Experimental Nanobiotechnology

Prof. P. Gopinath

Department of Biosciences and Bioengineering,

Indian Institute of Technology Roorkee

Lecture 14: Synthesis of Hydrogel and Nanogel

Hello everyone, today we are going to learn synthesis of hydrogel and nanogel. In today's lecture, we are going to learn what is hydrogel, how to synthesize and characterize hydrogels, and we are also going to learn about nanogels and their methods of preparation. At the end of this lecture, we will learn how to synthesize hydrogels and nanogels through a practical demonstration.

Let us see what is hydrogel. Hydrogels are three-dimensional, cross-linked polymeric networks capable of absorbing and retaining significant amounts of water or biological fluids. So, due to their unique ability to swell without dissolving, they are ideal for a variety of applications ranging from biomedical to industrial uses.

Hydrogels were first reported in 1960 for contact lens applications. Let us see the properties of hydrogels. Hydrogels have very good swelling capacity. They have the ability to absorb and retain water, and they are biocompatible materials. They do not induce any immune response.

So that is one of the important properties for medical applications. It is compatible with the biological system. It won't induce any adverse effects inside the body. So that's why it is highly biocompatible. And it also has very good mechanical properties.

And these mechanical properties can be adjusted from soft to tough. It depends on your final application. And the degradation properties can also be changed with respect to the final application. The last one is permeability. We can also control the diffusion of oxygen, nutrients, and drugs.

For example, these hydrogels are porous in nature. So they will allow oxygen, nutrients, and drugs to enter and pass through. So it is one of the important properties for various

biomedical applications. Let us see the factors involved in the fabrication of hydrogels. The first one is polymer type.

It can be a natural or synthetic polymer. Depending on your final application, we have to select the polymer. If you want to go for a biodegradable scaffold, we can use a natural polymer. And if you want more mechanical strength and long-term stability, we can use a synthetic polymer. The next one is cross-linking density.

If you have high cross-linking, it will increase the strength but decrease the flexibility and swelling. The next factors are pH and temperature. The pH and temperature affect the gelation and stability, mainly for sensitive polymers. The water content influences swelling, mechanical properties, and drug release rates. The final factor is the polymer concentration.

It affects the viscosity and network formation during gelation. We have to optimize all these parameters and understand these factors before fabricating our hydrogel. Let us see how we can classify these hydrogels. Hydrogels can be classified based on origin as natural and synthetic, and based on cross-linking, we can classify them into chemical-based cross-linking and physical cross-linking.

And the third one is degradability. So, it can be biodegradable or non-biodegradable. So, based on that, we can also classify. And the last one is porosity. It can be non-porous, microporous, mesoporous, or macroporous.

So, based on the porosity of hydrogels, we can also classify them. Let us see some examples of classification based on polymer type. So, based on natural polymers, we can classify them as natural hydrogels. Some examples are alginate, chitosan, and gelatin.

Under synthetic hydrogels, PEG (polyethylene glycol), PVA (polyvinyl alcohol), and polyacrylamide. These are some examples of synthetic hydrogel polymers. Let us see how we can classify hydrogels based on cross-linking. The first one is chemical cross-linking. Here, the hydrogels are cross-linked through chemical reactions, and the polymer chains are connected by covalent bonds.

Examples are ester bonds, amide bonds, and disulfide bonds. Some of the common reactions include free radical polymerization, condensation reaction, and click chemistry. An example is azide-alkane reactions. Here, this monomer will be cross-linked using the cross-linker, and it will form a chemically cross-linked hydrogel network. In this way, we can make the hydrogel, the porous hydrogel, based on chemical cross-linking.

Let us see some of the examples. The first one is gelatin, which can be cross-linked using glutaraldehyde. The next one is polyacrylamide gel, which can be cross-linked using bisacrylamide. These chemically cross-linked hydrogels have high mechanical strength due to strong covalent bonds. They are highly stable and do not degrade easily in an aqueous environment.

Their swelling behavior can be fine-tuned by adjusting the degree of cross-linking. We can increase the cross-linking or decrease the cross-linking. The biocompatibility depends on the material used and also on the cross-linker. For example, if you are using glutaraldehyde, it may reduce the biocompatibility if you use a high concentration of glutaraldehyde.

It may have some toxic effects, and it has many applications in the biomedical field. Especially in the case of drug delivery, because it has very good swelling and stability. It can be useful for controlled drug release, and it can also be useful for making scaffolds for tissue engineering applications. Let us see the next example, which is physical cross-linking. Here, the hydrogels are connected through non-covalent interactions, such as

ionic bonds, hydrogen bonds, Van der Waals forces, or hydrophobic interactions. These cross-links are reversible and environmentally sensitive. Some examples are alginate, Here, we can use ionic cross-linking with calcium ions. We can also use chitosan, where hydrogen bonding with polyacrylic acid can be used as a form of physical cross-linking.

The third one is PVA, which is polyvinyl alcohol hydrogen bonding during freeze-thaw cycling. These are some examples of physical cross-linking. Let us see some properties of physically cross-linked hydrogels. The first one is mechanical strength, which is generally lower than that of chemically cross-linked hydrogels, and stability-wise, it is also less stable due to reversible bonds, which make it

Biodegradable and swelling behavior, it is highly responsive to external stimuli. The fourth property is biocompatibility. Biocompatibility is better than chemically cross-linked hydrogels because here we are not using any toxic cross-linking agents. In the application part, we can use this hydrogel for various drug delivery applications, especially in smart drug delivery systems.

For example, we can make a pH-sensitive or temperature-sensitive responsive drug release. In the case of tumors, we have an acidic pH. We can make a smart drug release

system which is sensitive to acidic pH; it can release the anti-cancer drug and destroy the tumor cells.

We can also use it for wound dressing applications as it has very good biodegradability. It can be very useful for wound dressing applications. So, what is biodegradability? That means if the particular material is degrading inside this system, it should not induce any adverse effects. The degraded product should not induce any adverse effects.

And we can also use this for biosensor applications as it has very good stimuli responsiveness. We can also use it for biosensing applications. Let us see what the various methods available for hydrogel fabrication are. The first one is the physical method. Under the physical method, we have the freeze-thaw cycle.

So, we can use the freeze-thaw cycle to create physically cross-linked hydrogels. An example is PVA, that is, polyvinyl alcohol. And we can also use self-assembly. Self-assembly means it is a spontaneous organization of molecules into a structured network. An example is peptide-based hydrogels.

The next one is the chemical method. In the chemical method, we can use photo cross-linking. Photo means light. We can use UV or visible light. And this can induce polymerization or cross-linking.

An example is Gelma Hydrogel. That is gelatin methacrylate. The next one is ionic gelation. Here, polymers like alginate are used. We can use divalent cations.

These can cross-link the polymer chains. An example is calcium. The third one is click chemistry. It allows highly efficient and specific cross-linking reactions at room temperature. We can also use an emulsion polymerization process.

Here, two immiscible phases like oil and water are used to create microgels or nanogels. Let us learn about stimuli-responsive hydrogels in detail. These stimuli-responsive hydrogels undergo changes in their physical or chemical properties, such as swelling or shrinking, in response to specific triggers, such as pH, light, temperature, or electric or magnetic fields. Let us see some examples in detail.

The first example is temperature-responsive hydrogels. These hydrogels respond to changes in temperature, typically due to the balance between hydrophilic and hydrophobic interactions within the hydrogel. The example is poly(N-isopropyl

acrylamide). This polymer contains hydrophobic isopropyl groups and hydrophilic amide groups.

Temperature fluctuations cause hydrogen bonds to change, which results in swelling or shrinkage. This can be useful for smart drug delivery systems. With respect to temperature, it can release the drug. The next one is light-responsive hydrogels.

These hydrogels contain photochromic or photothermal molecules that react to specific wavelengths of light. Some examples are poly(acrylamide-co-spiropyran), where the spiropyran moiety undergoes reversible isomerization between hydrophilic and hydrophobic states under UV or visible light, leading to changes in swelling behavior. The applications include photo-controlled drug delivery or tissue engineering.

For example, we can use the light, and with respect to the light, we can control the release of the drug. So, this is a light-responsive hydrogel. The next example is electric field-responsive hydrogels. These hydrogels respond to electric fields through the electrophoretic movement of charged polymer chains, leading to swelling or contraction.

An example is polyacrylic acid hydrogel. It contains ionizable carboxyl groups, which dissociate under an electric field, causing electrostatic repulsion and hydrogel expansion. This can be useful for making actuators and biosensors. The fourth one is magnetic-responsive hydrogels. Here, we incorporate magnetic nanoparticles, which allow the hydrogel to respond to the external magnetic field.

We can use alginate and magnetic nanoparticles. The polymer network is inert, but when you add magnetic nanoparticles, with the help of an external magnetic field, we can induce structural changes. Some applications include controlled and targeted drug delivery with the help of an external magnetic field, or we can use it for soft robotics.

The next one is pH-responsive hydrogels. They contain acidic or basic functional groups that ionize or protonate at specific pH levels, altering the swelling behavior. An example is polyacrylic acid (PAA), and this PAA contains carboxyl groups that ionize at higher pH, increasing the electrostatic repulsion and swelling. This can be useful for various drug delivery applications, including the drug release system for the gastrointestinal tract.

for releasing the drugs at specific pH. Let us see how to characterize the hydrogel. The first one is we have to characterize the stretchability of the hydrogel using UTM, that is, a universal testing machine with a low-force load cell. This UTM measures the hydrogel's ability to elongate under tensile force.

The hydrogel samples are stretched until they break to determine parameters like elongation and tensile strength of the hydrogel. So, this tensile strength is very important for tissue engineering applications. How can we adjust these parameters? For example, if you increase the polymer concentration, it will reduce the stretchability.

Due to higher crosslink density, we can lower the crosslink density. It increases the stretchability but reduces the strength. The next one is compressibility. We can use the compression testing machine to measure the resistance to compression, which will be useful for load-bearing applications,

for example, if you are fabricating a scaffold for bone or cartilage repair, this compressibility is one of the important parameters. If you use a higher polymer concentration, it increases the compressive modulus but decreases the elasticity. And if you use less cross-linking agent, it will lower the compressive strength and stability. Let us see how to study the drug release from the hydrogel.

Here, we have to keep the drug-loaded hydrogel in a buffer solution at 37 degrees Celsius. Then, we have to use a UV-visible or fluorescence spectrophotometer or HPLC to measure the drug concentration released over time. This drug release kinetics is very important for a drug release system. If you have a higher crosslink density, the drug release is slower due to reduced pore size,

And if you have a low polymer concentration, fast drug release occurs but with poor control. The next one is rheology. The viscosity of hydrogels is evaluated using a cone-and-plate viscometer or rheometer. Because viscosity is an important parameter for injectable hydrogels. If you are making an injectable hydrogel, viscosity plays a very important role.

The viscosity is directly linked to the extent of cross-linking. So, we have to optimize the cross-linking if you are going for injectable hydrogels. The morphology of hydrogels can be studied using a scanning electron microscope or transmission electron microscope. We can also measure the pore size using SEM, but for that, we have to use ImageJ software. We can calculate the pore size, and the porosity can also be studied using various methods.

The pore size distribution can be studied using mercury intrusion porosimetry, or we can use BET analysis. This uses physical adsorption and desorption of gases to determine the porosity. Porosity is one of the important parameters for fluid exchange and drug release.

Based on the porosity, drug release can be slow or faster. So, that is why porosity is a very important parameter.

If you have an increased pore size, it enhances drug loading but weakens mechanical strength. And if you have a smoother morphology, it may reduce the surface interaction for bio-applications. The next one is swelling studies. The swelling study of hydrogels evaluates their ability to absorb fluids.

So that's why the swelling study is very important. So we have to use the dried hydrogel sample and rehydrate it using the swelling medium that is water, and we have to measure the weight at various time intervals to study the swelling kinetics. WD is the initial weight of the dried hydrogel, and WS is the weight of the hydrogel after swelling. The swelling properties are very important to predict the behavior of hydrogels for various biomedical applications.

Let us see some of the applications of hydrogels. The first application is tissue engineering. These hydrogels can act as scaffolds for cell growth and tissue regeneration. We can also use them for contact lenses, as their hydrophilic nature ensures comfort and hydration. And we can also use them for wound dressings.

It provides a moist environment and promotes healing while protecting the wound. We can also use it for drug delivery applications. As I mentioned earlier, we can use these hydrogels for controlled and sustained release. We can also use it for smart drug delivery. And we can also make an injectable hydrogel, which is one of the minimally invasive technique for drug release.

They are also useful for regenerative medicine applications. These hydrogels are responsive to external stimuli, so we can use them for biosensor applications. Let us see some of the challenges in the hydrogel synthesis and how to overcome those challenges. The first one is stretchability. If you have a brittle gel, reduce the cross-link density or polymer concentration.

If the hydrogel is weak under compression, increase the polymer concentration or add reinforcing fillers. If the drug release is too fast or too slow, optimize the pore size and cross-linking density. If the morphology has a poor surface structure, adjust the synthesis conditions, such as pH or temperature. If there is uneven pore distribution, you can optimize the polymer blending. If there is rapid degradation, increase the cross-linking density or add protective additives.

I hope you got an overall idea about the hydrogel. Let us learn about nanogels. Nanogels are nano-sized hydrogels. So whatever I explained about the hydrogel, those things can be applicable to nanogels as well. These nanogels are smaller in size, between 20 to 200 nanometers, and due to their high surface-to-volume ratio,

they are excellent carriers for targeted drug delivery applications and biomedical imaging applications. Let us see what various methods are available for nanogel preparation. The first one is emulsion polymerization. Here, the monomers are polymerized in a dispersed phase stabilized by surfactants. They are homogenized to produce uniformly sized nanogels.

The next method is ionic gelation. Here, we will be using cross-linking of charged polymers. An example is alginate nanogels. This is one of the simplest and most efficient methods for biopolymer-based nanogels. The third method is desolvation or nanoprecipitation.

Here, the polymer precipitates out as nanogel upon the addition of a non-solvent, which is the organic phase. The fourth one is reverse micelle polymerization. Here, the polymerization occurs inside surfactant micelles, forming nanogels and it requires the surfactant as well as the cross-linkers for making this kind of nanogel. Let us see the difference between hydrogels and nanogels.

These nanogels are nano-sized hydrogels, as I mentioned earlier. The size ranges from 20 to 200 nanometers, and since the size is smaller, it will have a high surface-to-volume ratio, enhancing its interaction with biological systems and also, due to its small size, it can be very useful for targeted drug delivery and these nanogels and hydrogels both have wide applications in the biomedical field.

I hope you understood the basics of hydrogels and nanogels. Let us go to the lab and learn how to synthesize hydrogels and nanogels. In this demonstration, we are going to make a guar gum hydrogel cross-linked with borax. For this, we require the following materials: guar gum powder, sodium tetraborate, a beaker with a magnetic bead, and a measuring cylinder.

ultra pure water, tissue paper, spatula, microtips, micropipette and a magnetic stirrer. We are going to make guar gum solution. So first weigh approximately 500 mg of guar gum powder then measure 50 mL of ultra pure water and add it to a beaker. Keep the beaker on stirring at 400 rpm and 37 degree Celsius.

Add the guar gum powder slowly to the beaker and wait for one hour to get a homogeneous solution of guar gum. You can see that a homogeneous solution of guar gum has been prepared. Now we will crosslink it with sodium tetraborate. For this purpose, we have already made a 5 percent weight by volume solution of borax. Add 250 microliters of this 5 percent borax solution drop wise to the guar gum solution.

You can see that instantly after addition of borax, the gelatin process has started. The guar gum solution will continue to gelate for the next 1 hour. A solid gel mass has been formed. We will leave it for 4 hours in a Petri dish at room temperature to allow the gel to set properly. After the incubation, the guar gum solution has completely set into a solid gel mass.

The gel is also able to hold its own weight. This guar gum hydrogel is a self-healing gel. So if we cut the gel mass into two parts and then allow the two parts to join together, the two parts will join together and again form a single hydrogel mass. You can observe that the two parts have joined together and it is also able to hold its weight.

So in this lab session, we have made guar gum hydrogel cross linked with borax. The scanning electron microscopic image of guar gum hydrogel reveals a well-defined porous structure. The porous nature of a hydrogel is important because it facilitates the efficient movement of fluids and molecules within the gel. This makes it ideal for applications such as drug delivery, tissue engineering and wound dressings where the ability to absorb and release substances as well as provide space for cell growth and migration is crucial.

The size and interconnectedness of force can be precisely controlled to optimize these functions depending on the specific application. In this experiment, we will learn how to synthesize a nano gel using sodium alginate and PAMAM dendrimer. The materials required for this experiment are sodium alginate, EDC, A vial with a magnetic bead, ultrapure water, 25 milligram dissolved in 2 mL PAMAM dendrimer solution,

10 percent calcium chloride solution, micropipettes, microtips, and a magnetic stirrer. First, we have weighed and kept 50 milligrams of sodium alginate, which will be dissolved in 2 mL of ultrapure water. Begin by adding 1 mL of ultrapure water to the vial. After that, add the weighed powder of sodium alginate, followed by another 1 mL of ultrapure water.

Now, set the temperature to 37 degrees Celsius and the RPM to 700. Let it stir for at least 1 hour to ensure that the sodium alginate completely dissolves. For safety, we are placing

cylindrical cardboard around the setup due to the high RPM. After one hour, we can see that the sodium alginate has dissolved completely. Now, we will switch off the RPM and add 20 mg of weighed EDC to the solution.

Once the EDC is added, switch on the RPM and keep it running for 1 hour to activate the carboxyl groups of the sodium alginate. After 1 hour, we will slowly reduce the RPM to 250. Once the RPM is reduced, add 2 mL of PAMAM dendrimer to the EDC-activated sodium alginate solution drop by drop. You will notice that the viscosity of the solution gradually increases as

The PAMAM dendrimer cross-links with the alginate chains. If we need drug-encapsulated gels, we should add the drug before this step. Now we have added 2 mL of PAMAM dendrimer. Close the lid and reduce the RPM to 100. Leave it for 1 hour as incubation time.

After 1 hour, we observe an increase in the viscosity of the solution. Next, carefully add 1 mL of 10% calcium chloride solution to the center of the vial. This is a crucial step, so it must be done with great care. Once the calcium chloride solution is added, switch off the RPM. We can now clearly see that gel formation has occurred.

Leave the solution for overnight incubation. After overnight incubation, The gel has formed, as we can clearly see in the vials. Using a spatula, we will take the gel and put it in this petri plate. We can wash the gel with ultrapure water and use it for further applications.

So in this experiment, we have learned how to synthesize nanogel. So this is the synthesized nanogel. So this can be characterized using FTIR. So this peak in sodium alginate corresponds to OH stretching vibration, and these two peaks correspond to carboxyl stretching vibration, asymmetric and symmetric

of the carboxyl salt groups, and this one corresponds to COC stretching of the saccharide structure of alginate. So, the next one is alginate gel with calcium chloride crosslinking; the peak at 1630 nm was intensified, and the symmetric carbonyl peak shifted to a higher wavenumber, that is 1430 nm. So, this 1300 nm and 800 nm represent the CO stretching and bending, and this one is the alginate gel formed with the help of PAMAM dendrimer.

After chemical crosslinking with EDC and PAMAM dendrimer, the carbonyl peak at 1630 nm redshifted to 1640 nm. Also, you can notice the amide linkage bond formation

at 1580 nm and internal CH2 groups of PAMAM can be observed at 1450 nm and 1410 nm. Here, you can observe the CN vibration as the amide group is introduced at 1450 nm.

AG-G5 structure, and this 720 nm represents the CH bending vibration. The formation of crosslinked AG-G5 nanogels was confirmed by this FTIR analysis. The mechanical strength of the nanogel or hydrogel can be measured using UTM. Elongation at break has an inverse relationship with tensile strength. The elongation at break value for AG-G5 was far less than that of AG nanogels.

Therefore, the chemical cross-linking of alginate with PAMAM dendrimer can significantly alter the mechanical strength of AG-G5 nanogels. Although nanogels and hydrogels appear similar when lyophilized, the dried nanogels can be dispersed in PBS to achieve the desired solution concentration. Then, the solution can be analyzed through electron microscopy to assess the

size, morphology, and structure of the nanogels. In summary, in today's lecture, we have learned about the basic overview of hydrogels and nanogels. We also learned about the properties of hydrogels and the various factors involved in hydrogel fabrication. We also learned how to characterize hydrogels. Through practical demonstration, we also learned how to synthesize hydrogels and nanogels.

Thank you for your kind attention. I will see you in another interesting lecture.