Experimental Nanobiotechnology

Prof. P. Gopinath

Department of Biosciences and Bioengineering,

Indian Institute of Technology Roorkee

Lecture 05: Synthesis Of Polymeric Nanoparticles

Hello everyone, today we are going to learn about the synthesis of polymeric nanoparticles. In today's lecture, we'll be learning about polymeric nanoparticles and their properties, and we'll also learn how to synthesize and characterize albumin nanoparticles, both theoretically as well as practically through demonstration. Let us see what is polymeric nanoparticles.

Polymeric nanoparticles are solid particles in colloidal form, and these polymeric nanoparticles are mainly composed of biocompatible and biodegradable polymers. Biocompatible means the particular polymer should be compatible with the biological system, and biodegradable means if the particular polymer degrades inside the body, it should not induce any adverse effects; that is a biodegradable polymer.

Mainly, these polymeric nanoparticles are useful for drug delivery applications. We can load therapeutic drugs into these polymeric nanocarriers and target these nanocarriers to specific locations, for example, the drug can be loaded inside the polymeric nanocarrier, which is called drug encapsulation. We can also add the drug on the surface through a physical process like adsorption.

So the drug can be attached on the surface and The third one is the drug can also be entrapped into the polymeric network, which is called drug entrapment. So we have to select the right polymers because selecting the right polymers will affect your biodegradability, biocompatibility, and also based on your polymer, it will improve the therapeutic efficacy and lower the side effects.

These polymeric nanoparticles can be categorized based on the methods of synthesis. For example, from preformed polymers in dispersion, we can use methods such as solvent evaporation, nanoprecipitation, emulsification, dialysis, and supercritical fluid technology for making nanoparticles from the preformed polymers. From monomers, we can make

polymeric nanoparticles by emulsification, mini-emulsion, and other techniques like radical polymerization and interfacial polymerization.

From hydrophilic polymers, we can make polymeric nanoparticles by ionic gelation or coacervation. So based on the polymers, we have to select the suitable method for synthesis. We can select any of the methods, depending on the polymer and your final application. Let us see the types of polymers used to synthesize polymeric nanoparticles. The first one is natural hydrophilic polymers.

We can use natural proteins like gelatin or albumin, or natural polysaccharides like alginate and chitosan. The other one is synthetic hydrophobic polymers. It can be preformed polymers like polystyrene and polycaprolactone, that is PCL, or it can be polymerized during the process, such as polymethylmethacrylate. The third one is amphiphilic block copolymers, which contains polymers with both hydrophobic as well as hydrophilic group in the polymeric molecule.

Some of the examples are diblock, triblock, and tetrablock copolymers. Let us see some examples of biodegradable polymers and their applications. The first one is polyester. Under polyester, we have polylactic acid. Under proteins, we have silk fibroin and albumin.

In today's lecture, we will also learn how to synthesize albumin nanoparticles. Under polysaccharides, we have examples like starch and cellulose. Under polyphenols, we have lignin and tannin. Under lipids, we have waxes and surfactants. These biodegradable polymers have wide applications including food packaging, drug delivery, tissue engineering, and also

sensors. Let us learn about protein based nanoparticles. Protein based nanoparticles have emerged as a potential candidate for various therapeutic as well as diagnostic application because it has a high biocompatibility and high water solubility and also it is easy to modify the surface of this protein nanocarriers.

So that is one of the important property. Abraxane, this is the first FDA approved albumin based drug nano formulation. So based on the success of this Abraxane, These albumin-based nanoparticles have gained overwhelming response and lot of research group is working on in developing this protein-based nanoparticle for various application including cancer therapy.

Another important property of this protein-based nanoparticle is the presence of functionally charged groups including amino and carboxy groups and that will be very very helpful for adding the functional groups and targeting these nano carriers to specifically to the cancer. As i told you earlier these protein-based nanoparticles have wide applications in drug delivery because of its biodegradable nature and easy to modify the surface

And also, it is easy to synthesize, cost-effective, and can hold hydrophobic molecules. As you know, most of your anti-cancer drugs are hydrophobic in nature. We can load hydrophobic drugs into these protein-based nanocarriers, and they also have enhanced circulation time. That means they can stay in your body for a longer amount of time and release the drug in a slow and controlled way. And also, it has preferential cellular uptake.

For example, these protein nanoparticles are taken up more by cancer cells, and there is no toxicity. So these are the advantages of these protein-based nanoparticles for drug delivery applications. As I told you earlier, Abraxane is the first albumin-based nanoparticle approved by the FDA. Based on the success of Abraxane, there are several albumin-based drugs and imaging agents on the market, and some are under clinical trials.

Let us see what Abraxane is and how it was made using nanoparticle albumin-bound technology. Abraxane is a solvent-free nano-version of Taxol and was approved by the FDA in 2005 for breast cancer therapy. You can see the difference between Taxol and Abraxane. In Taxol, we have only 6 mg of Paclitaxel, which is the anti-cancer drug. And to dissolve these 6 mg, we have to use Cremophor and ethanol.

In case of Abraxane, we have 100 mg of Paclitaxel in 900 mg of albumin. There is no use of surfactants or solvents in the preparation of this albumin based nanoparticle. That is the advantage of this Abraxane. It has more therapeutic effect when compared to the Taxol, which has only less concentration of your anti-cancer drug. Based on the success of Abraxane, various research groups developed tumor-targeted delivery of anticancer agents and imaging agents.

For example, we can target the drug and imaging agent specifically to the breast cancer by conjugating the albumin nanoparticle with folic acid. If a nanoparticle is conjugated with folic acid and the breast cancer have the receptor for the folic acid. So this nanoparticle will go and specifically bind to the breast cancer cells and it won't bind to

the normal cell. In this way, we can improve the therapeutic efficiency by specifically targeting to the tumor cells. And we can also add gold nanoparticles or gold-based nanostructures to this and we can use it for photothermal therapy.

Photothermal therapy means we can apply the light and that will generate the heat and it can destroy the cells. And we can also use iron oxide based nanoparticles. When you are using this iron oxide nanoparticle with the help of magnetic field and we can increase the temperature in the particular location that is called hyperthermia. By that way also we can kill the cancer cell. Let us see how to synthesize bovine serum albumin nanoparticles.

We can prepare BSA nanoparticles by the desolvation method. By the desolvation method, we can achieve a homogeneous distribution and less aggregated nanoparticles. In this method, we will be using ethanol as desolvating agent and glutaraldehyde as cross-linking agent. The nanoparticles prepared by this method show great stability in water as well as in cell culture medium. Let us see how glutaraldehyde is used as a cross-linking agent.

This glutaraldehyde will form a Schiff base upon reaction with protein, the positively charged amino groups of the protein react with the two carbonyl groups present in glutaraldehyde. In this way, it will cross-link and form stable protein-based nanoparticles. Let us see an overview of BSA nanoparticle synthesis by the desolvation method. Once we prepare the aqueous solution of albumin, we have to add the desolvating agent, which is ethanol.

Once you add ethanol dropwise, it will gradually reduce albumin solubility, leading to phase separation. In this step, if you want to add any anti-cancer drug, you can also do so. As I told you earlier, most of the anti-cancer drugs are hydrophobic in nature. We can dissolve the anti-cancer drug in ethanol or acetone and add it dropwise. Then, the drug will be encapsulated into the nanoparticles.

The albumin aggregates or nanoparticles formed at this stage are unstable. They may dissolve back into the solution. To avoid that, we have to add a cross-linking agent, which is glutaraldehyde. After the cross-linking step is over, we have to do centrifugation to remove the unreacted cross-linking agent. After centrifugation, discard the supernatant and add deionized water.

Repeat this centrifugation step at least three times to completely remove the unreacted cross-linking agent. Then, you can lyophilize the sample and add the powder to deionized

water to achieve the desired concentration of your protein nanoparticle. In this way, we can make stable albumin nanoparticles. In today's lecture, we will also learn how to synthesize BSA nanoparticles using this method in more detail through a practical demonstration. Let us examine the mechanism behind the cellular uptake of BSA nanoparticles.

Albumin is a key protein in cancer progression, supplying nutrients for tumor growth. This means tumor cells require more nutrients, and albumin protein is essential for their growth. When you add an anti-cancer drug to the albumin, the cells take up these albumin nanoparticles assuming they are nutrients for their growth. However, these albumin nanoparticles contain the anti-cancer drug.

So, it can enter the cell and kill the cancer cell. How is this albumin entering the cell? It enters through the GP60 receptor, which is a glycoprotein receptor present on the surface of the cells, and it leads to caveolae formation. Caveolae formation provides entry into the cell and allows the nanoparticle to enter the cell. Once it crosses the barrier,

It enters the cell, releases the anti-cancer drug, and induces cell death. These BSA nanoparticles interact with the secreted protein acidic and rich in cysteine (SPARC) protein to enter the tumor cells. These tumor cells overexpress the SPARC protein. So, these BSA nanoparticles can easily attach to the cancer cell, which overexpresses the SPARC protein.

By this way, we can target the nanoparticles to specifically to the tumor cells and we can kill the cancer cells. Let us see how this nanotechnology is improving cancer therapy. In traditional treatment, when using these anti-cancer drugs, they cannot distinguish between cancerous and noncancerous cells. They will attack all the cells.

In the end, all the cells will die. That leads to a lot of toxicity and side effects. In the case of nanotechnology, what happens is these nanoparticles are specifically targeted to the cancer cells. They will kill only the cancerous cells, and the non-cancerous cells remain intact. So that is the advantage of this nanoparticle-based tumor-targeted delivery.

There are two approaches to target the nanoparticles to the cancer cells. One is passive targeting, and the other is active targeting. Let us see what passive targeting is. This passive targeting is based on the retention effect of particles of a certain hydrodynamic size in cancerous tissue. For example, if you make a nanoparticle of size between 70 to 200 nanometers,

these nanoparticles will be trapped in the tumor location by an effect called the EPR effect. Enhanced permeability and retention effect. So this I will explain later. And the next approach is active targeting. Here we have to add a specific functional group or an antibody specifically for the cancer cell.

Once you add the antibody specific for the cancer cell, these nanoparticles will go and bind specifically to the cancer cell and destroy the cancer cell. This is called active targeting. Let us see what the EPR effect is. Tumors need blood to grow larger than 2 mm in size. What happens is these tumorous tissues suffer from the enhanced permeability and retention effect (EPR effect).

These nanoparticles injected into the bloodstream do not permeate through healthy tissues and will be trapped in the tumor region. This green color represents the tumor region. Here, you can see that there are many leaky blood vessels. Due to that, if you make nanoparticles between 70 to 200 nanometers, such as polymeric nanoparticles, they will be trapped in the tumor location and kill the tumor cells.

Let us see what active targeting is. In the case of active targeting, as I mentioned earlier, we have to add a specific ligand, an antibody, or a particular protein or peptide, and that will specifically bind to the cancer cell and once it binds to the cancer cell then it will kill the cancer cell whereas in case of normal cell we don't have that kind of receptors so

This nanoparticle cannot bind to the normal cell. It specifically binds only to the cancer cell and will kill the cancer cell. In addition to the anti-cancer drug, we can also add an imaging agent. In that case, we can call this nanoparticle a theranostic nanoparticle, which can be useful for both therapy and diagnostics. Let us see how to characterize these BSA nanoparticles.

We can characterize the BSA nanoparticles using a UV-visible spectrophotometer. In this UV-visible spectra, you can see two peaks for the niclosamide drug alone: one at 260 nanometers and one at 346 nanometers. Once this niclosamide drug is encapsulated into the BSA nanoparticles, you can see the peak at 346 nanometers for the drug-loaded BSA nanoparticle. The slight change in the spectra is due to the cross-linking between the amino acids of the protein and

the formation of a protein-drug nanoparticle complex. From this picture, we can understand the insolubility of niclosamide in water and the solubility of BSA-niclosamide nanoparticles in water. When you observe the niclosamide drug under a scanning electron

microscope, you see this kind of structure. Similarly, once the drug is loaded into the protein nanoparticle, you can see here the spherical-shaped nanoparticle loaded with niclosamide.

And the nanoparticles can also be observed under the AFM, which is an atomic force microscope. You can see that the protein nanoparticles are spherical in nature. By using dynamic light scattering (DLS), you can also determine the hydrodynamic size. The hydrodynamic size is roughly in the range of 200 nanometers. By using an electron microscope, an atomic force microscope, and DLS (dynamic light scattering),

we can determine the size as well as the shape of the protein nanoparticles. We can study the anti-cancer efficiency of niclosamide-loaded BSA nanoparticles compared to niclosamide by using a cell viability assay. In the cell viability assay, you can see that when you add niclosamide, which is a hydrophobic drug, it cannot enter the cell. It is unable to kill the cell. The cell viability is close to 100%.

But when you load this niclosamide drug into the protein nanoparticle, you can see that the number of cells percentage is going down with respect to the concentration. By using this cell viability assay, we can also calculate the IC50. IC50 means the concentration required to inhibit 50% of cell growth. In this case, we are using A549 that is the lung cancer cell line and you can see that the IC50, is 5 micromolar.

You can use the 5 micromolar to kill 50 percent of the lung cancer cells. In the case of breast cancer cells, you can see that the IC50 is roughly around 2.6 micromolar. The IC50 concentration will vary from cell to cell, which is why we have to use several cell lines to understand the therapeutic efficiency of your nanoparticles. Let us see some of the troubleshooting tips in the synthesis of BSA nanoparticles.

If you are observing particle aggregation, it may be due to clumping or large aggregates in the suspension. How to overcome that? You can check the pH of the solution and add the desolvating agent dropwise. Or you can use the optimum concentration of the polymer. The next one is if you are observing irregular particle size, that is, a wide distribution of particle size or inconsistent size.

How to overcome that? You can ensure consistent and vigorous stirring during solvent addition and add the crosslinking agent only after achieving uniform particles. The next one is if you are getting a low yield of nanoparticles, it may be due to low recovery of nanoparticles. You can adjust the centrifugation speed and the optimum polymer

concentration. Check batch-to-batch variation of all the reagents, and if you are facing increased turbidity,

That means the solution appears turbid, or particles are visible without magnification. You can use a 0.22-micrometer filter to remove any large aggregates. Wash the nanoparticles with distilled water to remove excess reagents. If you are facing reproducibility challenges, that means variation in the particle size or yield across batches, you can use consistent reagent quality, equipment, and environmental conditions.

Always prepare fresh polymeric solutions to avoid degradation effects. Hope you got the overall idea about how to synthesize BSA nanoparticles and also how to characterize BSA nanoparticles. Let us go to the lab and learn this technique in more detail. To synthesize BSA nanoparticles, we need BSA powder,

glutaraldehyde as a crosslinker, vials, magnetic beads, sterile ultrapure water, a spatula, absolute ethanol, a sterile syringe, tips, and a pipette. First, we will weigh 50 mg of BSA powder using a weighing balance. Then, add this BSA powder into a vial. Add 1 mL of sterile ultrapure water to it and keep it stirring at 400 rpm. Wait until the BSA is completely dissolved in the water.

After complete dissolution, we have to add 4 mL of absolute ethanol into the solution. For that, we will use a sterile syringe. Add absolute ethanol to the BSA solution drop by drop. A cloudy formation will be seen in the vial, indicating phase separation. You can see that the solution is getting slightly cloudy.

Now the solution is completely cloudy. Once the solution has turned cloudy, we will add 30 microliters of 8 percent glutaraldehyde to the BSA solution. You have to add it very quickly and keep it stirring overnight at 400 rpm. You can observe the color change in the solution, which denotes the successful synthesis of BSA nanoparticles. Moving on to the washing step, we will take out the magnetic beads from the solution and pour the solution into a centrifuge tube.

Similarly, prepare another tube with equal weight to balance the centrifuge tube with the sample. We have to centrifuge the solution for about 30 minutes at 8000 rpm. To balance the centrifuge, you should place the tubes of equal weight directly opposite each other in the rotor. Start the machine and wait for the centrifugation to complete. After centrifugation, carefully remove the centrifuge tube.

You can see that there is a pellet formation at the bottom, which contains BSA nanoparticles. We can discard or store the supernatant in a separate tube. Add sterile ultrapure water to the pellet for washing. Again, centrifuge the BSA nanoparticles at 8000 rpm for 30 minutes. Carefully take out the tube.

We will discard the supernatant. This is the washed nanoparticle pellet. After this, we will proceed to the lyophilization step. First, we have to turn on the compressor and wait until the temperature reaches minus 80 degrees Celsius. Remove the cover and place the centrifuge tube without the lid in the machine.

Turn on the vacuum pump for lyophilization. After lyophilization, carefully release the pressure and take out the tube. Now, we weighed 30 milligrams of lyophilized BSA powder. Now, we will add 1 mL of sterile ultrapure water into the tube to get a final concentration of 30 mg per mL. Here, we have made a stock solution of BSA nanoparticles.

As a summary, in today's lecture, we learned about what polymeric nanoparticles are and also how to synthesize and characterize BSA nanoparticles. We also learned about the applications of BSA nanoparticles in drug delivery. Through a practical demonstration, we also learned how to synthesize and characterize BSA nanoparticles. Thank you for your kind attention.

I will see you in another interesting lecture.