Experimental Nanobiotechnology

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Lecture 04: Synthesis Of Carbon Dots

Hello everyone, today we are going to learn synthesis of carbon dots. In today's lecture, we are going to learn what is carbon dots, what are the various applications of carbon dots and how to synthesize the carbon dots using hydrothermal and microwave assisted method. And we are also going to learn what are the various parameters involved in the hydrothermal and microwave assisted synthesis method.

Finally, we are also going to learn the synthesis of carbon dots using hydrothermal and microwave by practical demonstration. Let us see what is carbon dots. Carbon quantum dots, also known as carbon nanodots or simply carbon dots or CQDs, C Dots or CDs. These carbon dots are fluorescent nanomaterials that have emerged recently providing an alternate to the conventional toxic metal-based quantum dots.

These carbon dots are biocompatible and eco-friendly in nature and these carbon dots are small in size in the range of 2 to 15 nanometer mainly composed of element carbon and these carbon dots exhibit Unique optical properties such as efficient fluorescence, high photostability, broad excitation spectra and size dependent emission wavelength. These carbon dots come under the 0D materials.

That means all the dimensions like X, Y, Z, everything will be in the range of less than 100 nanometers. Let us see the difference between quantum dots and carbon dots. Mostly, the quantum dots are made up of heavy metal core which has the toxicity. Whereas, carbon dots are mainly made up of carbon sources; hence, they are biocompatible and non-toxic. Also, the synthesis of carbon dots is very simple, and it is easy to add functional groups.

Additionally, these are highly water-soluble when compared to quantum dots, which have poor solubility in nature. Let us see the unique properties of carbon dots. These carbon dots are inexpensive and biodegradable. These carbon dots can be made from any kind of

carbon source, and since they are made up of carbon, they are biocompatible as well as biodegradable. These carbon dots have high photoluminescence, are highly photostable, and are easy to functionalize.

Since these are made up of carbon, they are biocompatible as well as low in toxicity, and their fluorescent properties are also tunable. Let us see the various applications of carbon dots. Carbon dots have a wide range of applications. Out of these, I am going to briefly explain some of the important applications. Carbon dots can be useful for bioimaging due to their excellent fluorescent properties.

We can use it for bioimaging, and we can also use it for biomedicine. For example, if you want to use photothermal therapy, we can use these carbon dots, or we can conjugate the carbon dots with the drugs. And we can easily monitor the drug delivery. For example, these carbon dots have fluorescent properties.

When they are attached to the target cells, the cells can glow with fluorescence. We can monitor the fluorescence and understand the drug delivery. These carbon dots can also be useful for anticancer therapy. For example, we can conjugate the anticancer drug to the carbon dots. We can also tag antibodies specifically for the cancer cells.

Once they reach the cancer cells, which have the particular receptor ligand, they can bind only to the cancer cells, and the cancer cells will glow. At the same time, they will kill the cancer cells. The next application is that we can also use these carbon dots for sensing. For example, when you add the fluorescent carbon dots to hemoglobin, the fluorescence will be quenched.

That means the fluorescence is off. And when we add the cholesterol, it will efficiently bind to the hemoglobin. Then, the carbon dots will be released, so the fluorescence will turn on. By using this fluorescence on-and-off mechanism, we can detect the analyte. With this method, we can identify specific analytes in a given sample.

We can also use this carbon dots for photocatalysis and other various applications. Let us see how to synthesize carbon dots. Carbon dots can be synthesized via top-down or bottom-up approaches. Under the top-down approach, we have laser ablation. We can use the laser and vaporize the graphite target to obtain carbon dots.

Under the arc discharge, we can use the electric arc on the graphite. We can get the carbon dots. We can also use the electrochemical method or ultrasonic method to make

the carbon dots. In today's lecture, we will learn the hydrothermal method and the microwave pyrolysis method for carbon dot synthesis.

So let us see how to prepare the carbon dots using this hydrothermal and microwave assisted method. The first one is hydrothermal. The hydrothermal method will be synthesizing the carbon dots using high temperature and high pressure water. Or you can use other solvents to break down the carbon rich precursor. If you are using the water, then it is called as hydrothermal method.

If you are using the other solvent, then it is called as solvothermal method. This process will create a nano-sized carbon dots with very good fluorescence. And it is a simple and eco-friendly technique. And also it is a scalable method. It is mainly useful for making the carbon dots with the controlled size and functional properties.

So let us see why we have to use the hydrothermal synthesis for making carbon dots. The advantages are control over the particle size and morphology. The particle size can be controlled and uniform size carbon dots can be obtained by this method. Mild reaction condition. We can use this lower temperature and reduce energy consumption.

We can also obtain high purity and crystallinity. The other advantages are versatility. We can make complex products like core-shell types. We can make core and shell carbon dots by using this method. We can also have hierarchical assemblies.

And we can use a wide range of materials, including metal oxides and sulfides. So this is a low-cost and economical method; hence, the hydrothermal synthesis method is widely used for synthesizing carbon dots. Let us see what the various parameters involved in this hydrothermal synthesis are. The first one is the precursor type and concentration. If you use a high concentration of precursor, you get a high yield of carbon dots, and also the solvent, pH, stabilizer,

reducing agents, reaction temperature, and reaction time, which we will learn one by one in detail. So the first parameter is precursor type. The precursor type, based on the precursor we are using, will affect the size, surface properties, and optical characteristics of the carbon dots. Different precursors lead to varied carbonization rates.

if you are using this nitrogen or sulfur doping that leads to different fluorescent properties. So when we are having the different carbon sources, the carbonization rates will be varied. And also if you are using this nitrogen or sulfur doping, that leads to different optical and electronic properties of the carbon dots. Certain precursor types

provide a shift in the absorbance maximum of the carbon dots. That means there is a difference in the synthesized carbon dots.

Due to difference in the size, the absorbance maximum of carbon dots will also vary. By varying the precursors, parameters such as temperature and solvent will be varied, further which will affect the size and optical properties of the carbon dots. For example, if you are changing the precursor or if you are using a mixture of precursors, that will lead to change in the temperature and solvent and

that will affect the final size of the carbon dots as well as the optical properties of the carbon dots. The next parameter is reaction temperature. Higher temperature results in smaller and more crystalline carbon dots with strong fluorescence and when you are using this higher temperature which will favor the carbonization that means a process that converts the organic precursor into carbon rich structure.

And when we use high temperature, the carbonization process will be faster. But the problem is when you use high temperature, it leads to low yield. It may be due to the degradation of organic compounds or volatilization. The next parameter is pH. Acidic pH produces small and well-dispersed carbon dots.

So in most carbon dot syntheses, acidic pH is preferred for synthesizing carbon dots. The surface charge of carbon dots refers to the electrical charge present on their surface. And this charge is pH-dependent. That means the surface charge of carbon dots will vary with pH. The isoelectric point is the pH at which the net charge of a carbon dot is zero.

So when the net charge is zero, the carbon dots tend to agglomerate due to reduced electrostatic repulsion. If there is a uniform charge, whether negative or positive, the carbon dots will repel each other. And if there is no charge, the carbon dots tend to come closer and agglomerate. The next important parameter is reaction time. Using a longer reaction time leads to complete carbonization.

and we get the uniform carbon dots. So we can keep it for more amount of time. So it will have the complete carbonization. All the precursor will be converted into carbon dots. And we will get a uniform small size carbon dots.

As the reaction progresses, the organic precursors have more time to decompose and reassemble into carbon structure. That is why when we are keeping it for longer reaction time, we will get the uniform size carbon dots. Longer reaction time may also lead to increased fluorescent intensity due to the formation of more conjugated carbon structures.

However, sometimes the excessive reaction times may result in over carbonization and which leads to agglomeration of the carbon dots. For example, if you are keeping it for longer reaction time, even though it produces more amount of carbon dots, but however in some cases it may lead to agglomeration of your carbon dots which will reduce the yield and purity of your carbon dots.

So, the next parameter is precursor concentration. Higher concentration often provide better yield, but there is a chance of agglomeration. It will also affect the final size of the carbon dots. Higher concentration of precursor ensures that reacting species achieve the required stoichiometry for optimal carbon dots to be formed.

But sometimes it leads to the agglomeration of the carbon dots. And whenever we are synthesizing the carbon dots, uniform size carbon dots, in that cases, this will create a problem. And next parameter is solvent. The solvent which affects the solubility of the precursor and also it will influence the reaction environment. Solvents are mainly useful for solubilizing the precursor to ensure the formation of carbon dots.

Due to this they also dictate the reaction environment thus affecting the size and optical properties of the carbon dots. It means we have to select the right solvent to dissolve our precursor. Based on the solvent, the final product size as well as whether it is agglomerating or it is dispersed, everything will be decided based on the solvent. If you are using the water, then it is called as hydrothermal as I told earlier.

If you are using some other solvent, then it is called solvothermal synthesis. The next important parameter is stabilizers. Stabilizers like polyethylene glycol (PEG) or PVP, will improve the dispersion of carbon dots and prevent agglomeration. When carbon dots agglomeration occurs, what happens?

It will increase the size of the carbon dots. To synthesize monodisperse, uniform-sized carbon dots, we can use surfactants as stabilizing agents. These stabilizing agents can also functionalize the carbon dots for various applications. For example, in this case, we used chitosan and functionalized it with PEI (polyethyleneimine).

When you use PEI, it provides a positive charge to the carbon dots. When you use PEG (polyethylene glycol), it provides a negative charge to the carbon dots. Most of you know that cells are negatively charged. When you have positively charged carbon dots, they can easily bind to cells, which is useful for bioimaging applications.

The next parameter is reducing agent. And this reducing agent is not used in all the cases. It is used only in some of the cases for synthesizing the carbon dots. It will enhance the carbonization and also it will enhance the nucleation. Nucleation means the formation of first nanocrystal in the solution.

that will increase the carbon dots production and these reducing agents donate electrons to the precursor molecules promoting the formation of carbon rich structures. In the case of oxygen containing precursor these reducing agents can help to remove the oxygen atoms and which lead to more carbon rich product. So in some of the cases we have to use the reducing agent

for synthesizing the carbon dots and this reducing agent plays an important role in enhancing the carbonization as well as the nucleation and it will improve the yield of the carbon dots. So let us see the summary of the parameters for hydrothermal synthesis method. The first one is precursor type which controls the size, shape and optical properties and reaction temperature, high temperature is preferred. We can get the smaller and crystalline carbon dots.

However, when you are using the high temperature, there is a chance there may be a degradation of the precursor which leads to low yield also. And reaction temperature. Which impacts the size distribution of the carbon dots. We can keep more amount of reaction time. So that we can get the uniform and small size carbon dots.

And pH. As I told earlier. Acidic pH is preferred. We can get the small well dispersed carbon dots. And the precursor concentration.

We can get the better yield. If we are having the more amount of precursor. But the problem is agglomeration. And solvent type. So solubility of precursor.

It depends on the solvent we are using. We have to select the right solvent to dissolve the precursor and disperse the carbon dots. The next one is stabilizers. Stabilizers help in preventing agglomeration and also provide uniform carbon dots. They can also act as a kind of functional group to functionalize the carbon dots.

The last one is the reducing agent. Reducing agents are used only in some cases. They enhance carbonization, nucleation, and improve the yield of carbon dot production. Now, let us see the next method for synthesizing carbon dots: microwave-assisted synthesis. Here, we create quasi-spherical carbon nanomaterials of less than 10 nanometers in size.

The process involves irradiating a precursor solution in a microwave to create carbon dots. What is the advantage of this microwave-assisted synthesis? Rapid synthesis—we can synthesize carbon dots very quickly and enhance the reaction rate by using this microwave. This is an energy-efficient technique.

Direct energy transfer is happening and it will reduce the energy cost. We can also get the improved product quality. It can enhance the crystallinity. It will reduce the impurities. Also, this can be a green method.

We can use the mild reaction conditions, we can reduce the solvent use stage and we can also control the particle size and morphology that is the advantage of this microwave assisted synthesis similar to the hydrothermal synthesis method. Scalability also possible, scalable synthesis and consistent production quality is also possible

in the microwave assisted synthesis method similar to the hydrothermal method. Let us see what are the effective parameters involved in the microwave assisted synthesis method. The parameters are almost similar to the hydrothermal synthesis method except this microwave power and irradiation time. Let me briefly explain.

So let us see what the role of microwave power and irradiation time is. Higher microwave power and longer irradiation times can accelerate the carbonization process, leading to smaller particle size and enhanced fluorescence. But Excess energy can lead to over-carbonization, resulting in the loss of fluorescence and the formation of amorphous carbon.

That means when you use higher microwave power, you can produce carbon dots rapidly but at the same time, due to over-carbonization, you may lose all the carbon precursors and finally, instead of carbon dots, you may get amorphous carbon. What is the mechanism behind this microwave synthesis method? The mechanism behind microwave synthesis is that microwave heating involves the

interaction of microwave radiation with polar molecules in the reaction mixture. These polar molecules absorb the microwave energy and undergo rapid rotational and vibrational motions, which lead to localized heating. This localized heating creates hot spots within the reaction mixture, which can accelerate the carbonization process. Let us see an overview of how to synthesize carbon dots using hydrothermal and microwave-assisted synthesis methods.

The first step is to prepare the solution. For that, we need a precursor and a solvent. You have to select the right precursor and the right solvent. Once the solution is ready, transfer it to the Teflon-lined hydrothermal autoclave chamber. Properly seal the chamber.

Then, keep it in the muffle furnace. You have to keep it at 200 degrees Celsius for 12 hours. That is the hydrothermal synthesis method. In the case of the microwave-assisted method, we have to keep the solution in a conical flask and place it in the microwave. Keep it on for 20 seconds and then pause for 10 seconds until there is a color change.

Repeat the same process. 20 seconds on and 10 seconds off, and repeat this process until you see some color change. The complete solution will turn a dark brown color. That means complete carbonization has occurred. So, in the previous slide, we learned about the preprocessing during synthesis.

And here we are going to learn the post-processing that is after the synthesis of carbon dots. So once the carbon dots are synthesized by hydrothermal and microwave methods, collect the solution from the muffle furnace and microwave, allow it to cool down to room temperature, then perform centrifugation. Once you perform centrifugation two or more times,

you can remove all the precipitate and impurities and collect the carbon dots in the supernatant. These carbon dots can be further purified using a syringe filter. Use the syringe filter to filter and purify the carbon dots. The finally obtained carbon dots can be stored in an amber flask for further applications

or you can use them directly or store them in powder form by lyophilization. When you perform freeze-drying, the carbon dots can be made into powder form and this powder can be stored for later use. When you want to use them later, you can mix the carbon dots with deionized water and use them for bioimaging and other applications.

In this case, you can see here, the first one is the carbon dots synthesized using the hydrothermal method. And the second one is the control. So here, we are not using carbon dots; we are using only water, deionized water. You do not see any fluorescence in the second one. And the third one is

the carbon dots which we made using the microwave method and these carbon dots can be diluted or used as such for viewing under the UV transilluminator. So when you see under the UV transilluminator, the carbon dots which we made using the hydrothermal

method and the microwave-assisted method are giving fluorescence, whereas the water shows no fluorescence. I hope you understood the theory and the basis of how to synthesize carbon dots using hydrothermal and microwave-assisted methods.

Now, let us go to the lab and see the practical demonstration of how to synthesize carbon dots using hydrothermal as well as microwave-assisted methods. In this experiment, we will learn how to synthesize carbon dots from casein using the hydrothermal method. A simple and cost-effective approach to synthesize carbon dots. To begin, let us see the materials required for the synthesis.

We will be using casein as our carbon precursor, ultra-pure water, conical flask with magnetic bead, measuring cylinder and a Teflon-lined hydrothermal autoclave setup for the reaction. Additionally, we need a syringe and 0.22 micrometer membrane syringe filter to ensure the purity of our final product. Now we are going to weigh 1 gram of casein powder. To prepare the reaction mixture, we are going to measure 50 mL of water.

We have to add 25 mL water to the conical flask. and place it on the magnetic stirrer. Then add the powder slowly into the flask and stir vigorously. After complete dissolution, take out the magnetic bead from the conical flask and carefully transfer the solution into a Teflon lined hydrothermal autoclave.

Add the remaining water to the conical flask, gently mix it and pour the remaining solution also in the Teflon lined hydrothermal autoclave. Make sure that the Teflon lined hydrothermal autoclave is not filled beyond its maximum capacity. so that there will be enough space for the pressure buildup during heating. Now we are going to place the Teflon lined hydrothermal autoclave inside the stainless steel container

Once it is done, it is ready for the hydrothermal process. Place the autoclave inside the muffle furnace. Set the furnace temperature to 200 degrees Celsius and maintain it for 12 hours. This heat will promote the carbonization of casein. Leading to the formation of carbon dots.

Once the hydrothermal process is completed, turn off the furnace, take out the autoclave and allow it to cool naturally to room temperature. It is very important not to cool the autoclave rapidly, as sudden changes in temperature can cause dangerous pressure buildup. We are going to carefully open it.

Transfer the resulting solution into centrifuge tubes. Then centrifuge the solution for 15 minutes. This helps to separate any precipitate from the supernatant, which contains the

carbon dots. After centrifugation, carefully decant the supernatant into a clean container. To remove any remaining impurities, filter the supernatant through a 0.22-micrometer membrane syringe filter.

These steps ensure that the final solution of carbon dots is pure and free from contaminants. Now, we have transferred the solution into a centrifuge tube. And for the blank, we have added ultra-pure water to another centrifuge tube. We are going to view this under normal light and UV light. Here, you can observe that normal light does not have any effect,

whereas under UV light, the synthesized carbon dots show fluorescence. In the previous experiment, we learned how to synthesize carbon dots using the hydrothermal method. Now, we are going to learn how to synthesize passivated carbon dots using a microwave-assisted method. For performing the synthesis, we require chitosan as the carbon precursor, PEG 4000, which is polyethylene glycol, as the passivating agent, concentrated sulfuric acid,

ultra-pure water, a measuring cylinder, a conical flask with a magnetic bead, a glass pipette, and a micropipette. Now, we are going to weigh 0.2 grams of chitosan powder and 0.2 grams of PEG 4000 powder. To prepare the reaction mixture, we have to add 25 mL of water into a conical flask. Then, carefully add 4 mL of concentrated sulfuric acid to the water.

The sulfuric acid plays a critical role in breaking down the chitosan and facilitating the carbonization process. Stir the mixture thoroughly to ensure the acid is well dissolved in the water. For the surface-passivated carbon dots, first add 0.2 grams of chitosan into the solution. Stir continuously at 500 rpm. Until the chitosan is completely dissolved.

This will create a homogeneous solution, which is essential for the carbon dot formation. Now, once the chitosan is completely dissolved, add 0.2 grams of PEG 4000 to the mixture. PEG 4000 serves as a passivating agent, enhancing the dispersion and stability of the carbon dots, especially in aqueous solution. Stir the solution for another 15 minutes to ensure that the PEG-4000 is completely dissolved,

allowing for proper functionalization of the carbon dots. With the solution prepared, proceed with the microwave irradiation. Place the flask in a microwave oven and switch it on. Heat the solution for a few minutes, pausing in between, until the desired

carbonization is achieved. The microwave helps accelerate the reaction and leads to the formation of carbon dots.

Once the microwave irradiation treatment is complete, allow the solution to cool naturally to room temperature. After the solution has cooled, carefully transfer it to centrifuge tubes. Then, centrifuge the solution for 15 minutes. This helps to separate any precipitate from the supernatant, which contains the carbon dots.

After centrifugation, carefully decant the supernatant into a clean container. To remove any remaining impurities, filter the supernatant through a 0.22 micrometer membrane syringe filter. This step ensures that the final solution of carbon dots is pure and free from contaminants. Now, we have transferred the solution into a centrifuge tube, and for the blank, we have added

ultrapure water in another centrifuge tube. Then, similar to the previous experiment, we are going to view this under normal light and UV light. Here, you can observe that normal light does not have any effect, whereas under UV light, the synthesized carbon dots show fluorescence. We have successfully synthesized carbon dots using both the hydrothermal and microwave-assisted methods.

As a summary, in this lecture, we learned about carbon dots and their unique properties. We also learned about various applications of these carbon dots and how to synthesize these carbon dots using hydrothermal and microwave-assisted methods. We also discussed the various essential parameters involved in the synthesis of these carbon dots using hydrothermal and microwave-assisted methods.

Through practical demonstration, we also learned how to synthesize carbon dots using hydrothermal and microwave-assisted methods. Thank you all for your kind attention. I will see you in another interesting lecture.