecyl amine and dodecane thiol acted both as phase transfer agent and stabilizing agent. The nanoparticles synthesized via using dodecane thiol were not reproducible so the successful method here was ethanol mediated phase transfer via dodecyl amine.

3.3 Materials required during Synthesis

- Silver nitrate
- Sodium citrate
- Sodium boro hydride
- De ionized water
- Ethanol
- Toluene
- Methanol

3.4 Synthesis of nanoparticles

For synthesis of stable silver nanoparticles, three different approaches were tried. In the first approach we the Brust method was used in which initially ions were transferred through tetra octyl ammonium bromide (TOAB), dodecane thiol was added to solution and then reduced through sodium borohydride. But this method was not effective as the particles were not stable and settled down after 48 hours. In the second approach citrate capped nanoparticles were first synthesized in aqueous solution via Turkevich method and subsequently transferred to toluene by ligand exchange using dodecanethiol as a surfactant. The ligand exchange was accomplished at the interface of water and toluene in the presence of ethanol. In the third approach, the above procedure was repeated however, dodecanethiol was replaced with dodecylamine.

3.4.1 Synthesis of citrate capped silver nanoparticles

10 ml of 1 mM aqueous solution silver nitrate was synthesized and stirred at room temperature. 1 ml of 40 mM aqueous solution of sodium citrate was mixed with silver nitrate solution and both are stirred for 20 minutes. 112 mM of sodium borohydride aqueous solution was put drop wise in to the solution then vigorously stirred. For 10 ml of silver nitrate solution 100 µl of sodium borohydride was added to synthesized hydrosol of silver in which sodium citrate served as a

stabilizer. The solution was vigorously stirred for two hours at room temperature to finally obtain citrate stabilized silver nanoparticles.

3.4.2 Phase transfer of silver nanoparticle

For transfer of silver nanoparticles to toluene 200 µl of dodecyl amine was mixed in 10 ml of ethanol and mixed with 10 ml of silver nanoparticles solution. The mixture was stirred for 3 minutes at room temperature. After three minutes of stirring 5 ml of toluene was added to the mixture and stirring was continued for two more minutes. After addition of toluene change in the solution was clearly visible which indicated the transfer phenomenon. As a result of transfer from the solution containing water and ethanol became colorless. Solution was then transferred to separation funnel and both solutions were separated. After separation silver nanoparticles are stirred overnight at65 °C.

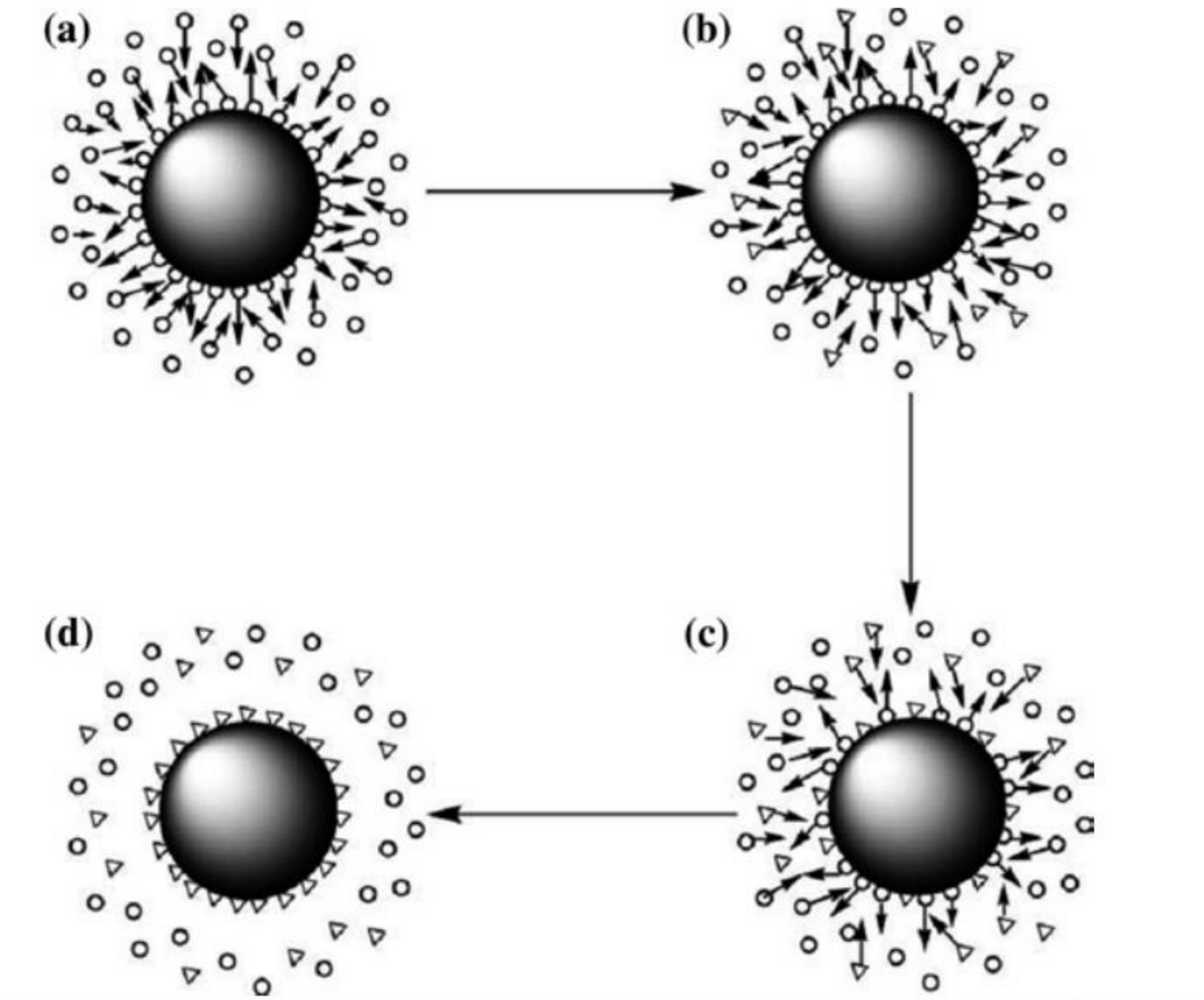

Fig3.2 Schematics of the process of displacing citrate from nanoparticle surface by NH 2 – (or SH–). Open circle citrate ions; triangle NH 2 – (or SH–).ig

3.4.3. Purification of silver nanoparticles

The prepared material contained free ligands and bigger aggregates. After synthesizing silver nanoparticles solution was mixed with methanol to get rid of free ligands and bigger aggregates which settle down. The ratio between nanoparticle and methanol has been kept at 1:10. Mixture was initially kept for 30 minutes to settle down and then transferred into centrifuge tube and centrifuged at 5000 rpm for 10 minutes. After centrifugation the supernatant was removed and remaining particles were suspended in toluene for further storage.

3.5 Polymer coating of silver nanoparticles

After purification, silver nanoparticles were coated with amphiphilic polymer. For this briefly, 150 µl of 0.8 M polymer solution in chloroform was mixed with 5 ml of silver nanoparticles in rotary evaporator for 60 min at a temperature of 65-70°C After 60 min the toluene was evaporated with the help of vacuum pump. The process was repeated three times. Finally the sample was mixed with sodium borate buffer pH 12 and suspended for further use.

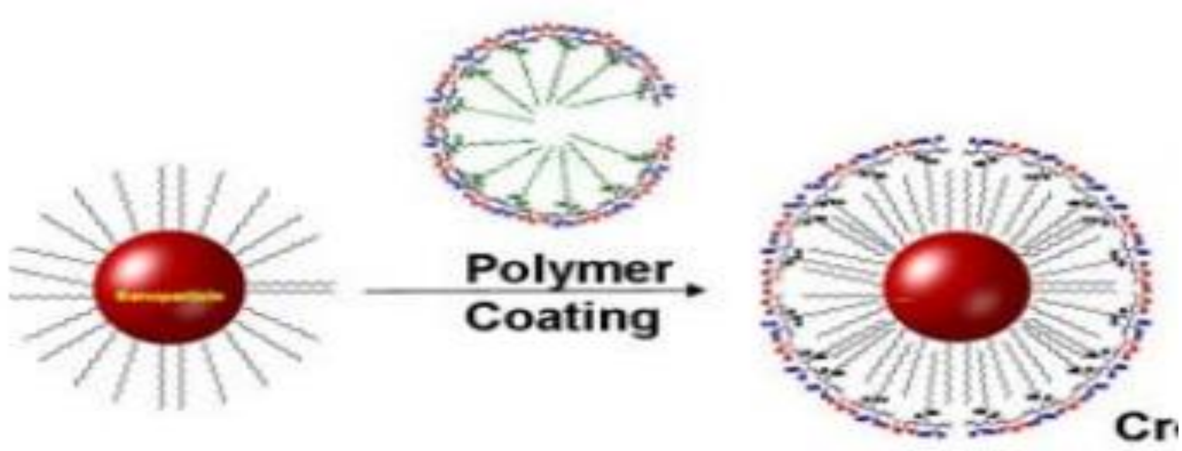

Fig 3.3 schematic of polymer coated silver nanoparticle

3.6 Drug attachment with silver nanoparticles

Polymer coated nanoparticles were attached with an anticancer drug viz. methatrecate. As here for linkage of nanoparticles with methatrexate a cross linker EDC (1-ethyl-3-(3-dimythylaminopropyl)carbodiimide) have been used. Aqueous solutions of EDC (0.768M) and metatrexate (0.5mM) were prepared in Sodium Borate Buffer (SBB, pH 9). For the purpose of conjugation, the concentration of EDC was optimized. For this different concentrations of EDC (refer to Table 3.1) were initially mixed with nanoparticles and the mixture was incubated for 45 minutes at room temperature in order to activate the carboxylic groups on the surface of nanoparticles. After 45 minutes drug solution was mixed with the activated particles and incubated for further one hour.

Nanoparticles concentration(10µl)	EDC concentration(10µl)	DRUG concentration(10µl)
2.3µM	0.3815M	0.5mM
2.3µM	0.1905M	0.5mM
2.3µM	0.0952M	0.5mM
2.3µM	0.0476M	0.5mM
2.3µM	0.0238M	0.5mM
2.3µM	0.0129M	0.5mM
2.3µM	0.0064M	0.5mM
2.3µM	0.0032M	0.5mM
2.3µM	0.0016M	0.5mM
2.3µM	0.0008M	0.5mM
2.3µM	0.0004M	0.5mM

Table 3.1 drug attached to polymer coated silver nanoparticles

Attachement of drugs with silver nanoparticles was further proved by gel electrophoresis.

Chapter 4

Characterization and application

4.1 Characterization of Silver Nano particle

4.1.1 UV-Vis spectroscopy

This technique is applied to determine the optical properties of materials. Absorbance, transmittance properties can be studied by this technique. It measures the absorption of light beam after it passes from a sample. This absorption can be of single wavelength or of extended spectral range.

A spectrophotometer used collimated electromagnetic (EM) radiations from ultraviolent (UV) region to far infrared region (IR) of electromagnetic spectrum. These radiations are made to fall on the sample. To calculate the transmitted beam intensity we use wavelength's range of incident radiations. A spectrometer is present in this instrument which records the amount of absorpotion of different wavelengths of sample. The resultant plot of absorbance (A) versus wavelength (λ) is obtained which is called a spectrum.

4.1.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FT-IR) technique is used to get information about the attached molecules on the surface of nanoparticles. The objective of any absorption spectroscopy is to quantify how fine a sample absorbs light at each wavelength. The most straight forward approach to do this is by shining a beam of monochromatic light at the specimen, and measure how much part of light is retained, and repeat for each diverse wavelength. As we can observe that a monochromatic beam of light having full spectrum of wavelength is obtained from Michelson interferometer. Distinctive wavelength is squeezed at various rates so that every beam coming out has distinctive spectrum. As shown in given fig. raw data obtained is called "interferogram" and then this data is processed through a computer to get desired results.

4.2. Uv visible spectroscopy

Uv visible spectroscopy of nanoparticles were recorded at room temperature using molecular probe spectromax spectrometer, using 1 cm path length glass cuvette and fixed slit width 2nm. For citrate capped silver nanoparticles baseline was corrected with the help of deionized water and spectra was recorded and absorbance peak observed between 300 and 500nm. The same was done for dodecyl amine capped silver nanoparticle but the base line was set with the help of organic solvent. Spectra here was observed between 200-1000nm and absorbance peak was observed between 300 and 600nm.

As we can clearly see in cit-Agnps spectrum that it has highest peak at 399nm and its width shows us that size distribution of nanoparticles is minimal.

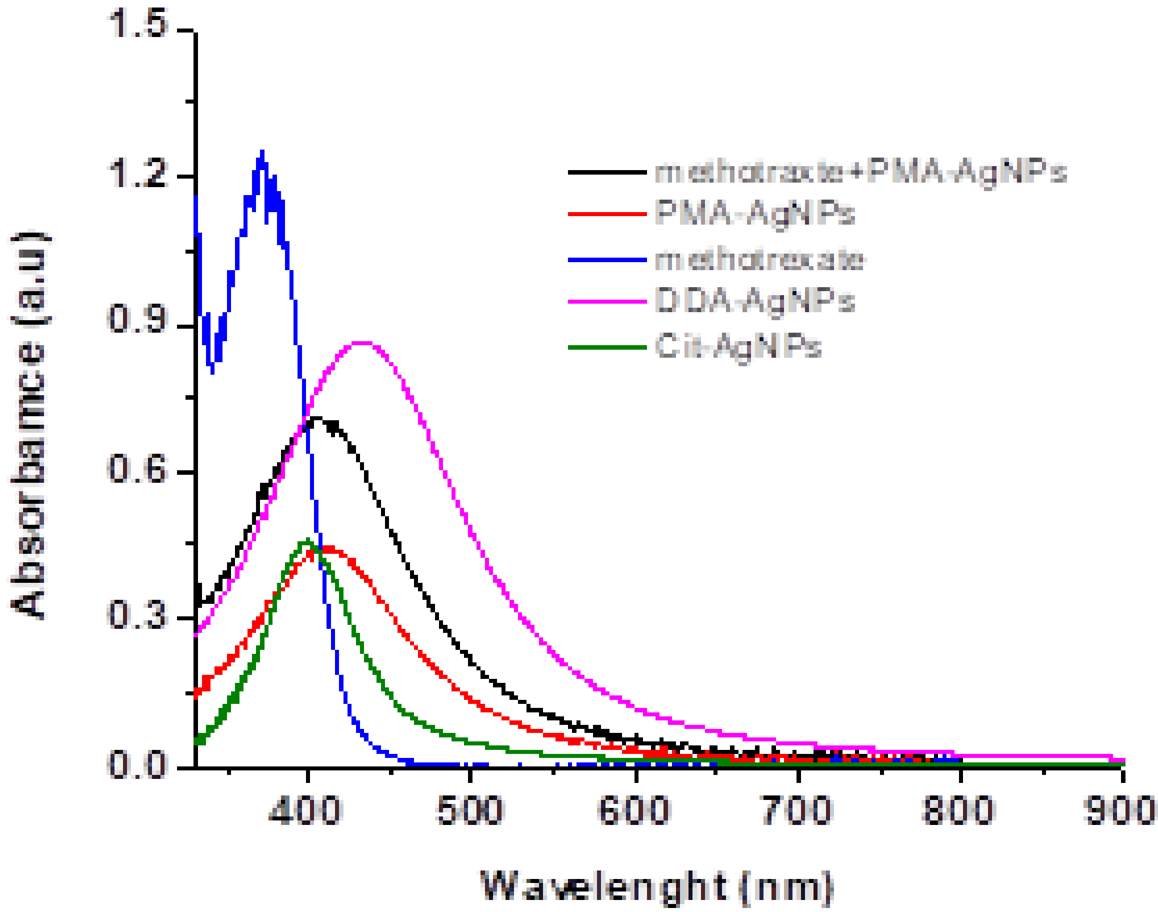

fig4.1 comparison uv-spectrum PMA-agnps , MTT+PMA-agnps, methatrexate

As the particles are transferred from water to toluene there is a phenomenal change observed in the spectrum. There is strong red shift occur in the spectrum from 399 nm to 433 nm which shows an increase in the size of nanoparticles. It can also be observed that width of the spectrum increases which mean that size spread of nanoparticles also increases. After polymer coating again we can observe a blue shift as absorbance is observed at 410 nm which means that nanoparticles of small size are coated and also width of peak decreased. From the spectrum of drug attached polymer coated silver nanoparticles it can be observed that there are two peaks one at 299 nm and 407 nm. From it can be seen a little blue shift comparing its peak with methotrexate drug spectrum it can be said that other peak belong to methotrexate.

4.2. Gel elecroporysis

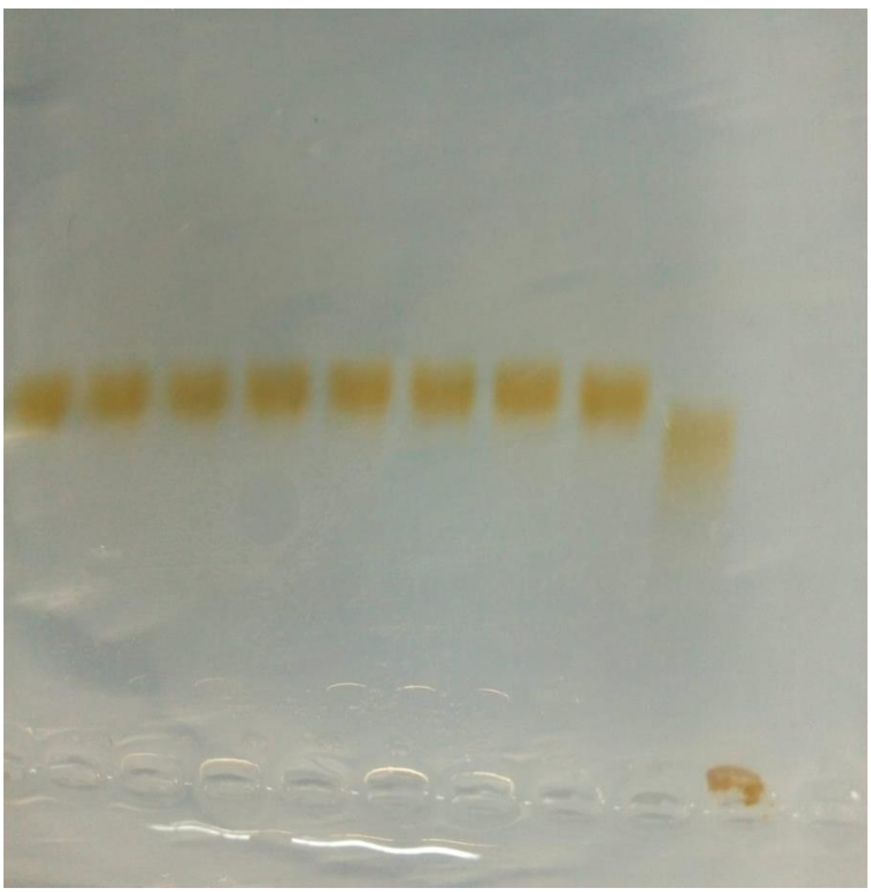

Fig4.2 gel electrophoresis of meth+Agnps with different concentrations of EDC

As we have discussed previously that polymer coated silver nanoparticles are attached with methotrexate drug with the help of EDC different concentration. To verify the attachment sample were loaded to gel and voltage was applied. In the figure we can easily see the movement of particle from positive to negative point. We have find that how different concentration effect the drug attachment. High concentration of EDC which in our case is 0.3815M cannot able to attached the particle with drug. But as concentration of EDC halved the effect can clearly be observed. In figure the last sample to the right is only Ag nps.

4.3. Ftir analysis

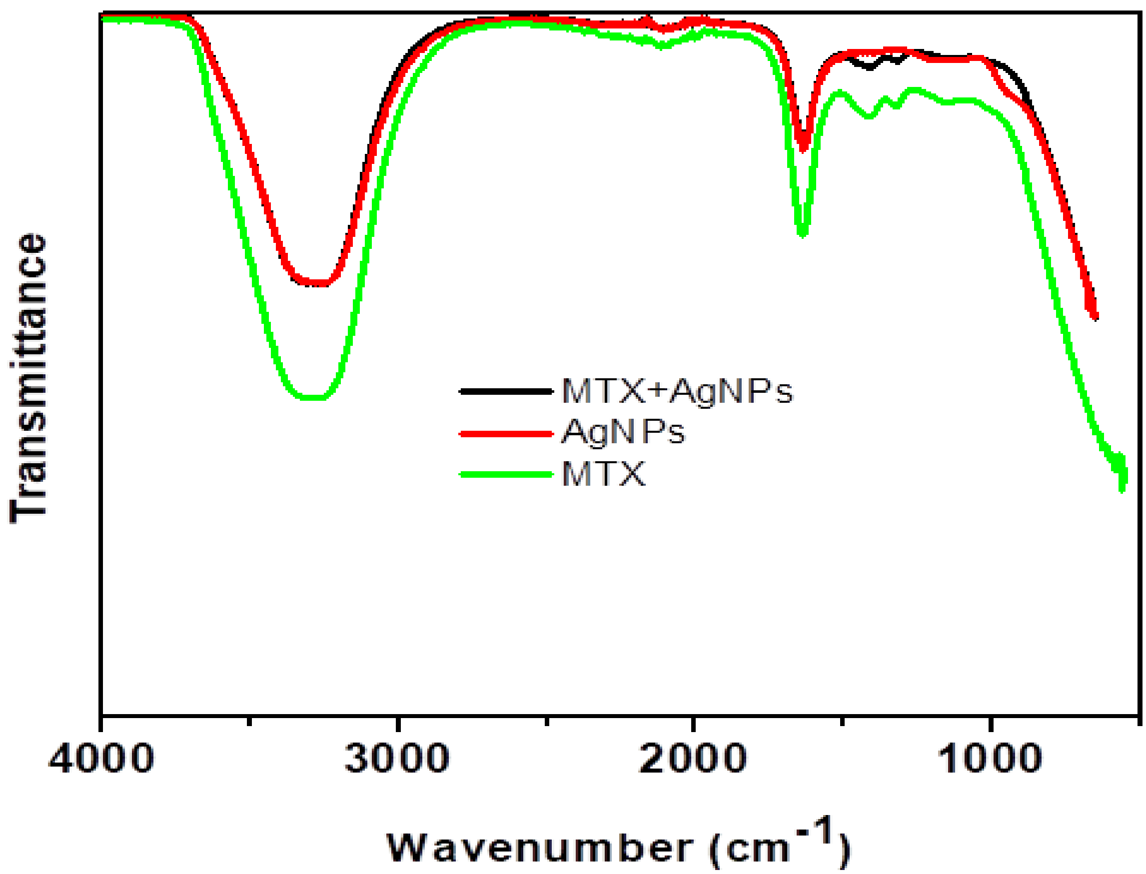

fig4.9 ftir comparison graph between methotrexate, silver nps and meth+agnps

Ftir spectroscopy is very important for the characteristic of the amphiphilic polymer coated and methatrexate attached polymer coated silver nanoparticles it can easily be observed the three main peaks in all three samples which are at 3728cm^{-1}, 1634.92cm^{-1} and 2103.5cm^{-1}. The band at 1634cm^{-1} shows bending of amines band of vibration and their corresponding stretching is at

3728cm^{-1}. by comparing spectra of methotrexate and methotrexate attached silver nanoparticles there is actually no difference between them beside that the width of peaks in methotrexate are broadened but when they are attached to silver naoparticles their width shrinks. While comparing these both with polymer coated silver nanoparticles it can clearly be observed that there two extra peak both in methotrexate and methotrexate attached silver nanoparticles which are at1451cm^{-1} and 1321cm^{-1}. While polymer coated silver nps have a shows a little bump at 960cm^{-1} which is nowhere in others two spectrum.

4.4. Anti bacterial analysis

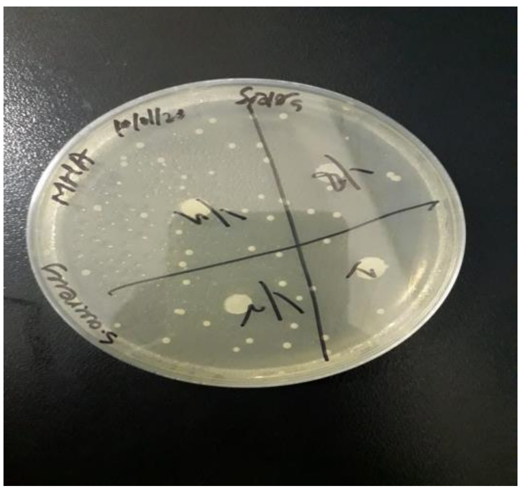

Fig4.10 S.aureus bacteria

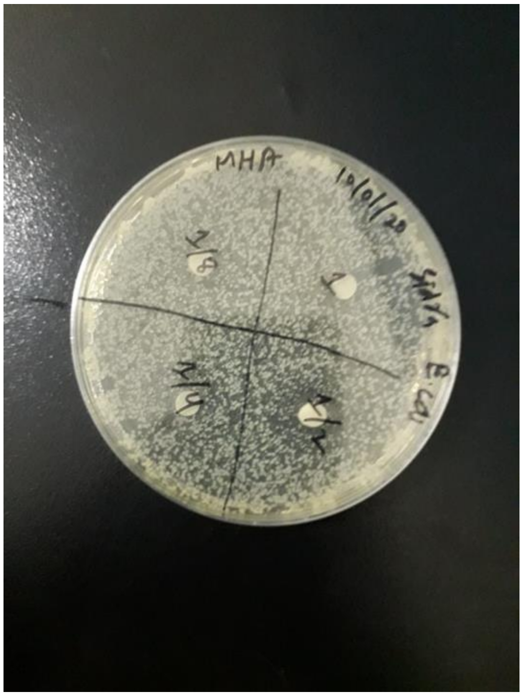

Fig4.11 E.coli becteria

Fig4.12 Klebesiela pneumonia

Silver nanoparticles were applied to three different bacteria E.coli, S.aureus and klebsiella pneumonia and were assesed for 24 hours.it was found that the applied cocentrations of methatraxate loaded silver nanoparticles have no effect on the becteria as can be observed from figures. Hence it can concluded from the figure that polymer coated silver nanoparticles has no effect on these becterias. The study has been done at three different concentration of nanoparticles i-e 2.3 micrometer, 1.15 micrometer and 0.57 micrometer the result for all were same and there was no effect on bacteria.

4.5. MTT analysis

4.5.1. Materials And Method

The MTT analysis is the most common type of Viability Assay performed involving cell lines. MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) a dye which is used to measure in vitro cell proliferation. Tetrazolium salts is commonly used in cell biology for determining the metabolic activity of cells from microbial origin to mammalian cells. For this purpose, HuH-7 cells cultured, using DMEM with the solution having 10% FBS and 1% Penicillin-Streptomycin, at 37°C for 24 hours. When the cultures reached desired confluence, 10,000 cells were plated in every well of a 96-well culture plate which was then incubated in CO_2 incubator for 24 hours. The concentrations of the compounds were prepared by dissolving them in deionized water and making subsequent dilutions. The drug dilutions along with the solvent control were loaded in the 96-well culture plate with the final volume of 200ul per well and the plate was incubated for another 24 hrs. MTT was prepared in the medium to a final concentration of 5 mg/ml. The plate was then inoculated with 15ul of prepared MTT in each well and incubated for 3 hours at 37°C, until intracellular purple formazan crystals became visible under microscope. Then MTT was removed and solubilizing solution i.e., 150 ul DMSO was added in every well. The Incubation at room temperature was done for few minutes while pipetting up and down the materials of each well, so that the cells had been lysed and purple crystals had been dissolved. The plate was covered in foil to protect from light and immediate measurement of the absorbance in Spectrophotometer was carried out at 550nm wavelength. The experiment was performed in triplicates and the results are the average of the three experiments.

4.5.2. Results

Following data was obtained by testing varying concentrations per microlitre of each compound on HuH7 cells.

4.5.2.1. Nanoparticles

Nanoparticles	**Control**	**1pM**	**10pM**	**20pM**	**Borate Buffer (12pH)**	**Borate buffer (9pH)**
Percentage viability of cells	**100%**	**7.2%**	**7.22%**	**7.12%**	**23.7%**	**23.1%**

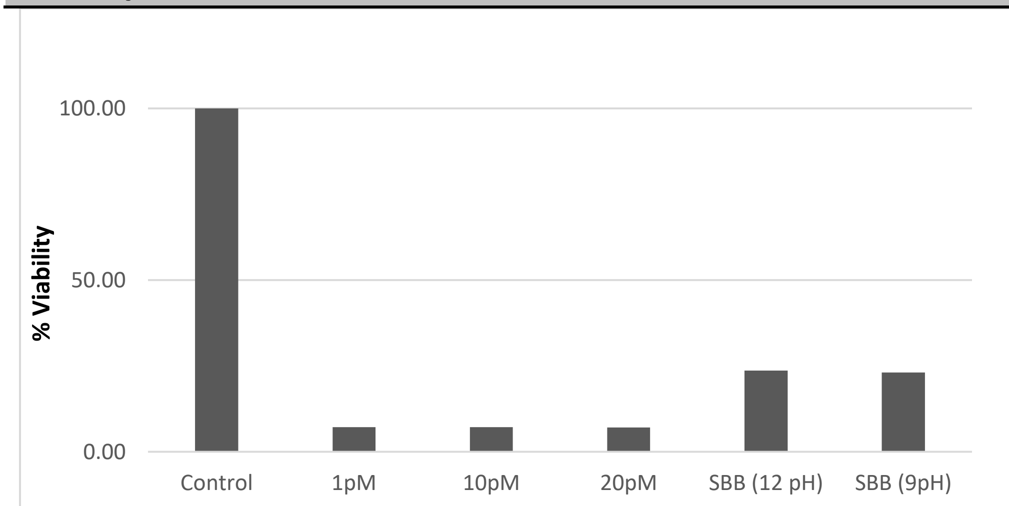

Fig.4.13 Nanoparticles activity on cells. The increasing concentration of the particles shows cytotoxic effect on the cells.

4.5.2.2 Methatrexate Drug + Nanoparticles

Drug loaded NP	Control	0.5pM	1pM	2pM
Percentage viability of cells	**100%**	**7.15%**	**7.7%**	**5.6%**

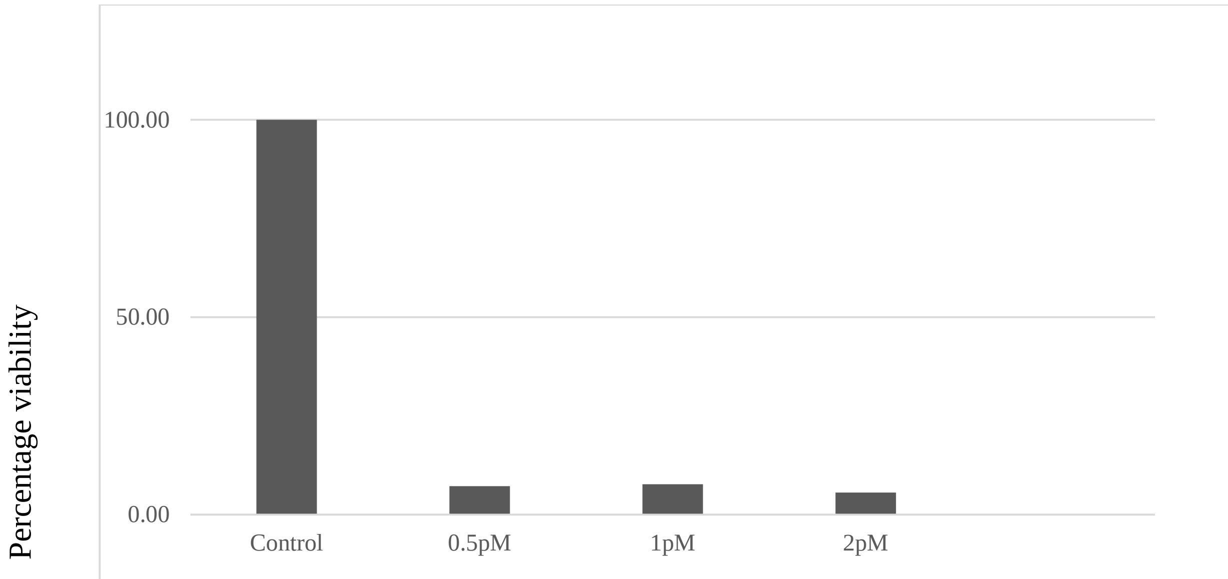

Fig 4.14: The activity of Drug plus Nanoparticles on huh7 cells. The compound shows cytotoxic effect on the cells.

4.5.2.3. Methatrexate Drug

Methatrexate Drug	Control	0.25mM	0.5 mM	1mM
Percentage viability of cells	**100%**	**5.5%**	**5.5%**	**3.88%**

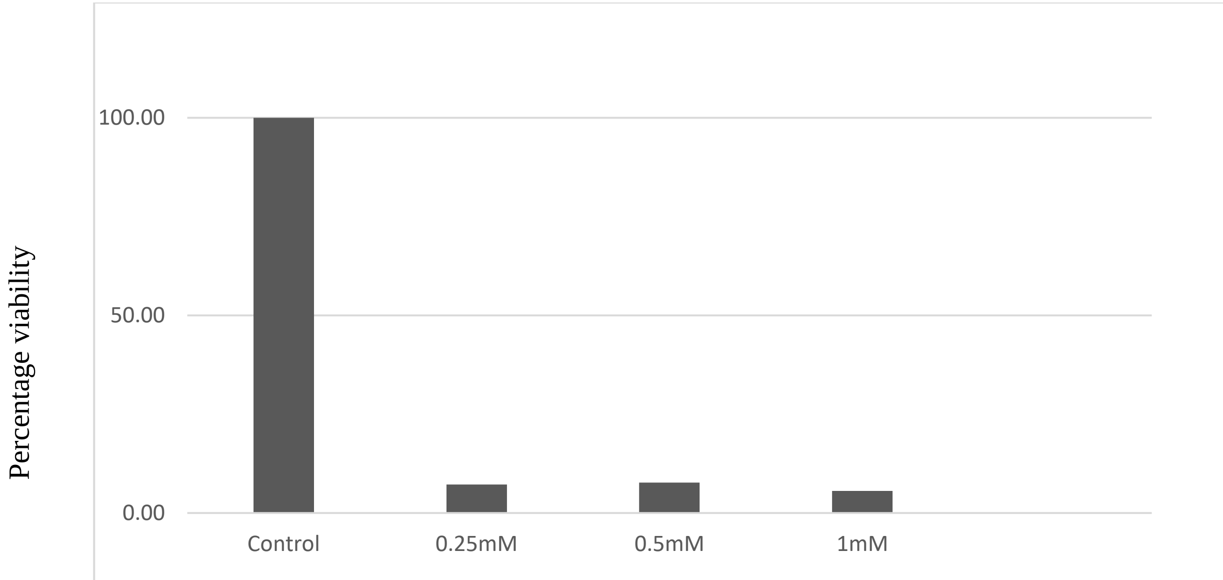

Fig 4.15: The drug shows cytotoxic effect on cells even at the minimum concentration (0.10M)

The afore-mentioned results were obtained from Spectrophotometric analysis after normalized interpretations. The normalization has been done by eliminating the blank absorbance from all the samples. After subtracting blank absorbance reading from both sample and control the sample result is divided by control result and multiplied by 100 to find percentage as mentioned in below formula. The value greater than control indicates cell proliferation and lower value suggest cell death.

$$\% \text{ viable cells} = \frac{(abs_{sample} - abs_{blank})}{(abs_{control} - abs_{blank})} \times 100$$

Chapter 5

Conclusion and future recommendation

To use metal nanoparticles in application it needs long term stability which is can achieved by synthesizing the colloidal nanoparticles through phase transfer approach. Silver nanoparticle has been successfully synthesized via phase transfer method which was confirmed from Uv-spectroscopy. For further use and stabilization nanoparticles were coated with hydrophilic polymer which enables it for further functionalizing. By comparing the Uv-spectrum of all three types of silver nanoparticles which are cit-AgNps, DDA-AgNps and PPA-Agnps it can be concluded the size distribution curve has been improved. Polymer coated nanoparticles were applied on bacteria which does not have any effect on bacteria. In MMT analysis it can be easily proved that with the help of nanoparticles less concentration of drug can be effective and cell death can occurred. For future we can use these methotrexate attached nanoparticles against other cancer cells like breast cancer etc.

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