Experimental Nanobiotechnology

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Lecture 06: UV-Visible and Fluorescence Spectroscopy

Hello everyone, today we are going to learn UV visible and fluorescence spectroscopy. These two techniques are very important techniques in nanomaterial characterization. In today's lecture, we will be learning about what is spectroscopy and we will also learn what is the working principle of UV visible and fluorescent spectroscopy both theoretically as well as practically through demonstration. Let us see what is spectroscopy. Spectroscopy is the study of the interaction between electromagnetic radiation and matter.

It involves analyzing how a substance observes, emits, or scatters light to provide information about the composition, structure and physical properties of substances. There are various types of spectroscopic techniques like observation spectroscopy, emission spectroscopy and scattering spectroscopy. In today's lecture, we are going to learn about UV visible spectroscopy and fluorescent spectroscopy in detail. Let us briefly learn the history of spectroscopy.

In 19th century, Dr. William and Dr. Joseph made a groundbreaking discovery of absorption spectra. In 1941, the first commercial UV visible spectrophotometer was introduced. Currently, the spectroscopic techniques are a standard analytical tools for real-time and high-precision measurements. Let us see what is UV visible spectroscopy. UV visible spectroscopy is a versatile analytical technique which is used to measure the absorption of light by a sample in the ultraviolet and visible range of the electromagnetic spectrum.

So that is why it is called UV-visible spectroscopy. It can measure the absorption of light by a sample in both the ultraviolet and visible ranges of the electromagnetic spectrum. Some spectrophotometers have an extended wavelength range and can measure even in the near-infrared range. That is from 800 to 3200 nm. The measurement is based on the

absorption of light due to the excitation of electrons in molecules from lower to higher energy states.

Let us see the principle of this technique. The principle is that when light passes through a sample, some of the energy is absorbed by the molecules in the sample. The electrons in the molecule get excited and move from a lower energy level (the ground state) to a higher energy level (the excited state) by absorbing energy of a particular wavelength. Let us learn about transmittance and absorbance.

Transmittance is defined as the ratio of the transmitted intensity to the incident intensity. It has a value between 0 and 1.

$$T = \frac{I}{I_0}$$

where: I = Intensity of transmitted light
I_0= Intensity of incident light

Absorbance is the measure of the amount of light absorbed by the sample at a specific wavelength.

$$A = \log 10 \frac{I_0}{I} \quad \text{or} \quad A = -\log(T)$$

Transmittance and absorbance are inversely related and have a logarithmic relationship. For example, if you have an absorbance value of 0, that means 100% transmittance. If the absorbance value is 1, that is 10% transmittance.

Absorbance value = 0 → 100% transmittance
Absorbance value = 1 → 10% transmittance

Let us learn about Beer-Lambert law. The Beer-Lambert law is a linear relationship between the absorbance, concentration of the analyte, molar extension coefficient and optical path length of a solution

$$A = \varepsilon cl$$

Where,
A = Absorbance (unitless)
ε = Molar absorptivity or molar extinction coefficient (L $mol^{-1}cm^{-1}$)
c = Concentration of the analyte (mol L^{-1})
l = path length of light through the sample (cm), usually the width of the cuvette (typically 1 cm).

if you are using a sample with higher concentration that leads to greater absorption which reduces the transmittance and if you are using the longer path length that increases the interaction with the light leading to more absorption longer path length refers to the distance that light travels through the sample in the keyword or cell this path length is typically measured in centimeters

And the molar absorptivity is the intrinsic property of a particular chemical species. And molar absorptivity is a measure of how strongly a substance absorbes light at a particular wavelength. Let us learn about the parts of spectrophotometer. The first one is light source which provides the radiation. And we have two light source.

One is deuterium lamp which is for UV range. And we have tungsten lamp which is for the visible range. I will explain about this light source in my next slide and we have monochromator which separates the light into its component wavelength and selects the desired wavelength and here we have a sample holder which holds the sample we can use the quartz cuvette if you want to measure the your sample in UV range

And you can use the glass cuvette for the visible range, and in some cases, we can also use microplates for high-throughput analysis. For example, if you want to have multiple samples, like a 96-well plate, and want to analyze all the samples simultaneously, for that, we have to go for a microplate holder. Then we have the detector, which measures the intensity of the transmitted light after passing through the sample. Then we have the processor unit, which converts the signal from the detector into absorbance or transmittance values. Then we have the display unit, which displays the data as spectra, tables, or graphs.

And in this picture, what we are seeing is a double-beam UV-visible spectrophotometer. So, the advantage of using this double-beam UV-visible spectrophotometer is that we can measure the absorbance of the sample as well as the reference simultaneously. And most labs use this double-beam UV-visible spectrophotometer. Let us see the details of the light source. The first one is the deuterium lamp.

So, the deuterium lamp is mainly useful for measuring the absorbance of the sample in the UV range, which is 200 to 400 nanometers. The deuterium lamp uses an arc discharge

from deuterium gas. So, one drawback is that it has a short half-life. So, we have to frequently replace the deuterium lamp. The next one is the tungsten halogen lamp.

It is mainly useful to measure the absorbance of a sample in visible range that is from 400 to 800 nanometer and the filament emits light when heated. So, that is why before we perform the experiments we have to switch on the equipment and allow some time so that the lamp get warm up and it has a very low noise and low drift and it has a longer life. The third one is mainly in the near IR region that is the xenon lamp. So, it can measure your sample from UV to near IR region that is from 185 to 2500 nanometer. It emits short flashes of high energy light and it is mainly useful for photosensitive materials.

And here there is no need for warm up time of the lamp. Let us learn about the cuvette. The cuvette for UV visible spectroscopy is done based on the factors like material transparency, path length, volume and chemical inertness. The quartz cuvette is mainly used for measuring the absorbance of sample in the UV range. The reason is the quartz cuvette is transparent to UV radiations and the glass cuvette is mainly used to measure the samples absorbance in the visible range because the glass absorbes the UV radiation.

So, that is why we have to use the glass cuvette for measuring the absorbance in the visible range and we have to use the quartz cuvette for measuring the absorbance in the UV range. And these cuvette are usually frosted on two opposite sides to increase the accuracy of absorption measurements. You can see here, so two sides are opaque and two sides are transparent. The cuvette also supplied as matched pairs which have identical optical properties.

So this minimizes the variation between the sample and reference in a double-beam spectrophotometer. So the cuvettes are always available in pairs to avoid batch-to-batch variation as well as to minimize the variation between the sample and reference in a double-beam spectrophotometer. Let us see the difference between single-beam and double-beam spectrophotometers. In a single-beam spectrophotometer, it uses one light path, and the reference and sample are measured separately. Whereas in the case of a double-beam spectrophotometer, it splits the light into two paths for simultaneous measurement of the sample and reference.

And the measurement process requires manual switching between the sample and reference in a single-beam spectrophotometer. Whereas in the case of a double-beam spectrophotometer, it measures the sample and reference simultaneously. So that is the advantage of this double-beam spectrophotometer. And speed-wise, it is slower due to

separate measurements, and it is faster. As both paths are measured in real-time, and accuracy-wise, it is more prone to fluctuations in light source intensity.

Whereas in the case of a double-beam spectrophotometer, it compensates for light source fluctuations and provides better accuracy. So that is why most labs use these double-beam spectrophotometers. Let us learn about the importance of blank and baseline calibration. The blank calibration eliminates instrumental noise. And when we measure the absorption of the sample at a particular wavelength, the blank calibration ensures that the absorbance of the solvent is subtracted from the final reading.

And this way it improves the measurement accuracy. The next one is baseline calibration. When we do the spectral scan of the sample, the baseline calibration basically subtracts the absorption spectrum of the solvent used for making this sample solution to improve the quality of the obtained data. So it is similar to how we zero the weight of the container when weighing something.

For example, whenever we go to the fruit markets, so the vendor removes the weight of the basket before he puts the fruits. So that means we are getting the exact weight of the fruits. not with the basket similarly when you are using this baseline calibration or the blank calibration it will remove the absorbance of solvent and it will provide only the absorbance of your particular sample let us see some examples for understanding how we can use this UV-vis spectrophotometer for characterizing the metal nanoparticles

the first example is gold nanoparticles when you use the gold nanoparticle you get a single peak And when you are using these gold nanorods, you get two peaks. One is for transverse plasmon band and the other one is for longitudinal plasmon band. Let us see another example for understanding how we can use this UV-vis spectrophotometer for measuring the absorbance of silver nanoparticles. And in this figure you can see that, so this is the colloidal silver in various stages of aggregation.

you see the silver nanoparticle being yellow color and to get the proper silver nanoparticle we have to use the metal precursor and reducing agent that is sodium borohydride in the right ratio. If you are using the ratio of 2, you will get a highly stable silver nanoparticle. If the ratio is 2.1, the nanoparticle is stable for 30 minutes. If the ratio is 1.9, it is stable for 20 minutes. If the ratio is 1.8, the silver nanoparticle is stable only for 5 minutes.

You can see here with respect to the aggregation, the colour is also varies because as the particle size is bigger with respect to the aggregation. And when we measure the absorbance of silver nanoparticle using the UV-visible spectrophotometer, so you get the peak around 400 nanometer for silver nanoparticles. And based on the previous literature, we can get approximate idea about the size. For example, if you are getting a peak around 420 nanometer, the size is between the 35 to 50 nanometer. And the size of the silver nanoparticle can be confirmed by using the transmission electron microscope.

Let us see the applications of UV-visible spectroscopy, we can use it for characterization of nanoparticles as we learnt in the previous slides like we can use it for studying the metal nanoparticles, so as it exhibit SPR peaks in the UV-visible spectrum and we can use this spectroscopy for understanding whether the nanoparticle is in the dispersed state or in the aggregation state. And we can also use it for measuring the concentration. Nanoparticle concentration in a colloidal solution can be measured using the spectrophotometer.

For example, we can calculate the silver nanoparticle concentration using its extinction coefficient at 400 nanometers. And we can also use it for analyzing the size and shape. So, this UV-visible spectrum can provide insights into the nanoparticle size and shape. For example, if you have rod-shaped nanoparticles, as I told you, you get two peaks: one for the transverse and one for the longitudinal. And you can also use it for understanding the optical properties. So, the optical bandgap of semiconductors and quantum rods can also be determined.

So, we can measure the bandgap between the conduction band and the valence band. We can also use this in aggregation studies to monitor the changes in UV-visible absorption peaks to evaluate the nanoparticle stability. For example, if the nanoparticle is aggregated, there is a redshift or broadening of peaks. So, that indicates the aggregation of nanoparticles. Usually, for gold nanoparticles, you get a sharp peak.

If the nanoparticle is aggregated, you get a peak shift as well as a broadening of peaks. And we can also use it for drug release studies. It measures the drug release kinetics from nanocarriers like liposomes or polymeric nanoparticles. For example, if you are loading your nanoparticle with an anti-cancer drug or a drug like curcumin, we can measure the absorbance of the particular drug. In the case of curcumin, we can measure the absorbance at 425 nanometers.

So, when you are using this curcumin-encapsulated nanocarrier, With respect to time, we can understand the release of the drug by using this visible spectrophotometer. And we can also use it for understanding the functionalization, which means the additional functional group on the nanoparticle. So, it confirms the binding of biomolecules like proteins or DNA to the nanoparticle surface. For example, you have a positively charged nanoparticle and your DNA is negatively charged.

So, once the nanoparticle attaches to the DNA, we can see the changes in the SPR peak and confirm the binding of biomolecules to the nanoparticle surfaces. We can also use the visible spectrophotometer for studying the photocatalytic activity of nanomaterials. For example, here we can use these TiO2 nanoparticles for the photocatalytic degradation of methylene blue dye. So, that can be monitored by measuring the absorbance decrease at 665 nanometers. And when we are using dyes like methylene blue, we can use disposable cuvettes, or if you are using glass or plastic cuvettes, make sure they are cleaned properly.

Otherwise, that will interfere with the next sample. Let us see some of the common issues we face when using the reusable spectrophotometer for measuring the absorbance of samples and how to overcome those challenges. The first problem is sample preparation issues. So, the possible causes may be contamination or improper mixing. We can use uniform volumes or filter the samples to remove impurities or contamination and ensure proper mixing.

And if you are facing a problem like noise, it may be due to the instrument instability, the cuvette not being clean, or environmental interference. So, calibrate the instrument, clean the cuvettes, and ensure there is a vibration-free environment. And if you are facing a problem in the blank or zero calibration, the possible cause may be a contaminated blank, an unclean cuvette, or improper zeroing. So always use a fresh blank, clean the cuvettes as I mentioned earlier in the previous slides. So always zero the instrument.

And if you are facing a problem like too high concentration, when the sample concentration is too high, it exceeds the linear range and deviates from the Beer-Lambert law. So in this case, we have to dilute the sample or use shorter path-length cuvettes. I hope you got the overall idea about the UV-vis spectrophotometer. The next technique we are going to learn in today's lecture is fluorescence spectroscopy.

Fluorescence spectroscopy is an analytical technique used to measure the fluorescence emitted by a sample when it absorbs light. Fluorescence is a type of luminescence. It

occurs when a molecule, known as a fluorophore, absorbs light at one wavelength (the excitation wavelength) and then re-emits light at a longer wavelength (the emission wavelength). Fluorescence spectroscopy is widely used in chemical, biological, and environmental analysis. Let us see the principle of fluorescence.

So the fluorophore absorbs light, causing an electron to jump to a higher energy level, which is the excited state. The molecule loses energy through internal conversion or vibration and returns to a lower energy excited state. When returning to the ground state, the molecule emits a photon of lower energy. So that is fluorescence.

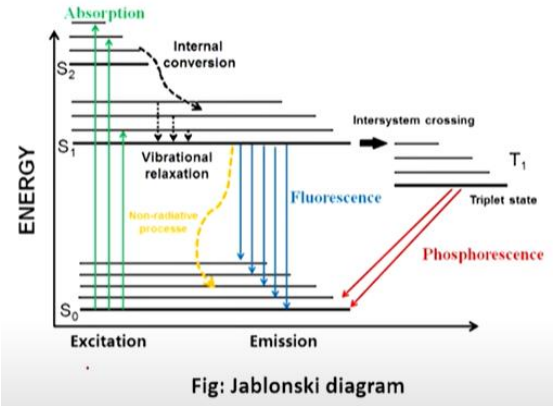
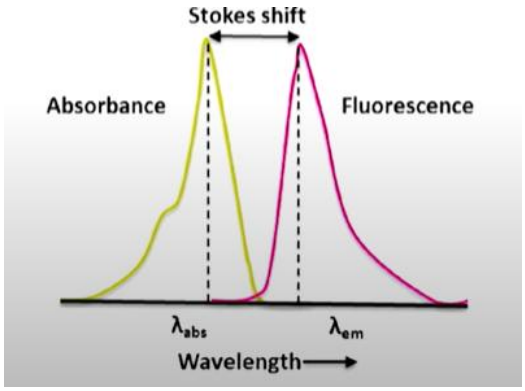
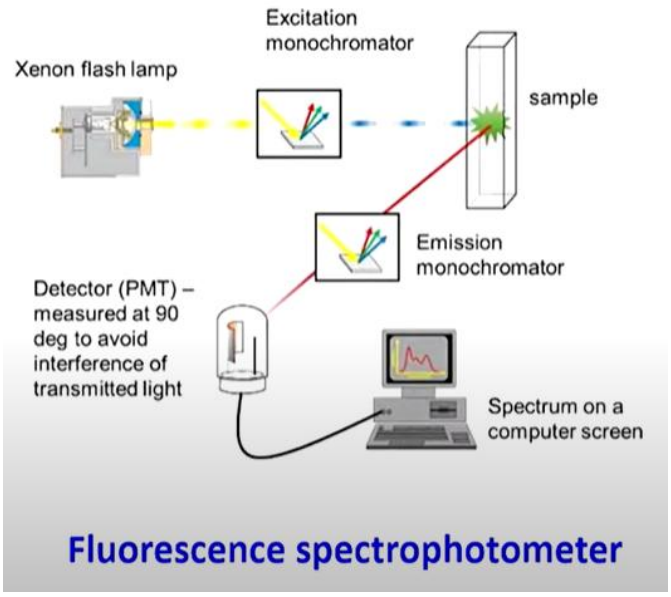


Fig: Jablonski diagram



Let us learn about the parts of a fluorometer.



In the fluorescence spectrophotometer, we have a light source, which is the xenon flash lamp. It provides the energy needed to excite the sample. Following that, we have the excitation monochromator. It selects the exact wavelength of light used to excite the sample. Then we have the sample holder.

Usually, we use a quartz cuvette, transparent on all four sides. We also have the emission monochromator, which selects the emission wavelength, similar to the excitation monochromator but for the emitted light. Then we have the PMT detector, which is the photomultiplier tube. It can amplify the light and helps detect even faint signals. This detector is kept at 90 degrees to avoid interference from the transmitted light.

So when you have this light source and your cuvette is here, and the detector is placed at a 90-degree angle to your light source. So, in this way, it can avoid the interference of the transmitted light. And we have the computer and software, which can collect and analyze the data to produce the fluorescence spectrum. Let us see the working of the instrument in detail. The light source emits light, which is then passed through an excitation monochromator.

Then, the excitation monochromator selects the desired wavelength. The excitation light then illuminates the sample, exciting the electrons in the fluorophores to a higher energy state, as I explained in the principle of fluorescence. So, the fluorescence light is emitted when the high-energy electrons return to their ground state. The emitted light is of a higher wavelength because of the vibrational energy loss suffered by the electrons. The emission passes through a monochromator, which filters out the emission wavelength.

The emitted light is detected by the detector, and the fluorescence spectrum is recorded, which shows the intensity of the emitted light at various wavelengths. Whenever we synthesize fluorescent nanomaterials, we have to calculate the quantum yield. The quantum yield is one of the important parameters in deciding the fluorescent nanomaterials for various applications. So, let us learn how we can calculate the quantum yield. So, the fluorescent quantum yield is the ratio of the number of photons emitted by a fluorophore to the number of photons absorbed.

$$\Phi = \frac{\text{Number of photons absorbed}}{\text{Number of photons emitted}}$$

It measures the efficiency of fluorescent emission, and it is a key factor in determining the brightness and sensitivity of fluorophores. In applications like fluorescent microscopy, diagnostics, and sensors. So, that is why whenever we synthesize carbon dots or quantum dots, we have to check whether they have high fluorescent quantum yield or not. So, if we have a high quantum yield, that makes the carbon dots bright and sensitive. So, this is one of the essential parameters for applications in the biomedical field, especially in cases of bioimaging and biosensing, where strong fluorescent signals are required to detect low concentrations of analytes.

The quantum yield can be calculated by comparing the fluorescent intensity of a sample to a reference material. So, usually, most labs use quinine sulfate, or we can also use fluorescein with a known quantum yield.

$$Q_S = Q_R \times \frac{I_S}{I_R} \times \frac{A_R}{A_S} \times \frac{\eta_S^2}{\eta_R^2}$$

Where:

- Φ_{sample} : Quantum yield of the sample
- Φ_{ref} : Known quantum yield of reference
- I_{sample} , I_{ref} : Integrated fluorescence intensities of the sample and reference.
- η_{sample} , η_{ref} : Refractive indices of the solvents for the sample and reference.
- A_{sample} , A_{ref} : Absorbance values of the sample and reference at the excitation wavelength.

So, the quantum yield of these two chemicals is already well known. So, we can use this as a kind of standard reference material, then we can calculate the quantum yield for our sample. The first step is to prepare solutions for both the sample and reference in the same solvent such that the absorbance values at the excitation wavelength for both solutions are less than 0.1.

This is done to eliminate the inner filter effect, where the sample absorbs the emitted light due to high concentration. The next step is to record the fluorescence spectrum at the same excitation wavelength for both and calculate the integrated area under the fluorescence spectrum. To calculate the quantum yield of the particular nanomaterials, we can use this equation. Let us see some of the nanotechnological applications of fluorescence spectrophotometry. The first one is that we can use this fluorescence spectrophotometer for the characterization of fluorescent nanomaterials to assess the optical properties and quantum yield of nanomaterials such as carbon dots or quantum dots, and we can also use it for biosensing and diagnostics.

If you are having a fluorescent nanomaterials, so it can be act as a kind of fluorescent nanosensor. So once it bind to the particular cancer cell and it can glow. So based on that we can use it as a kind of specific fluorescent nanosensor for detecting the specific diseases. And we can use it for study of energy transfer, so it can be useful for application in detecting molecular interaction and conformational changes. If there is any conformational change, so the fluorescent intensity will go down or it can go high.

So, based on the intensity, we can understand the conformational changes. We can also use it for nanostructure surface studies for studying the surface interaction between nanoparticles and biological molecules using fluorescence quenching or enhancement. For example, if you are having a fluorescent nanoparticle and once it binds to the the particular analyte. These nanoparticles can give fluorescence or the fluorescence can be quenched. So that means the fluorescence can be on or the fluorescence can be off.

So by this way we can understand the surface interaction between nanoparticles and biological molecules. We can also use it for studying the photocatalysis. For example, we can use it for evaluating the efficiency of photocatalysts like doped nanoparticles under light exposure. When you are using the light, we can see whether the photocatalyst efficiency is improved or not. And we can also use it for nanotoxicology for detecting the release of ROS, that is the reactive oxygen species, by using the fluorescent-based assays, such as DCFH-DA.

If ROS is more, then fluorescent intensity will be high. No ROS then no fluorescence. With respect to the fluorescence, we can quantify the ROS. Let us see some of the common issues which we face when we use the fluorescence spectrophotometer and how to overcome those challenges. The first problem is weak or no fluorescence.

So, it may be due to the low fluoropore concentration or it may be due to the incorrect excitation wavelength. So, we can overcome by increase the concentration or we can check the absorption spectrum and use the correct wavelength. And next one is high background signal. It may be due to the solvent fluorescent or impurities or it may be due to the improper blank or it may be due to the contaminated cuvette. So we can use the high purity solvents or you can subtract the blank spectrum from the sample spectrum or you can use the clean cuvettes.

And third one is the fluorescence quenching. So the fluorescence quenching may be due to the presence of quenches like oxygen or heavy metals. We can remove the oxygen by purging with nitrogen or using deoxygenated solvents. And it may be due to energy

transfer to nearby molecules. So we have to identify and remove the potential quenches from the solution.

And the fourth one is inner filter effects. It may be due to the high sample absorbance. So we always have to dilute the sample to maintain the absorbance. Or it may be due to poor calibration. Calibrate the instrument regularly using standard fluorophores.

And the next one is photobleaching. Photobleaching may be due to prolonged excitation light exposure. So you can use reduced light intensity, a pulsed light source, or switch to more stable fluorophores. The last one is temperature effects. This may be due to temperature fluctuations.

It can be overcome by maintaining constant temperature conditions throughout the analysis. Hope you got the overall idea about the fluorescence spectrophotometer. Let us go to the lab and learn this technique in more detail. Now, we are going to learn UV-visible spectroscopy. For this, we have already synthesized two materials.

One is gold nanoparticle synthesized using sodium citrate and another one is tannic acid cap gold nanoparticles. We will need a set of quartz cuvette, ultra pure water for preparing dilution, tips and pipette, tissue paper and beaker for discarding the waste. first turn on the machine and wait for the lamp to be warm up then set the machine on a spectral scan and keep the wavelength from 400 to 800 nanometer select a step size of 5 nanometer first we have to calibrate the baseline for that we have to use ultra pure water While adding any sample, hold the cuvette at an angle to avoid any bubble formation in the cuvette. Now place the cuvette in the machine.

Ensure that the clear sides of the cuvette in line with the light source and detector. Now we have to select the blank calibration option. You can see that the machine will take a spectral scan from 800 to 400 nanometer which will be the baseline for our measurement. After the calibration has been done, we will take a blank reading of the solvent that we have used for the preparation of gold nanoparticles. In this case, it is ultra pure water.

So we will place the cuvette in the machine and press the zero option on the software. After blank reading, discard the blank solvent and add 2 ml of gold nanoparticle into the cuvette. The cuvette holds approximately 3 ml of the sample. Here first we are going to take UV reading for gold nanoparticles synthesized through turkevitch method.

You can see the ruby-red color of the gold nanoparticles. Add the sample to the cuvette and place it carefully, ensuring proper orientation. Now press start to initiate the

measurement. You can see that the peak is going above 1 absorbance. So, we will dilute the sample to get a reading in the proper range.

So, we will take out the cuvette, remove 1 ml from it, and store the extracted sample back in the vial. Now add 1 ml of ultra-pure water to the cuvette to achieve a half dilution of the gold nanoparticle sample. Mix it properly and ensure there are no bubbles in the cuvette, then place it in the cuvette holder facing the light source and detector. Once again, press start and take the reading. Now you can see that after dilution, the peak intensity has decreased, and the gold nanoparticles synthesized through the Turkevich method show a peak around 520 nanometers.

Now we'll take the absorbance for tannic acid-capped gold nanoparticles. First, we will discard the previous sample and clean the cuvette using ultra-pure water. Gently tap the cuvette on the tissue paper to dry it. Then add 2 ml of the gold nanoparticle sample to the cuvette. Since the solvent is the same for this sample as well, we are not repeating the blank calibration.

Place the cuvette in the machine and press start. You can see that it is taking a reading and you can observe a broad peak around 530 to 550 nanometer. This shift in the peak towards right side is due to the capping of tannic acid on gold nanoparticles. For saving the file, you can click on the save as option and save the file with proper sample name and date.

In this demonstration, we are going to perform fluorescence spectroscopy to analyze the photoluminescence spectrum for the two carbon dot samples that we have synthesized in one of the previous lectures. The first carbon dot sample was synthesized using the hydrothermal method and the other one using a microwave assisted method. The materials required are a beaker to discard waste, a four-sided quartz cuvette, a wash bottle, tissue paper, micro tips and micro pipette. First, switch on the machine and wait for 15 to 20 minutes for the light source to warm up.

Once the warm up is done, Open the software and select the spectrum option and within that select the emission option for the first carbon dot sample synthesized using the hydrothermal method. The excitation wavelength is around 270 nanometers. So we will set the emission wavelength at 290 nanometers and the end wavelength for the spectrum at 700 nanometers. Add 2 ml of the carbon dots sample into the 4 sided quartz cuvette.

Ensure that the cuvette is clean and there are no bubbles in the sample. Now, place the cuvette inside the sample holder of the machine. After closing the chamber, press 'Run' to start the PL spectrum measurement. You can see that it has started collecting the emission data from the sample. To save the file, we'll enter the sample name and click 'OK' to save it at our desired location.

You can see the PL spectrum on the screen for the carbon dots synthesized using the hydrothermal method. Now, for the next carbon dots sample synthesized using the microwave-assisted method, the excitation wavelength is around 280 nm. So, we will set the emission wavelength at 300 nm and the end wavelength of the spectrum at 700 nm. Discard the previous sample from the cuvette. and wash it 2 to 3 times using distilled water. Ensure that the cuvette is completely clean. Dry the cuvette using tissue paper.

Fill 2 mL of the carbon dots sample in the cuvette. Ensure that there are no bubbles in the sample and the cuvette is clean. Place the cuvette in the sample holder. Press 'Run' to measure the PL spectrum. You can see the PL spectrum for the carbon dots synthesized using the microwave-assisted method on the screen. There is an extra peak in the spectrum due to the lower purity of the synthesis in the microwave method, but it has more fluorescent intensity than the hydrothermal method.

After taking the measurement, save the file, exit the software, and turn off the machine. As a summary, in today's lecture, we learned the principle of UV-visible and fluorescence spectroscopy. We also learned how to use UV-visible and fluorescence spectroscopy for characterizing nanomaterials, both theoretically and through practical demonstration. Thank you for your kind attention. I will see you in another interesting lecture.