Natural Synthesis and classification of Silver Nanoparticles utilizing Bacillus thuringiensis with Nanotechnology

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INTRODUCTION

Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructure materials, play an ever increasing role in science, research and development as well as also in every day’s life, as more and more products based on nanostructure materials are introduced to the market. Nanotechnology deals with materials with dimensions of nanometers, i.e. nanostructure materials. The sizes of nanometer dimensions determined the physical and chemical properties of materials undergo extreme changes, which open up a wide range of future, but partly already realized applications. There are two more forces driving towards nanotechnology, mostly biomolecules and other bioentities are of nanometer size; thus the nanoscale provides the best opportunity to study such bio-entities [1].

Silver Nanoparticles:

The aspect of current nanotechnology research is silver nanoparticles can be synthesized by several physical, chemical and biological methods. One of such promising process is green synthesis. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality [2]. Silver nanoparticles have unique optical, electrical, and thermal properties and are incorporated into products that range from photovoltaic to biological and chemical sensors, including pastes, conductive links and fillers which utilize silver nanoparticles for their high electrical conductivity, stabilization and low sintering temperatures.

Due to optical properties of nanoparticles, these are mainly used in molecular diagnostics and photonic devices.
An increasingly application is the use of silver nanoparticles for antimicrobial coatings, and many textiles, wound dressing, and biomedical devices contain silver nanoparticles that continuously release a low level of silver ions to provide protection against bacteria.

**History of Nanotechnology:**

The development of the concepts and experimental work is the broad category of nanotechnology. Recently nanotechnology is a development in scientific research, the development of its central concepts happened over a longer period of time. Since, people have been preparing the glass windows with tiny colored metal particles especially silver which provide glassy yellow color

**Concept of Nanotechnology:**

The history of nonmaterial’s is quite long and major developments within nanoscience have taken place during the last two decades. The idea of Nanotechnology was first highlighted by Noble laureate Richard Feynman, in his famous lecture at the California Institute of Technology, 29th December, 1959. In one of his articles published in 1960 titled, discussed the idea of nanomaterials. He pointed out that if a bit of information required only 100 atoms, then all the books ever written could be stored in a cube with sides 0.02 inch long. Norio Taniguchi first defined the term Nanotechnology, in 1970.

**SILVER NANOPARTICLES**

The optical properties of silver nanoparticles were used by glass founders as far back as in the time of the Roman Empire. That evidenced, so-called Lycurgus cup (4th century AD) now exposed in the British Museum. A detailed study of the composition of its bronze-mounted insets of stained glass, carried out in the late 20th century, revealed the presence of metal nanoparticles (with the average diameter of 40 nm) that consists of silver (70 %) and gold (30 %) alloy. It explained the remarkable feature in bowl to change its color from red in transmitted light to grayish green in reflected light. Before the 1980s, the scientific and practical interest in silver nanoparticles was exclusively caused by the possibility of their use as highly dispersed supports for enhancing the signals from organic molecules in the Raman spectroscopy. Fundamental studies carried out in the last three decade shows that silver nanoparticles exhibit a rare combination of valuable properties including, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc. For that reason silver nanoparticles serve as a material in the development of new-generation electronic, optical and sensor devices. In the past 20 years, the trend miniaturization and the necessity of modernization of technological processes led to the substantial increase in the number of scientific publication devoted to the synthesis and properties of silver nanoparticles [3].

**Use of plants to synthesize Nanoparticles:**

Group of researchers developed silver nanoparticles being extensively synthesized using various Plant. Different types of plants are being currently investigated for their role in the synthesis of nanoparticles. Gold nanoparticles with a size range of 2- 20 nm have been synthesized using the live alfa alfa plants Nanoparticles of silver, nickel, cobalt, zinc and copper have also been synthesized inside the live plants of *Brassica juncea* (Indian mustard), *Medicago sativa* (Alfa alfa) and *Helianthusannus* (Sunflower). Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyper-accumulators [4].

**CATALYTIC ACTION:**

Due to high surface area and high surface energy predetermine metal nanoparticles for being effective catalytic medium. Growing small particles of silver have been observed to be more effective catalysts than stable colloidal particles. These growing particles catalyzed the borohydride reduction of several organic dyes. The reduction rate catalyzed by growing particles is distinctly faster compared to that of stable and larger silver particles, which are the final products of growing particles. Catalysis is due to efficient particle-mediated electron transfer from the BH4- ion to the dye.

**Materials and Methods are following:**

Chemicals required: Silver nitrate (AgNO3), Nutrient Broth, Nutrient agar wasof analytical grade obtained from Hi media, India.

Glasswares: All glass wares (Conical flasks, Measuring cylinders, Beakers, Petri plates and Test tubes etc.) were purchased from borosil, India.

Strains used: Bacillus thrunigenesis, Bacillus subtilis, Staphylococcus aureus were used to test antimicrobial activity of AgNPs.

**MIC (Minimum inhibitory concentration) test:** MIC is the lowest concentration of an antimicrobial agent that will inhibit the growth of microorganisms after overnight incubation. It is important for the determination or to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents [5].
Initially, two bacterial strains like *B. thuringiensis* and *B. subtilis* were grown in nutrient broth medium and were screened against silver nitrate to find out the minimum concentration of silver nitrate, which inhibit the growth of bacteria. From the analysis it was found that lowest conc. of AgNO₃ given moderate activity against *B. thuringiensis*.

**Characterization of silver nanoparticles:**

Several techniques are used for characterizing different nanoparticles. Here we have discussed the basic principles of few techniques that have been used for the characterized the silver nanoparticles in this project work. They are Absorption spectrophotometer (UV-VIS), Particle size analyzer, X-Ray diffraction (XRD) and Scanning electron Microscope (SEM) and Fourier transforms infrared spectroscopy [7].

**SEM:**

SEM is the scanning electron microscope that creates various images by focusing a high energy beam of electrons onto the surface of a sample and detecting signals from the interaction of the incident electron with the sample's surface. SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material [9]. Magnification is of order 10,000 X and resolution 10 nm.

**Scanning process:** In electron surface when stream of electrons is formed and accelerated towards the specimen using positive electric potential. Using metal apertures and magnetic lenses the stream confined and focused into a thin focused monochromatic beam.

**XRD:**

In XRD a large fraction of the X-rays that are not simply absorbed or transmitted by the object but are scattered.

When an X-ray beam hits an atom, the electrons around the atom start to oscillate with the same frequency as the incoming beam creating an electric field.

**FTIR:**

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique useful for identifying organic chemicals in a whole range of applications although it can also characterize some inorganic include paints, adhesives, resins, polymers, coatings and drugs.

It is powerful tool for isolating and characterizing organic contamination. FTIR is the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum.

This absorption corresponds to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range 4000 – 600 cm⁻¹.

The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place.

The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum [6].

**Results and Discussion:**

![Fig 1: MIC result of AgNO₃ against B.thuringiensis and B.subtilis](image-url)

[The fig1 contained X-Axis = Concentration of AgNO₃ and Y-axis = Optical Density (absorbance)]

The MIC test was performed to find out the minimum inhibitory concentration of AgNO₃ against two organisms. According to the fig-3 the MIC results shows that lowest conc. Of AgNO₃ gives moderate activity against *B. thuringiensis*. So I have taken *B. thuringiensis* as potential bacteria for synthesis of silver nanoparticles.
Synthesis of Silver Nanoparticles:

Fig 2: Synthesis of silver nanoparticles by B. thuringiensis.

Fig 2- shows the over-night culture of B. thuringiensis along with AgNO3 of concentration 35 µM. Color of the solution clearly confirm the reduction of AgNO3 by B. thuringiensis forming silver nanoparticles.

Characterization of silver nanoparticles

UV-Vis spectroscopy Analysis

Fig 3: UV-Vis spectra of silver nanoparticles synthesized by B.thuringiensis

Fig 3 Shows the UV-Vis spectra of silver nanoparticles synthesized by B. thuringiensis. The peak of the above spectra was found at 421nm and this peak is due to Surface Plasmon Resonance (SPR) property of silver nanoparticles.
SEM Analysis

The morphological features of synthesized silver nanoparticles were studied by SEM analysis shown in fig-4. SEM analysis suggested that most of the particles are spherical in shape.

Fig 4: SEM image of silver nanoparticles synthesize by *B. thuringiensis*

XRD Analysis

XRD spectrum of synthesized silver nanoparticles (Fig.5) shows distinct diffraction peaks arround 32°. These sharp Bragg peaks might have resulted due to crystalline nature of silver nanoparticles.

Fig 5: XRD spectra of silver nanoparticles
FTIR Analysis.

The Fig 6 shows the FTIR image of silver nanoparticles synthesized from *B. thuringiensis*. FT-IR analysis revealed the strong bands at 3383, 2352, 1601, and 1404, 1113, 675, 518 cm\(^{-1}\). The band at 2352 for O-H stretching corresponds to carboxylic acid.

1601 cm\(^{-1}\) for stretching C=C corresponds to aromatic amino groups. The band at 675 cm\(^{-1}\) corresponds to C-H stretching of phenyl ring of substitution band, whereas the stretch for Ag-NPs was found around 518 cm\(^{-1}\).

Antibacterial activity test:

The Fig 7 shows the antibacterial activity of silver nanoparticle against *B. subtilis*, *S. aureus*, *E. coli*. According to the figure-7, the growth of *S. aureus* and *B. subtilis* and *E. coli* is inhibited in the presence of nanoparticles as compared control. From the results, we can conclude that silver nanoparticles have significant antibacterial activity against *B. subtilis* and *S. aureus* whereas less against *E. coli*.
CONCLUSION:

Silver nanoparticle was successfully synthesizing by biological method from *Bacillus thuringiensis*. The Surface Plasmon Resonance (SPR) property of synthesized nanoparticle was studied by UV-Vis spectroscopy and the peak of the spectra was found to be at 421 nm. The morphological study of AgNPs using SEM suggests that the nanoparticles are spherical in shape with diameter around 450 nm to 1000 nm. The physiochemical properties of silver nanoparticles using FTIR, XRD conclude that the nanoparticle form in the process is crystalline with miller index of 011 and angle of diffraction of $2\theta = 32^\circ$. The antibacterial activity of silver nanoparticles concludes that the silver nanoparticles shows significant antibacterial activity against *B. subtilis* and *S. aureus* whereas less activity against *E. coli*.d [8]

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