

SILVER NANO PARTICLES-INDUCED CYTOTOXICITY

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Abstract: The demand for silver nanoparticles (AgNPs) is growing rapidly in many streams such as medical, pharmaceutical, healthcare, food, consumer, cosmetic, etc. Due to its use, it is used for several applications such as antibacterial properties, household, medicine. equipment and food industry, wound dressings, in diagnostics, orthopedics and anti-cancer agent. It has been found that these nanoparticles are unique in nature and are also able to change their “physical, chemical and biological properties and therefore can be used for various purposes. The preparation methods for the preparation of AgNPs are physical, chemical and biological. . Among all the three methods, the biological method is found to be a simple, eco-friendly, commercial and one-step method and does not require elevated temperature, pressure, force and lethal chemicals. Before applying nanoparticles for any purpose such as medicine, human welfare or healthcare, it is very important to characterize the prepared nanoparticles to verify the safety of any of the prepared nanoparticles. Analytical techniques that are used for the analysis of these AgNPs are UV-Vis spectroscopy, XRD, FTIR, DLS, XPS, SEM, TEM, AFM, etc. AgNPs have applications such as anticancer, antifungal, antibacterial, anticancer.

Keywords: Biological, cancer, diagnosis, nanoparticles, silver nanoparticles.

Synthesis of Silver Nanoparticles:

Using Physical and Chemical Methods:

As I talked about, AgNPs can be prepared by three methods; Physical, chemical and biological methods. Using the evaporation-condensation method with a tube furnace at ambient temperature, these nanoparticles are prepared by a physical method. Before some conventional methods have been used to prepare nanoparticles, they are pyrolysis and spark discharge. Since no toxic chemicals are used in this method, it is found to be safe.[8] Another advantage is speed and radiation, i.e. use as a reducing agent. In addition, there are some disadvantages such as low yield, contamination in the solvent, high energy consumption, and sometimes lack of uniform distribution.

In the "top-down" method with the use of a colloidal protective agent, mechanical grinding of large-sized metals is used, followed by stabilization. The second method, i.e. "bottom-up" methods, includes chemical reduction, sono-decomposition and electrochemical methods. In this case, the main advantage of the chemical method is the high yield, but the disadvantage is that the methods are very expensive when mature. Second, the materials that are used to synthesize AgNPs using this chemical method are very toxic and dangerous.

Characterization: to check or evaluate the functional properties and properties of the synthesized nanoparticles, imaging is very important. As I mentioned earlier, there are many techniques that are used for the characterization of these nanoparticles, it can be done by different techniques such as XRD, FTIR, DLS, UV-Vis spectroscopy, TEM, SEM, XPS, so by using these technologies- we are able determine various properties of the synthesized nanoparticles.[15] Techniques for characterization are shown in Figure 1.

X-Ray Diffraction Spectroscopy (XRD):

Basically, XRD is the primary or main technique to identify the crystalline nature of a product. In addition, this method is used to measure phase identification, perform qualitative analysis, and also determine structural imperfections in many streams such as pharmaceutical, environmental, geological, and sometimes forensic sciences.

The disadvantage of this XRD technique is that there are sometimes difficulties with crystal growth. This is the only drawback of XRD.

the study of molecular and crystalline structure, determination of quantitative movement in chemical substances, degree of crystallinity, particle size, etc. can be carried out using this technique, i.e. XRD. The

structural properties of a wide variety of compounds such as glasses, superconductors, inorganic catalysts, polymers can be produced using this XRD technique.[17] When light falls on a crystal, many diffraction patterns are formed, which are then able to reflect the physico-chemical properties of the crystal structure. In case the sample is used in powder, then the rays that are diffracted usually come from the sample and then this beam will be able to reflect the physico-chemical structure of the product.

Introduction: silver has been widely used for its antibacterial nature since 5000 years ago. Ag is preferred as nanoparticles

for the reason that it has antibacterial properties and is not toxic to humans. Either killing or limiting the growth of bacteria without affecting surrounding cells is known as antibacterial activity. Various methods such as physical, chemical and biological are used for the preparation of silver nanoparticles. The demand for silver nanoparticles is growing rapidly in many streams such as medical, pharmaceutical companies, healthcare, food, consumer, cosmetic, etc. Due to its use, it is used for several applications such as antibacterial properties, household, medical devices, and food industry, dressings wounds, in diagnostics, orthopedics

and anticancer agent.[1] These nanoparticles have been found to be unique in nature and are also capable of changing their physical, chemical and biological properties and hence can be used for various purposes. To meet the requirements of AgNPs, various methods are used to synthesize these AgNPs

As we all know about cancer, cancer is a complex disease in which there is an uncontrolled growth of cells, which may be due to many reasons such as a combination of genetic, internal or may be external factors. It is well known that cancer treatment includes chemotherapy, surgery, radiation therapy; targeted therapy is also very expensive and painful.[6] Therefore, it is necessary to find effective, cheap and sensitive molecules for cancer treatment. AgNPs have been found to have applications in cancer as an anticancer agent as well as in diagnostics. In addition, AgNPs have other applications such as antifungal, antibacterial, anticancer, etc.

after the synthesis of these nanoparticles, their particle characterization is important, because the biological properties can be strongly influenced by the physicochemical properties of the particle. Before applying nanoparticles for any purpose such as medicine, human welfare or healthcare, it is very important to characterize the prepared nanoparticles to check the safety issue of any of the prepared nanoparticles[3]. Before determining toxicity or biocompatibility, it is important to check the characteristic properties of any nano material such as

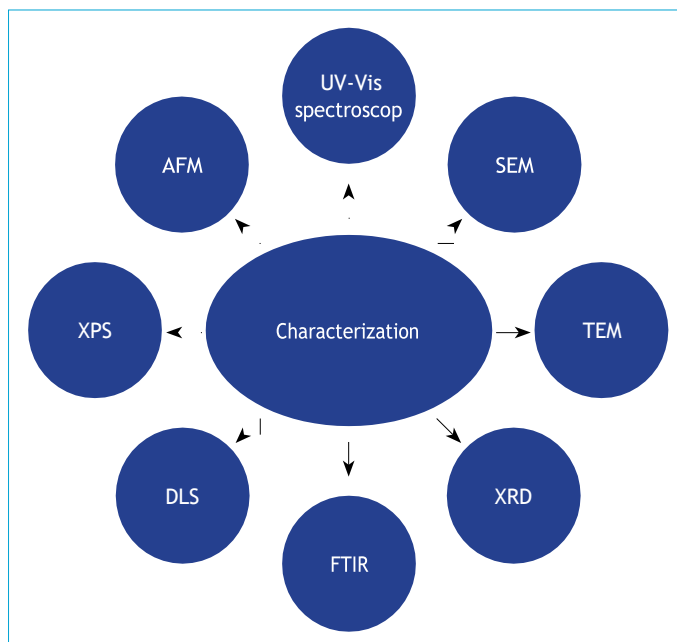
particle size, particle size distribution, shape, surface area, aggregation, solubility, etc.

Analytical techniques used for the analysis of these AgNPs are UV-Vis spectroscopy, XRD, FTIR, DLS, XPS, SEM, TEM, AFM.

The development of AgNPs with controlled structure, i.e. uniformity of size, morphology and functionality, are important for various biomedical applications. The bioavailability of any drug after both systemic and local administration of AgNPs can be enhanced by the physicochemical properties of the drug.

Normally, physical and chemical methods are found to be expensive and dangerous. But nanoparticles which are prepared by biological method show high yield, high solubility and also high stability. Among the three methods, the biological method is found to be a simple, ecological, commercial and one-step method and does not need elevated temperature, pressure, force and deadly chemicals.

UV-Vis Spectroscopy: The assimilation of AgNPs from the sample mainly depends on three things, which are particle size, dielectric environment and chemical environment. In the case of SPR, the peak observation is well assigned for particles that are in the 2-100 nm range.



Characterization techniques of AgNP.fig1

For the primary characterization of prepared nanoparticles, this UV-Vis technique is one of the simplest and most reliable methods, in addition, the synthesis and stability of nanoparticles can also be controlled using this

technique. In this technique, prepared AgNPs are able to interact with a specific wavelength of light. In the case of a colloidal suspension, no calibration of the sample or the suspension is necessary.[16] Therefore, this technique is found to be easy, reliable, sensitive, simple and efficient and selective in many nanoparticles. In AgNPs, studies have found that the valence band and conduction band are very close to each other, which results in the free movement of electrons, and these free electrons then lead to surface plasmon resonance (SPR). band, which is generally caused by the combined electron fluctuation of AgNPs in resonance with the light wave.

Dynamic Light Scattering (DLS): DLS can be used to determine particle sizes ranging from submicron to one nanometer. Particles ranging from 2 to 500 nm can be determined using this DLS, meaning that smaller particles can be easily determined.[18] This method essentially depends on the interaction between light and particles. It is the most commonly used technique in measuring particle size and particle size distribution.

an important parameter is the study of biological activities using radiation scattering techniques, physico-chemical parameters or the evaluation of synthesized nanoparticles. DLS can be used to determine particle sizes ranging from submicron to one nanometer. With this DLS, particles ranging from 2 to 500 nm can be determined, which means that smaller particles can be easily determined with this method.

his method essentially depends on the interaction between light and particles. It is the most commonly used technique in measuring particle size and particle size distribution. DLS measures that light that is scattered from a laser that will be able to pass through the colloid and will rely mainly on Rayleigh scattering from the fact that the nanoparticles are on the edge. The modulation of the scattered light force acting as the purpose of time is then analyzed and the hydrodynamic size of the particles can be determined.

Fourier Transform Infrared Spectroscopy (FTIR):

This method provides both accuracy and reproducibility, and also with the help of the method we are able to determine whether biomolecules are involved in the synthesis of nanoparticles. Apart from FTIR, it is a non-invasive technique. FTIR is also used in some other aspects such as authentication of functional molecules that are grafted onto silver, gold, silver and graphene nanoparticles, carbon nanotubes, etc. FTIR is capable of

providing strong data, fast data collection, large signal to noise ratio and also less amount of sample heating. Overall, this FTIR is a simple, accurate, valuable, cost-effective, non-invasive technique used to confirm the function of biomolecules. Using FTIR, it is possible to determine small changes in absorbance, i.e. up to 10^{-3} , which will ultimately help in performing differential spectroscopy,

which will then serve to determine the small combination bands of functionally dynamic residues left over from the large absorption bands of proteins.

Applications: Silver has been widely used since the last 5000 years for its antibacterial nature. Ag is preferred as a nanoparticle because it has antibacterial properties and is non-toxic to humans. Either killing or limiting the growth of bacteria without affecting surrounding cells is known as antibacterial activity. In addition to their antibacterial activity, AgNPs are widely used for other purposes, such as their antiviral activity, antifungal, antiangiogenic, cosmetics, water treatment, health care, antioxidant, biosensing, anti-inflammatory, drug carrier, imaginary purpose and textiles, etc. The applications of silver nanoparticles are listed as in Figure 2.

Atomic Force Microscopy (AFM): AFM is generally used to find the dispersion and aggregation of nanomaterials. In addition to their size, shape and structure, there are three other different scanning modes available and these three modes are known as contact mode, non-contact mode and intermittent sample contact mode. The communication of nanomaterials with their lipid bilayer support can be characterized using AFM, which cannot be done by other electron microscopy techniques.

A limitation of AFM is that due to the size of the canilever, there is an overestimation of the lateral dimensions of the sample, so much more attention should be paid to eliminating the error.

Various Silver Nanoparticles Prepared Till Date:

List of silver nanoparticles prepared till date are shown in [Table 1](#).

Conclusion:

Of all three biological methods, it is mainly used because it is ecological, non-toxic and environmentally friendly. It has been found to be safe and site-specific in the case of cancer treatment. Thus, it can be said that these AgNPs are a safe, simple and reliable treatment for many diseases.

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Silver nanoparticles are now widely used due to their numerous applications in many industries. Extremely used as an antibacterial, Ag-NPs also have their uses as antiviral, anti-inflammatory, gene therapy, antifungal, for diagnostic and imaging purposes, and many others.

AgNPs ia an area of explore in caseof cancer therapy as an alternative to other conventional.

Table 1. Various silver nanoparticles prepared till date

Drug	Part taken	Reference
Pedaliu murex	Leaf extract	27
White sugar		28
Carpesium cernuum	Extract	29
Coriander sativum	Leaf extract	30
Salicornia brachiata	Aqueous extract	31
Amaranthus gangeticus	Leaf extract	32
Ocimum sanctum (Tulsi)	Leaf extract	33
Parthenium	Slurry	34
Gum acacia	Gum	35
Red apple	Fruit extract	36
Coffea Arabica	Seed extract	37
Syzygium aromaticum (Clove)	Bud extract	38
Putranjiva, drypetes roxburghii (Wall)	Fruit extract	39
Lippia nodiflora	Aerial extract	40
Origanum vulgare		41
Soymida febrifuga	Aqueous fruit extract	42

Antibacterial Activity:

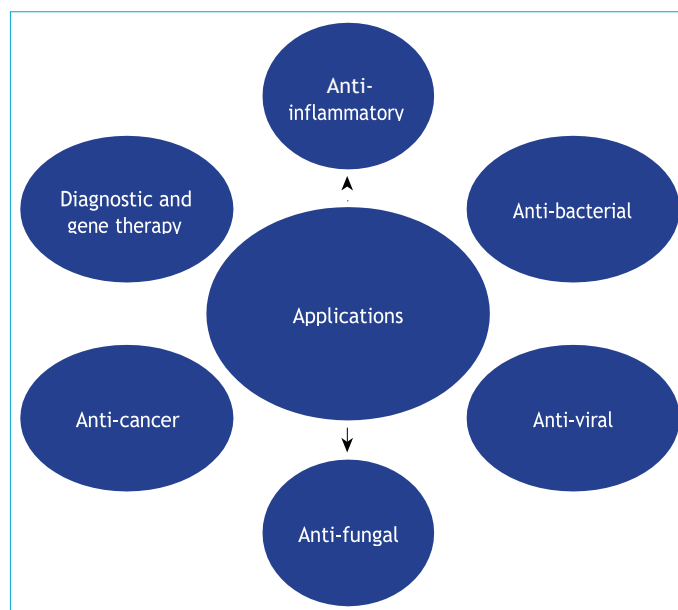


Fig.application of AgPS.

This is due to the presence of cell wall i.e. peptidoglycan layer which is thick in gram positive bacteria. i.e. 30 nm, in case of gram-negative bacteria it is 3-4 nm, which is very thin compared to gram-positive bacteria. Another reason is that the cell membrane is negatively charged, which may be due to the presence of carboxyl, phosphate, and amino groups, while the positive charge in bacteria causes an attraction between AgNPs and the cell membrane, i.e., negatively charged, which will lead to the attachment of AgNPs to the cell membrane. membrane, which will change the antibacterial activity of AgNPs[14] Since the one-pot synthesis promotes the formation of small polymer-bound AgNPs that can be distributed in a pH 6.3 medium, the antimicrobial activity of the chitosan-Ag-nanoparticle composite was found to be higher than the activity of its components at their respective concentrations.

Killing or limiting the growth of bacteria without affecting surrounding cells is known as antibacterial activity. Ag is preferred as a nanoparticle because it has antibacterial properties and is non-toxic to humans. Ag-NPs are able to overcome the resistance that was caused by antibiotics. It has been observed that due to the presence of their large surface-to-volume ratio as well as the crystallographic structure of the surface AgNPs, they are found to be potential antibacterial agents.[21] AgNPs are able to destroy many drug-resistant strains, suggesting that these AgNPs have the potential to act as an antibacterial agent.

Anti-inflammatory Activity:

Since AgNPs are known for their antibacterial and antimicrobial activities, their response to act as an anti-inflammatory agent is limited, but they also play an important role in this anti-inflammatory field.

Inflammation is a condition where some part of the body becomes swollen, red, hot and sometimes painful, and this can occur due to a certain injury or sometimes an infection. Inflammation has also been found to provide an immunological response that is against certain foreign particles

Anticancer Activity:

There are many treatments such as chemotherapy, radiation therapy, etc. that are given to patients who are suffering from cancer, but the side effects of these treatments are very harsh and the process is also very painful. It is well known that cancer treatment includes chemotherapy, surgery, radiation therapy; targeted therapy is also very expensive and painful.[7] Therefore, it is necessary to find effective, cheap and sensitive molecules for cancer treatment. Several studies have been conducted to know the promising result of AgNPs.[6] It has been found to be the most suitable as well as an alternative for other cancer treatments. They have the ability to target specific cells or a tumor at that location just by encapsulating a therapeutic agent in a nanoparticle and then using it as a drug delivery system.

Cancer is basically the uncontrolled growth of cells in a certain area in the body. Every 1 in 3 people in the world suffer from one type of cancer or another

After 6 hours of exposure to *Albizia adianthifolia* leaf extract synthesized AgNPs (AA-AgNPs), A549 cells showed

21% and 73% cell viability and normal peripheral lymphocytes showed 117% and 109% viability. This means that AgNPs are not harmful to normal PLs cells. At 43 g/mL AA-AgNPs, 50 percent cellular inhibition of A549 cells was achieved and cell death was induced by ROS generation, resulting in apoptosis. After 48 hours of Hoechst staining, MCF-7 cells treated with AgNPs mediated by *Sesbania grandiflora* (20 g/ml) show nuclear condensation, cell shrinkage and fragmentation. These changes mean that DNA repair has been made possible as a result of the cleavage.

Challenges for Cancer therapy Using AgNPs:

AgNPs are used as an alternative treatment in cancer therapy due to their site-specific use, reduced toxicity, better efficacy and some other advantages, but there are some limitations of AgNPs for cancer therapy. These limitations are physiological barriers, increased permeation and retention effect (EPR), limited load capacity and mainly production problems.

Antiviral Activity:

In the case of HIV, AgNPs were found to give an acceptable result. In this case, AgNP acts as an antiviral in the early phase of viral replication, acts mainly as virucidal or can inhibit virus entry. AgNP binds to gp120 and prevents de-CD-4

dangling virion attachment and infectivity that will result in acting as an effective virucidal agent against cell-free virus. In addition to these AgNPs, they inhibit the post-entry phases of the HIV-1 life cycle.

Viral infections and diseases are found to be very common worldwide, so it is very important to produce antiviral agents that show significant results. AgNPs were found to be prominent in showing such results due to their very small size as well as their shape. Silver has been found to be relatively non-toxic to both humans and animals and has been found to be effective against viruses.

Antifungal Activity:

Antiviral drugs should be biocompatible, non-toxic and environmentally friendly. AgNPs have been found to be prominent against many fungal diseases[7] By inhibiting the germination of conidia, the biologically synthesized AgNPs showed good antifungal activity against *Bipolaris sorokiniana*. Indoor fungal species such as *Penicillium brevicompactum*, *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Chaetomium globosum*, *Stachybotrys chartarum* and *Mortierella alpine* cultured on agar medium are also inhibited by AgNPs

People with lower immunity are more prone to fungal infections. To overcome diseases related to fungi, this process has been found to be very tedious in nature. There is a very limited number of antiviral drugs available on the market.

Scanning Electron Microscopy (SEM):

The disadvantage of SEM is that we are not able to identify the internal structure of the particle with this technique, but the advantage is that the purity and degree of aggregation of the particles can be determined using SEM.

To learn much more about nanotechnology and nanoscience, many techniques are used, including many electron microscopy techniques. Among which is SEM

SEM is a technique that is basically used to determine the morphology of particles. AS SEM is a surface imaging method, so with SEM we are able to determine particle size, particle size distribution, surface morphology and shapes of nanomaterials. Using SEM, we are able to determine the morphology of the particles and subsequently we are able to either draw a histogram or count the number of particles either manually or using other software. SEM is used together with EDX to determine the morphology of the silver powder and to perform chemical composition analysis.

Transmission Electron Microscopy (TEM):

The ratio of the distance between the objective lens and the specimen to the distance between the objective lens and the image plane will help determine the TEM magnification. TEM is able to provide better resolution and better analysis than SEM.[23]

The limitation of SEM is that it requires a high degree of vacuum, the sample should be very thin and sometimes also time consuming.

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Transmission Electron Microscopy (TEM):

The limitation of SEM is that it requires a high degree of vacuum, the sample should be very thin and sometimes also time consuming

TEM is one of the most common and important techniques

i.e. it is used for particle characterization. Using this TEM technique, we can find the quantitative dimension of particle size, particle size distribution and particle morphology

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Atomic Force Microscopy (AFM):

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A limitation of AFM is that due to the size of the canilever, the lateral dimensions of the sample are overestimated, so eliminating the error should require much more attention.

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