

## Role of Silver Nanoparticles in Colorimetric Detection of Biomolecules

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**Abstract:** Nanotechnology deals with the study and manipulation of materials on an atomic or a molecular scale almost less than 100 nm. Nanotechnology encompasses the synthesis and applications of biological, chemical and physical systems which ranges from individual atom to molecular or submicron level. It is the more interesting field for the scientists due to various applications in different fields of science e.g., biomedical engineering, material science, medicine and electronics at nanoscale level. The particles ranges from 1 to 100 nm by a surrounded interfacial layer are basically called nanoparticles. Ag nanoparticles are the most fascinating and vital nanoparticles amongst the other metallic nanoparticles like gold, tin, palladium etc. In this review literature, the synthesis and properties of Ag nanoparticles have been discussed. Herein, main area of study interest is to find biomolecules by using conventional as well as colorimetric detection methods with silver nanoparticles. Synthesis and characterization of Ag nanoparticles is an immense attention in nanotechnology due to its wide range applications in industries. Ag nanoparticles are synthesized by different methods (physical, chemical, biological and photochemical methods), amongst these methods green synthesis method is most favorable method, because it is cost effective and eco friendly method. Ag nanoparticles have various significant properties such as antibacterial, antifungal, antiviral, anti-cancer, and anti-angiogenic etc. Nanoparticles possess many applications in different fields of life e.g., medicine, biomedical devices, multicolor optical coding, manipulation of biomolecule (proteins, DNA, cysteine), environmental remediation and cosmetics. The aim of prospective applications is to investigate the colorimetric and optical detection of biomolecule by Ag nanoparticles, e.g., dopamine, proteins, DNA etc. because of its highly sensitivity and selectivity. The colorimetric detection of various biomolecules by Ag nanoparticles has inherent chirality. Ag nanoparticles provides high surface to volume ratio and function for detection of proteins, nucleic acid, ions and molecules. Ag nanoparticles as colorimetric probes and chiral selector are helpful in determining citalopramenantiomers. However, we can easily read out the assay described in this work with naked eye and by use of ultra violet spectrometer.

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**Key words:** silver nanoparticles; colorimetric detection; biomolecule; applications

### Introduction

Nanotechnology deals with the study and manipulation of materials on an atomic or a molecular scale almost less than 100 nm (Ali et al., 2016). It involves the study and development of different materials at nano scale (less than 100 nm). It is the more interesting field for the scientists due to various applications in different fields of science e.g., biomedical engineering, material science, medicine and electronics at nanoscale level (Bhattacharyya, Singh, Satnalika, Khandelwal, & Jeon, 2009). It enables the scientists to control properties at nanoscale and the material formed has distinct properties about 1 to 100 nm (Williams, 2008).

The particles having size b/w 1 to 100 nm by a surrounded interfacial layer are basically called as nanoparticles (Zong et al., 2005). Nanoparticles possess a wide range of applications in different fields

of life e.g., medicine, biomedical devices, multicolor optical coding, manipulation of biomolecule (proteins, DNA, cysteine), environmental remediation and cosmetics (De, Ghosh, & Rotello, 2008). Ag nanoparticles have fascinated significance due to define physico-chemical and biological properties (Tran & Le, 2013). Nanoparticles are natural, incidentally or artificially prepared at nanoscale in an agglomerate state, as an aggregate and an unbound state with dimension and size range of 1-100 nm (Flanagan, Uyarra, & Laranja, 2011).

The aim of this perspective review to investigate the colorimetric and optical detection of biomolecule by Ag nanoparticles, e.g., dopamine, proteins, DNA etc. because of its highly sensitivity and selectivity. Nanotechnology involves the nanomaterials with the attention of different biomolecule such as proteins, carbohydrates at cellular level. The biological system

have dynamic self-assembly process so a DNA molecule replicates itself within the cell (Eijkel & van den Berg, 2006).

The colorimetric detection of various biomolecules by Ag nanoparticles has inherent chirality. Ag nanoparticles provides high surface to volume ratio and function for detection of proteins, nucleic acid, ions and molecules. Therefore, Ag nanoparticles have a very few access for the recognition that based on inherent chirality. For example, Citalopram is an antidepressant drug that is used primarily for the treatment of CNS diseases, and for the treatment of depression like, panic disorder, social phobia etc. (Halas, Lal, Chang, Link, & Nordlander, 2011). The visual chiral recognition methods of CIT by using Ag nanoparticles as colorimetric probes and chiral selector are helpful in determining citalopram enantiomers. These have low cost, easy to handle, and ready availability. However, we can easily read out the assay described in this work with naked eye and by use of ultra violet spectrometer (Tashkhourian, Afsharinejad, & Zolghadr, 2016).

### Background

About 2000 years ago, nanotechnology was used during prominent sword of Tipu Sultan when Indian artisans and craftsmen use carbon nanoparticles to make long lasting cave painting (Kingdom of Mysore, Ajanta paintings). In 1902 the first observation and size measurement of nanomaterials were practiced by Richard Zsigmondy by using ultra microscope. Two inventions in the fields of nanotechnology was made that permit the imaging of individual atoms, molecules as well as their treatment that led to significant progress in nanotechnology (Ochekpe, Olorunfemi, & Ngwuluka, 2009). In, Eric Drexler worked began more popular to nanotechnology through books or speeches "Engines of Creation: Coming era of Nanotechnology". Saumio Iijima in 1991, discovered 'C' nanotubes, so the national nanotechnology institute is launched in 2000 (Ochekpe et al., 2009). There is unreliable evidence for the use of Ag nanoparticles in Egypt and Rome (Reidy, Haase, Luch, Dawson, & Lynch, 2013).

The Ag nanoparticles were used by Macedonians for the treatment of ulcer, wound healing and Hippocrates (Damschroder et al., 2009). Ag nitrate solution was to treat the eye infection in new borns by Crede (McGillicuddy et al., 2017). The purity of liquids such as water, milk, vinegar, and wine was historically increased by use of Ag vessels (Damschroder et al., 2009). Ag dollars were used by American pioneers to prevent milk spoilage and in 1954 Ag material, colloidal, suspension were registered as a biocidal materials used in medications

(Reidy et al., 2013). This wide history and a variety of implications of Ag nanoparticles has led to interesting field for the researcher on its environmental and chemical properties (Michel et al., 2011).

### Synthesis of Silver nano particles

There are available a number of synthesis methods of Ag nanoparticles, e.g. by physical, chemical, biological, electrochemical, radiation methods (Yin, Ma, Wang, & Chen, 2003). Due to the variations in particle size, size distribution, toxic effect, cost, scalability, environmental impact, and energy consumption, so each method has some advantages and disadvantages and the green synthesis approach is more reliable and preferable (Ankamwar, Damle, Ahmad, & Sastry, 2005). There are basically present two main approaches for the synthesis of nanoparticles 1) Bottom-Up approach and 2) Top-Down approach.

#### Bottom-up approach

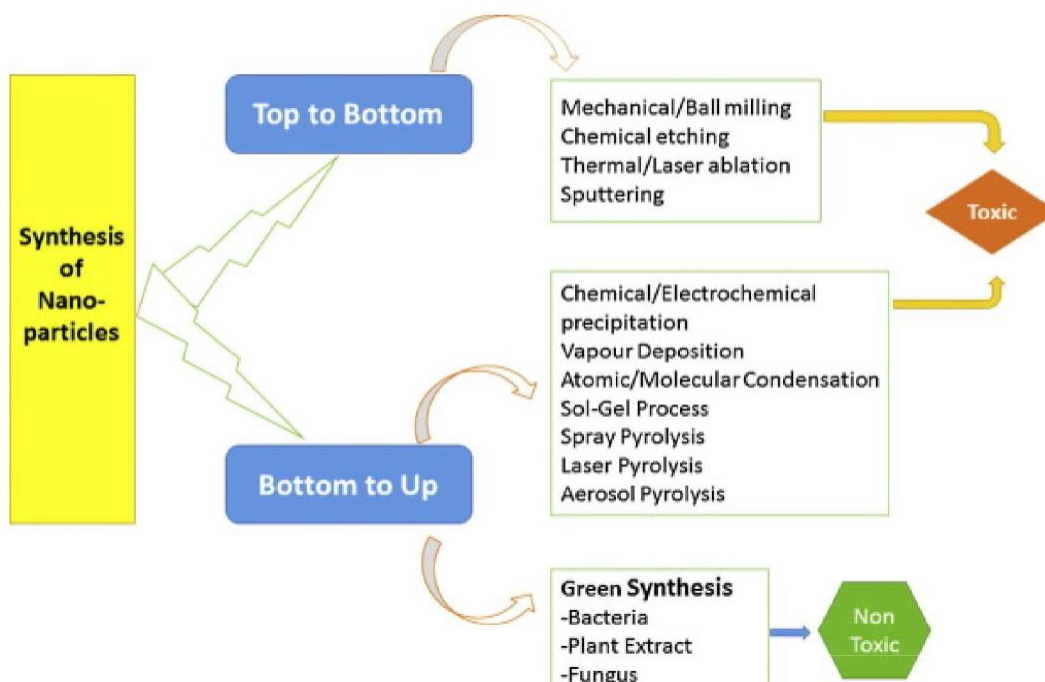
The Ag nanoparticles are achieved by growing atoms or molecules, are basic building blocks of nanomaterials and are self assembled, manipulated through chemical reactions. Atoms or molecules can be assembled by templating or non-templating under specific sequence, structure, pattern, special constraint, external force. Nano particles below 100 nm are prepared by a technique known as "Self Assembly Lithography" which is cost effective technique. However, the random movement of atoms is one of the peculiar challenge of the Bottom Up Approach (Ochekpe et al., 2009).

#### Top-Down approach

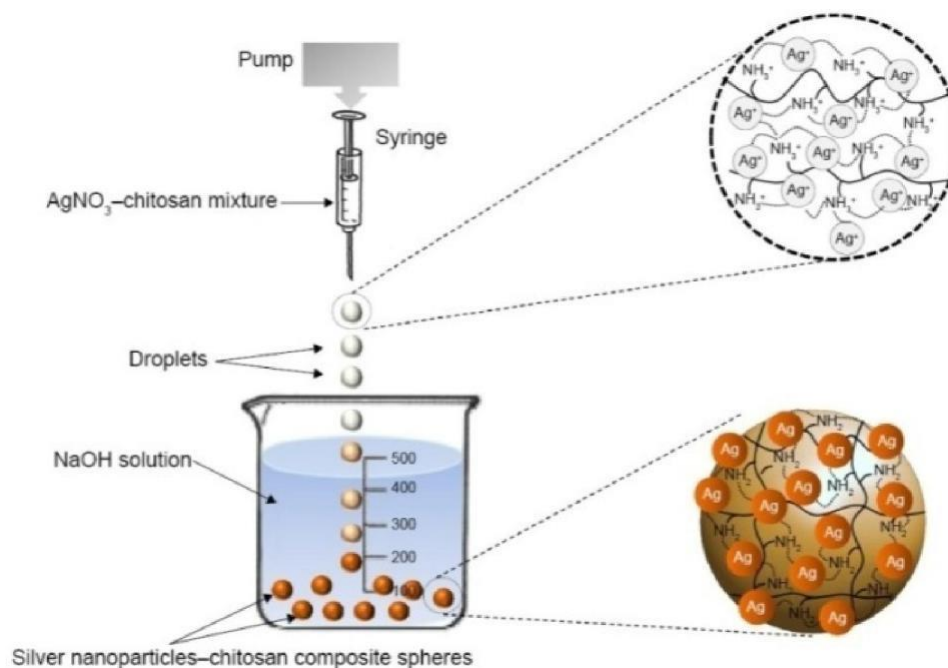
In this approach, the bulk materials reduced by some techniques that result the formation of nanostructures. This process involves the breaking, cutting, and etching of bulk material and the whole procedure is involved step by step employing lithography e.g., film machining, surface machining, and mold machining, x-ray, electron beam lithography etc (Ochekpe et al., 2009).

### Chemical synthesis

Chemical methods are mostly used for the synthesis of Ag nanoparticles. Monodisperse samples of Ag nanocubes are synthesized by reducing Ag Nitrate with Glycol and treated with PVP; this process is known as polyol process (Y. Sun & Xia, 2002). ETHYLENE GLYCOL acts as both "reductant" as well as "solvent". The size and geometric shape of the product is dependent on PVP and its molar ratios with Ag nitrate. We can synthesize the required size of Ag nanoparticles by polyol process or with the modified precursor injection technique by controlling the experimental conditions (D. Kim, Jeong, & Moon, 2006).



**Fig.1** Different approaches of synthesis of silver nanoparticles(Cao, Jin, & Mirkin, 2001).



**Fig. 2** Chemical method for synthesis of Ag nanoparticles(D. Kim et al., 2006).

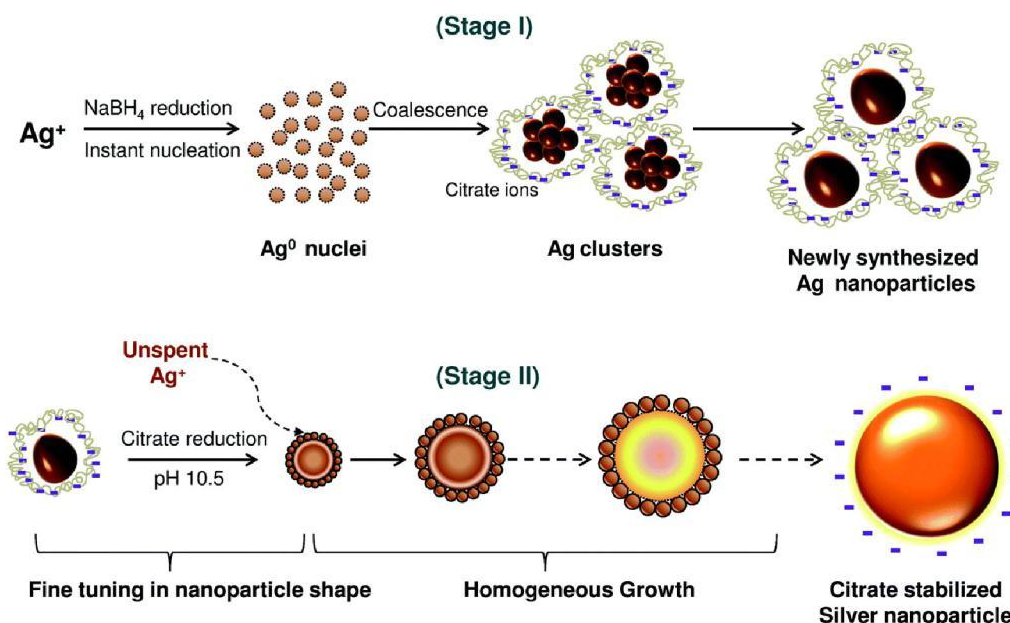
Monodisperse Ag nanoparticles are prepared by oleylamine-liquid paraffin. Basically herein 3 different chemical are used: Ag Nitrate, liquid paraffin and oleylamine. This process consists of three stages; 1) Growth 2) Incubation, and 3) Ostwald ripening. Higher boiling point of Paraffin helps to yield at controllable size otherwise size of Ag nanoparticles is

not controllable by changing with heating temperature, or ripening time (M. Chen et al., 2007).

The synthesis of Ag nanoparticles in solution has following main steps: a) metal precursor, b) reducing agents, c) stabilizing or capping agents. The reduction of Ag salt is reduced into following 2 steps nucleation and Subsequent growth and these steps control the size

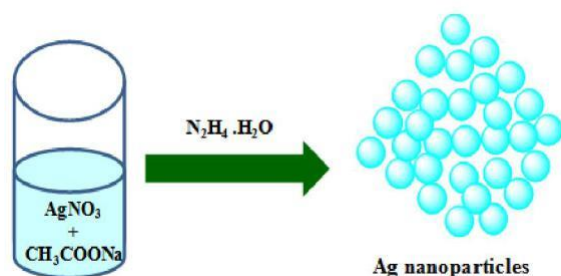
and shapes of Ag nanoparticles. Moreover, mono dispersed Ag nanoparticles with uniform nuclei and size distributions have same time and also have the same subsequent growth (M. Chen et al., 2007). The

initial nucleation process is controlled by pH, reducing or stabilizing agents and precursors (S.-F. Chen & Zhang, 2012).



**Fig. 3** Size controlled synthesis of Ag nanoparticles by Co-reduction method (Rao, Kulkarni, Thomas, & Edwards, 2002).

### Physical synthesis



**Fig. 4** Synthesis of Silver nanoparticles by Evaporation-condensation method (a Physical method) (Kruis et al., 2000)

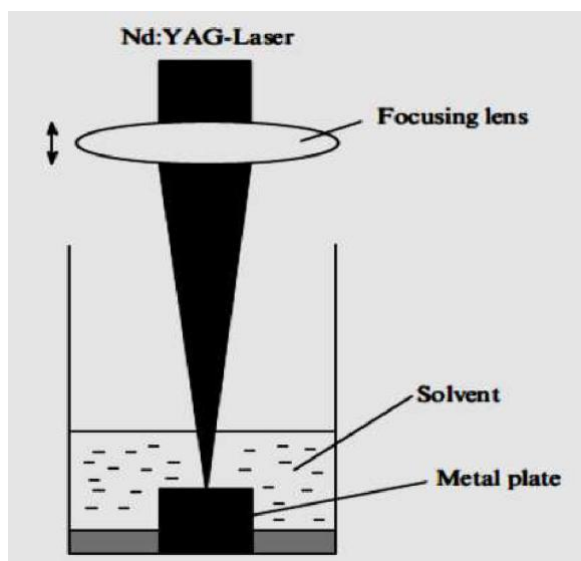
Evaporation-condensation and laser ablation methods are the most significant and former physical methods. Condensation-evaporation method involves the use of tube furnace at (atm P). By using tube furnace, there is some drawbacks e.g. High energy consumption, higher environmental temperature near to the source and large space of tube furnac, and too much time required for thermal stability (D. K. Lee & Kang, 2004). The synthesis of silver nanoparticles through physical methods involves the uniform

distribution of metallic nanoparticles and also the thin film prepared in the absence of solvent contamination. A solution mixture of 'Ag' nitrate and 'Na' acetate is heated in tube furnace and it converts the liquid into gas. Then there will be the formation of Ag nanoparticles after cooling process. A several kilowatts of power is required by a typical furnace and also required a preheating time to attain a stable temperature (Kruis, Fissan, & Rellinghaus, 2000).

Silver nanoparticles can also be synthesized by using a small ceramic heater and local heating area. The heating surface is maintained and temperature fluctuation made stabilized and that's why the evaporated vapors are cooled down at rapid rate and the generation of Ag nanoparticles is very stable. For long term experiments this method is useful as a measurement equipment for Ag nanoparticles and for inhalation toxicity studies (Jung, Oh, Noh, Ji, & Kim, 2006).

Ag Nanoparticles can be synthesized by laser ablation method. This method is more efficient and it depends upon the different parameters e.g. duration of laser pulses (in Femto, Pico, and Nanosecond), wavelength of laser interrupt the metallic target, presence or absence of surfactants, and effective liquid medium (Jung et al., 2006).





**Fig. 5** laser ablation method (Jung et al., 2006).

Laser ablation technique has command over as other techniques because it produces of metallic nanoparticles in the absence of chemicals and pure, uncontaminated product is formed. Ag nanospheroids (20-50 nm) are prepared with “Femtosecond Laser” in water at pulse rate of 800 nm. The size and the

effectiveness of colloidal metallic particles formation prepared in femtosecond are compared with nanosecond laser pulses. Therefore, the efficiency of Nanosecond Pulses is greater than that of Femtosecond Pulses. However results deducted that “The size of colloids prepared by Nanosecond Pulses are more dispersed than the colloids prepared by femtosecond pulses” (Tien, Tseng, Liao, Huang, & Tsung, 2008).

#### Photochemical synthesis

There are actually present two synthetic strategies which are categorized into two different approaches **a)** Photophysical Top Down & **b)** Photochemical Bottom Up. In case of photophysical method, the metallic nanoparticles are prepared by the breaking bulk metals, while the photochemical method develops metallic nanoparticles by the ionic precursors. The photochemical or photophysical methods involve “reduction” of metallic source. The photoreduction of metal ions generates intermediates like excited molecules or free radicals and such whole method is called as photosensitization process for the Ag nanoparticles synthesis (Christy & Umadevi, 2012).



**Fig. 6** Ag nanoparticles synthesis by photochemical method(Li, Jiang, Jia, & Wang, 2016).

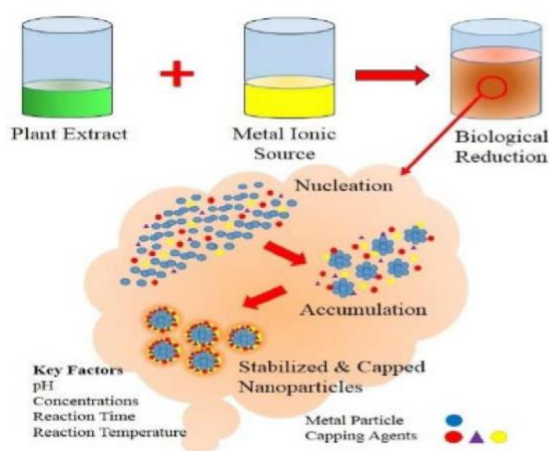
The stable Silver Nanoparticles also produced by UV photo activation method in aqueous triton x\_100. It improves the size of nanoparticle distribution by increasing surface tension at the interface of solvent nanoparticles. Ag nanoparticles, in other studies are synthesized by alkaline aqueous solution of AgNO<sub>3</sub>/carboxymethylated chitosan (a biocompatible chitosan derivative and water soluble served as reducing agent) with UV light irradiation. It acts as reducing and stabilizing agent for Ag nanoparticles synthesis (Huang et al., 2008).

The main advantages of this method are: **a)** it has a high spatial resolution and clean process. **b)** great

versatility (make possible to fabricate the nanoparticles in different mediums such as Surfactant Micelles, Polymer Films, Emulsions, Cells) (Sakamoto, Fujistuka, & Majima, 2009).

#### Biological Synthesis

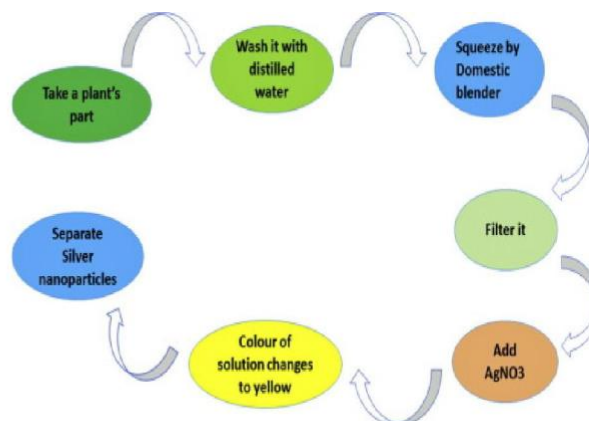
In case of “biological synthesis” of Ag nanoparticles, living organisms are used by replacing the reducing agents or the stabilizer. The stabilizing or reducing compounds are used by yeast, bacteria, plants, fungus and algae (Sintubin, Verstraete, & Boon, 2012).



**Fig. 7** Schematic diagram for Synthesis of Ag nanoparticles by biological method (Shah, Fawcett, Sharma, Tripathy, & Poinern, 2015).

In summary, it provides broad resources for silver nanoparticles synthesis. This method is low cost method and environmentally friendly. By using biological agents at suitable Temperature and Pressure, so there will be an increase in the rate of reduction of metal ions. For intracellular synthesis of Ag nanoparticles, the cell wall of the microorganism plays an important role under following strategy. The +vely charged metal ions will interact electrostatically with the -vely charged cell wall and it reduces the “metal ions” into nanoparticles (Thakkar, Mhatre, & Parikh, 2010). Ag nanoparticles are synthesized by using *Salicornia* extracts with a dimension of 1-50 nm and without any composition of foreign chemical reagents. This method is simple, inexpensive and eco-friendly (Khatami, Noor, Ahmadi, & Aflatoonian, 2018). Other Ag nanoparticles synthesis by using the plant extracts as reducing agents. This synthesis method involves the minimum use of dangerous materials and it is environmental friendly (Amaladhas, Sivagami, Devi, Ananthi, & Velammal, 2012).

Incubation of Ag ions with microorganisms also leads to the formation of extracellular Silver nanoparticles. So it involved a defense mechanism against the toxic effect of metal. This mechanism has significant plus points against conventional methods like chemical synthesis method, used as Ag nanoparticles synthesis. However, this method does not produce large amount of Ag nanoparticles (Shah et al., 2015).



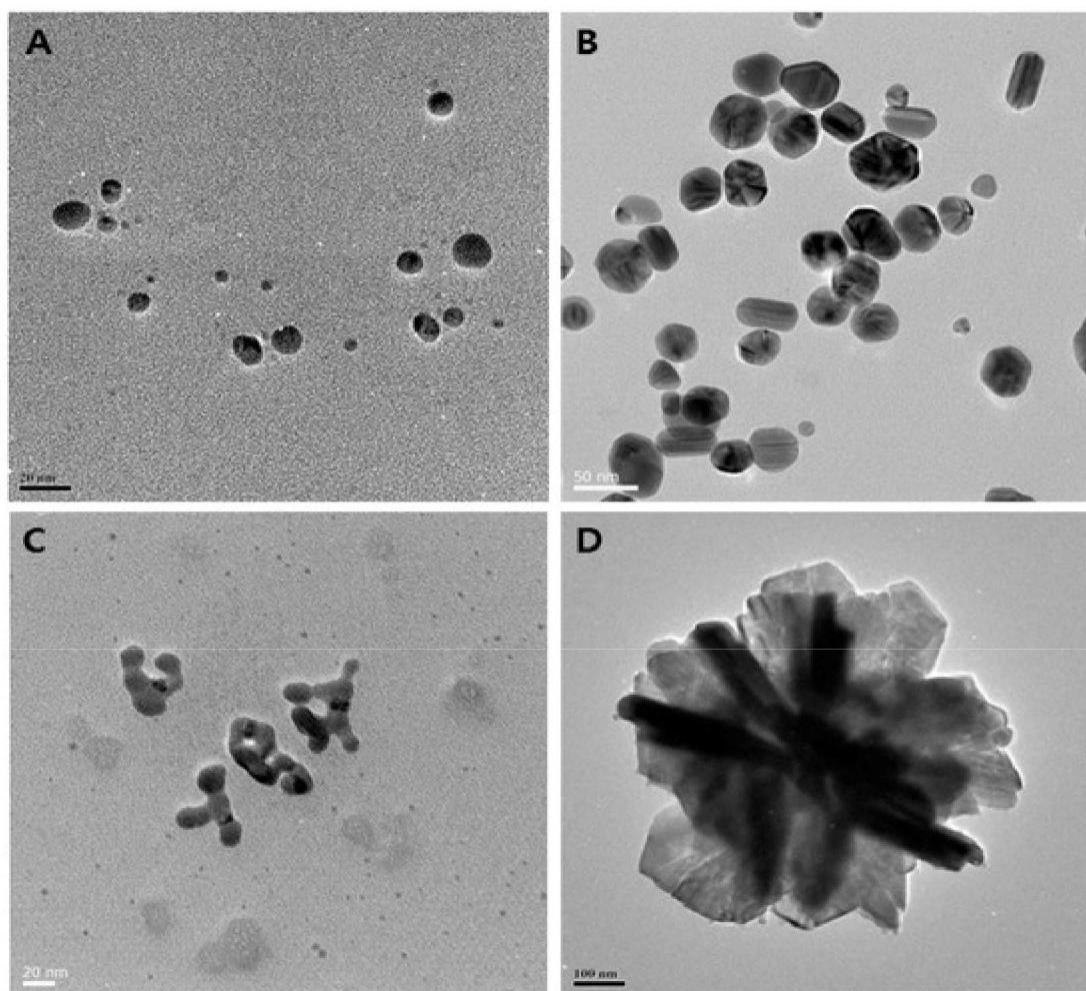
**Fig. 8** green synthesis of Ag nanoparticles (Thakkar et al., 2010)

### Properties of silver nanoparticles

Ag nanoparticles have distinctive physical properties such as electrical, optical and thermal properties. Ag nanoparticles have high electrical conductivity, low sintering temperature and great stability due to which they have been extensively used for preparation of various chemical and biological sensors. Furthermore, these have many properties such as antimicrobial, antifungal, optical, biomedical, bioanalyzers, used in wound dressing, in keyboards etc (Michel et al., 2011).

Furthermore, Ag nanoparticles have large surface to volume ratio, controlled drug release, therapeutic compounds, transfection vectors, and anti-microbial agent's surface functionalization. Ag nanoparticles also possess various physico-chemical properties like strong plasmon resonance absorption (SPR), biocompatibility, surface-enhanced Raman scattering, thermal conductivity, preeminent electrical, chemical stability, catalytic activity and non linear optical behavior (Dave et al., 2001).

The advantages of nanotechnology is that it stops changes in body, Immortality, painless child births etc and has important implications like reproducing extinct animals and plants, molecular food synthesis, and in industrial field like automatic pollution cleanup, by making computerized machines in a smaller size (Ahamed, AlSalhi, & Siddiqui, 2010). The disadvantages of nanotechnology is that it loss of jobs because the engineered robots work instead of human being and the nanoparticles released in the atmosphere and penetrated into animal body and these affect the performance of animal cells. It damage the agriculture fields, global monetary crises, oil becomes worthless. The risk of inhaling the nanoparticles is very dangerous can cause death (Abdel-Hafez, Nafady, Abdel-Rahim, Shaltout, & Mohamed, 2016).



**Fig. 9** Different shapes of Ag nanoparticles synthesized by biological method using *Bacillus* species. **a)** spherical **b)** mixed shapes (octagonal, rod, hexagonal, and icosahedral) **c)** highly branched shape **d)** flower shape (Shah et al., 2015).

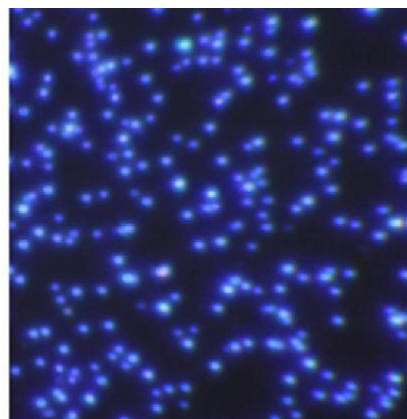
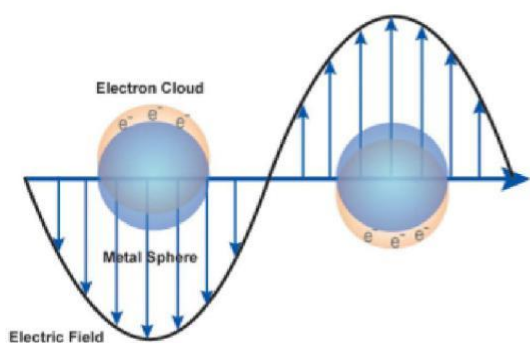
### Optical Properties of Ag Nanoparticles

Ag nanoparticles have unique optoelectronic properties. These distinctive optical properties initiate with combined oscillation of conduction electrons, such term is called “Surface Plasmon Resonance”. Following factors which are responsible for oscillations:

**a)** Acceleration by electric field of incident radiations with Conduction electrons. **b)** Induced polarization generation in the presence of restoring forces both in surrounding medium as well as in particles. **c)** Imprisonment of the Electrons with dimension smaller than wavelength of light (Krutyakov, Kudrinskiy, Olenin, & Lisichkin, 2008).

Such properties depend upon their composition, size, and their environment. It explores how the size dependent absorbance of Ag nanoparticles that adjust optical properties (Mock, Barbic, Smith, Schultz, & Schultz, 2002). According to Emory and Nie, Ag nanoparticles exhibit enhanced signals in the order of  $10^{14}$  to  $10^{15}$  that vary with size and shapes. The result is that, it supports the proposal of Surface Plasmon Resonance” (SPR) on size distribution which strongly make a contribution to surface enhanced Raman (SER) signals that detect signal molecules (Nie & Emory, 1997).





**Fig. 10** surface Plasmon resonance (Nie & Emory, 1997).

By using plasmonic sensors, we can detect different biomolecular analytes. Their properties actively participate with surface enhanced resonance to observe large intensities. Ag nanoparticles have hot spots regions such as gaps and junctions that enhanced signal by Raman signals of analyte of interest that helps to detect enough single-molecule (Potara, Baia, Farcau, & Astilean, 2012). “Raman Scattering” with Ag nanoparticles makes a highly sensitive tool to detect trace molecules. Ag nanoparticles have proved its role in Raman scattering as it is more accepted SERS active substrate (Nie & Emory, 1997).

#### Antimicrobial properties of Ag nanoparticles

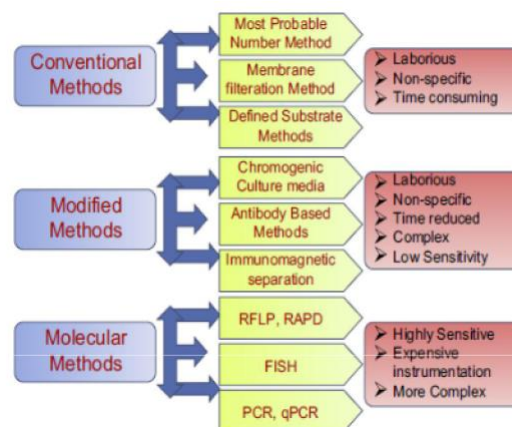
Ag has a wide range antimicrobial activity against fungi, protozoa, and certain viruses, gram positive and gram negative bacteria etc (Nair & Laurencin, 2007). Understanding the antibacterial mechanism, it is possible to design the nanoparticles to achieve the synthetic effects with biomolecules. The release of reactive oxygen species by Ag nanoparticles is involved the mechanism of cellular toxicity. Their Antibacterial properties are related with **a)** slow oxidation, and **b)** liberation of  $\text{Ag}^+$  ions and acts as biocidal agent to the environment (Nel et al., 2009). The small size of Ag nanoparticles helps to pierce into the bacterial cell membranes. It changes the intracellular environment inside of cell membrane, and provides maximum contact due to large surface area with the environment to the developed surface of these particles (Nel et al., 2009). Ag nanoparticles damage the membrane due to contact into the bacterial cell membrane, in the end the death of the bacteria (Vigneshwaran, Kathe, Varadarajan, Nachane, & Balasubramanya, 2006).

#### Detection Methods

##### Conventional methods

Conventional techniques have been used for decades for the detection and identification of microbes. For a particular time period and at a specific temperature, microbes grow on specific culture media.

The colorimetric detection now overcomes the above restrictions. The dopamine possesses amine groups get attached with ascorbic acid based Ag nanoparticles or gold and the hydrogen bonding b/w the adjacent dopamine. It causes the aggregation of Ag and Au nanoparticles. The solution color changes from red to blue or orange to red (Snozzi, Koster, Bartram, Ronchi, & Fewtrell, 2013). Recently we used different colorimetric nanoprobes and assays e.g., paper based, quantum dots based, magnetic bead based, and photochemical immune assays etc (Sharma, Dhillon, & Kumar, 2018). The colorimetric detection of dopamine was reported using Ag or gold nanoparticles or Ag-Au NPs. The color changed due to dispersion and aggregation of nanoparticles. The method used for the detection of dopamine was spectrometry. It is simple, selective and less sensitive (Iswarya, Daniel, & Sivakumar, 2017).



**Fig. 11** Different methods of pathogen detection (Bevan, 1999).

##### Most Probable Number Method

The most probable number method is an important method for the estimation of microbes present in soil, foods, and agricultural products and in



water. The exact cell number of an organism is not possible to determine. To estimate the population of organism, the most probable method is feasible where heterotrophic counts are difficult. It does not depend on quantitative estimation of individual cells. This method is used only to count the microbial population size. It takes 24 to 48 hours and this method is uninteresting method (Baptista et al., 2008).

#### **Membrane Filtration Method**

This method is used to test large volumes of samples and it yields rapid results than most probable method and is useful method in monitoring drinking water. The coliform group consists of all aerobic and facultative anaerobic, rod shaped bacteria; gram negative bacteria produce a red colony with a metallic shine. It may develop some new nucleated colonies with dark red color without a metallic sheen. A positive  $\beta$ -galactosidase and negative cytochrome oxidase reaction takes place by these coliform bacteria. When there was present no membrane filter technique for verification of typical coliform colonies, hence the similar tests were conducted with multiple-tube fermentation technique to express applicability (Boyer, Tamarat, Maali, Lounis, & Orrit, 2002).

The nanoparticles interact with biomolecules in a control way, so they have significant applications in biology field such as **a)** biological imaging **b)** biosensing and **c)** hyperthermia treatment. This process is compatible and favorable with the environment and the valid biological systems. A variety of approaches are accessible which facilitate the formation, modification, and the organization of Ag Nanoparticles. Some methods are attributed by Ag Nanoparticles onto glassy surface, incorporating bivalent linker compounds, biomolecule as linker molecules, and the participation of nanoparticles on prepared surfaces (Sperling & Parak, 2010).

#### **Colorimetric detection of various biomolecule Colorimetric and Optical Detection of Cysteine (Cys)**

The colorimetric and optical detection has been extensively used for the detection of biological and many hazardous molecules (Rajamanikandan & Ilanchelian, 2017). Cysteine is an important amino acid and is detected with optical spectral probe; an exact amount of cysteine solution is mixed with  $\beta$ -CD silver nanoparticles by addition of aliquot amount in standard flask at specific temperature. Colloidal  $\beta$ -CD silver nanoparticles mixed in cysteine solution with constant stirring and allowed to rest almost 10 minutes and the absorption spectra is recorded that ranges from 200 to 800 nm. Cysteine can be visualized with naked eye and the fine Amino acid amount mixed in  $\beta$ -CD silver nanoparticles with colloidal dispersion and shakes well, allowed for 10 minutes at constant

equilibrium time. The change in colour of  $\beta$ -CD silver nanoparticles in the suitable amount of Amino acids present is visualized by naked eyes. However, Cysteine is a universal genetic code and is an important amongst 20 amino acids. Other amino acids are investigated by using this method and same experiments are performed at least “3” times to get authenticate precision value at 25°C. Usually, Ag nanoparticles are used as chemo-biosensors that monitor the shifting and changes in “SPR” band. Colorimetric detection principle of Ag Nanoparticles is showed in Fig 21 (Rameshkumar, Viswanathan, & Ramaraj, 2014).

#### **Colorimetric Detection of Dopamine by Using Reduced Graphene Ag Nanoparticles**

A colorimetric probe based on Ag nanoparticles has been attracted due to their distinctive Surface Plasmon Resonance properties, simplicity, and unique sensitivity as well as cost effectiveness. Ag nanoparticles have some disadvantages because of low reproducibility and insufficient stability that originates from nanoparticles aggregation. However, Graphene is used to solve this problem and it is used to prepare Ag nanoparticles (Golsheikh, Huang, Lim, & Zakaria, 2014). “Graphene Nanosheets” have strong Van der Waals and large surface area and interactions with silver nanoparticles that help to decrease agglomeration. In addition, interfacial interactions increase the reproducibility and stability of Ag nanoparticles (Basiri, Mehdinia, & Jabbari, 2018). So graphene oxide is decorated with pre synthesized Ag nanoparticles and this technique is very complex involve several steps. DA is prepared in PBS and 500  $\mu$ L of different concentration (10mM, pH=7) mix with (250  $\mu$ L of rGO/Ag nanoparticles) and (1750  $\mu$ L of PBS) buffer solution (10mM, pH=7) and incubated for 15 minutes. After some time the colour change is examined by bare eye. The mixture is taken into “Quartz cuvette” for UV-visible recordings. Silver nanoparticles get prepared by mixing Graphene oxide with Ag salts through chemical reduction method. In advance studies, Ag nanoparticles are combined with graphene is synthesized by reduction of silver ions onto the graphene oxide surface (Khodaveisi, Shabani, Dadfarnia, Moghadam, & Hormozi-Nezhad, 2016).

#### **Antifungal Activity of AgNPs**

Fungal infections are very tedious to patients who are immune suppressed. There are present a number of drugs against fungal disease, so these should be biocompatible, eco-friendly and non toxic in order to develop urgent antifungal agents. Ag nanoparticles have an important role against antifungal diseases. An inert matrix of Ag nanoparticles with average size of 20nm into soda-lime glass that shows enhanced biocidal activities (K.-J. Kim et al., 2008).

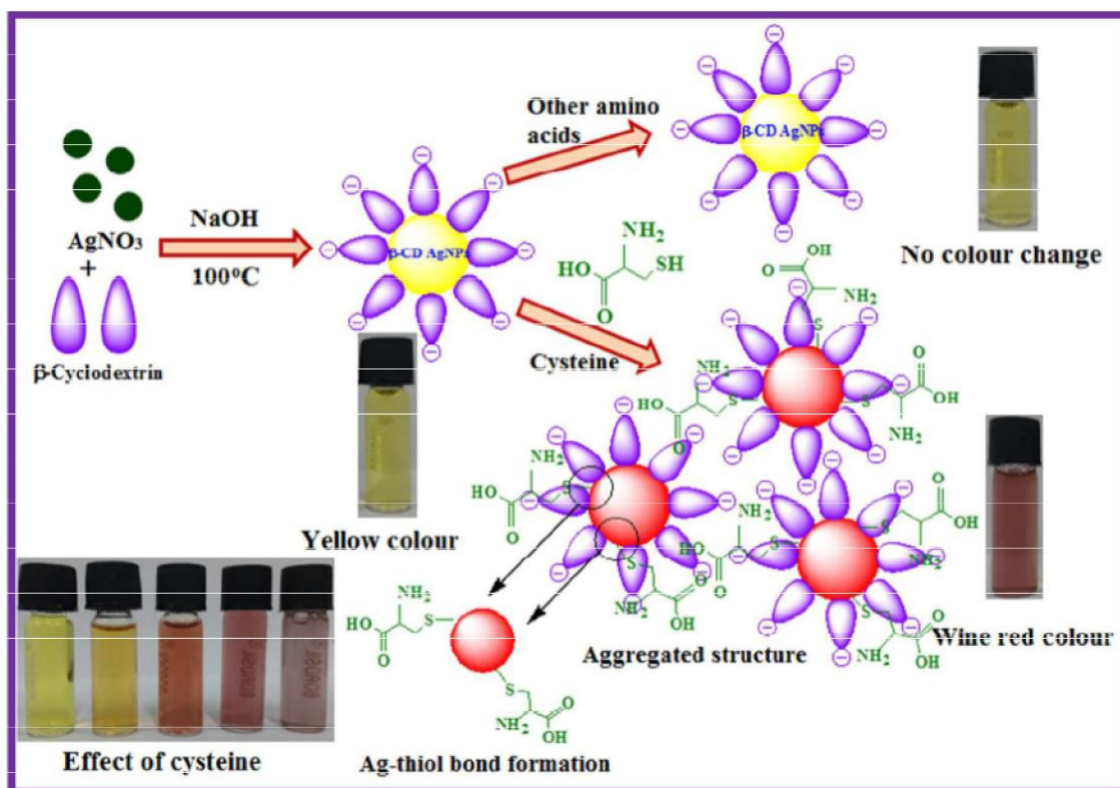


Fig. 12  $\beta$ -CD AgNPs by sensing strategy determine of cysteine

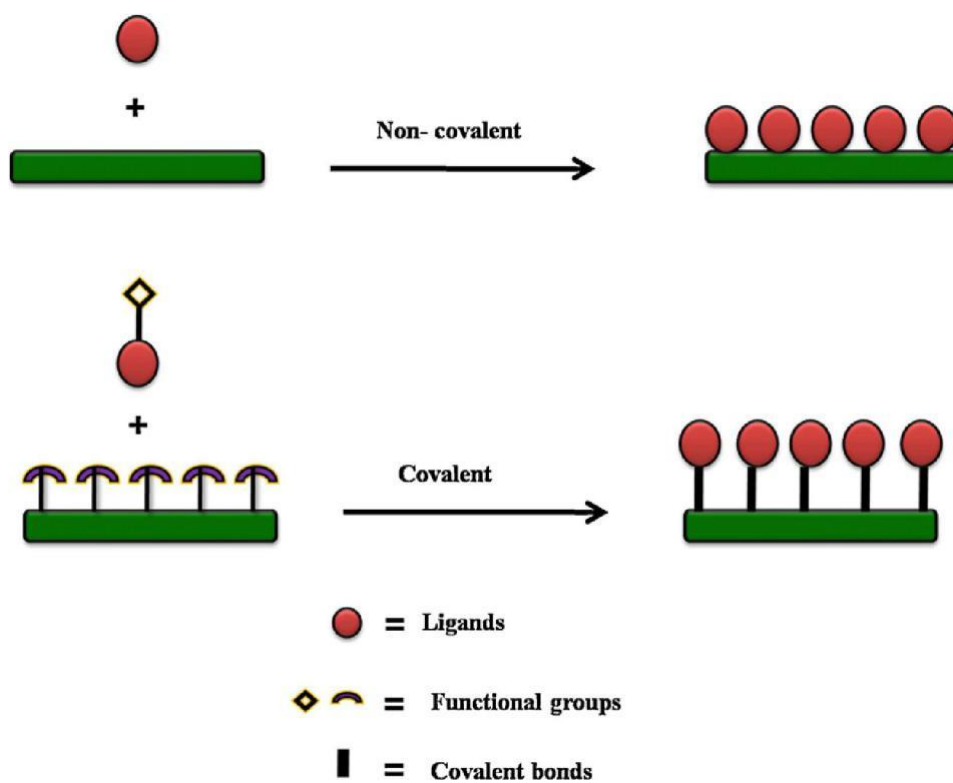


Fig. 13 Covalent and non-covalent approaches for surface modification of Ag nanoparticles (Ravindran, Chandran, & Khan, 2013).

### Proteins and antibodies

Ag nanoparticles are very specific and efficient for nanoparticle based delivery systems. Proteins are made of amino acids that are linked together with amide bond and furthermore proteins with special classes comprise of enzymes and antibodies (Di Marco et al., 2010). Enzymes are specific as for metabolism and catalysis the “Biochemical reactions”. Proteins are conjugated with nanoparticles has great applications in **i**) sensing **ii**) delivery **iii**) imaging activity and **iv**) catalysis. Several approaches are available for protein-nanoparticles interactions. For example in non-specific adsorption mechanism, nanoparticles are incubated with proteins that are adsorbed on the surface of nanoparticles due to electrostatic attractions that are provided by oppositely charged partner molecule. Other cases are studied, like these are connected through **a**) Hydrogen Bridge, **b**) Ag-thiol bonds, **c**) van der Waals forces or **d**) by hydrophobic interactions, e.g., when iso electric point of nanoparticle or proteins is related to pH then reduction in electrostatic repulsions is examined.

Sastry et al. used amino acid cysteine to change the surface properties of nanoparticles by the development of “H<sub>2</sub>O dispersible Nanoparticles” (Selvakannan, Mandal, Phadtare, Pasricha, & Sastry, 2003). They observed that Amino Acid capped nanoparticles can be bonded by ‘H’ bonding b/w carboxylic acid and amino functional groups. The attachments of nanoparticles with biomolecule by covalent and non-covalent linking mechanism are shown in Fig. 25.

In physisorption, ligands get adsorbed through non-covalent interactions to the surface of nanoparticles, including H-bonding, electrostatic interactions, and hydrophobic interactions etc. Another process that used is steric stabilization process that helps in binding capping agents through non-bonding interactions such as surfactants, polymers to nanoparticles surface. In such type of interactions biomolecule get linked directly with Ag nanoparticles by “Exchange reactions” with the help of binding agents. The process of agglomeration is inhibited by steric repulsion so it kept the nanoparticles dispersion intact (Ravindran et al., 2013).

Flocculation assay is adopted to find out the optimal coupling ratio, so addition of electrolytes to Ag Nanoparticles produce a shield of “Repulsive double layer” that is responsible for the flocculation. Steric repulsions prevent flocculation of nanoparticles while adsorption stabilize proteins on the Ag nanoparticles surface (Shenton, Davis, & Mann, 1999).

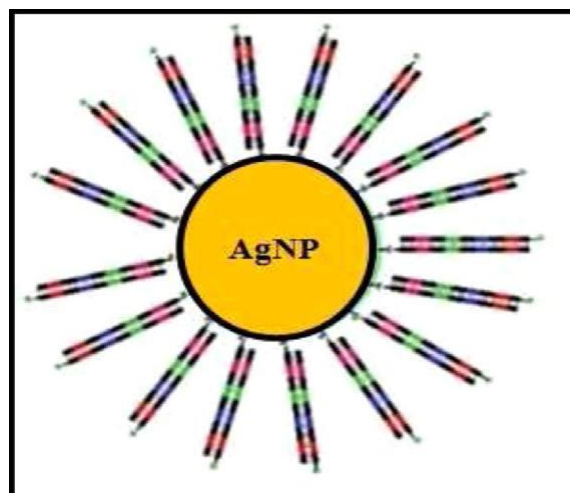
In order to determine amount of stabilizers that avoid flocculation, so increase the concentration of electrolytes when Ag nanoparticles prepared. These

are coated with specific amounts of proteins formerly. Ag nanoparticles are bonded through ester, amide, disulfide which are covalently linked to thiol, amines, carboxylic acid surface. This method is mostly used for coating of Ag nanoparticles to immune globins and serum albumins in which cysteine residues get reached for heterogeneous interphase coupling (Selvakannan et al., 2003).

For recognition of biomolecule, antibodies are commonly used for plasmonic nanoparticles modification, but some limitations are existed here like expensiveness, difference in quality of various batches, and instability. Now over the years, a series of biomolecule are recognized, e.g., bacteriophage, vancomycin (Cheng et al., 2015), aptamers (Bayrac, Eyidoğan, & Öktem, 2017), and lectin used to detect bacteria because of their great stability (Mikaelyan, Poghosyan, Hendrickson, Dzantiev, & Gasparyan, 2017).

### DNA Based Nanoparticle Systems

Nucleotide and DNA interactions with Ag nanoparticles have an exhaustive field of study. DNA is a specific molecule and is responsible for the construction of genetic material in nanosciences. DNA has enormous specificity and simplicity, so bonding is specific b/w adenine-thymine and guanine-cytosine that programming in a convenient way of artificial DNA receptors. DNA used as building block of artificial structures and their sequences are synthesized and amplified from “Microscopic to macroscopic” quantities by programmed methods which enhanced their command as a molecular tool (L. Sun et al., 2006).



**Fig. 14** Conjugate DNA-silver nanoparticle (Ravindran et al., 2013).

DNA has great rigid property due to double helical structure and due to highly physiochemical stability, so it is most suitable for the modification of

nanoparticles surfaces. It acts as a counter ion and it interacts with metal ion through non covalent bond to equalize the (-ive charge of Phosphate) backbone. Other interactions like covalent bonds may occur through special binding sites of DNA like sugars, nucleobases, or phosphates. DNA based studies, a researcher Eichorn proposed that silver ions ( $\text{Ag}^+$ ) strongly bound with nucleobases rather than Phosphates. By using analytical techniques, like SEM, TEM, CD, fluorometry and UV-spectroscopy,  $\text{Ag}^+$  ions interactions mechanism with DNA were studied (Marcelis & Reedijk, 1983).

#### Bacterial and fungal surface layers

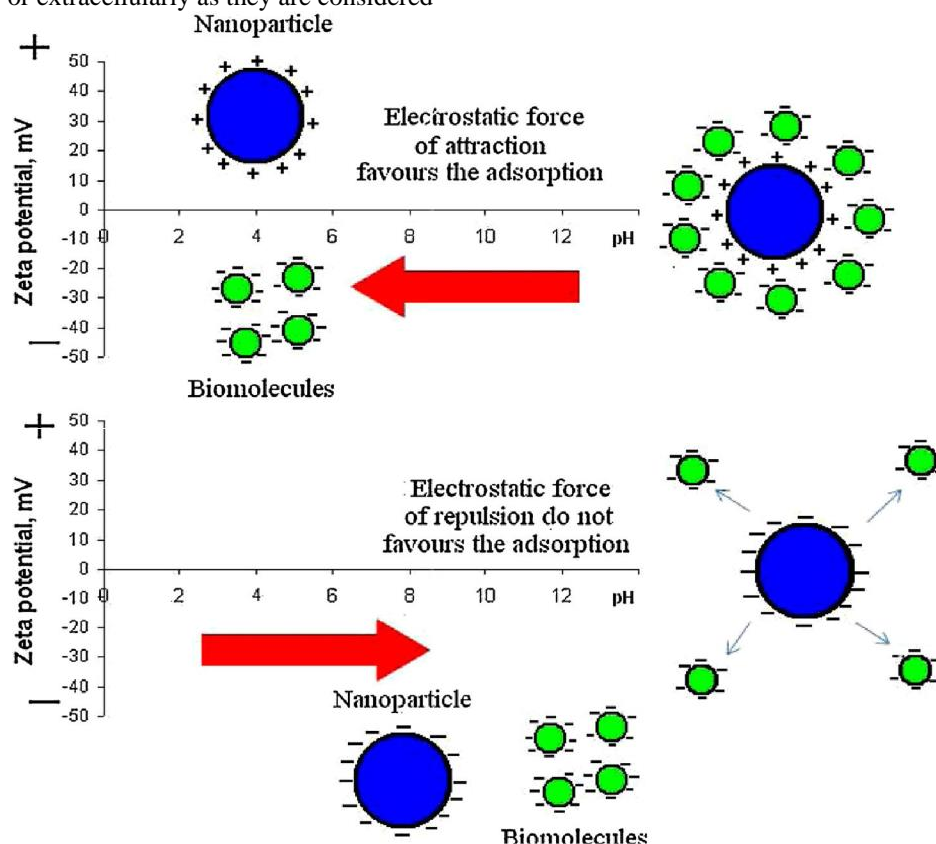
The colorimetric detection of various microorganisms like bacteria is based on inhibition of aggregation of Ag nanoparticles and MPBA. A well dispersed solution of MPBA and Ag nanoparticles was set off to aggregation in the absence of bacteria so the color was changed from yellow to brown by adding excess MPBA. MPBA linked with Ag nanoparticles through a planer six membered boroxine ring within the MPBA molecule. In such process, boronic acid group get dehydrated and condensed by itself (Zhou et al., 2014).

Bacteria produce inorganic substances through intracellularly or extracellularly as they are considered

as potential biofactory of Ag nanoparticles synthesis or the other metal ions. For example in the absence of MPBA and Ag nanoparticles S-layer bacteria produce gypsum or  $\text{CaCO}_3$ , and Magnetotactic bacteria construct magnetic nanoparticles (Aryal, KC, Bhattarai, Kim, & Kim, 2006).

The following mechanism shows the adsorption of biomolecule on the surface of nanoparticles at different pH which actually influence zeta potential. Adsorption process is preferential at low pH while nanoparticle possesses a net positive charge. Other studies revealed that “*Aeromonas punctata*” acts as a (capping agent) that helps to stabilize of the nanoparticles and produces exo polysaccharides (Ravindran, Mani, Chandrasekaran, & Mukherjee, 2011).

Exo polysaccharides Capped Nanoparticles’ shows less toxicity to **a)** “*Staphylococcus aureus*”, **b)** “*Micrococcus luteus*”, and **c)** *E. coli* as compared to uncapped ones. It suggests that capped nanoparticles produced by bacteria produce EPS that has a strong physiological defense mechanism (Ravindran et al., 2013; Sudheer Khan, Bharath Kumar, Mukherjee, & Chandrasekaran, 2011).



**Fig. 15** Effect of zeta potential by adsorption of biomolecule on Ag Nanoparticles at various pH (Ravindran et al., 2013).



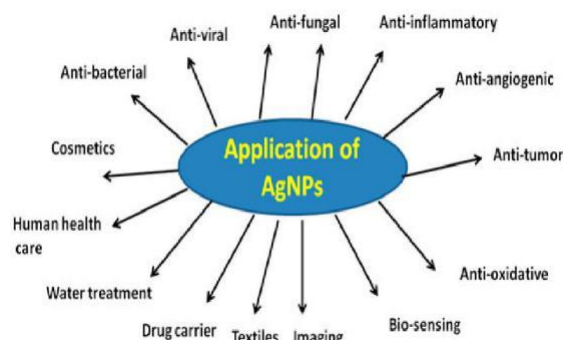
### Applications of silver nanoparticles

Ag nanoparticles have many applications such as anti-bacterial, anti-viral, anti-fungal properties. Ag is safe in small concentration for human body cells but deadly for the bacteria and viruses. Usually used in the preservation of foods and water in daily life (Batareseh, 2004).

Due to their unique potential, Ag nanoparticles have received much attention in advanced Sensor and Photonic applications (McFarland & Van Duyne, 2003). Ag has SPR and fabrication properties, so it is widely used in nanotechnology, medicine, and electronics, bioengineering, optoelectronics and in advanced fields (Aarthi et al., 2017).

### Ag nanoparticles in anti fungal therapy

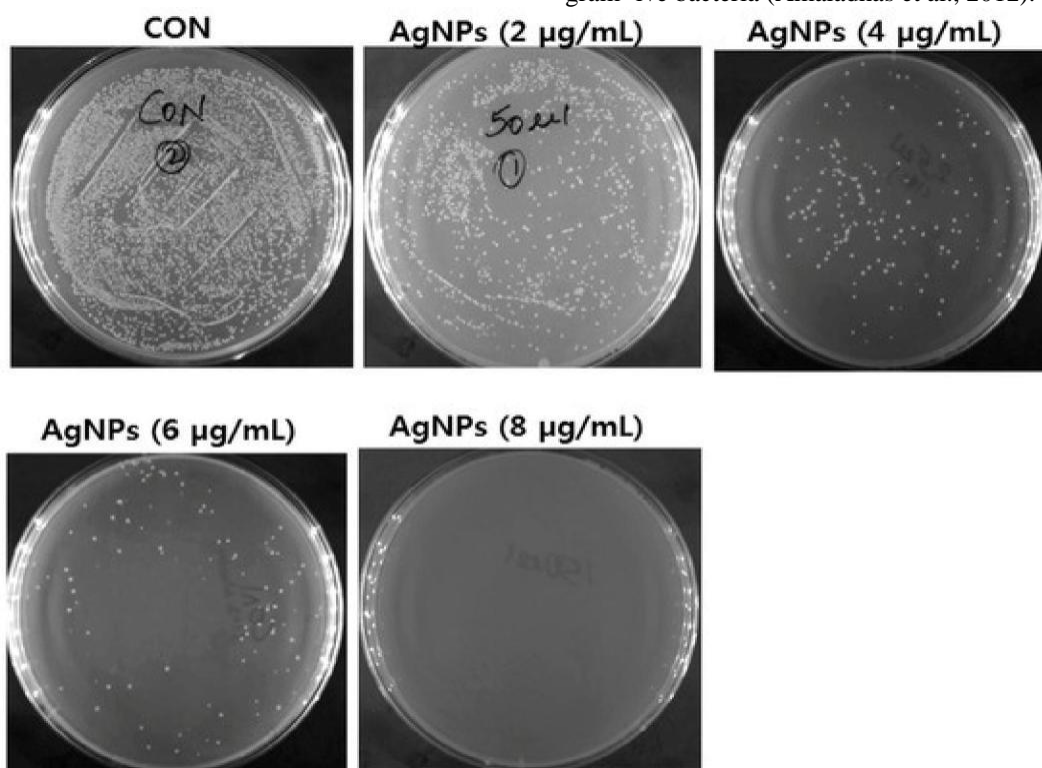
In the recently years, fungal infections are more common and AgNPs have come out as anti-fungal agent. Fungal infections are found in immune system of living organisms because the human immune system is affected by viral infections (P. Lee & Meisel, 1982). Ag Nanoparticles have significant effect on *Candida albicans* by piercing into the cell membrane and by inhibited the normal budding process (K.-J. Kim et al., 2009).



**Fig. 16** Different applications of AgNPs (McFarland & Van Duyne, 2003).

### Antibacterial Activity of AgNPs

Ag nanoparticles required antibacterial agents to overcome the resistance against antibiotics. The “Antibacterial Activity” of Ag Nanoparticles significantly depend on the size and shape (Sondi & Salopek-Sondi, 2004). Ag nanoparticles show high antimicrobial activity against gram +ive and gram -ive bacteria such as Methicillin resistant *Staphylococcus aureus* that is multi-resistant strain (Hanaa & Khaled; Lin & Mao, 2011). Ag nanoparticles not only dependent on size for the determining efficiency, but also depend on shape dependent interactions with the gram -ive bacteria (Amaladhas et al., 2012).



**Fig. 17** Biologically synthesized Ag nanoparticles dose dependent antibacterial activity of in *E. coli* (Besinis, De Peralta, & Handy, 2014).

## Conclusion

The development in the nanotechnology areas include the study of materials with significant properties developed from their nanoscale dimensions. Nanoparticles are natural, manufactural or incidental material with size ranges from 1nm to 100nm. Ag nanoparticles have many advantages in the nanotechnology, nanomedicines, and nanochemicals.

Ag nanoparticles are synthesized by different methods like physical, chemical, electrochemical, photochemical, radiation, and biological also known as green synthesis that is eco-friendly synthesis process. They have distinctive physico-chemical properties such as highly thermal or electrical conductivity so it used in the microelectronics medical imaging fields. The process of agglomeration, interactions of live cells with a high illuminating system is examined by Ag nanoparticles. AgNP is as a biocide, attributed to the well-documented antimicrobial properties and by using silver nano particles, we can detect biomolecules. For a particular time period and at a specific temperature, microbes grow on specific culture media. The colorimetric detection now overcomes the above restrictions. The dopamine possesses amine groups get attached with ascorbic acid based Ag nanoparticles or gold and the hydrogen bonding b/w the adjacent dopamine. It causes the aggregation of Ag and Au nanoparticles. The change in the color is visualized by naked eye or under spectrophotometer. Cysteine is an important amino acid and it is detected with optical spectral probe; an exact amount of cysteine solution is mixed with  $\beta$ -CD silver nanoparticles by addition of aliquot amount in standard flask at specific temperature. Ag nanoparticles as colorimetric probes and chiral selector are helpful in determining biomolecules. However, we can easily read out the assay described in this work with naked eye and by use of ultra violet spectrometer.

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