



Author

Shikha Behera

P.L.Nayak Research Foundation, Neelachal Bhavan,
Cuttack- 753004, Odisha, India

Ashutosh Debata

Principal, Stewart Science College, Cuttack-753001,
Odisha

P.L.Nayak

P.L.Nayak Research Foundation, Neelachal Bhavan,
Cuttack- 753004, Odisha, India

Email: nayakpl@sify.com

Biomedical Applications Of Silver Nanoparticles

Abstract

Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale, stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. Nano-silver is used in an increasing number of products. Some of the applications have resulted in the concern of governments and the public, since little is known about the potential risks of nano-silver. In this review, an inventory is made to identify knowledge gaps that have to be filled before risks for both man and the environment can be assessed as reliable as for 'non-nanosized' chemicals. It is hypothesized that the toxic effects of nano-silver are due to a combination of the specific properties of silver nanoparticles and the generation of ions from them. The main topic for future research is validation of our '0-hypothesis' that toxic effects of nano-silver are proportional to the activity of free silver ions released by the nanoparticles. Furthermore, it must be determined whether or to what extent nano-silver particles will enter the body. The outcomes of these tests will determine the requirements for further toxicity testing.

1.Introduction

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronics and medicine^[1]. Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100 nm. A new branch of nanotechnology is Nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation. These methods of synthesis can be divided on intra cellular and extracellular^[2].

Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, the pharmaceutical companies and the researchers are searching for new antibacterial agents^[3]. In the present scenario nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area to volume ratio and its unique chemical and physical properties^[4,5]. Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level^[6]. A nanoparticle is by definition a particle where all the three dimensions are nanometer in scale. Nanoparticles are known to exist in diverse shapes such as spherical, triangular, cubical, pentagonal, rod-shaped, shells, ellipsoidal and so forth. Nanoparticles by themselves and when used as building blocks to construct complex

nanostructures such as nanochains, nanowires, nanoclusters and nanoaggregates find use in a wide variety of applications in the fields of electronics, chemistry, biotechnology and medicine, just to mention few: For example, gold nanoparticles are being used to enhance electroluminescence and quantum efficiency in organic light emitting diodes ; palladium and platinum nanoparticles are used as efficient catalysts ; glucose sensors are developed based on silver nanoparticles ; and iron oxide nanoparticles are used as contrast agents in diagnosing cancer in Magnetic Resonance Imaging (MRI).

Nanoparticles contain small enough a number of constituent atoms or molecules that they differ from the properties inherent in their bulk counterparts. However, they contain a high enough a number of constituent atoms or molecules that they cannot be treated as an isolated group of atoms or molecules .Therefore, nanoparticles exhibit electronic, optical, magnetic and chemical properties that are very different from both the bulk and the constituent atoms or molecules. For example, the striking colors of metallic nanoparticle solutions (such as gold and silver) are due to the red shift of the plasmon band to visible frequencies, unlike that for bulk metals where the plasmon absorption is in the UV region (a plasmon is a quantum of collective oscillation of free electrons in the metals). This red shift of the plasmon occurs due to the quantum confinement of electrons in the nanoparticle, since the mean free path of electrons is greater than the nanoparticle size. Additionally, the optical properties of nanoparticles depend significantly on their size and shape as well as on the dielectric constant of the surrounding medium. For example, in spherical gold nanoparticles, the plasmon absorption red shifts with increasing diameter of the nanoparticle . Likewise, quantum dots (semiconductor nanoparticles such as CdSe and CdTe) exhibit red shift in their band gap (emission) as their size increases . Silver nanoparticles of spherical, pentagonal and triangular shape appear blue, green and red respectively under a dark field microscope, suggesting strong correlation between optical property and shape of the nanoparticles . Gold nanorods exhibit different optical properties than their spherical counterparts. Gold nanorods show two plasmon resonances, one a transverse plasmon at 520 nm and the other a longitudinal

plasmon at longer wavelengths. Unlike the transverse plasmon mode, the wavelength of the longitudinal plasmon mode increases with increasing aspect ratio of the nanorods . Additionally, gold nanoparticles dispersed in different solvents exhibit plasmon absorption at different wavelengths suggesting the effect of surrounding media . Nanoparticles have large surface to volume ratio, thus surface related phenomena/properties are drastically affected with slight modification of size, shape and surrounding media of nanoparticles. Therefore, the optical properties desired of nanoparticles depending on application can be tuned by generating the nanoparticles of definite size and shape in preferred media and henceforth, develop new effective nanomaterials and nanodevices.

Nanoparticles (1-500 nm) are much smaller than human cells which are about 10-20 μm (Figure 1.). However, nanoparticles have sizes similar to that of the biomolecules encountered at the cellular level. This unique size of nanoparticles facilitates development of nanodevices/nanosensors that can travel into cells to probe proteins (enzymes and receptors) or the DNA inside the cell or outside the cell. Consequently, the first step involved in developing nanodevices/nanosensors is to produce hybrid nanoparticles: nanoparticles labeled with molecules that can investigate or target the specific cellular entities. Though a plethora of hybrid nanoparticles labeled with peptides and proteins have been produced and investigated for their potential applications in biological field , gold hybrid nanoparticles specifically have emerged as favorites in biomedical applications owing to their exciting chemical, electronic and optical properties along with their biocompatibility, dimensions and ease of characterization.

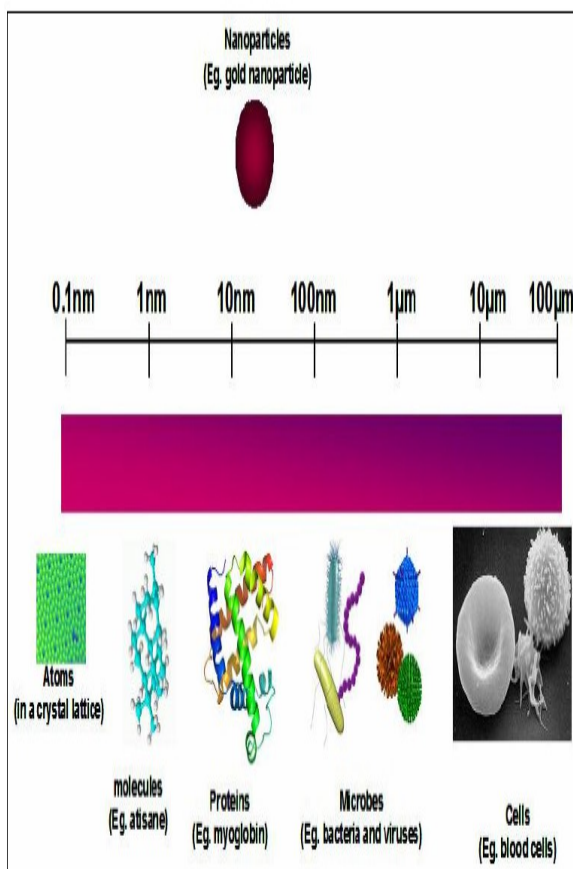


Figure 1- Nanoparticles in comparison with other biological entities^[145]

Nanoparticles may provide advanced biomedical research tools based on polymeric or inorganic formulations or a combination of both. They have the potential to be used in many different biological and medical applications as in diagnostic tests assays for early detection of diseases, to serve as tools for noninvasive imaging and drug development, and to be used as targeted drug delivery systems to minimize secondary systemic negative effects.

Nanoparticles used in biomedical applications include liposomes, polymeric micelles, block ionomer complexes, dendrimers, inorganic and polymeric nanoparticles, nanorods and quantum dots. All have been tested pre-clinically or clinically for targeted drug and gene delivery and as agents to enhance diagnostic imaging output like in MRI^[7-9]. Properties present only on the nanoscale level, like the increased intensity of

fluorescent light emission of semiconductor crystals (quantum dots) or switchable magnetic properties of superparamagnetic nanoparticles (SPIONs), make these materials unique and useful for applications in the biomedical field of medical imaging and cell tracking. Other nanoparticles like water-soluble synthetic polymers (dendrimers) were tested in pre-clinical models for the delivery of drugs, genes, and as imaging agents showing a rich versatility for tailoring their binding properties to several requirements, among them facilitation of cellular uptake of drugs (e.g. cancer drugs)^[10-12].

Particles with diameters in the range of 2 to 100 nm, so-called *nanocrystalline* materials, have become a major interdisciplinary area of research during recent decades. In fact, since the seventeenth century, noble metallic nanomaterials, though not understood, have been obtained and used to give rise to a brilliant rose color throughout Europe in stained glass windows of cathedrals and by the Chinese in coloring vases and other ornaments^[13,14]. The scientific preparation of nanoparticles dates back to the nineteenth century, with Faraday reporting the preparation of colloids of relatively monodispersed gold nanoparticles.

The high-resolution TEM (HRTEM) and low-resolution (LRTEM) allow one to observe a substance at a nanometer scale directly. The magic machines make it possible to investigate the nanomaterials with respect to size, size distribution, shape evolution, and shape uniformity and even the structure. The past couple of decades have witnessed an exponential growth of activities in this field worldwide, driven both by the excitement of understanding new science and by the potential hope for applications and economic impacts. Indeed, many efforts have been devoted to investigation into the synthesis, characterization, and application of nanomaterials. In general, nanomaterials can be classified into three groups: zero-dimensional materials, so-called nanoparticles, with variations in shape and diameter, one-dimensional materials, including nanorod and nanowire, and two-dimensional materials, including nanobelts, nanodisks, films, and nanosheets. Herein we focus on the nanoparticles (NPs), especially the metallic ones.

The intense interest in the metallic NPs derives from their unique chemical and electronic

properties arising from the small volume to big surface area ratio and the separation in the electronic energy level. The change in the properties at this length scale from their bulk counterparts is not a result of only scaling factors. It results from different causes as far as different materials are concerned. In semiconductors, it results from the further confinement of the electronic motion to a length scale that is comparable to or smaller than the length scale characterizing the electronic motion in bulk semiconducting material (called the electron Bohr radius, which is usually a few nanometers). As noble metals are reduced in size to tens of nanometers, a new very strong absorption is observed, resulting from the collective oscillation of the electrons in the conduction band from one surface of the particle to the other. This oscillation has a frequency that absorbs the visible light. This is called the *surface plasmon absorption*. In the case of transition metal nanoparticles, the decrease in the particle size to the nanometer length scale increases the surface to volume ratio. This, together with our ability to make them in different sizes and shapes, makes them potentially useful in the field of catalysis. The absorption spectra of many metallic nanoparticles are characterized by a strong broad absorption band that is absent in the bulk spectra. Classically, this giant dipole (or surface plasmon) band is ascribed to a collective oscillation of the conduction electrons in response to optical excitation^[15]. The presence of this band in the visible region of the spectrum is responsible for the striking colors of dilute colloidal solutions of noble, alkali, alkaline Earth (Ca, Sr, Ba), and rare Earth (Eu, Yb) nanoparticles [16]. Mie's theory predicts that below a certain size, less than one-tenth of the optical wavelength, the position and width of this band should remain constant, independent of size^[17]. Experimental evidence, however, together with a dramatic increase in width with decreasing size^[18], indicates a slight but significant shift to lower energy. Frangstein and Kreibitz and others^[19-21] have investigated the optical absorption spectra theoretically in the case of free-electron metals and advanced the theory that when the particle diameter becomes small enough, with diameter less than the electronic mean-free path in the bulk metal (~20 nm for gold), the scattering of free electrons with the particle surface begins to have an influence on optical excitation. Such a simple and practical

theory succeeds in terms of spectra of relatively large particles (>3 nm for gold) but does not agree with the experimental results when applied to the smaller sizes. It is anticipated that at one point the phenomenological description of free electrons, as well as inherent fundamental assumptions of infinite lattice periodicity and a continuous energy level spectrum, must fail, because it is widely known that the continuous energy level band will separate into individual levels since in the cluster the number of atoms is so small. Experimental measurements for the small nanoparticles are often degraded by a lack of size and shape uniformity that renders comparison with theory questionable^[22], and this is what makes it so difficult to unequivocally identify quantum size effects in the optical spectra of metal nanoparticles prepared in macroscopic quantities, although such effects are well known from experiments on metal-cluster beams and from conductance measurements on single-metal nanostructures^[23]. Hence, it is a prerequisite to take a good grasp on the control of the size distribution of the metallic nanoparticle. In later discussion, we see the same problem in the case of magnetic metallic nanoparticles. So much effort has been devoted to finding an approach that can afford us nanoparticles with a narrow size distribution. Besides noble metal nanoparticles, nanoparticles with novel magnetic properties are attracting more and more attention with regard to bioapplications. As far as magnetism is concerned, magnetic particles with diameters smaller than some certain critical value usually show properties different from their bulk counterparts. At room temperature, the measured M-H curve, namely the hysteresis loop, recorded on the magnetic nanoparticles shows saturation magnetization but no coercive field or magnetic remanence synchronously. In other words, from the viewpoint of the coercive field or magnetic remanence, they behave similarly to paramagnetic materials; on the other hand, the magnetization can reach the saturation state and is larger than paramagnetic ones. The scientists describe this behavior as *superparamagnetic*. This indicates that magnetic moments in the particles are free to align with the field during the measuring time at room temperature. This phenomenon is caused by the fact that in such small particles, because of the thermal fluctuation the magnetic moments can rotate freely despite the magnetic energy barrier. This characteristic allows a promising future in

application of the magnetic nanoparticles in biomedicine, particularly in magnetic resonance imaging (MRI), tissue engineering, and drug delivery. Because of both the excitement for understanding a new science and its potential for applications having economic impact, a great deal of effort has been devoted to investigations into the metallic nanoparticles in terms of synthesis, size and shape control, surface modification, and the primary applications. Significant breakthroughs have been achieved in the synthesis of nanoparticles of varying diameters and in obtaining a narrow size distribution. Based on these, highly ordered self-assembly structures with large area of metallic nanoparticles have been obtained. For the purpose of bioapplication, some kinds of organic molecules, including biomolecules of different lengths and functional groups, have been coated or linked onto the surface of metallic nanoparticles. In this review, we discuss the synthesis and properties of zero-dimensional metallic nanomaterials, including the noble metal nanoparticles and the magnetic ones. We start with a discussion of some effective methods aimed at the synthesis of the nanoparticles. After that their novel properties are introduced. The application of the metal nanoparticles in biotechnology is presented in the final section. We focus on the synthesis of metallic nanoparticles by the wet chemistry method. Chemical vapor deposition (CVD) and the physical techniques such as using electron, ion, or photon beams in lithography to make nanostructures are not discussed. In general, this chapter is constructed based on the remarkable work reported in the past decades. Because of the limitation of our knowledge and experiences, there must be some incorrect sayings or points in this overview. Due to the explosion of publications in this field, we do not claim that this review includes all of the published work, but rather an exposure to the methods. We apologize to other authors who have contributed to the synthesis, characterization, and application of the metal NPs whom we have unintentionally left out.

The application of nanoscale materials and structures, is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment .

Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts .Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest .Gold, silver, zinc,iron and copper have been used mostly for the synthesis of stable dispersions of nanoparticles, which are useful in areas such as photography, catalysis, biological labeling, photonics, optoelectronics and surface-enhanced Raman scattering (SERS) detection .Moreover, functionalized, biocompatible and inert nanomaterials have potential applications in cancer, diagnosis and therapy .

The biological application of nanoparticles is a rapidly developing area of nanotechnology that raises new possibilities in the diagnosis and treatment of human cancers. In cancer diagnostics, fluorescent nanoparticles can be used for multiplex simultaneous profiling of tumour biomarkers and for detection of multiple genes and matrix RNA with fluorescent in-situ hybridisation. In breast cancer, three crucial biomarkers can be detected and accurately quantified in single tumour sections by use of nanoparticles conjugated to antibodies. In the near future, the use of conjugated nanoparticles will allow at least ten cancer-related proteins to be detected on tiny tumour sections, providing a new method of analysing the proteome of an individual tumour. Supermagnetic nanoparticles have exciting possibilities as contrast agents for cancer detection in vivo, and for monitoring the response to treatment. Several chemotherapy agents are available as nanoparticle formulations, and have at least equivalent efficacy and fewer toxic effects compared with conventional formulations. Ultimately, the use of nanoparticles will allow simultaneous tumour targeting and drug delivery in a unique manner. In this review, we give an overview of the use of clinically applicable nanoparticles in oncology, with particular focus on the diagnosis and treatment of breast cancer.

2.Silver Nano Particles

Silver nanoparticles (AgNPs)(figure 2) have emerged as an arch product from the field of nanotechnology. Over the last few years due to its good conductivity, chemical stability,

catalytic and antibacterial activity silver has gained much of the interest. The diversity and importance of their applications has generated a great deal of interest in developing versatile methods to synthesize silver nanoparticles with well-defined and controlled properties. Although various chemicals and biochemical methods are being explored for production of AgNPs. Microbes are exceedingly effective in this process biosynthesis of silver nanoparticles from bacteria, fungi, yeast, plants, fruits and so on have been reported. Colloidal silver solutions (CSSs) have an increased interest due to their antimicrobial properties, with large applications including pharmacology, human and veterinary medicine, food industry, water purification. For a long time silver has been known to have a disinfecting effect and has found applications in traditional medicines culinary items silver has known to be a metal that come into use even before Neolithic revolution. Even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during 8th century Thus nanoparticles of silver have aptly been investigated for their antimicrobial properties Nanoparticles of silver have thus been studied as a medium for antibiotic delivery. Antimicrobial activity of silver nanoparticles have been studied by various researchers especially on *E. coli*, *S. aureus*.

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics. Ag nanoparticles can be applied effectively in the control of microorganisms and the prevention of deleterious infections. Nanometer sized silver particles synthesized by inert gas condensation or co-condensation techniques showed antibacterial activity against *E. coli*. The antibacterial efficiency of the nanoparticles was investigated by introducing the particles into a media containing *E. coli* and it was found that they exhibited antibacterial effect at low concentrations. In addition it was observed a relationship between the antibacterial properties and the total surface area of the nanoparticles.

Smaller particles with a larger surface area were more efficient in the antibacterial activity tests.

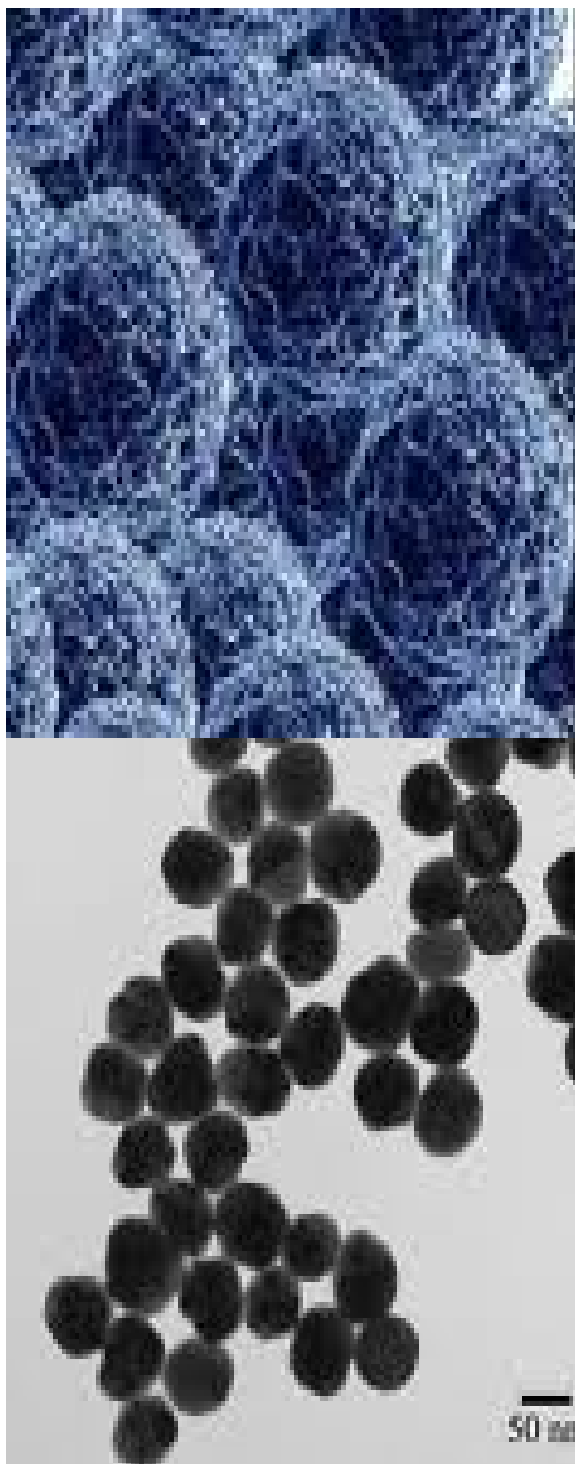


Fig 2:Image of silver nano particles ^[146]

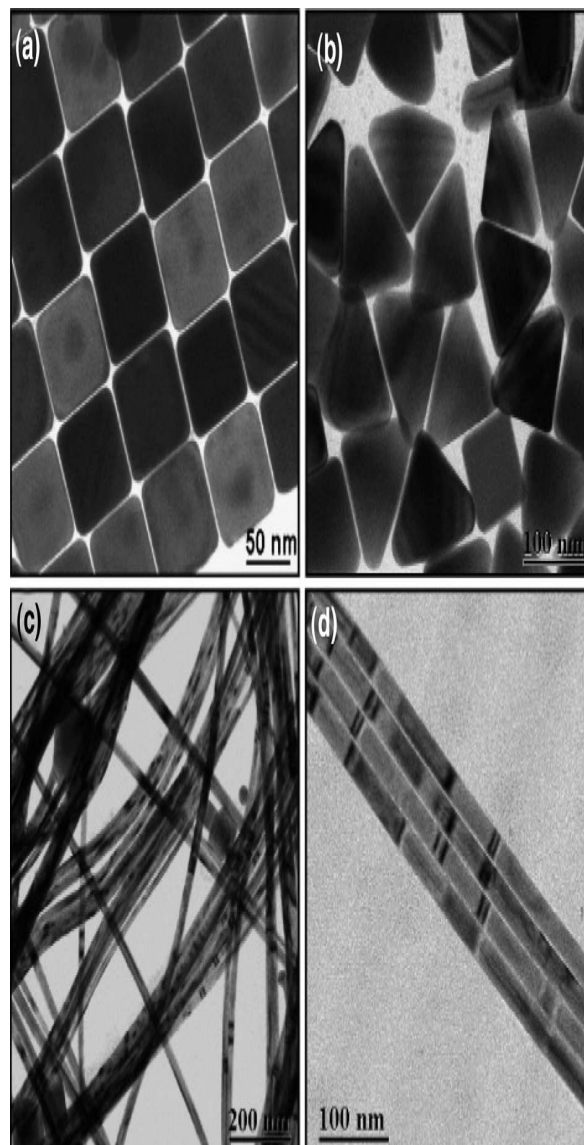


Fig 3: TEM images of silver nanoparticles: (a) cubes; (b) triangles; (c) wires; (d) an alignment of wires ^[146]

In the last few years, the market for antimicrobial textiles has shown double digit growth. This growth has been fuelled by the increased need of consumers for fresh, clean, and hygienic clothing. Extensive research is taking place to develop new antimicrobial finishes. The Nanosilver is a powerful and natural antimicrobial agent that has been proven highly effective in fighting a whole range of microbes. Acting as a catalyst, it reportedly disables the

enzyme that one-celled bacteria, viruses, and fungi need for their oxygen intake without causing corresponding harm to human enzymes or other parts of the human body chemistry. The result is the destruction of disease-causing organisms without any detrimental effects on the surrounding human tissue.

The bactericidal effect of silver ions on microorganisms is very well known; however, the bactericidal mechanism is only partially understood. It has been proposed that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. Experimental evidence suggests that DNA loses its replication ability once the bacteria have been treated with silver ions. Other studies have shown evidence of structural changes in the cell membrane as well as the formation of small electron-dense granules formed by silver and sulfur. Silver ions have been demonstrated to be useful and effective in bactericidal applications, but due to the unique properties of nanoparticles nanotechnology presents a reasonable alternative for development of new bactericides. Metal particles in the nanometer size range exhibit physical properties that are different from both the ion and the bulk material. This makes them exhibit remarkable properties such as increased catalytic activity due to morphologies with highly active facets. We can apply several electron microscopy techniques to study the mechanism by which silver nanoparticles interact with these bacteria. We can use high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM), and developed a novel sample preparation that avoids the use of heavy metal based compounds such as OsO₄.

3. Biosynthesis Of Silver Nano Particles

3.1: Synthesis of silver nanoparticles by bacteria

An important part of work in nanobiotechnology concerns the synthesis of nanoparticles of different chemical compositions, sizes, shapes, and polydispersity. Many microorganisms produce inorganic materials either intra- or extracellularly. Well-known example is magnetotactic bacteria which are able to synthesize magnetic nanoparticles^[24]. Magnetotactic bacteria are motile, prokaryotes that move along geometric field lines. They produce

magnetosomes, unique intracellular structure contains a magnetic particle, in narrow range of very low oxygen concentration. Magnetotactic bacteria usually mineralize either oxide magnetite Fe₃O₄ or iron sulfide Fe₃S₄ – greigite. An extracellularly preparation of metal nanoparticles generally involves the reduction of metal ions in solution. The formation of extracellular and intracellular silver nanoparticles by bacteria (*Pseudomonas stutzeri*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus*) has been investigated^[25]. Various microbes are known to reduce metal ions to the metals. The formation of extracellular silver nanoparticles by photoautotrophic cyanobacterium *Plectonema boryanum* had been described. The procedure of synthesis was as follows: 5 mL of silver solution (approximately 560 mg/L) was added to 5 mL of washed cyanobacteria culture (approximately 10 mg dry weight). The synthesis was conducted at 25, 60 and 100°C for up to 28 days in the dark. Only, at 100°C, the soluble silver was completely precipitated from solutions within 28 days. A greyish-black silver particles adhered to bacterial cells were observed macroscopically. The reaction products were analyzed using transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS). The addition of AgNO₃ caused the precipitation both inside and outside the microbial cells. At 60°C, silver nanoparticles were deposited at the cell surface. At 100°C, the cyanobacterial cells were encrusted by silver nanoparticles. The size of nanoparticles inside the cell was ranging from 1 to 40 nm. The size of nanoparticles of silver which were precipitated outside the bacterial cells was in the range of 1 -200 nm.

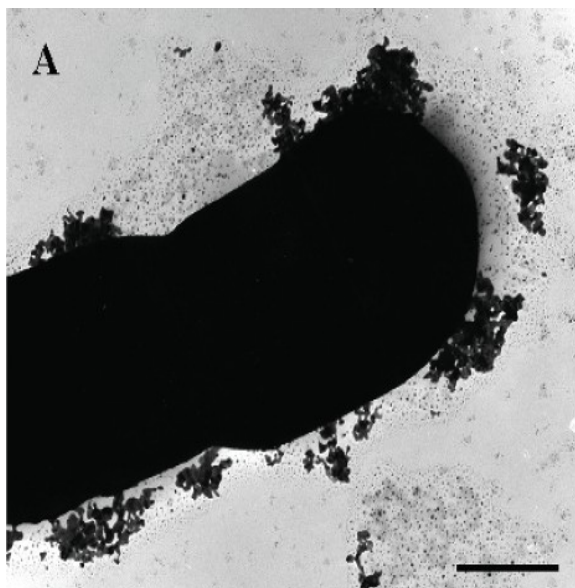
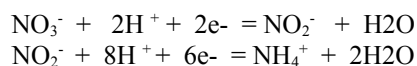


Fig. 4. Precipitated silver nanoparticles on the cyanobacteria cell surface ^[25]

The bioreduction of the Ag⁺ ions could be associated with metabolic processes utilizing nitrate by reducing nitrate to nitrile and ammonium. Cyanobacteria commonly use nitrate as the major source of nitrogen. Nitrate was reduced by cyanobacteria metabolic process.



It suggests that Ag⁺ ions could be reduced by an intracellular electron donor ^[25].

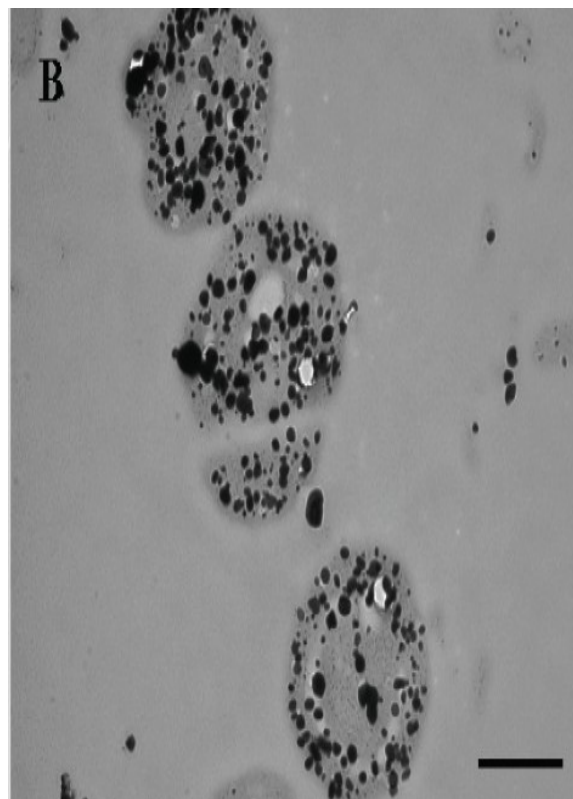


Fig. 5. Cyanobacteria cells with nanoparticles of silver inside the cell ^[25]

Silver nanoparticles in the range of 50 nm were synthesized by supernatant of *Bacillus licheniformis* ^[26] *Bacillus licheniformis* is a gram positive, thermophilic bacterium, commonly found in the soil. As was showed previously, during the visual observation culture supernatant incubated with silver nitrate showed a color change from yellow to brown. The appearance of brown color suggested the formation of silver nanoparticles. The XRD pattern shows four characteristic peaks in the whole spectrum. The peaks at 2θ values of 38.48°, 44.48°, 64.69° and 77.62° corresponding to 111, 200, 220 and 311 planes for silver crystal, respectively.

Recently, a rapid method for synthesizing silver nanoparticles by treating the aqueous silver nitrate solution with culture supernatants of different strains of *Enterobacteria* such as *Klebsiella pneumonia* has been described ^[27,28]. The process of synthesis was quite fast. As it has been presented, the silver nanoparticles were formed within 5 min of the silver ions coming in contact with the culture supernatant. The particle size histogram of silver nanoparticles

showed the particle range in size from 28.2 nm to 122nm with the average size value of 52.5 nm. *Enterobacteria* is a Gram-negative bacteria, usually associated with intestinal infections. The investigations which have been realized by Mokhtari and coworkers showed that piperitone (3 methyl-6-1 methylethyl)-2 cyclohexan-1-one) can be responsible for the silver ions reduction to metal ^[28]. This conclusion supports the hypothesis that nitroreductase enzymes may be involved in silver ions reduction process.

The effect of visible-light irradiation on the synthesis of silver nanoparticles has been recently investigated ^[28]. The following procedure was used for the silver nanoparticles formation using a supernatant of *B. pneumonia* in the presence of light. The first step was silver chloride suspension preparation. The sodium chloride solution (50mL, 140 mg/L) was added to 50 mL of silver nitrate solution (340 mg/L) in a dark pale. The sediment fraction of AgCl was separated, cleaned and redispersed in distilled water. Then 1mL of culture supernatant of *K. pneumonia* was added to the suspension. The silver nanoparticles fabrication was realized in the presence of various visible light intensities, generated by a 75 W halogen lamp. The experimental results confirmed a proposed mechanism involving the conversion of AgCl into Ag nanoparticles. This conversion of AgCl to silver nanoparticles by culture supernatant of *K. pneumonia* in a bright condition is presented at Fig. 7. Generally, AgCl is treated as the main intermediate compound during the bioreduction of the silver ions.

Kalimuthu and coworkers ^[31] have investigated the process of synthesis silver nanoparticles using bacteria *Bacillus licheniformis* and sonification of reacting mixture. *Bacillus licheniformis* were isolated from sewage collected from municipal wastes. Ultrasonic destruction of bacteria cell was carried out with ultrasonic processor over three 15 s periods. The interval between periods was 45 s. Fabricated silver nanoparticles had a size ranging from 2 nm to 100 nm. The average particle size was found to be around 50 nm.

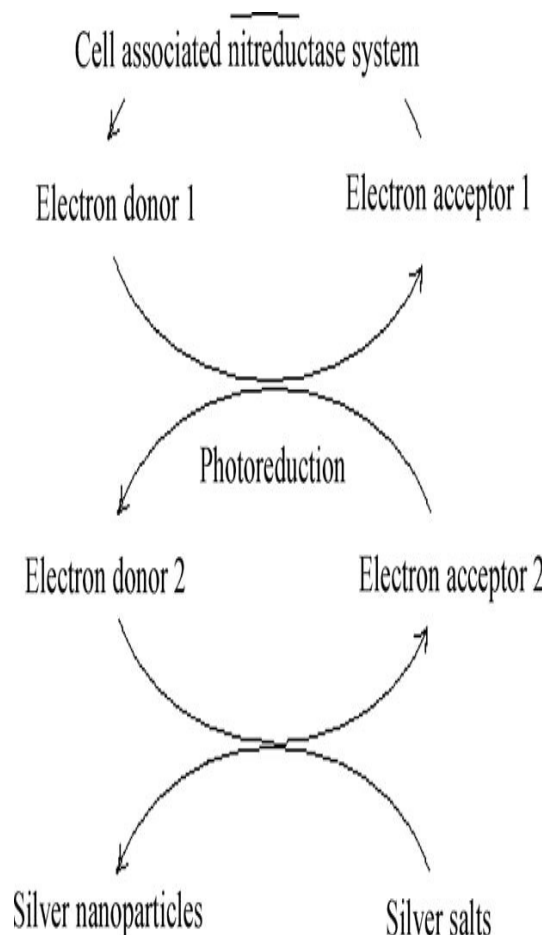


Fig.6. Hypothetical mechanism of silver nanoparticles synthesis using the culture of *B.licheniformis* ^[28]

The enzyme involved in the fabrication of nanoparticles can belong to nitrate reductase, presented in *B.licheniformis*. This enzyme reduces the silver ions to metallic silver. It is known that NADH dependent nitrate reductase enzyme are important factors in the biosynthesis of metal nanoparticles. The possible mechanisms of reduction of silver ions is using nitrate reductase, as it was presented in Fig.5.

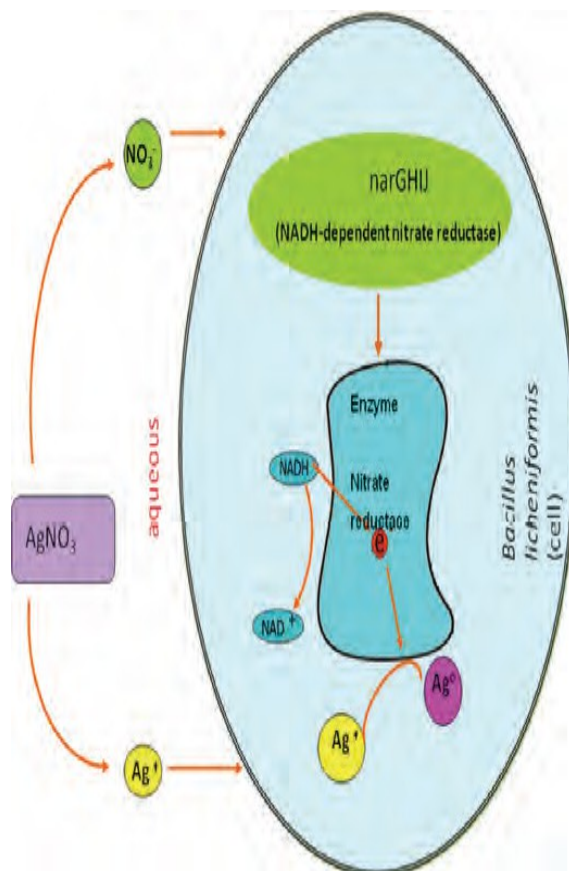


Fig. 7. Possible mechanism for silver nanoparticles synthesis using *Bacillus licheniformis* [31]

Minaeian and coworkers [29] used different cultures which were sterilized and inoculated with fresh culture of the strains (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacterdoacae*, *Staphylococcus aureus*, *Candida albicans*). The biosynthesized silver nanoparticles have the size range 50-100 nm. Novel method of biosynthesis of silver nanoparticles using a combination of culture supernatant of *Bacillus subtilis* and microwave irradiation was proposed by Saifuddin and coworkers [33]. The formation of nanoparticles by this method was extremely rapid. The samples (supernatant and $AgNO_3$ solution) were subjected to several short burst of microwave irradiation at the frequency of 2.45 GHz, at power output of about 100 W in a following cyclic mode on 15 s off 15 s to prevent of overheating. The synthesized nanoparticles had the size range of 5 -50 nm.

3.2: Synthesis of silver nanoparticles by the fungal systems

The fungi are extremely good candidates in the synthesis of metal nanoparticles. The synthesis of silver particles using two *Aspergillus niger* strains was investigated [32]. These strains were isolated from a soil. Inoculated fungi were prepared in Petri dishes at the room temperature using 2% malt extract with 0.5% yeast extract. Fungal biomass preparation was grown aerobically in the liquid medium containing (g/L): KH_2PO_4 7.0; K_2HPO_4 2.0; $MgSO_4 \cdot 7H_2O$ 0.1; $(NH_4)_2SO_4$ 1.0; yeast extract 0.6 and glucose 10.0. After the incubation, the biomass was separated and extensively washed with distilled water. Fresh and clean biomass was collected with 100 mL of Milli-Q deionized water and new incubation was carry out. After the incubation, the supernatant was obtained by passing suspension through Whatman filter paper No. 1. For synthesis of silver nanoparticles, $AgNO_3$ 1mM solution of the final concentration was mixed with 50mL of cell filtrate in an Erlenmeyer flask and agitated at 250 C in the dark. Sample 1 mL was with drown at different time intervals and absorbance was measured using UV-visible spectrophotometer. The spectra recorded at different times of biosynthesis is presented in Figure 9.

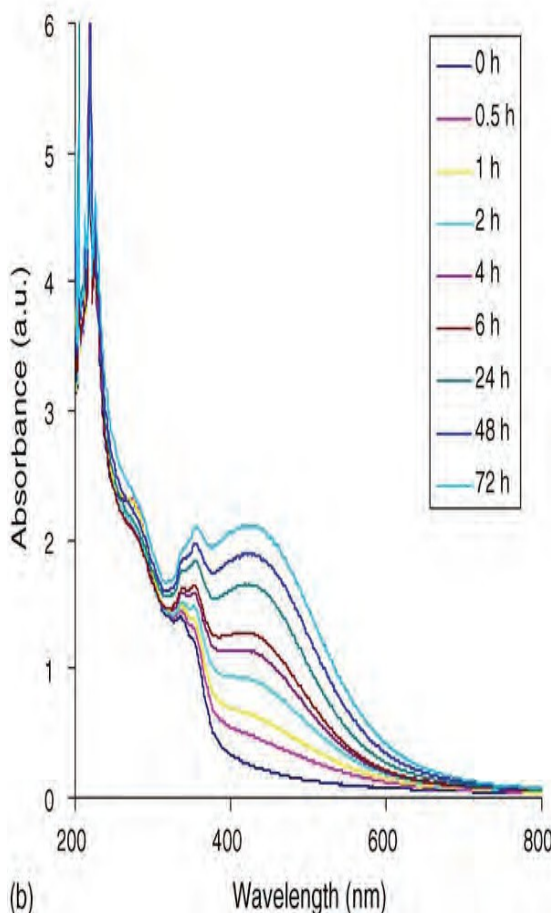


Fig. 8. UV-vis spectrum of aqueous medium during the synthesis of silver nanoparticles [32]

The electrokinetic measurements indicated that zeta potential of silver nanoparticles was negative value [32].

The extracellular synthesis of silver nanoparticles by fungus *Fusarium oxysporum* biomass had a contribution on the formation of nanoparticles [34]. The reduction of silver ions by *Fusarium oxysporum* strains has been attributed to a nitrate-dependent reductase and a shuttle quinone extracellular process. In a typical biosynthesis, 10g of fungal biomass was taken in Erlenmeyer flask containing 10 ml of distilled water. A corresponding quantity of AgNO_3 was added to Erlenmeyer flask to yield the concentration of Ag^+ ions equal 10^{-3} M. The reaction was carried out in the dark. Periodically, 5 mL of the reaction solution was removed and

subjected to UV-vis spectroscopic measurements. Independently, it was observed that the biomass suspension has a yellow color before reaction with the silver ions and brown color on completion of the reaction.

The extracellular biosynthesis of silver nanoparticles using the filamentous fungus *Aspergillus fumigatus* has been investigated [35]. This study included kinetics of synthesis, spectroscopic and microscopic characterization of the silver nanoparticles. The fungus *Aspergillus fumigatus* (NCIM 902) was grown aerobically in a liquid media containing (g/L) KH_2PO_4 , 7.0; K_2HPO_4 , 2.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $(\text{NH}_4)_2\text{SO}_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The biomass was harvested after 72 h of growth, then it was extensive washing with distilled water. 20g of fresh biomass was contacted with 200 mL of deionized water for 72 h and agitated in the same condition as first sample. After incubation, the suspension was filtered using Whatman filter paper No. 1. The mechanism of leading to formation of silver nanoparticles is not definitely understood at the moment. One hypothesis supports that a first step involve trapping of the Ag^+ ions onto the surface of the fungal cells. It can be realized by electrostatic interaction between Ag^+ and a negative charged carboxylate groups on the cell surface. The reduction of metal ions occurs on the surface by the enzymes presented in the cell wall [30]. The extracellular enzymes such as naphthoquinones and anthraquinones showed an excellent redox properties, they can act as electron shuttle in silver ions reduction. It was presented [36] that enzyme hydrogenase is present in a filtrate broth obtained from *Fusarium oxysporum* growth. The silver nanoparticles production capacity has been depended on the reductase/electron shuttle relationships. Next paper presents the extracellular synthesis of stable silver nanoparticles using the fungus *Penicillium brevicompactum* WA 2315 [38]. The analysis of data obtained from transmission electron microscope showed the average size of nanoparticles to be 58.35 ± 17.88 nm. Figure 8 shows the FTIR spectrum of the freeze-dried powder of silver nanoparticles formed after 72 h of incubation with the fungus supernatant. The band seen at 3356 cm^{-1} and 2922 cm^{-1} were assigned to the stretching vibration of primary and secondary amines, respectively. The bands at 1622 cm^{-1} and 1527 cm^{-1} correspond to the

stretch molecule vibration. The two bands existing at 1412 cm^{-1} and 1029 cm^{-1} can be assigned to the C-N stretching vibrations of aromatic and aliphatic amines. This FTIR spectrum supports the presence of proteins in the synthesis of silver nanoparticles. The use of fungus *Fusarium semitectum* for the extracellular synthesis of silver nanoparticles has been reported by Basavaraja and co-workers^[37] The formation and stability of the reduced silver nanoparticles in colloidal solution was monitored by using UV-vis spectral analysis. It was observed from spectra that the silver surface plasmon resonance band occurred at 420nm and this absorption steadily increased in intensity as a function of time of reaction. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration.

The mean particles diameter of silver nanoparticles was calculated from the XRD pattern using Scherrer equation. The calculated average particles size of silver nanoparticles was found to be 35 nm. The extracellular synthesis of silver nanoparticles by a marine fungus *Penicillium fellutanum* has been described by Kathiresan and coworkers^[39]. The fungus *P. fellutanum* was isolated from a coastal mangrove sediments. The procedure of biosynthesis of silver nanoparticles was analogous with the procedure has early described. For synthesis of silver nanoparticles AgNO_3 1mM solution was mixed with 50 mL of cell filtrate and agitated in dark. The present of silver nanoparticles in reacting mixture was confirmed by absorption peak at 430 nm. The obtained silver nanoparticles were spherical in shape with size ranging from 5 to 25 nm. The TEM micrograph of silver nanoparticles synthesized by *P. fellutanum* is presented in Fig.10.

Cladosporium cladosporioides is a commonly available fungus found in marshland regions. This fungus was employed to biosynthesis of silver nanoparticles^[40]. To prepare biomass for the synthesis, the fungus was grown aerobically in liquid medium containing (g/L): K_2HPO_4 , 2.5; KNO_3 , 5.0; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 1.00; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.001; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.003; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; $\text{Na}_2\text{MoO}_3 \cdot 2\text{H}_2\text{O}$ 0.0015; FeCl_3 0.02 and glucose, 40.0. The fungus was grown for 1 week then the broth was

filtered and washed with distilled water. 10 mL of pure solution were brought in contact with 100 mL of double distilled water containing 0.01 mL Ag^+ metal ion solution. The mixture was agitated and kept on a shaker at 27°C for 78 h. Size and morphology of obtained nanoparticles were analyzed by employing TEM and Fourier transform infrared spectroscopy (FT-IR).



Fig. 9. TEM micrograph of silver nanoparticles synthesized by *Penicillium fellutanum*.^[39]

The study on edible mushroom as reducing and protective agent for silver nanoparticles has been carried out by Philip^[41]. Edible mushroom *Volvarella volvacea* was used for the metal

nanoparticles synthesis. In the case of silver nanoparticles biosynthesis, 35mg of AgNO_3 dissolved in 250 mL of water was contacted with a various volume (from 6 to 25 mL) of mushroom extract. The bioreduction was complete in 6 h. Ag-Au bimetallic nanoparticles were prepared by the simultaneous reduction of Au^{3+} and Ag^+ ions using excess of mushroom extract.

3.3: Synthesis of silver nanoparticles using plant extracts

An important branch of biosynthesis of nanoparticles is the application of plant extract to the biosynthesis reaction. Fig. 10 shows some popular plants using to the extract preparation. A rapid reduction of the silver ions was observed when the silver nitrate solution was contacted with geranium (*Pelargonium graveolens*) leaf extract^[43]. The extract used for reduction of Ag^+ ions to Ag^0 was prepared by taking 20g of thoroughly washed and finely cut geranium leaves in a 500 mL Erlenmeyer flask with 100 mL of Fig. 10.



Fig. 10. Plants used for biosynthesis of metal nanoparticles

distilled water. The suspension was boiling for 1 min. 5 mL of pure broth was added to 100 mL of 10⁻³ M aqueous solution of AgNO_3 . The bioreduction of the Ag^+ ions was monitored by measuring the UV-vis spectra of the solution.

In the case of Neem leaf extract a competition reduction of Ag^+ ions presented simultaneously

in solution was observed. Silver nanoparticles ranging from 55 to 80 nm in side and triangular were fabricated using the novel sundried biomass of *Cinnammum canphora* leaf^[42]. A simple procedure applying *Aloe vera* leaf extract has been used for spherical silver nanoparticles synthesis^[44].

Eclipta (known as Bhingraj) belongs to the family Asteraceae. It is a common weed growing mostly in a shade area^[45]. Extract from *Eclipta* leaf has used as medically important herb. The plant is rich in flavonoids, belonging to the group of phenolic compounds. The sample of 5 g of freshly collected leaves of *Eclipta* was washed for 10 min and ringed briefly in distilled water. Prepared biomaterial was taken in 250 mL capacity beaker having 200mL of 50% Et-OH and was placed on boiling steam bath for 15 to 20 min. till color of the solvent changes to dark green. The cold extract was treated with 20 ml of 0.025 (M) AgNO_3 solution and warmed again on the steam bath for 10 min until the color of solution changes. The formation of silver nanoparticles was monitored by UV-vis spectrophotometry and X-ray diffractometer. Biosynthesis of silver nanoparticles was also conducted using *Cycas* leaf extract. *Cycas* belongs to the *Cycadaceos* family. It is a common gymnospermic plant and is a commercial source of sago^[45]. This plant is rich in flavonoids broadly belonging to the lass of phenolic compounds. The procedure of *Cycas* leaf broth preparation was like to made *Eclipta* extract. The *Cycas* extract solution was treated with 20 mL of 0.25 M AgNO_3 solution and wormed on the steam bath for 20 min until the color of solution changes to brown. The particle size histogram obtained silver nanoparticles shows broad distribution of particle size. The size particle ranged from 2-6 nm and the average particle size comes out to be 3.29 ± 0.22 nm. The X-ray diffraction patter obtained for silver nanoparticles synthesized by *Cycas* leaf broth shows that the silver nanoparticles are crystalline in nature. Silver nanoparticles were successfully synthesized using the latex of *Jatropha curcas*. The plant, *Jatropha curcas* is commercially important one as bio diesel is extracted from it seeds on industrial scale. Crude latex was obtained by cutting the green stems of *J.curcas* plants. Milky white latex was stored in the refrigeration. For biosynthesis, 20 mL of 3% of latex solution was mixed with 20 mL of 5 10⁻³ M aqueous silver nitrate solution. The reacting

mixture was heated at 85°C with constant stirring for 4h. The bark powder and water extract from *Cinnamomum zeylanicum* tree were used for silver synthesis. The bark was cut into small pieces powdered in a mixer and then sieved using a 20-mesh sieve. The final sieved fraction of powder was used for further experiments. For the production of extract, 2.5 g of powder was added to a 500 mL Erlenmeyer flask with 100 mL distilled water and then boiled for 5 min. For the silver nanoparticles synthesis 100, 500 and 1000 mg of powder were added to 50 mL of 1mM aqueous AgNO₃ solution in a 250 mL Erlenmeyer flask. The flasks were then incubated in the dark at 25°C. In the second way 1, 2.5 and 5 mL of extract were used for the biosynthesis of Ag nanoparticles from 50 mL of 1 mM aqueous AgNO₃ solution. The alfalfa seeds were soaked to avoid fungal contamination in 3 % formaldehyde for 15 min. and washed three times with deionised water. Approximately 100 seeds were transferred to a mason jar and autoclaved for a sterile conditions. The nutrient solution had a composition: Ca(NO₃)₂ 4H₂O (3.57 10⁻⁴ M); H₃BO₃ (2.31 10⁻⁵ M); CaCl₂ 2 H₂O (2.14 10⁻³ M); KH₂PO₄ (9.68 10⁻⁴ M); KNO₃ (2.55 10⁻⁴ M); MgClO₄ (1.04 10⁻³ M); FeCl₃ (6.83 10⁻⁵ M); MnSO₄ H₂O (7.69 10⁻⁶ M); MoO₃ 1.0 10⁻⁵ M) ZnSO₄ H₂O (7.69 10⁻⁵ M); CuSO₄ 5H₂O (1.6 10⁻⁶ M), and agar-agar 1g per 200 mL. Gold(III) ions from potassium tetrachloroaurate was used at concentrations of 0, 5, 10, 20, 40, 80, 160, and 320 ppm. Also, the alfalfa plants were harvested after two weeks of growth.

It has also been published that living alfalfa plants have the capability to take up silver from liquid media. The alfalfa plant samples grown in silver ions rich media were embedded in a synthetic resins and dried in an oven at 65°C for 24 h. TEM analysis suggested that silver atoms accumulated inside the alfalfa plant tissue under nucleation and nanoparticles formation as a correlated processes. The extract from Black Tea has been employed as a reducing agent for the synthesis of Au and Ag nanoparticles. Three different extracts were prepared from Black Tea: (i) tea leaf broth, (ii) ethyl acetate extract and (iii) CH₂Cl₂ extract. Metal nanoparticles were synthesized by adding aqueous solution of AgNO₃ or HAuCl₄ to any of the three extracts. The formation and growth of the nanoparticles was monitored with the help of absorption

spectroscopy and transmission electron microscopy.

Demberelnyamba Dorjnamjin et. al. [147] reported they developed a new one phase method for the synthesis of uniform monodisperse crystalline Ag nanoparticles in aqueous systems that has been developed by using newly synthesized mono and dihydroxylated ionic liquids and cationic surfactants based on 1,3-disubstituted imidazolium cations and halogens anions. The hydroxyl functionalized ionic liquids (HFILs) and hydroxyl functionalized cationic surfactants (HFCs) also simultaneously acts both as the reductant and protective agent. By changing the carbon chain length, alcohol structure and anion of the 1,3-imidazolium based HFILs and HFCs the particle size, uniform and dispersibility of nanoparticles in aqueous solvents could be controlled. Transmission electron microscopy (TEM), electron diffraction, UV-Vis and NMR, were used for characterization of HFILs, HFCs and silver nanoparticles. TEM studies on the solution showed representative spherical silver nanoparticles with average sizes 2-8 nm, particularly 2.2 nm and 4.5 nm in size range and reasonable narrow particle size distributions (SD-standard distribution) 0.2 nm and 0.5 nm respectively. The all metal nanoparticles are single crystals with face centered cubic (fcc) structure. The silver nanoparticles surface of plasmon resonance band (λ_{max}) around 420 nm broadened and little moved to the long wavelength region that indicating the formation of silver nanoparticles dispersion with broad absorption around infrared (IR) region. Silver complexes of these HFILs as well as different silver nanoparticles dispersions have been tested *in vitro* against several gram positive and gram negative bacteria and fungus. The silver nanoparticles providing environmentally friendly and high antimicrobial activity agents.

There has been an increased awareness about the role of contaminated environmental surfaces within the home and health care settings as a potential vector of disease transmission. Many disinfectants contain nonvolatile antimicrobial agents such as quaternary ammonium compounds (QACs) that can leave an antimicrobial residue on treated surfaces. The potential of these agents to prevent bacterial colonization is limited because of their lack of persistence on surfaces after some environmental

insult, such as water contact or rubbing. These moist environments and physically contacted surfaces are the most likely to be contaminated, to allow bacterial proliferation, and to act as a pathogenic reservoir. For a given disinfectant technology to realize a significant residual antimicrobial benefit, it must persist under such conditions. A unique new silver based material has been shown to provide a persistent antimicrobial benefit that can provide environmental control of pathogenic bacteria on treated surfaces. Research showing colloidal silver's superior performance in fighting multiple disease causing pathogenic microorganisms including anthrax has attracted the attention of leading scientists, given the current concerns over terrorism. Colloidal silver have been known for a long time to possess antimicrobial properties and also to be non-toxic and environmentally friendly. Silver is used as a biocide to sterilize recycled drinking water aboard of the NASA space shuttle and MIR space station. In fact, the real gold standard among antimicrobial agents turns out to be not any type of silver, but a new, patent pending form of stabilized colloidal silver particles that kills virtually any germ^[46-47]. Silver may have an important advantage over conventional antibiotics in that it kills all pathogenic microorganisms, and no organism has ever been reported to readily develop resistance to it. Researchers believe that the potential of colloidal silver is just beginning to be discovered. Nanoparticles have been extensively investigated due to the attraction of their unique physical properties, chemical reactivity, and potential applications with high academic and industrial impacts^[48-50]. Usually when metal nanoparticles are prepared by chemical methods, the metal ions reduced by the reducing agents and a protective agents or phase transfer agents are also added to stabilize the nanoparticles^[51-53]. Several types of toxic reducing agents containing boron commonly have been employed to produce metal nanoparticles from inorganic salts, the resulting metal nanoparticles are contaminated with borides. This allows for the preparation of boride free metal nanopowders specially for use in biological and medical purposes. The use of quaternary ammonium for stabilizing metal nanoparticles was proposed by Bonnemant^[51] and the particles are not dispersible in water because their anionic particle's surface is surrounded by the hydrophobic tetraoctyl quaternary ammonium

ions. Particles synthesized in organic solvents are water-immiscible, which limits their applicability. Many applications require for nanoparticles to be dispersible and stable in water^[54]. However, water-based synthesis of nanoparticles are fraught with many problems such as ionic interactions, low reactant concentration and stabilizers are difficult to remove^[55]. The particles synthesized in organic solvents can be made at relatively high concentrations^[56], with predefined size and shape and with improved monodispersity^[57] when compared with those prepared in aqueous media. Here we first used mono and dihydroxyl functionalized ionic liquids (ILs) and cationic surfactants for preparation silver nanoparticles can be readily dispersed into water. The formation and stabilization efficiency values of HFILs and HFCSs can be tailored by varying the ring structure, the substituting groups in the cations and the counter anions. The anions also plays a key role in formation and stabilization of nanoparticles and can be rationalized by considering the difference in the anion size of surfactants. Based on the same principle, we also envision the potential uses of HFILs and HFCSs containing different functional groups as selective reagents used for the formation and stabilization of nanoparticles. In this research, we synthesized Ag nanoparticles by using newly synthesized mono and dihydroxyl functionalized ILs and cationic surfactants. These ILs and surfactants were designed to have one, and two alcohol groups, different long chain structure, the former serving to produce small nanoparticles with different sizes dependence of number of alcohol groups, chain length and their position in molecules. Herein, we report our preliminary results on the preparation of novel ionic liquids and cationic surfactants terminated alcohol ligands in cation and one pot synthesis of Ag nanoparticles stabilized by these ligands, their antimicrobial properties.

I. H. Maliszewska et. al^[148] the study of biosynthesis of nanomaterials offers a valuable contribution into materials chemistry. The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticles should be employed in the search for new materials^[58]. Biosynthetic methods have been investigated as an alternative to chemical and physical ones. These methods can be divided into two categories depending on the place where the nanoparticles or nanostructures are

created as many microorganisms can provide inorganic materials either intra- or extracellularly [59]. For example, bacteria *Pseudomonas strutzeri* isolated from silver mine materials is able to reduce Ag⁺ ions and accumulates silver nanoparticles, the size of such nanoparticles being in the range 16–40 nm, with the average diameter of 27 nm^[60]. The examples also include magnetotactic bacteria which produce magnetite (Fe₃O₄) or greigite (Fe₃S₄) and diatoms which produce siliceous material^[61]. The intracellular methods need a special ion transportation system into the microbial cell. Formation of magnetite particles proceeds through a sequence of events: reduction of Fe(III) to Fe(II), precipitation of amorphous oxide and subsequent transformation to magnetite^[61]. Gold nanoparticles have also been synthesized in human cells, both in cancer and non-cancer ones^[62]; the scanning microscopic images confirmed that their morphologies differed significantly. This behaviour can have an implication to cancer diagnostics. In contrast, extracellular synthesis of nanoparticles occurs in alkalothermophilic actinomycete, *Thermomonospora* sp., which reduces gold ions. The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for such processes. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp.^[58] or *Aspergillus fumigatus* has been reported^[63]. A novel biological method for synthesis of silver nanoparticles using *Vericillum* was proposed by Mukherjee et al.^[64, 65]; a two-step mechanism was suggested. The first step involves trapping of Ag⁺ ions at the surface of the fungal cells. In the second step, enzymes present in the cell reduce silver ions. The extracellular production of metal nanoparticles by several strains of the fungus *Fusarium oxysporum* has been described by Duran et al.^[66]. The presence of hydrogenase in the *F. oxysporium* broth was demonstrated. This extracellular enzyme shows excellent redox properties and it can act as an electron shuttle in metal reduction. It was evident that electron shuttles or other reducing agents (e.g., hydroquinones) released by microorganisms are capable of reducing ions to nanoparticles. The Neem (*Azadirachta indica*) leaf broth and aqueous solution of silver nitrate or chloroauric acid were used for the extracellular synthesis of pure metallic silver and gold particles^[67]. The

time required for Ag⁺ and Au³⁺ ions to reduce was 4 h and 2 h, respectively, being extremely short compared to both bacteria and fungi (24 h and 120 h). Surface active constituents of the leaf broth stabilize nanoparticle suspensions – an aqueous suspension showed stability even after 4 weeks.

Mishra et al.^[149] have reported diabetes mellitus is most common disease of the altered glucose homeostasis. Diabetics have impaired wound healing and impaired formation of coronary collaterals. The abnormal apoptosis or angiogenesis may cause many of the clinical manifestations of diabetes. Silver has been known to have effective bactericidal properties for centuries. Nowadays, silver-based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers. Silver nanoparticles are novel nanosized and highly crystalline antibacterial agent which carries Ag⁺ ions by ion exchanging. Silver has been used in the clinical setting as an antimicrobial for over a century, and silver nitrate is still a common antimicrobial used in the treatment of chronic wounds^[68]. Silver nitrate causes a significant amount of staining of virtually any surface with which it comes into contact^[69] and can also cause irritation to tissues. Silver sulfadiazine was introduced in the 1960s to overcome some of the shortcomings of silver nitrate, but both are limited in the clinic due to the need for a high frequency of application, inactivation of much of the silver by wound fluid and the formation of a pseudo-eschar. New silver-impregnated dressings such as Acticoat were designed to overcome these limitations, in particular the rapid inactivation of silver. In these dressings, as silver is consumed by interaction with target cells or inactivated by protein and anion complexes in wound fluid, additional silver is released, thus producing a sustained, steady supply of active silver. Silver exerts its antimicrobial effects by interfering with the respiratory chain at the cytochromes^[70]. Silver ions also interfere with components of the microbial electron transport system, bind DNA and inhibit DNA replication^[71-73]. Silver is effective against a broad range of aerobic, anaerobic, Gram-negative and Gram-positive bacteria, yeast, filamentous fungi and viruses^[68, 74-78]. In addition to the antimicrobial properties, silver also appears to have anti-inflammatory properties, as suggested by the loss of rubor in

chronic wounds treated with colloidal silver^[75]. An ideal Ag⁺ donor site dressing material would promote healing, cause minimal pain to the patient, prevent infection, result in minimal scarring, and be inexpensive and easy to use. A dressing which possesses all of these qualities has yet to be developed, but currently many dressing materials meet some of these criteria to varying degrees.

Rakel et al.^[79] concluded that dressings which provide a moist healing environment such as calcium alginate, transparent films, or hydrocolloids were associated with the fastest healing times. Furthermore, hydrocolloid and transparent film dressings were more likely to result in a smooth stable epithelial surface than air exposure, xenografts, gauze, or calcium alginate dressings. Donor site pain was lowest with transparent film dressings, and, in increasing order, higher with hydrocolloids, Biobrane, Tulle gauze, and Scarlet Red. Thus, based on the literature published over the last 30 years, it would appear that transparent films and hydrocolloids come closest to meeting the criteria of fast, stable healing and reduced donor site pain.

M.Raffi et al.^[150] reported silver nanoparticles of mean size 16 nm were synthesized by inert gas condensation (IGC) method. Crystalline structure, morphology and nanoparticles size estimation were conducted by X-ray diffraction (XRD) and transmission electron microscopy (TEM). Antibacterial activity of these silver nanoparticles as a function of particles concentration against gram-negative bacterium *Escherichia coli* (*E. coli*) was carried out in liquid as well as solid growth media. Scanning electron microscopy (SEM) and TEM studies showed that silver nanoparticles after interaction with *E.coli* have adhered to and penetrated into the bacterial cells. Antibacterial properties of silver nanoparticles are attributed to their total surface area, as a larger surface to volume ratio of nanoparticles provides more efficient means for enhanced antibacterial activity. Nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of atoms at the grain boundaries. They represent an important class of

materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications^[80-83]. Synthesis of nanosized drug particles with tailored physical and chemical properties is of great interest in the development of new pharmaceutical products^[84]. Emergence of new resistant bacterial strains to current antibiotics has become a serious public health issue, which raised the need to develop new bactericidal materials^[85]. However, the phenomenon of enhanced biological activity and certain material changes resulting from nanoparticles is not yet understood fairly. Investigations have shown encouraging results about the activity of different drugs and antimicrobial formulation in the form of nanoparticles. Activity of nanoemulsions of oil droplets against bacteria and spores has been analyzed^[7]. Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 type of diseases causing microorganisms^[87]. Silver has been described as being 'oligodynamic' because of its ability to exert a bactericidal effect at minute concentrations^[88]. It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory^[89]. Silver ions (Ag⁺) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells. Therefore, silver ions, being antibacterial component, are employed in formulation of dental resin composites, bone cement, ion exchange fibers and coatings for medical devices^[90-91].

Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of changes in local electronic structures of the surfaces due to smaller sizes. These effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surfaces. Ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. It has been suggested that DNA loses its replication ability once the bacterium are treated with silver ions^[92]. Two dimensional electrophoresis and proteins identification analysis of antibacterial action of silver nanoparticles have disclosed accumulation of envelope proteins precursors.

Silver nanoparticles destabilize plasma membrane potential and depletion of levels of intracellular adenosine triphosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death^[93]. Compounds of silver such as silver nitrate and silver sulfadiazine are used to prevent bacterial growth in drinking water, sterilization and burn care.

It is economical to consolidate silver in polymers, composites, fabrics and catheters for antibacterial functionality^[94-97]. The present study was conducted to synthesize silver nanoparticles by inert gas condensation (IGC) process and characterization of their crystalline structure, morphology, estimation of mean size and distribution by XRD and TEM techniques. Antibacterial activity of these silver nanoparticles as a function of particles concentration has been demonstrated for inhibiting the growth and killing of *E. coli* ATCC-15224 strain in liquid as well as solid growth media. The ultimate objective was to study the interaction between bacteria and silver nanoparticles.

Kim, Keuk-Jun et. al. ^[151] reported spherical silver nanoparticles (nano-Ag) were synthesized and their antifungal effects on fungal pathogens of the skin were investigated. Nano-Ag showed potent activity against clinical isolates and ATCC strains of *Trichophyton mentagrophytes* and *Candida* species (IC80, 1-7 µg/ml). The activity of nano-Ag was comparable to that of amphotericin B, but superior to that of fluconazole (amphotericin B IC80, 1-5 µg/ml; fluconazole IC80, 10-30 µg/ml). Additionally, we investigated their effects on the dimorphism of *Candida albicans*. The results showed nano-Ag exerted activity on the mycelia. Thus, the present study indicates nano-Ag may have considerable antifungal activity, deserving further investigation for clinical applications.

Skin infections caused by fungi, such as *Trichophyton* and *Candida* species, have become more common in recent years ^[108]. In particular, fungal infections are more frequent in patients who are immunocompromised because of cancer chemotherapy, or organ or human immunodeficiency virus infections ^[104]. This upward trend is concerning, considering the limited number of antifungal drugs available because prophylaxis with antifungals may lead to the emergence of resistant strains. Therefore,

there is an inevitable and urgent medical need for novel antifungals. Since ancient times, it has been known that silver and its compounds are effective antimicrobial agents ^[100, 105, 106].

In particular, because of the recent advances in research on metal nanoparticles, nano-Ag has received special attention as a possible antimicrobial agent ^[98, 101, 102, 107]. Therefore, the preparation of uniform nanosized silver particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products ^[99, 103]. Many studies have shown their antimicrobial effects, but the effects of nano-Ag against fungal pathogens of the skin are mostly unknown. In this study, nano-Ag was synthesized and its antifungal effects on clinical isolates and ATCC strains of *Trichophyton mentagrophytes* and *Candida* species were investigated.

D. Jain et. al. ^[136] reported there is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO₃ solution through the extract of papaya fruit as reducing as well as capping agent. Nanoparticles were characterized using UV-Vis absorption spectroscopy, FTIR, XRD and SEM. X-ray diffraction and SEM analysis showed the average particle size of 15 nm as well as revealed their cubic structure. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route.

The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and

compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

Prabhu et. al. ^[144] reported fungal infections remain an important cause of morbidity and mortality in hospitalized patients and others who in contact. The primary factor driving the emergence of antifungal resistance appears to be selective pressure. Resulting from increased research on alternative system of medicine including nanotechnology given the seriousness of fungal infections and difficulties linked with their diagnosis, restricting empiric antifungal therapy may not be the best solution for combating resistance. Furthermore, when resistance does occur it is generally after prolonged exposure to relatively low concentrations of drug such is the case when antifungal prophylaxis is given to immune compromised patients ^[109-111]. Dermatophytosis is one of the most common infectious diseases in humans which are mainly caused by invasion of stratum corneum dermatophytic fungi ^[112]. The various forms of a disease include tinea corporis, tinea pedis, oncomycosis, candidiasis etc. For which many antifungal drugs including terbinafine, triazoles, butenafine have been used clinically for the topical treatment of dermatophytosis ^[113]. The current antifungal armamentarium recommends as first line therapy showed high incidence of toxicity wide adverse events and observable resistancy. This upward trend is considering limited number of antifungal drug available because prophylaxis with antifungal may lead to emergence of resistant strains ^[113,114]. Therefore there is an inevitable and urgent medical need for novel antifungal formulations. In recent advances in research on metal nanoparticles, silver based fungal nanoparticles have received special attention as a possible antimicrobial agent ^[115,116,117,118]. Since ancient times it has been known that silver and its compounds are effective antimicrobial agents ^[114,119]. There, the preparation of nanosized silver particles with specific requirements in terms of

size, shape and chemical properties is of great interest in the formulation of new pharmaceutical products.

Jun Sung Kim et. al. ^[133] reported the antimicrobial effects of silver (Ag) ion or salts are well known, but the effects of Ag nanoparticles on microorganisms and antimicrobial mechanism have not been revealed clearly. Stable Ag nanoparticles were prepared and their shape and size distribution characterized by particle characterizer and transmission electron microscopic study. The antimicrobial activity of Ag nanoparticles was investigated against yeast, Escherichia coli, and Staphylococcus aureus. In these tests, Muller Hinton agar plates were used and Ag nanoparticles of various concentrations were supplemented in liquid systems. As results, yeast and E. coli were inhibited at the low concentration of Ag nanoparticles, whereas the growth-inhibitory effects on S. aureus were mild. The free-radical generation effect of Ag nanoparticles on microbial growth inhibition was investigated by electron spin resonance spectroscopy. These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics ^[120]. The antibacterial effects of Ag salts have been noticed since antiquity ^[121], and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds ^[122,123]. In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including E. coli ^[124]. Recently, Mecking and co-workers showed that hybrids of Ag nanoparticles with amphiphilic hyperbranched macromolecules exhibited effective antimicrobial surface coating agents ^[125].

Reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. In fact, nanotechnology helps in overcoming the limitations of size and can change the outlook of the world regarding science [126]. Furthermore, nanomaterials can be modified for better efficiency to facilitate their applications in different fields such as bioscience and medicine. In this study our research team, consisting of experts in several disciplines, has investigated the antimicrobial effects of Ag nanoparticles against representative microorganisms of public concern. A possibility of free-radical involvement near the Ag nanoparticles surface in the antimicrobial activity of Ag nanoparticles was discussed based on electron spin resonance (ESR) measurements. Here, we report that Ag nanoparticles can be applied effectively in the control of microorganisms and the prevention of deleterious infections. Our results support the hypothesis that Ag nanoparticles can be prepared in a simple and cost-effective manner and are suitable for formulation of new types of bactericidal materials.

Mritunjai singh et. al. [84] reported nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale, stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. In all the nanomaterials with antibacterial properties, metallic nanoparticles are the best. Nanoparticles increase chemical activity due to crystallographic surface structure with their large surface to volume ratio. The importance of bactericidal nanomaterials study is because of the increase in new resistant strains of bacteria against most potent antibiotics. This has promoted research in the well known activity of silver ions and silver-based compounds, including silver nanoparticles. This effect was size and dose dependent and was more

pronounced against gram-negative bacteria than gram-positive organisms.

Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging [128], sensing [129], targeted drug delivery [130] and gene delivery systems [131] and artificial implants [132]. The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer [133] and fight human pathogens like bacteria [134-138]. The development of new resistant strains of bacteria to current antibiotics [139] has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [10]. Bacteria have different membrane structures which allow a general classification of them as Gram-negative or Gram positive. The structural differences lie in the organization of a key component of the membrane, peptidoglycan. Gram negative bacteria exhibit only a thin peptidoglycan layer (~2-3 nm) between the cytoplasmic membrane and the outer membrane [140]; in contrast, Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer of about 30 nm thick micro-organisms; for this reason silver-based compounds have been used extensively in many bactericidal applications. Silver compounds have also been used in the medical field to treat burns and a variety of infections. Several salts of silver and their derivatives are commercially employed as antimicrobial agents. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size dependent interaction of silver nanoparticles with bacteria [137]. Nanoparticles of silver have thus been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials. However, the bactericidal property of these nanoparticles depends on their stability in the growth medium, since this imparts greater retention time for bacterium-nanoparticle interaction. There lies a strong challenge in preparing nanoparticles of silver stable enough to significantly restrict bacterial growth. Studies were carried out on both antibiotic resistant (ampicillin-resistant) and nonresistant strains of

gram-negative (*Escherichia coli*) and a non-resistant strain of gram-positive bacteria (*Staphylococcus aureus*). A multi-drug resistant strain of gram-negative (*Salmonella typhus*, resistant to chloramphenicol, amoxycilin and trimethoprim) bacteria was also subjected to analysis to examine the antibacterial effect of the nanoparticles]. Efforts have been made to understand the underlying molecular mechanism of such antimicrobial actions. The effect of the nanoparticles was found to be significantly more pronounced on the gram-negative strains, irrespective of whether the strains were resistant or not, than on the gram-positive organisms. We attribute this enhanced antibacterial effect of the nanoparticles to their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the bacterial proteins and arrests bacterial growth.

The bactericidal effect of silver ions on microorganisms is very well known; however, the bactericidal mechanism is only partially understood. It has been proposed that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. Experimental evidence suggests that DNA loses its replication ability once the bacteria have been treated with silver ions. Other studies have shown evidence of structural changes in the cell membrane as well as the formation of small electron-dense granules formed by silver and sulfur. Silver ions have been demonstrated to be useful and effective in bactericidal applications, but due to the unique properties of nanoparticles nanotechnology presents a reasonable alternative for development of new bactericides. Metal particles in the nanometer size range exhibit physical properties that are different from both the ion and the bulk material. This makes them exhibit remarkable properties such as increased catalytic activity due to morphologies with highly active facets. We can apply several electron microscopy techniques to study the mechanism by which silver nanoparticles interact with these bacteria. We can use high angle annular dark field (HAADF) scanning transmission electron microscopy (STEM), and developed a novel sample preparation that avoids the use of heavy metal based compounds such as OsO₄. High resolutions and more accurate x-ray microanalysis were obtained.

4. Silver Nano Particles Against Hiv Pathogens

Jose Luis Elechiguerra et. al.^[116] reported the interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. Within this field, an area that has been largely unexplored is the interaction of metal nanoparticles with viruses. Silver nanoparticles undergo a size-dependent interaction with HIV-1, with nanoparticles exclusively in the range of 1–10 nm attached to the virus. The regular spatial arrangement of the attached nanoparticles, the center-to-center distance between nanoparticles, and the fact that the exposed sulfur-bearing residues of the glycoprotein knobs would be attractive sites for nanoparticle interaction suggest that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells, as demonstrated in vitro.

Among noble-metal nanomaterials, silver nanoparticles have received considerable attention due to their attractive physicochemical properties. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling^[141], where phenomena such as surface enhance Raman scattering (SERS) can be exploited. In addition, the strong toxicity that silver exhibits in various chemical forms to a wide range of microorganisms is very well known^[142-144], and silver nanoparticles have recently been shown to be a promising antimicrobial material^[145].

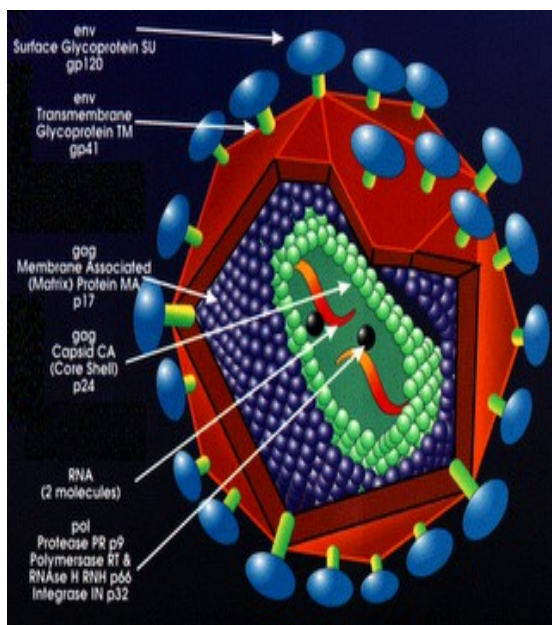


Fig 11 .structure of HIV

5. Conclusion

Synthesis and characterization of silver nano particles has attracted attention in recent years because of their biomedical applications .Silver nano particles have been synthesised using chemical, photochemical and biological methods. The reduction of the metal ions through the green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc leading to the formation of silver nanoparticles of fairly well-defined dimensions. Applications of such eco-friendly silver nanoparticles in bactericidal, fungicidal, wound healing and other medical applications makes them potentially emerging ones.

References

- [1]. R.W Glomm, " Functionalized Nanoparticles for Application in Biotechnology", *J.Dispersion Sci. Technology*, 26, 2005, pp 389-314.
- [2]. A Ahmad, P Mukherjee, S Senapati, D Mandal, M.I Khan, R Kumar and M Sastry . "Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium*

oxysporum" , *Colloids Surfaces, B: Biointerfaces*, 27, 2003, pp 313-318 .

[3]. M. Rai, A. Yadav, and A. Gade, *Biotechnol. Advances*. 27, 2009, pp 7-83 .

[4]. J. R. Morones, J. L. Elechiguerra, A. Camacho and J. T. Ramirez, *Nanotechnol.* 1, 2005, pp2346-2353 .

[5]. J. S. Kim, E. Kulk, K. N. Yu, J. H. Kim, S. J. Park and H. J. Lee , *Nanomed Nanotechnol Biol Med*. 3, 2007 ,pp 95-101.

[6]. M. A. Albrecht, C.W. Evan and C. R. Raston, *Green Chem*. 8, 2006, pp 417-432 .

[7]. JL Gilmore, X Yi, L Quan and AV Kabanov. "Novel Nanomaterials for Clinical Neuroscience" , *J Neuroimmune Pharmacol*. Jan 22, 2008.

[8]. TJ Deerinck , "The application of fluorescent quantum dots to confocal, multiphoton, and electron microscopic imaging", *Toxicol Pathol.*, 36(1), 2008, pp 112-6.

[9]. SJ Son, X Bai and SB Lee. , "Inorganic hollow nanoparticles and nanotubes in Nanomedicine" Part 1. Drug/gene delivery application , *Drug Discov Today*. Aug;12(15-16), 2007, pp 650-6.

[10]. R Duncan, and F Spreafico, Polymer conjugates. "Pharmacokinetic considerations for design and development", *Clin Pharmacokinet*. Oct;27(4), 1994, pp290-306.

[11]. BJ Boyd., "Past and future evolution in colloidal drug delivery system" , *Expert Opin Drug Deliv*. Jan;5(1), 2008, pp 69-85.

[12]. R Duncan. "Designing polymer conjugates as lysosomotropic Nanomedicine", *Biochem Soc Trans*. Feb;35(Pt 1)56-60.

[13] T. Trindade, P. O'Brien, and N. L. Pickett, "Anocrystalline Semiconductors: Synthesis, Properties, and Perspectives", *Chem. Mater.*, . 13, 2001, pp3843.

[14] C Burda , "Chemistry and Properties of Nanocrystals of Different Shapes", *Chem.Rev.*, 105, 2005, pp 1025.

- [15] C. F. Bohren., and D. R. Huffman, "Absorption and Scattering of Light by Small Particles", New York: Wiley, 1983.
- [16] J. A. Creighton., and D. G. Eadon, "New Mixed Quantum/Semiclassical Propagation Method", J. Chem. Soc. Faraday Trans., 87, 1991, pp3881.
- [17] W. Ostwald, *Theoretical and Applied Colloid Chemistry*, New York: Wiley, 1922, p. 200.
- [18] U. Kreibig, and L. Genzel, "Optical Absorption of Small Metallic Particles", Surf. Sci., 156, 1985, pp 678.
- [19] C. V. Fragstein, and U. Z. Kreibig, "Studies of Intersystem Crossing Dynamics in Acetylene", Phys. 224, 1969, pp 306.
- [20] R. H. Doremus, "Optical Properties of Small Silver Particle", J. Chem. Phys., 42, 1965, pp 414
- [21] W. T. Doyle, "Absorption of Light by Colloids in Alkali Halide Crystals", Phys. Rev. 111, 1958, pp 1067.
- [22] D. R. Huffman, "An Introduction to Physics and Technology of Thin Films", Wagendristerl, A., and Wang, Y., (eds.), New Jersey: World Scientific, 1988.
- [23] D. C. Ralph, C. T. Black, and M. Tinkham, "Spectroscopy of the Superconducting Gap in Individual Nanometer-Scale Aluminum Particles", M. Phys. Rev. Lett., 74, 1995, pp 3241-3244.
- [24] A. B. Bazyliniski and B. R. Frankel, "Magnetosome formation in Procarayotes", Nature reviews- Microbiology, 2, 2004, pp 213-230.
- [25] F. M. Lengke, E. M. Fleet and G. Southam, "Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver(I) nitrate complex", Langmuir, 23, 2007, pp 2694-2699.
- [26] K. Kalishwaralal, V. Deepak, S. Ramkumar, P. N. H. Nellaiah and G. Sangiliyandi, "Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis". Materials Letters, 62, 2008, pp 4411-4413.
- [27] R. H. Shahverdi, S. Minaeian, H. Jamalifar and A. A. Nohi, "Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria", A novel biological approach. Process Biochemistry, 42, 2007, pp919-923.
- [28] M. Mokhtari, S. Deneshpojuoh, S. Seyedbagheri, R. Atashdehghan, K. Abdi, S. Sarkar, S. Minaeian, R. H. Shahverdi and R. A. Shahverdi, "Biological synthesis of very small silver nanoparticles by culture supernatant of Klebsiella pneumonia, The effects of visible-light irradiation and the liquid mixing process", Materials Research Bull., 44, 2009, pp1415-1421.
- [29] S. Minaeian, R. A. Shahverdi, S. A. Nohi and R. H. Shahverdi, "Extracellular biosynthesis of silver nanoparticles by some bacteria", J. Sci. I.A., 17, 66, 2008, pp 1-4.
- [30] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, R. S. Sainkar, I. M. Khan, R. Parishcha, V. P. Ajaykumar, M. Alam, R. Kumar and M. Sastry, "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelia matrix". A novel biological approach to nanoparticle synthesis, Nano Letters, 1, 10, 2001, pp 515-519.
- [31] K. Kalimuthu; S. R. Babu, D. Venkataraman, M. Bilal and S. Gurunathan. "Biosynthesis of silver nanoparticles by Bacillus licheniformis", Colloids Surfaces B: Biointerfaces, 65, 150-153 (2008).
- [32] Z. Sadowski, I. Maliszewska, I. Polowczyk, T. Kozlecki and B. Grochowalska, "Biosynthesis of colloidal-silver particles using microorganisms", Polish J. Chem., 82, 2008, pp 377-382.
- [33] N. Saifuddin, W. C. Wang and A. A. Nur Yasumir "Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation", EJournal Chem., 6, 2, 2009, pp 61-70.
- [34] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar and M. Sastry, "Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum", Colloids Surfaces, B: Biointerfaces, 27, 2003, pp313-318.

- [35] C K Bhainsa and F S D'Souza, "Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus funigatus*", *Colloids Surfaces B: Biointerfaces*, 47, 2006, pp 160-164.
- [36] N Duran, D P Marcato, L O Alves, G De Souza and E Esposito, "Mechanical aspect of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains". *J Nanobiotechnology*, 3, 2005, pp 8-15.
- [37] S Basavaraja, D S Balaji, A Lagashetty, H A Rajasab and A Venkataraman, "Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*", *Materials Research Bulletin*, 43, 2008, pp 1164-1170.
- [38] S N Shaligram, M Bule, R Bhambure, S R Singhal, K S Singh, G Szakacs and A Pandey, "Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungus", *Process Biochemistry*, 44, 2009, pp 939-943.
- [39] K Kathiresan, S Manivannan, A M Nabeel and B Dhivya, "Studies on silver nanoparticles synthesized by a marine fungus *Penicillium fellutanum* isolated from coastal mangrove sediment", *Colloids Surfaces B: Biointerfaces*, 71, 2009, pp 133-137.
- [40] S D Balaji, S Basavaraja, R Deshpande, B D Mahesh, K B Prabhakara and A Venkataraman, "Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus", *Colloids Surfaces B: Biointerfaces*, 68, 2009, pp 88-92.
- [41] D Philip, "Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract", *Spectrochimica Acta Part A*, 73, 2009, pp 374-381.
- [42] J Huang, Q. Li, D Sun, Y Lu, Y Su, X Yang, H Wanh, Y Wang, W Shao, N He, J Hong and C Chen, "Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum canphora* leaf", *Nanotechnology*, 18, 2007, pp 1-11.
- [43] S Shiv Shankar, A Ahmad and M Sastry, "Geranium leaf assisted biosynthesis of silver nanoparticles", *Biotechnol. Prog.*, 19, 2003, pp 1627-1631.
- [44] P S Chandra, M Chaudhary, R Pasricha, A Ahmad and M Sastry, "Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract", *Biotechnology Prog.*, 22, 2006, pp 577-583.
- [45] K.A Jha and K Prasad, "Biosynthesis of silver nanoparticles using *Cycas* leaf broth". *Bioprocess Biosystems Eng.*, in press. 2009.
- [46] M J Brady, C M Lisay, A V Yurkovetskiy and S P Sawan, "Persistent silver disinfectant for the environmental control of pathogenic bacteria". *Am. J. Infect. Cont.* 31, 2002, pp 208-214.
- [47] J Nuorti, J Butler, J Crutcher, "An outbreak of multidrug-resistant pneumonia and bacteremia among unvaccinated nursing home residents". *New Engl. J. Med.*, 338, 1998, 1861-1868.
- [48] R F Service. "Atom-Scale Research Gets Real". *Science*, 290, 2000, pp 1524-1531 (special issue on nanotechnology)
- [49] H G Craighead. "Nano electromechanical systems". *Science*, 290, 2000, pp 1532-1535.
- [50] S R Quake, A Scherer, "From Micro- to Nanofabrication with Soft Materials". *Science*, 290, 2000, pp 1536-1540.
- [51] H Bonnemann, W Brijoux, R Brinkmann, A S Tilling, T. Schilling, B. Tesche, K Seevogel; R Franke; J. G Hormes, J.R Pollmann and W. Vogel, "Selective oxidation of glucose on bismuth promoted Pd-Pt/C catalysts prepared from NOct4Cl-stabilized Pd-Pt colloids". *Inorg. Chim. Acta*, 270, 1998, pp 95-110.
- [52] L N Lewis, "Chemical catalysis by colloids and clusters". *Chem. Rev.*, 93, 1993, pp 2693-2730.
- [53] J H Fendler, "Nanoparticles and nanostructured films"; Wiley: Weinheim, 1998.
- [54] D V Goia and E Matijevic, "Preparation of monodispersed metal particles". *New J. Chem.*, 22, 1998, pp 1203.
- [55] H Bonnemann and W Brijoux, "In Advanced Catalysts and Nanostructured Materials"; Moser, W., Ed.; Academic Press: New York, USA, 1996.

- [56]. M Green and P O Brien, "Recent advances in the preparation of semiconductors as isolated nanometric particles: new routes to quantum dots". Chem. Commun., 1999,pp 2235-2236.
- [57]. M P Pileni, "Optical properties of nanosized particles dispersed in colloidal solutions or arranged in 2D or 3D superlattices". New J. Chem., 22, 1998,pp 693-702.
- [58] D Mandal ,M E Bolander ,D Mukhopadhyay ,G Sarkar and P Mukherjee , Appl. Microbiol. Biotechnol. 69 ,2006,pp 485.
- [59] S. Mann (Ed.), Biomimetic Materials Chemistry, VCH, New York, 1996..
- [60] T Klaus-Joerger ,R Joerger ,E Olsson and C G Granqvist , Trends Biotechnol., 19,2001,pp 15.
- [61] S Mann ., Biomineralization, Principles and Concepts in Bioinorganic Materials Chemistry, Oxford University Press, 2001.
- [62]J A Sai Vankataraman ,C Subramaniam ,R R Kumar ,S Priya ,R T Santhosh , V.R. Omkumar ,A John ,and T Pradeo , Langmuir, 21 , 2005,pp 11562.
- [63] K C Bhanska and S F D'souza ., Coll. Surf. B, 47 ,2006, pp 160.
- [64] P Mukherjee ,A Ahmed.,D Mandal ,S Senapati ,S E Saikar ,I M Khan ,R Ramani , R Parishcha ,P V Ajaya Kumar ,M Alam ,M Sastry and R Kumar , Angew. Chem. Int. Ed., 40, 2001, pp 3585.
- [65] P Mukherjee ,A Ahmad ,D Mandal ,S Senapati ,S R Saikar , I M Khan ,R Parishcha ,P V Ajaya Kumar ,M Alam ,R Kumar and M Sastry , Nano Lett., 1 , 2001, pp 515.
- [66] N Duran ,D P Marcato ,L O Alves ,I H G DeSouza and E Esposito ., J.Nanobiotechnol., 3 , 2005, pp 8 .
- [67] S Shankar ,A Rai ,A Ahmad and M Sastry , J. Colloid Inter. Sci., 275, 2004,pp 496,
- [68] J B Wright ,K Lam , D Hanson and RE Burrell , Am J Inf Cont 27, 1999,pp 344 .
- [69] D G Klein ,DE Fritsch and SG Amin , Crit Care Nursing Clin NA7, 1995,pp 627.
- [70] P D Bragg and DJ Raimie , "The effect of silver ions on the respiratory chain of E coli". Can J Microbiol 20, 1974, pp 883 .
- [71] C Cervantes and S Silver , "Metal resistance in Pseudomonas: genes and mechanisms",Edited T Nakazawa ,K Furukawa,D Haas,S Silver, Molecular Biology of Pseudomonas. American Society for Microbiology, Washington 1996.
- [72] Modak SM and Fox CL, " Binding of silver sulfadiazine to the cellular components of Pseudomonas aeruginosa". Biochem Pharmacol 22, 1973,pp 2391 .
- [73]HS Rosenkranz and S Rosenkranz , "Silver sulfadiazine: interaction with isolated deoxyribonucleic acid". Antimicrob Agents Chemother 2, 1972,pp 373.
- [74]N Grier , Silver and its compounds. In: Block SS, editor. Disinfection, Sterilization and Preservation, 3rd ed. Philadelphia, PA: Lea & Febiger, 1983.
- [75]R H Demling and L DeSanti , "Effects of silver on wound management". Wounds 13, 2001,pp 4.
- [76]W W Monafu and CA Moyer , The treatment of extensive thermal burns with 0.5% silver nitrate solution. Ann NY Acad.Sci. 150, 1968,pp 937 .
- [77]C L Fox , Silver sulfadiazine - a new topical therapy for Pseudomonas in burns. Arch. Surg. 96, 1968,pp 184 .
- [78]H Q Yin ,R Langford ,and RE Burrell , Comparative evaluation of the antimicrobial activity of ActicoatTM antimicrobial barrier dressing. J Bum Care and Rehab. 20, 1999, pp 195 .
- [79] B A Rakel ,MA Bermel ,LI Abbott ,SK Baumler ,MR Burger and CJ Dawson . Split-thickness skin graft donor site care: a quantitative synthesis of the research. Appl. Nurs Res 11,1998,pp 174 .
- [80] R.W.Sigel: Mater. Sci. Eng. B, , 19, 1993, 37.

- [81] C.Suryanarayana: *Int. Mater. Rev.*,40(2), 1995, pp 41.
- [82] H.Gleiter: *Acta Mater.*, 48(1),2000,pp 1.
- [83] H.J.Lee, S.Y.Yeo and S.H.Jeong: *J. Mater. Sci.*, 38(10), 2003,pp 2199.
- [84] Mritunjai Singh, Shinjini Singh, S. Prasada and I. S.Gambhir, "Nanotechnology In Medicine And Antibacterial Effect Of Silver Nanoparticle" , *Digest Journal of Nanomaterials and Biostructures* 3,3, 2008, pp 115 – 122.
- [85] J.R.Morones, J.L.Elechiguerra, A.Camacho, K.Holt,J.B.Kouri, J.T.Ramirez and M.J.Yacaman: *Nanotechnology*, 16(10),2005,pp 2346.
- [86] P.K.Stoimenov, R.L.Klinger, G.L.Marchin and K.J.Klabunde: *Langmuir*, 18, 2002,6679
- [87] S.H.Jeong, S.Y.Yeo and S.C.Yi, *Mater. Sci.*, , 40,2005, pp 5407.
- [88] S.L.Percivala, P.G.Bowler and D.Russell, *J. Hosp. Infect.*, , 60(1),2005, pp 1.
- [89] P.L.Taylor, A.L.Ussher and R.E.Burrell,*Biomaterials*, , 26(35), 2005, pp 7221.
- [90] A.Panacek, L.Kvitek, R.Prucek, M.Kolar, R.Vecerova,N.Pizurova, V.K.Sharma, T.Nevecna and R.Zboril, *J.Phys. Chem. B*, , 110(33),2006,pp 16248.
- [91] V.Alt, T.Bechert, P.Steinrucke, M.Wagener, P.Seidel, E.Dingeldein, E.Domann and R.Schnettler, *Biomaterials*, , 25(18),2004,pp 4383.
- [92] J.R.Morones, J.L.Elechiguerra, A.Camacho, K.Holt,J.B.Kouri, J.T.Ramirez and M.J.Yacaman: *Nanotechnology*,16(10),2005,pp 2346.
- [93] C.N.Lok, C.M.Ho, R.Chen, Q.Y.He, W.Y.Yu,H.Z.Sun, P.K.H.Tam, J.F.Chiu and C.M.Che, *J. ProteomeRes*, 5, 2006, pp 916.
- [94] A.Oloffs, C.Grossesiestrup, S.Bisson, M.Rinck,R.Rudolph and U.Gross, *Biomaterials*, 15(10),1994,pp 753.
- [95] P.Li, J.Li, C.Z.Wu, Q.S.Wu and J.Li, *Nanotechnology*, , 16(9), 2005, pp 1912.
- [96] S.T.Zhang, R.W.Fu, D.C.Wu, W.Xu, Q.W.Ye andZ.L.Chen, *Carbon*, 42(15), 2004,pp 3209
- [97].C Baker, A. Pradhan, L. Pakstis, D. J. Pochan, and S. I. Shah.. "Synthesis and antibacterial properties of silver nanoparticless", *J. Nanosci. Nanotechnol.* 5,2005,pp 244-249.
- [98]. I C Brigger, C. Dubernet, and P. Couvreur, Nanoparticles in cancer therapy and diagnosis,*Adv. Drug Deliv. Rev.* 54,2002,pp 631-651.
- [99].H J Klasen, A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. *Burns* 26,2000, pp 131-138.
- [100].B U Lee, S. H. Yun, J.-H. Ji, and G.-N. Bae., "Inactivation of *S. epidermidis*, *B. subtilis*, and *E. coli* bacteria bioaerosols deposited on a filter utilizing airborne silver nanoparticless". *J. Microbiol. Biotechnol.* 18,2008, pp 176-182.
- [101].A Melaiye, Z. Sun, K. Hindi, A. Milsted, D. Ely, D. H. Reneker, C. A. Tessier, and W. J. Youngs., "Silver(I)-imidazole cyclophane gem-diol complexes encapsulated by electrospun tectophilic nanofibers: Formation of nanosilver particles and antimicrobial activity". *J. Am. Chem. Soc.* 127, 2005,pp 2285-2291
- [102].E Merisko-Liversidge, G. G. Liversidge, and E. R. Cooper, "Nanosizing: A formulation approach for poorly-watersoluble compounds". *Eur. J. Pharm. Sci.* 18,2003,pp 113-120
- [103] P N Mirmirani, A. Hessel, T. A. Maurer, T. G. Berger, P. Nguyen and A. Khalsa, "Prevalence and predictors of skin disease in the Women's Interagency HIV Study" (WIHS). *J. Am. Acad. Dermatol.* 44,2001,pp 785-788.
- [104]. A D Russell and W. B. Hugo. "Antimicrobial activity and action of silver". *Prog. Med. Chem.* 31,1994,pp 351-370.
- [105]. S Silver, "Bacterial silver resistance: Molecular biology and uses and misuses of silver compounds". *FEMS Microbiol. Rev.* 27,2003, pp 341-353.

- [106].I Sondi and B. Salopek-Sondi. "Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria". *J. Colloid Interface Sci.* 275,2004, 177-182.
- [107]. Woodfolk, J. A.. Allergy and dermatophytes. *Clin. Microbiol. Rev.* 18,2005,pp 30-43.
- [108].H E Jones :*Journal . Amer Acad. Dermatol* ,28 1993,pp 512- 8 .
- [109] K Uchida and H Yamaguchi .*Japanese Journal of Antibiotics*,47, 1994,pp 1401–6.
- [110] H Wakabayashi ,T Hiratani , K Uchida and H Yamaguchi, *Journal. Infec. Chemotherapy*; 1,1996,pp 185–9.
- [111] I Weitzman,R.C Summerbell, *Clinical Microbiology Reviews*,; 8,1995,pp 240–59.
- [112] S Watanabe, *Japanese Journal of Medical Mycology*, 40,1999,pp 151–5.
- [113] Kim.,Keuk – Jun.,Woo Sang Sung.,Seok – ki Moon.,Jong – Soochoi.,Jong Guk kim.,Dong Gun Lee, *J. Microbiol. Biotechnol.*; 18(8),2008,pp 1482-1484.
- [114] N Mclain , R Ascanio. ,C Baker. R. A Strohaber. *J M Dolan* , 44, 2000,pp 2873-2875.
- [115] A Melaiye ,Z Sun ,k Hindi ,A. Milsted,D Ely,K.H Reneker ,C.A Jessier, W.J Youngs , J. Am. Chemo Soc.,; 127,2005,pp 2285-2291.
- [116] Jose Luis Elechiguerra, Justin L Burt, Jose R Morones, Alejandra Camacho-Bragado, Xiaoxia Gao, Humberto H Lara and Miguel Jose Yacaman, " Interaction of silver nanoparticles with HIV-1", *Journal of Nanobiotechnology*, 3,2005, pp 6 .
- [117]H.J Klasen , *Burns*, 26,2000,pp 131-138.
- [118] S A Jones , PG Bowler ,M Walker and D Parsons , "Controlling wound bioburden with a novel silver-containing Hydrofiber dressing". *Wound Repair Regen*;12(3),2004,pp 288- 94.
- [119] S Silver and LT Phung. "Bacterial heavy metal resistance: new surprises". *Annu Rev Microbiol*;1996,50,pp 753- 89.
- [120] M Catauro, MG Raucci, FD De Gaetano and A Marotta . "Antibacterial and bioactive silver-containing Na₂O- CaO -2SiO₂ glass prepared by sol-gel method". *J Mater Sci Mater Med*;15(7),2004,pp 831 - 7.
- [121]J H Crabtree, RJ Burchette , RA Siddiqi , IT Huen ,LL Handott and A Fishman , "The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections". *Perit Dial Int*;23(4),2003,pp 368- 74.
- [122] G Zhao and Jr S E Stevens , "Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion". *Biomaterials*;11,1998,pp 27 – 32.
- [123] C Aymonier , U Schlotterbeck ,L Antonietti ,P Zacharias , R Thomann and JC Tiller , "Hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibiting antimicrobial properties". *Chem Commun (Camb)*; 24,2002,pp 3018- 9.
- [124] CA Mirkin and TA Taton . *Semiconductors meet biology. Nature* ; 405,2000,pp 626- 7
- [125] A. Curtis and C. Wilkinson, *Trends Biotechnol.* 19, 2001,pp 97 .
- [126] C. W. Warren and S. Nie, *Science* 281, 1998, pp 2016 .
- [127] A. Vaseashta and D. Dimova-Malinovska, *Sci. Technol. Adv. Mater.* 6 2005,pp 312 .
- [128] R. Langer, *Science* 293, 2001,pp 58 .
- [129] K. Roy, H. Q. Mao, S. K. Huang and K. W. Leong, *Nat. Med.* 5 1999,pp 387
- [130] E. Sachlos, D. Gotoro and J. T. Czernuszka, *Tissue Eng.* 12 2006,pp 2479
- [131] O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie and R.Langer, *Proc. Natl Acad. Sci. USA* 103, 2006, pp 6315 .

- [132] P. K. Stoimenov, R. L. Klinger, G. L. Marchin and K. J. Klabunde, *Langmuir* 18, 2002, pp 6679 .
- [133] Jun Sung Kim, Eunye Kuk, Kyeong Nam Yu, Jong-Ho Kim, Sung Jin Park, Hu Jang Lee, So Hyun Kim, Young Kyung Park, Yong Ho Park, Cheol-Yong Hwang, Yong-Kwon Kim, Yoon-Sik Lee, Dae Hong Jeong, Myung-Haing Cho, Antimicrobial effects of silver nanoparticles ,*Nanomedicine: Nanotechnology, Biology, and Medicine* 3 ,95– 2007,101.
- [134] A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V. K. Sharma, T. Nevecna and R. Zboril, *J. Phys. Chem. B* 110, 2006, pp 16248 .
- [135] J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramirez and M. J. Yacaman, *Nanotechnology* 16, 2005, pp 2346 .
- [136] D. Jain, H. Kumar Daima, S. Kachhwaha and S. L. Kothari, *Synthesis OF Plant-Mediated Silver Nanoparticles Using Papaya Fruit Extract And Evaluation OF Their Anti-Microbial Activities Digest Journal of Nanomaterials and Biostructures* 4, 3,2005, pp 557 – 563.
- [137] S. V. Kyriacou, W. J. Brownlow and N. Xu X-H, *Biochemistry* 43,2004, pp 140 .
- [138] R. G. E. Murray, P. Steed and H. E. Elson, *Can. J. Microbiol.* 11, 1965,pp 547
- [140].Schultz S, Smith DR, Mock JJ and Schultz DA: “Single-target molecule detection with nonbleaching multicolor optical immunolabels”. *Proceedings of the National Academy of Sciences of the United States of America* FIELD Publication Date: Feb 1 97,2000,pp 996-1001
- [141].S Y Liao ,D C Read ,W J Pugh ,J R Furr and A D Russell : Interaction of silver nitrate with readily identifiable groups: Relationship to the antibacterial action of silver ions. *Lett Appl Microbiol*, 25,1997, pp 279-283.
- [142].A Gupta and S Silver ,“Silver as biocide:Will resistance become a problem?” *Nat Biotechnol* 16,1998, pp 888.
- [143]. K Nomiya, A Yoshizawa, K Tsukagoshi, NC Kasuga, S Hirakawa and J Watanabe ,”Synthesis and structural characterization of silver (I), aluminium (III) and cobalt(II) complexes with 4-isopropyltropolone (hinokitiol) showing noteworthy biological activities”. *Action of silver(I)-oxygen bonding complexes on the antimicrobial activities. J Inorg Biochem* 98,2004,pp 46-60.
- [144]. Prabhu N, Ayisha Siddiqua , Yamuna Gowri k , And Divya T. Raj, *Antifungal Activity Of Silver Fungal Nanoparticles – A Novel Therapeutic Approach ,Arch Pharm Sci & Res* 2 , 2 ,2010,pp 355-359
- [145]. Vijayalakshmi kattumuri : *Gold Nanoparticles For Biomedical Applications: Synthesis, Characterization, In Vitro And In Vivo Studies* , December 2006
- [146] Yu D,Yam VW-W.*J Phys Chemm B* 109 (12) ,2005,pp 5497.
- [147] Demberelnyamba Dorjnamjin, Maama Ariunaa and Young Key Shim, “Synthesis of Silver Nanoparticles Using Hydroxyl Functionalized Ionic Liquids and Their Antimicrobial Activity”. *Int. J. Mol. Sci.*, 9,2008,pp 807-820 .
- [148] Z. Sadowski, I. H. Maliszewska, B. Grochowalska, I. Polowczyk and T. Koźlecki, “Synthesis of silver nanoparticles using microorganisms ,*Materials Science-Poland*”, 26,2008,pp 2 .
- [149] Manish Mishra, Hemant Kumar and Kamlakar Tripathi, “Diabetic Delayed Wound Healing And The Role Of SilverNanoparticles”,*Digest Journal of Nanomaterials and Biostructures* 3, 2,2008,pp 49 – 54.
- [150] M.Raffi, F.Hussain,, T.M.Bhatt, J.I.Akhter, A.Hameed and M.M.Hasan ,*Antibacterial Characterization of Silver Nanoparticles against E: Coli ATCC-15224, J. Mater. Sci. Technol.*, 24,2008,pp 2.
- [151] Kim, Keuk-Jun, Woo Sang Sung, Seok-Ki Moon, Jong-Soo Choi, Jong Guk Kim, and Dong Gun Lee, “Antifungal Effect of Silver Nanoparticles on Dermatophytes”, *J. Microbiol. Biotechnol.*, 18(8), 2008,pp 1482–1484.

TABLE OF CONTENTS

1. INTRODUCTION	2-8
2. SILVER NANOPARTICLES	8-10
3. BIOSYNTHESIS OF SILVER NANOPARTICLES	
<i>3.1 Synthesis of nanoparticles by bacteria</i>	11-16
<i>3.2 Synthesis of nanoparticles by the fungal system</i>	16-20
<i>3.3: Silver nanoparticles synthesis using plant extracts</i>	20-22
4. SILVER NANO PARTICLES AGAINST HIV PATHOGENS	32-33
5. CONCLUSIONS	33-34
6. REFERENCES	

