Experimental Nanobiotechnology

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Lecture 03: Biological Synthesis of NanomaterialS

Hello everyone, today we are going to learn about the biological synthesis of nanomaterials. In today's lecture, we are going to learn about what biological synthesis is, what the advantages of biological synthesis of nanomaterials are, and we are also going to learn about the role of bacteria, fungi, algae, and plants in the synthesis of nanomaterials. In today's lecture, we are also going to learn about viral nanotechnology.

At the end of the lecture, we will also have a practical demonstration to understand the biological synthesis of nanomaterials. Let us see what biological synthesis is. Biological synthesis is an eco-friendly and sustainable method for synthesizing nanoparticles using biological entities such as plants, bacteria, fungi, algae, or viruses. This method utilizes natural biomolecules.

For example, we can use enzymes, proteins, polysaccharides, or secondary metabolites which can act as reducing agents and can also act as stabilizing or capping agents during the synthesis of nanoparticles. When compared to chemical synthesis, in biological synthesis, we are going to use biological molecules. These can be enzymes, proteins, or polysaccharides. That is the advantage of this biological method.

And this biological synthesis has four important phases. The first one is the initial phase. In the initial phase, a metal precursor is added to the microbes or plant extract. Then, in the activation phase, it consists of metal ion reduction and nucleation, mediated by microbial enzymes that can be detected by the color change in the reaction mixture.

The third one is the growth phase. In the growth phase, it leads to the amalgamation of the nucleated metals into varying sizes and shapes of nanoparticles that are thermodynamically stable. And the fourth phase is the termination phase. This decides the final morphology of the nanoparticles,

which is greatly affected by different factors, including temperature, pH, time, the capping agent, and also the agitation frequency. So, this is the bioreduction process for synthesizing nanoparticles through a biological method. As I mentioned earlier, to synthesize nanoparticles through a biological method, we can use these microbes, or we can use plant phytochemicals.

And these phytochemicals and enzymes act as reducing agents as well as capping agents. When we add the metal salt as a precursor, it will be reduced into metal nanoparticles. The size and shape of the nanoparticles depend on the reactant concentration, pH, kinetics, as well as the mixing ratio. Once the nanoparticles are formed, we can purify and characterize them using various characterization techniques. The purified nanoparticles can be in colloidal form or in powder form through lyophilization.

We can perform freeze-drying to convert them into powder form. We can use them for various applications, including imaging and drug delivery. Not only microbes or phytochemicals, but we can also use food waste, prepare food extracts, and use them to synthesize nanoparticles. Let us learn about biological reduction in detail.

Similar to the chemical method, here we also use metal salt as the precursor, and we can use microbes or phytochemicals from plant extracts, or we can use food waste extracts, which act as a source of biomolecules such as alkaloids, enzymes, flavonoids, proteins, or polysaccharides. These can reduce the metal salts into metallic nanoparticles, and they act as reducing agents as well as stabilizing or capping agents.

So, as you know from the previous lecture, the first step is nucleation. This is followed by the growth of the nanoparticles, and these nanoparticles should be stabilized with the help of capping agents or stabilizing agents. So, as I told you earlier, here, the enzymes or the proteins act as capping agents as well as stabilizing agents. Finally, what you get is the capped and stable nanoparticle.

Let us learn about the advantages of biological synthesis. Biological synthesis is an eco-friendly and green method because it avoids the use of toxic chemicals. Additionally, it provides biocompatible nanoparticles because we are using biological agents as the coating agents or stabilizing agents. Moreover, it is a cost-effective technique because we use inexpensive raw materials like plant extracts.

It is also easily scalable since we use simple plant extracts or microbes. Thus, we can easily scale up to industrial production. Furthermore, we can use this biological method to synthesize a variety of nanoparticles, including metals and metal oxides. We can also make nanoparticles of different shapes and sizes. Additionally, it is an energy-efficient technique because it requires mild conditions.

And here, the reduction and stabilization occur in one step. So, it simplifies the synthesis process and can also provide better functionalization because we are using biomolecules as functional groups for capping or stabilizing. And the nanoparticle synthesis through biological methods has wide applications, including medicine, agriculture, and environmental remediation.

Let us learn about how we can use bacteria for nanoparticle synthesis. Here, the bacteria can be termed as microfactories for nanoparticle synthesis because Large quantities of bacteria can be easily cultured in routinely used growth mediums, And they produce various enzymes and biomolecules that can act as reducing agents And stabilizing agents for synthesizing nanoparticles.

Here, the bacteria can synthesize nanoparticles in two ways. The first one is intracellular synthesis, and the second one is extracellular synthesis. In intracellular synthesis, bacterial cells absorb metal ions and Reduce them into nanoparticles using enzymes like nitrate reductase. In the case of extracellular synthesis, bacteria secrete enzymes or metabolites into the culture medium.

And these extracellular reagents reduce the metal ions and stabilize the nanoparticles. Let us learn how bacteria synthesize nanoparticles through intracellular and extracellular routes. To synthesize nanoparticles using bacteria, the bacteria must be grown in a petri dish under optimum conditions. Then inoculate the bacteria into the culturing flask and

incubate under optimum conditions. Once the bacteria have grown, collect them and centrifuge in a centrifuge tube.

After centrifugation, you obtain a cell pellet. That is the biomass. This biomass can be collected for synthesizing nanoparticles through the intracellular route, and the supernatant can be collected for synthesizing nanoparticles through the extracellular route. Here, wash the pellet with sterile distilled water,

then add the metal precursor, incubate under optimum conditions, and collect the nanoparticles through centrifugation. In the intracellular route, the nanoparticles are synthesized inside the bacteria. To retrieve these nanoparticles, the bacterial cell wall must be lysed. This is an additional step in the intracellular route. In the extracellular route, only the supernatant is taken, then the metal precursor is added.

Then, incubate at optimum conditions, and you can collect the nanoparticles by centrifugation. Here, there is no additional step. You can directly obtain the nanoparticles as you have this reducing agent and stabilizing agent in the supernatant. So, this is the difference between the intracellular route and the extracellular route. Let us learn about the mechanism of extracellular and intracellular synthesis of nanoparticles by bacteria.

So, once you add the metal precursor salt, the metal ions will attach to the bacteria through electrostatic interaction, and these metal ions will enter the bacterial cell. Inside the bacteria, you have the reductase enzyme, and this reductase enzyme will reduce the metal ions into metal nanoparticles. These metal nanoparticles can be stabilized with the help of proteins, enzymes, or polysaccharides, which act as stabilizing agents or capping agents.

So, this is the intracellular synthesis of metal nanoparticles. In extracellular synthesis, the metal ions are reduced into metal nanoparticles through membrane redox proteins. Another approach is that the reductase enzymes are secreted out of the bacteria, and once the enzymes are secreted out of the bacteria, they can reduce these metal ions into metal nanoparticles. These can act as reducing agents and also as stabilizing or capping agents.

So, this is the difference between extracellular and intracellular synthesis of nanoparticles by bacteria. So, these are some of the examples of bacteria used for nanoparticle synthesis.

For example, if we use this bacteria, we can synthesize silver nanoparticles of size between 10 to 30 nanometers. Let us learn about the biosynthesis of magnetic nanoparticles. To synthesize magnetic nanoparticles, we have to use magnetotactic bacteria.

These magnetotactic bacteria orient and migrate along geomagnetic field lines based on intracellular magnetic structures called magnetosomes. So, these magnetosomes are membrane-bound magnetite particles. For example, we can use Magnetospirillum magneticum bacteria to synthesize magnetite nanoparticles. Here, this bacteria absorbs iron ions from the environment and reduces them to form magnetic nanoparticles. The advantage of magnetotactic bacteria is

once the magnetic nanoparticle is synthesized inside the bacteria, with the help of an external magnetic field, we can arrange these magnetic nanoparticles in any shape we want. So, once the bacteria are arranged, we can add detergent and lyse the bacterial cell wall, then we can obtain the arranged magnetic nanoparticles. So, that is the advantage of using magnetotactic bacteria for synthesizing magnetic nanoparticles.

Let us learn about nanoparticle synthesis by fungi. So here the fungi act as a natural environmentally friendly bio factories for synthesizing nanoparticles due to their ability to secrete enzymes and metabolites that facilitate the nanoparticle formation. So most of you know the fungi is a very good source for producing the proteins and enzymes so it is a act as a eco-friendly agent for synthesizing the nanoparticles

and using the fungi we can synthesize various nanoparticles for various applications. And similar to the bacteria the fungi also synthesize the nanoparticles by intracellular as well as the extracellular process. And the fungal biomass has lot of proteins and polysachharides which act as a reducing agent as well as a stabilizing agent similar to the bacteria. Whenever we synthesize nanoparticles through extracellular route, there are some challenges. For example, the growth medium also contains glucose and protein, which can also act as reducing agent and stabilizing agent.

So to overcome that, we can use a synthetic media and sucrose. But using the synthetic media reduces the number of reducing agent, but the residual glucose or hydrolyzed sucrose can still contribute to the nanoparticle formation. So if you want to understand the

nanoparticle synthesis through the fungi-originated components, we have to wash and process the mycelia to remove the growth media remnants.

And by using the intracellular extracts derived from the cell reception, we can confirm the role of fungi-originated compounds. such as metabolized enzymes in the synthesis of nanoparticles. So, in this research article, they have used three approaches to confirm and prove the synthesis of nanoparticles through fungi by the extracellular fluid, autolysate, and intracellular fluid. So, once the fungi are grown on the agar plate,

they collected them using centrifugation. So once you have done the centrifugation, they used the supernatant for synthesizing the gold nanoparticles. So this is the extracellular fluid method. So then the next method is autolysate. So here they wash the biomass.

Then resuspend in the acetate buffer with antibiotics. So this antibiotic will prevent bacterial growth and contamination. And they kept it for 8 days. Then after 8 days, they centrifuged and took the supernatant for synthesizing the gold nanoparticles. And the third one is the use of intracellular fluid.

So, here they taken the biomass in the acetate buffer and using the cell disruptor they disrupt the complete cell then by centrifugation they collected the supernatant. then they added to the gold metal salt to prepare the gold nanoparticle. So, by this way they confirm the role of the fungi originated compounds in the synthesis of gold nanoparticles

and they also confirm the size of reducing and capping agent. For example, the reducing agent in the fungal extract are smaller than the 3 kilodaltons, such as glucose, amino acids and cofactors. And which can act as a reducing agent, but they do not stabilize the nanoparticles. So, by stabilizing nanoparticles, larger biomolecules such as proteins are used as a stabilizing or capping agent through the Van der Waals forces.

or bonding with the nitrogen or sulfur atoms. So, in this study they have concluded that the fungal metabolites can be effectively used for green synthesis of nanoparticles, but careful processing of mycelia is necessary to distinguish the contribution of fungal compounds from those of the growth media. So, that is why whenever we synthesize the nanoparticles

through the biological method, we have to distinguish the difference between the synthesis of nanoparticles through the growth media

And the synthesis of nanoparticles through particular microbes. So, these are some examples of nanoparticle synthesis by fungi. Let us learn about the synthesis of nanoparticles using algae. So, this algae naturally tolerates heavy metals. So, it can be a very good bio-agent for removing heavy metal pollution

and also it can be useful for synthesizing various metallic nanoparticles. This algae assimilates low concentrations of heavy metals for essential cellular processes such as enzyme functioning, electron transport, and nitrogen assimilation. But high concentrations negatively impact their growth and morphology. So, the mechanism of nanoparticle formation in algae is that this algae combats heavy metal stress

by producing metal-chelating agents and reactive oxygen species. These interact with the metal nuclei to facilitate the biosynthesis of metallic nanoparticles. So, this algae synthesizes nanoparticles in three ways. One is intracellular. So, once you add the aqueous metal salt, this algal biomass will uptake the metal salt solution and synthesize the metal nanoparticles inside the algae.

Using the polysaccharides or proteins, which can act as reducing agents as well as stabilizing agents, you can obtain these stable nanoparticles. The second approach is synthesizing the nanoparticles by the extracellular method, that is, secreting some enzymes or proteins extracellularly, and these can act as reducing agents and reduce the metal ions into metal nanoparticles. The third approach is

Algae have many pigments, such as chlorophylls, phycobilins, and carotenoids, which can act as reducing agents as well as stabilizing or capping agents. With the help of sunlight, they can convert metal ions into metal nanoparticles. Let us learn about heavy metal removal by algae-based nanoparticles. The first step is biosorption.

Here, the adsorption of heavy metals on the cell surface is assisted by metabolic or physicochemical means. Then, one of these metal ions is converted into metal

nanoparticles. These can accumulate in the vacuoles and cytoplasm. The third step is biotransformation, which converts these metals into non-toxic metal nanoparticles.

by the activity of chelating agents such as peptides, enzymes or organic acid. The nanoparticles synthesized using this algal biomass has dual benefits because it is converting the heavy metals into the nanoparticles and these nanoparticles also has the efficiency in degrading the hazardous pollutants like dyes or nitro compounds from the aquatic environment.

So, when you are using these algae-based green biosynthesis, it can address the heavy metal pollution. At the same time, it is also producing the nanoparticle which can degrade the toxic metabolites in the wastewater and which will be useful for water purification application. So these are some of the examples of nanoparticles synthesized using algae. Let us learn why do microorganism synthesize nanoparticles.

Microorganism synthesize nanoparticles as part of their natural metabolic processes, this phenomenon termed biogenic synthesis. It is a result of interaction between microorganisms and their surrounding environment let us learn some of the reasons and mechanism behind this ability. The first one is defense mechanism the microorganism synthesize nanoparticles to protect themselves from the environmental stresses including toxic metal ions and oxidative stress. So, these microbes will convert the toxic metal ions into less soluble and less toxic nanoparticles

So that the microorganism can survive in environments with high metal concentrations. And the next one is oxidative stress. The nanoparticles, like silver nanoparticles, exhibit antimicrobial properties that may prevent competition from other microorganisms. So it won't allow other microorganisms to grow in that particular environment. And the next one is energy and metabolism.

Most microorganisms reduce metal ions during respiration or metabolism to obtain energy. And some microbes use metal ions as electron acceptors, indirectly leading to the synthesis of nanoparticles. The third one is detoxification. So here, the microbes synthesize nanoparticles to regulate or detoxify excess metal ions in their surroundings. This mechanism helps maintain ionic balance and protect cellular structures.

The fourth one is survival and adaptation. The nanoparticles can enhance the surface characteristics of microbial cells, which helps them adhere to surfaces or resist harsh conditions. And also, it provides an evolutionary advantage, meaning these nanoparticles might confer a selective advantage, such as creating a microenvironment unfavorable to predators or competitors.

And the last one is The nanoparticle synthesis by this microbe may be due to the enzyme activity and secondary metabolites because the microbes have enzymes like nitrate reductase or superoxide dismutase which can act as reducing agents and lead to nanoparticle formation. It also has many secondary metabolites like proteins or polysaccharides

which can act as reducing as well as capping agents for nanoparticle synthesis. Let us learn about the synthesis of nanoparticles using plant extracts. The synthesis of nanoparticles using plant extracts is an eco-friendly and green method because plant extracts provide a sustainable and environmentally friendly alternative for synthesizing nanoparticles. They act as natural reducing and stabilizing agents,

eliminating the need for harmful chemicals in traditional chemical synthesis methods. Again, this is a cost-effective and efficient method because plant-mediated synthesis is relatively inexpensive as we use readily available plants. The process is very simple. It requires very few resources and can be scaled up for industrial applications.

The third one is the bioactive compounds. The presence of bioactive compounds like flavonoids, terpenoids, and alkaloids plays a key role in reducing the metal ions to metal nanoparticles. The nanoparticles synthesized using plant extracts have wide applications, especially in the biomedical field. These are some examples of the biosynthesis of nanoparticles using plant extracts. Whenever we synthesize nanoparticles using plant extracts,

it has an additional advantage. For example, if you use medicinal plants to synthesize metal nanoparticles, such as silver nanoparticles, they have very good antibacterial properties. When you use medicinal plants like neem, which also have antibacterial properties, and

combine these two to synthesize nanoparticles, they will have a synergistic effect. For example, if silver nanoparticles can kill 5 bacteria and neem can kill 5 bacteria,

synthesizing nanoparticles using neem extract can kill more than 20 bacteria. That is called a synergistic effect. Suppose, instead of 20 bacteria, if it kills only 5 plus 5, where the silver nanoparticles kill 5 bacteria and neem kills 5 bacteria, and in total, it kills only 10 bacteria, that means it is an additive effect.

But mostly, when we use plant extracts for synthesizing nanoparticles, it always shows a very good synthetic effect for antibacterial or anti-cancer activities. In this way, we can improve the therapeutic efficiency of nanomaterials. Let us learn some of the important parameters for the synthesis of metal nanoparticles through biological methods. Whenever we synthesize metal nanoparticles through biological methods,

we have to optimize the conditions. For example, the size and shape of nanomaterials depend on pH, agitation, temperature, and the metal salt precursor concentration. and the ratio of reactants and the incubation period. By optimizing these parameters, we can successfully synthesize nanoparticles through biological methods. Let us learn about viral nanotechnology—how we can use viruses for making nanomaterials.

Viral capsids, devoid of their nucleic acid genomes, can be thought of as nanocontainers. That means when you have a virus, this viral capsid is made up of proteins. Most of you know the size of viruses is in the nanometer range. When you remove the genomic material, it can be DNA or RNA. Once you remove the nucleic acid, we can use this protein as a nanocontainer.

And once you remove the nucleic acid, we can add anti-cancer drug. And we can use it for cancer therapy. So this is called as viral-like particles or viral nanoparticles. And this is inspired by nature, that is a biomimetic approach. And we can also use this virus capsid to serve as a size-constrained reaction vessel.

For example, you can see here, so the metal ions can enter into the viral capsid. And if the size of the virus is in the range of 20 nanometres, And the metal ions can enter into the

viral capsid through diffusion. So once it enters, it will be reduced into metal nanoparticle and it will grow. The maximum growth it can attain is it can reach up to 18 or 19 nanometer.

So it will have a uniform size nanoparticle because the virus capsid act as a kind of size constrained reaction vessel. So we can easily synthesize uniform size nanoparticle. If you want to make uniform size nanoparticle, we can use the virus as a kind of nano container to synthesize the nanoparticles. And once the nanoparticle is synthesized, we can remove this viral capsid by

Treating with a detergent or using the protease enzyme. We can remove the viral capsid, and what you get is intact nanoparticles. So that is the advantage of using this virus for making nanomaterials. Let us learn about viral nanoparticle functionalization. In the viral nanoparticle, once you remove the nucleic acid,

we can add an anti-cancer drug or any therapeutic molecule inside the virus. And in this subunit interface, we can add gadolinium metal, which can be useful for imaging and will be helpful for cancer diagnostics. To make this nanoparticle specific to cancer cells, we can add RGD. This is a three-amino-acid peptide: arginine, glycine, and aspartate.

Most cancer cells overexpress the receptor for RGD. So this can easily bind specifically to cancer cells. In this way, we can make multifunctional nanoparticles. Inside, we have the anti-cancer drug. We also have gadolinium metal, which can be useful for cancer diagnostics through imaging.

On the outside exterior, we have the RGD, which will specifically bind to the cancer cells. So in this way, we can target these nanoparticles specifically to the cancer cells, and we can increase the therapeutic efficiency and reduce the side effects. So the next question is, what kind of virus do we have to use for synthesizing the nanomaterials? We have to use the plant virus.

Why do we have to use the plant virus? Because it minimizes the chance of the virus interacting with human proteins and causing toxic side effects, infection, and immune responses. To avoid that, we have to use plant viruses. Additionally, plant viruses are easy to produce in large quantities, and they are available in various sizes and shapes.

So here also, you can see that they are available in various sizes and shapes. Shapes—so that is the advantage of plant viruses, and again, plant viruses can hold approximately 10 cubic nanometers of particles, so they have very good drug-loading efficiency. They can carry many molecules of cancer drugs and can be very useful for cancer therapy, so that is the advantage of using plant viruses.

additionally when we are using the rod shape plant virus such as the tobacco mosaic virus the amino acid composition of this tobacco mastic virus is different for exterior and interior so in this case we can synthesize different kind of nanoparticles inside the TMV and outside the TMV. For example, we can synthesize the silver nanoparticle inside the TMV virus and we can synthesize the gold nanoparticle or some other material on the exterior of the TMV virus.

So in this case this can act as a template for nano rod synthesis. By this way we can make a novel and new materials. Let us learn how to produce viral nanoparticles for various therapeutic application. So to produce the viral nanoparticles we have to add the virus to the plant leaf and once we add the virus it will make millions of copies of plant virus.

Then these viruses can be chemically modified. We can remove the nucleic acid and we can add the anti-cancer drug or any therapeutic molecules inside the viral capsid. Then we can also add antibody or the ligand which can specifically bind to the cancer cell. Once the viral nanoparticle is ready, then you can evaluate the efficiency of viral nanoparticle

In in-vitro as well as in-vivo conditions. For example, we can use cancer cells that express a particular receptor, and when we add the particular viral nanoparticle, it can bind to the cancer cell and kill it. Then, in the in vivo condition, we can use a mouse model that has the tumor. When we inject this nanoparticle, it can bind to the tumor cells and destroy them.

In this way, we can evaluate the therapeutic efficiency of viral nanoparticles both in vitro and in vivo. I hope you got the overall idea about the biological synthesis of nanomaterials. Let us go to the lab and learn how to synthesize nanomaterials through a biological method. Today, we will learn how to synthesize silver nanoparticles using neem extract. To proceed with the experiment, we need

freshly plucked neem leaves, silver nitrate, glassware, tissue paper, a spatula, scissors, a micropipette, and micro tips. In the first step, we will prepare the neem extract. We have freshly plucked leaves here that have been washed with distilled water and chopped into smaller pieces. This is approximately 40 grams of neem leaves, which we will add to 200 ml of distilled water. Cover this with aluminum foil to prevent evaporation while boiling.

Place it on the heating mantle and let it boil for around 15 to 20 minutes. Here, you can see that the color of the water has changed. Turn off the heater and set the beaker aside to cool. As the solution cools, you can observe the color change due to the neem extract. Once cooled, we will pour the solution into a separate bottle.

Now, we will filter the neem extract. We have already prepared the neem extract solution by filtering it with Whatman filter paper. To ensure further purification, we will filter the solution using a syringe filter membrane with a 0.22-micrometer pore size. Now that we have prepared the neem extract, let us proceed with the synthesis of silver nanoparticles. For synthesizing silver nanoparticles, we need neem extract,

ultrapure water, two conical flasks with beads—one for the blank and one for synthesizing silver nanoparticles, 10 millimolar silver nitrate solution, a micropipette, microtips, and a measuring cylinder. First, we will add 50 ml of ultrapure water to the blank, which is the negative control conical flask. Set the magnetic stirrer to 40 degrees Celsius and 300 rpm. Next, add 50 ml of 10 millimolar silver nitrate solution to the second conical flask.

Cover the flask with aluminum foil to prevent the photoreduction of silver nitrate. Set the same temperature and RPM on the stirrer. Leave both flasks for a while. Now we will add 5 mL of neem extract to both flasks. Allow the reaction to continue for at least an hour

to ensure the complete reduction of silver nitrate ions. As you can see, there is a slight color change, indicating that the reduction process has begun. The further color change signifies the formation of silver nanoparticles. We will leave this solution to complete the reduction process. Once the reaction is complete, turn off both stirrers.

As you can observe, the color of the solution in the blank flask containing ultra-pure water and neem extract is different from the one in the flask containing 10 millimolar silver

nitrate solution and neem extract, indicating the formation of silver nanoparticles. To confirm the formation of silver nanoparticles, we will use a UV-visible spectrophotometer. First, turn on the UV-visible spectrophotometer and allow it to warm up.

Open the software on the system and select the new file option. Choose spectral scan and set the wavelength range from 350 to 800 nanometers. We will first calibrate the baseline using ultrapure water as the reference. When adding samples, ensure you hold the cuvette at an angle to avoid bubble formation. Place the cuvette in the machine, ensuring the clear sides align with the light source and detector.

Select the blank calibration option. After calibration, perform a blank reading with ultrapure water, which is the solvent we used. And press the zero option on the software. Once completed, discard the water and add the sample to the cuvette. First, we will take the UV reading of the 10 millimolar silver nitrate solution.

Press start. As expected, there is no peak for the silver nitrate solution. Now wash the cuvette and add the neem extract. Place the cuvette inside the machine and press start. Again, no peak is observed in the range of 350 to 800 nanometers.

Next, we will add the synthesized silver nanoparticles. Since it is highly concentrated, we will prepare a 1:4 dilution of the stock solution. To do this, we will mix 250 microliters of nanoparticle solution with 750 microliters of ultrapure water to achieve the desired dilution. Mix well and transfer the solution to the cuvette. Place it in the spectrophotometer and take the reading.

We can now observe a peak around 450 nm, confirming the synthesis of silver nanoparticles. Save the data. Finally, wash the cuvette. This concludes our demonstration on the synthesis of silver nanoparticles. through the biological method using neem extract.

As a summary, in today's lecture, we learned about the biological synthesis of nanoparticles and the advantages of biological synthesis. And also, through a practical demonstration, we learned how to synthesize nanoparticles by the biological method. Thank you for your kind attention. I will see you in another interesting lecture.