Experimental Nanobiotechnology

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Lecture 02: Synthesis Of Gold Nanoparticles

Hello everyone, today we are going to learn about the synthesis of gold nanoparticles. In today's lecture, we will be learning about noble metal nanoparticles. We will also be learning about the physical and chemical properties of metal nanoparticles. At the end of the lecture, through a practical demonstration, we will learn how to synthesize gold nanoparticles. Let us see what is noble metal nanoparticle.

Noble metal nanoparticles include nanoparticles synthesized from salts of noble metals like gold, silver, and platinum. The noble metals, when it is in bulk, it will show different kind of properties. The same metal, when reduced to the nanoscale, shows remarkable properties. For example, the bulk gold, which we are using it for making ornaments or jewels, when reduced to the nanoscale, can be useful for various biomedical applications.

For example, it can be useful for nanobiosensors, for point-of-care disease detection, and it can also be useful as a nanoprobe for in vivo cell imaging and tracking. It can also be useful for a drug delivery system or for theranostic management of cancer. What is theranostic? Theranostic means these are multifunctional nanoparticles which can do therapy and diagnostics.

That is called theranostic nanoparticle. The nanoparticle which can do the multiple function, which can do therapy as well as diagnostics simultaneously. And the commonly used nanoparticles for biomedical applications are gold and silver nanoparticles. In today's lecture, we will be learning about how to synthesize gold nanoparticle and also we will be learning about the applications of gold nanoparticle.

As I told you in the previous lecture, we can synthesize the metal nanoparticles by both top-down as well as bottom-up approach. In the previous lecture, we learnt about the ball milling, how to use the ball milling to make nanomaterials. We can also use the ball milling to make the metal nanoparticles and under the bottom-up approach, we can make

nanoparticles through chemical synthesis. In today's lecture, we are going to learn about the chemical synthesis method, how to prepare gold nanoparticle by chemical synthesis as well as through microwave method. To synthesize metal nanoparticles, we need three important components.

The first one is metal salt. The next one is reducing agent. The reducing agent, which will reduce your metal salt into metal nanoparticle, but these metal nanocollides, it will be highly unstable. To make it more stable nanoparticle, we have to add capping agent or stabilizing agent, which will stabilize these metal nanoparticle to stay for longer duration. Some of the examples of reducing agents is sodium citrate, sodium borohydrate and ascorbic acid. And some of the examples for capping agents are thiols citrate and polymers like PVA that is polyvinyl alcohol.

By using this, we can make the metal nanoparticles. The growth of nanocrystals in solution involves two important processes: nucleation and growth. Let us see what is nucleation and growth. Nucleation is the phenomenon of the initiation of the formation of the first nanocrystalline solution. It involves the appearance of very small particles or nuclei of the new phase, which are capable of growing.

Once a nuclei is formed, then followed by that there will be a growth and we get the nanoparticle. To get small-sized nanoparticles, we need strong nucleation and slow growth. Let us learn about nucleation and growth in detail with the example of a precipitation reaction. Once we add the metal salt and reducing agent, all the metal salt will be reduced into metal atoms. These atoms combine and form clusters.

These clusters combine and form crystallites. This step is nucleation. Once nucleation is over, there will be a growth process, and these crystallites combine to form primary particles, and we have to control the growth. Otherwise, the particles keep growing, leading to agglomeration.

These are big size particles which are not useful. So we have to control the growth of particles by adding a stabilizing agent or capping agent. As I mentioned in the previous slide, once the nucleus is formed, the growth starts. So there are two types of nucleation. The first one is homogeneous nucleation.

That means all the nuclei will form simultaneously. If all the nuclei form simultaneously, you get monodispersed particles. That means you get uniformly sized nanoparticles. The other type is heterogeneous nucleation. That means nucleation occurs at different times.

In this case, you get polydispersed particles. You can see that the particle sizes are different. Some are small, some are large. But in the case of homogeneous nucleation, you can obtain uniformly sized nanoparticles. Let us see how to control nanoparticle growth in solution.

We can control the nanoparticle growth in solution by arrested precipitation. That is precipitation under starving conditions. We can create a large number of nucleation centers by vigorous mixing of reactant solutions. Following that, we can keep the growth concentration small. In this case, the nucleus growth is stopped due to lack of material.

This is called arrested precipitation. And the next one is Oswald ripening. Here, the growth mechanism is that small particles get dissolved and are consumed by larger particles. As a result what happens is the average particle size increases with time, the particle concentration decreases. Once the particle size increases, that will decrease the solubility.

We have to control the particle size by adding a stabilizer. We have to protect the particles from Oswald ripening by adding a stabilizing agent. Let us see how to tune the size of nanoparticles. The size of nanoparticles can be controlled by controlling the nucleation and growth rates. For example, if you have fast nucleation, you get smaller nanocrystals.

In case of slow nucleation, you get larger particles. From this picture, you can understand. In case of fast nucleation, assume that here there are seven nuclei, and if you have like 14 metal atoms, assume that you have 14 metal atoms. What happens is the seven nuclei each will get only two metal atoms, and the size will be small; the growth will be less.

And the size of the nanoparticle will be small. But in the other case, where there is slow nucleation, and you have the same number of metal atoms for the growth, and the nuclei are only two, in this case, each will get seven metal atoms, so due to that, the particle size will be large. So by controlling the nucleation and growth, we can fine-tune the particle size. Let us see the role of the stabilizing agent.

The stabilizing agents are also called capping agents or passivating agents. The stabilizing agent prevents uncontrolled growth of particles, prevents particle aggregation, and controls the growth rate. And controls the particle size, and it also allows particle solubility in various solvents. The stabilization of nanoclusters against aggregation can be broadly divided into two types: electrostatic stabilization and steric stabilization. Let us learn about what is electrostatic stabilization and steric stabilization.

In electrostatic stabilization, there will be an adsorption of ions onto the surface. which creates an electrical double layer and that leads to repulsion force between the individual

particles. For example, if we have a nanoparticle carrying a uniform charge, these particles repel each other. In this case, the particles can be stabilized and remain dispersed for a longer time without aggregation. The next type is steric stabilization.

Here the metal nanoparticles are surrounded by layers of material that are sterically bulky. For example, we can use polymer or surfactants and which can form like a layer and it can prevent the nanoparticle from aggregation. Let us see what are the various parameters affect the particle growth, shape and structure. The first one is the type of capping agents or stabilizers. So what kind of stabilizing agent you are using based on the the size and shape of the particles may differ.

And again, it depends on the reducing agent. In some cases, we have to use a strong reducing agent. In some cases, we have to use a weak reducing agent. The third one is the concentration of the reactants and the pH value of the solution. Finally, the duration of the heat treatment.

The heat treatment favors the more and fast nucleation. Let us learn the basics of synthesis of gold collides. To synthesize gold collides, we need tetrachloroauric acid this is a metal precursor and we need trisodium citrate that is the reducing agent

by this method we can make gold colloid with nanoparticles between 10 to 20 nanometer in size in the reaction this citrate will act as a weak reducing agent and it also act as a kind of stabilizing agent how it is acting as a stabilizing agent it forms a layer of citrate anions which adsorbs around the each nanoparticle and prevent this nanoparticle from aggregation due to the electrostatic repulsion of the nanoparticle. Once the nanoparticle is formed, you get this kind of ruby red color.

Once you get the ruby red color, you can confirm that you made the gold nanoparticles. Whenever we synthesize gold or silver colloids, we get different colors with respect to shape and with respect to the size. The reason is surface plasmon resonance. Here also you can see here, With respect to the size and with respect to the shape, you are getting different colors. Let us see what is surface plasmon resonance.

When a nanoparticle is much smaller than the wavelength of light, there will be a coherent oscillation of conduction band electrons. It is induced by interaction with an electromagnetic field. This resonance is called surface plasmon resonance. Let us learn about the surface plasmon resonance more in detail. Most of you know whenever we go to the jewellery shop, we can see that the bulk metals are shiny in nature.

So what is the reason? The bulk metal is shiny due to the electron cloud. And these electrons have dual nature. When we have light of matching wavelength that produce the resonance, then the electron cloud start vibrating. For nanoparticle, this wavelength lies in the visible region.

When you have the incoming radiation that leads to oscillation of conduction electrons in the nanoparticle and that leads to surface plasmon resonance. Here the peak position depends on the metal size and shape. Whenever we make the nanoparticle we measure the absorbance using UV visible spectrophotometer and we get the absorbance maximum peak. And this peak position depends on the metal size and shape. There will be a red shift with increasing in the particle size.

Let us see what the factors are which control the size as well as the stabilization of the nanoparticle. The average size is controlled by the reducing agent concentration, stirring rate, and temperature. The size distribution is controlled by the rate of reducing agent addition and the stirring rate. You always have to use a fresh filter solution to get small-sized nanoparticles. And also the stabilizing agent, what kind of stabilizing agent you are using.

That also decides the stability as well as the size and shape of your nanoparticle. Let us see how to synthesize gold nanoparticles by the chemical reduction method. In today's practical session, you are going to learn how to make gold nanoparticles by the sodium citrate reduction method. This is the protocol we are going to follow in today's lab session. Let me briefly explain the principle of this chemical reduction method.

To synthesize gold nanoparticles by the chemical reduction method, as I mentioned earlier, we need a metal precursor, reducing agent, and capping agent. Here, we are going to use tetrachloroauric acid as the metal precursor. We are going to use trisodium citrate as the reducing agent. And here we are not going to use the stabilizing agent because this trisodium citrate, it can act as both reducing agent as well as capping agent. Once you see the gold nanoparticles, you will observe this kind of ruby-red color.

Then, you can characterize these gold nanoparticles using UV-visible spectroscopy. We can also characterize the nanoparticles by electron microscopic techniques, such as transmission electron microscopy or scanning electron microscopy. Let us see how to characterize these citrate-capped gold nanoparticles using UV-visible spectroscopy as well as by TEM analysis, that is, transmission electron microscopic analysis. In UV-visible spectroscopy, you get the absorption maximum around 520 nanometers, which confirms

the formation of gold nanoparticles. By using electron microscopy, you can see that the gold nanoparticles are of uniform size, almost uniform in size.

Using UV-visible spectroscopy, we can also confirm whether the nanoparticles are in a dispersed state or an aggregated state. For example, in this case, you can see here the dispersed gold nanoparticles, which give this kind of sharp peak, whereas in the case of agglomerated gold nanoparticles, the peak is shifted. Normally, we get the gold nanoparticle peak around 520 to 550 nanometers. Here, you can see that the peak is shifted. That means the particles are aggregated.

Not only by spectroscopy but also by visual observation, you can see here the dispersed gold nanoparticles are ruby red in color, whereas these agglomerated gold nanoparticles are in the range of purple or blue color. If you keep it for more time, it will aggregate and form a black precipitate, leaving a clear solution. Eventually, all the gold nanoparticles will aggregate and settle at the bottom. Today, we are also going to learn how to synthesize gold nanoparticles by the microwave method.

And this is the protocol we are going to follow for making the gold nanoparticles by the microwave method. Let us see the overview of how to synthesize gold nanoparticles by the microwave method. Here, we are going to use tetrachloroauric acid as a metal precursor, and we are going to use tannic acid as a reducing agent. This tannic acid acts as both a reducing agent and a capping agent. Once we synthesize the gold nanoparticles, you can observe the ruby-red color.

Then, we can characterize these gold nanoparticles by UV-visible spectrophotometer and also by electron microscopes such as scanning electron microscope and transmission electron microscope. Let us see how to characterize the tannic acid-capped gold nanoparticle. To synthesize the tannic acid-capped gold nanoparticle, we have to use the metal precursor and tannic acid, which acts as a reducing agent and also as a stabilizing agent. By using the microwave-assisted method, we can obtain the tannic acid-capped gold nanoparticle. By using UV-visible spectroscopy, we can characterize the tannic acid-capped gold nanoparticle.

You can see that there is an absorption maximum at 530 nanometers. Through different synthesise routes, we understood how to synthesize the gold nanoparticle. One is the chemical reduction method, and the other one is the microwave-assisted method. Let us learn about the various problems we face when we synthesize gold nanoparticles and how to overcome those issues. The first one is no color change.

If there is no color change, it may be due to the incorrect gold precursor. So for that, you have to check the concentration of the salt solution. Or it may be due to the incorrect ratio of gold precursor to citrate. Adjust the reducing and capping agent ratio. Or it may be due to incomplete or improper heating.

So make sure that you have proper heating in the particular gold nanoparticle synthesis setup. And if you are getting unstable or polydispersed nanoparticles, it may be due to inconsistent heating or stirring. So try to maintain the consistency throughout the experiment. And if there is an incorrect or insufficient capping agent, optimize the concentration. Or if there is any contamination in the glassware or chemicals, then check for contamination.

Always use clean and new glassware for synthesizing the nanoparticles that you are using for biomedical applications. Some of the other problems include the aggregation of nanoparticles. The aggregation of nanoparticles may happen due to insufficient capping agent, high ionic strength of the solution, or impurities. In this case, you have to increase the capping agent concentration or work in a low-salt environment during synthesis and storage. And store the solution in a dark and clean container to minimize light-induced aggregation.

And if you are not able to get the results properly and if you are getting non-reproducible results, it may be due to variability in precursor or reagent quality or changes in the reaction conditions. Standardize and document all the parameters, for example, temperature, reagent volumes, and stirring speed, so that you can follow that and understand at which step you are making a mistake. Use the same batch of metal precursor and capping agent for consistency.

Some of the other observations are: if you are getting a blue-gray or cloudy solution, it may be due to the overgrowth or aggregation of nanoparticles. Or it may be due to excess capping agent or insufficient reduction. So, check the reducer-to-gold molar ratio and also shorten or optimize the heating time. If the particles appear aggregated, sonicate the solution briefly to break them apart. And if you are getting insufficient yield, that may be due to the loss of material during handling or improper reaction conditions.

Scale up carefully and maintain the same ratio and conditions. And also, you can use a spectrophotometer to monitor the SPR peak around 520 to 530 nm to confirm particle formation. I hope you got the overall idea about how to synthesize gold nanoparticles. Let

us see how we can use these tannic acid-capped gold nanoparticles for sensing polyamines in saliva. Polyamines are more abundant in the saliva samples of oral cancer patients.

Can we use simple gold nanoparticle based sensor for detecting the presence of polyamine in the saliva? For that, we are going to use the tannic acid capped gold nanoparticles. Once you add the polyamine, in this case, we are adding spermine, that is the polyamine. What happens is, this amine group in the polyamines interact with the phenolic groups of the tannic acid cap gold nanoparticle through hydrogen bonding or electrostatic interaction that leads to the aggregation of tannic acid cap gold nanoparticle. And that results in the red shift of SPR.

That means, the SPR band will be shifted from 530 nanometer to 560 nanometer. From this picture, we can clearly understand with respect to concentration, the aggregation also increased. Then you can see the color difference. The color will also increase. That is due to the aggregation of gold nanoparticles. By using a simple colorimeter app in the smartphone, we can measure the RGB values.

From this video, you can understand Here we are using the blank sample that means we are not adding any polyamine. This is only gold nanoparticle. In this case we are adding the polyamine that is the spermine. Once we add the polyamine and leave it for few minutes then you can see that there is a change in the color due to the aggregation of tannic acid cap gold nanoparticles.

You can observe here there is a change in the color. It is due to the aggregation of nanoparticles that can be simply measured using the colorimeter app in the smartphone. Here we have used commercially available artificial saliva with known concentration of spermine for understanding the sensing efficiency of tannic acid cap gold nanoparticle. Let us go to the lab and learn how to synthesize these gold nanoparticles in details. We are going to learn gold nanoparticle synthesis through turkevitch method.

Materials required are 0.25 millimolar of tetrachloroauric acid as a gold precursor solution, trisodium citrate as the reducing agent, conical flask and magnetic bead, measuring cylinders, pipette and tips. In the first step, we are going to measure 50 mL of the precursor solution. Pour the precursor solution into a conical flask. Then we are going to heat the solution to 100 degree Celsius.

Cover the flask with a glass lid or plate to avoid evaporation. Set the stirrer at 100 degree Celsius and 400 rpm. Wait till the solution is completely heated. After the solution is

heated, we will add 3 mL of reducing agent into the gold precursor solution. The solution will slowly start to change its color from clear to slightly black color and then to ruby red color.

You can see that the solution is changing color. The color of the solution is slowly changing to a dark purple. The color is slowly changing into a ruby red. Here we can see the comparison between the color of the solution and the color of the precursor solution after reduction. So we have successfully synthesized gold nanoparticles through the Turkevich method, which is confirmed by the ruby red color of the solution.

Now we are going to learn how to synthesize tannic acid-capped gold nanoparticles through the microwave synthesis method. For that, we need a 5% tannic acid solution, a gold precursor solution, and an empty vial as a reaction vessel. First, we are going to add the gold precursor solution into the empty vial, which means we have to add 990 microliters of 0.1 millimolar tetrachloroauric acid into the empty vial. Then, heat this vial in a microwave oven for 10 seconds. Take out the vial and add 10 microliters of 5% tannic acid solution so that the final concentration will be 0.05%.

After adding, gently shake the vial to mix it properly and heat it again in a microwave oven for 10 seconds. We have successfully synthesized tannic acid-capped gold nanoparticles, as observed by the color change. As a summary, in today's lecture, we learnt about what is noble metal nanoparticle and how to synthesize gold nanoparticles using chemical reduction and microwave method. And we also learnt the applications of gold nanoparticles in sensing polyamines in saliva samples. Thank you, everyone. I will see you in another interesting lecture.