Experimental Nanobiotechnology

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Lecture 08: Fourier Transform Infrared Spectroscopy

Hello everyone, today we are going to learn about Fourier transform infrared spectroscopy. So, this is an important technique in nanometallic characterization. In today's lecture, we are going to learn what infrared radiation is and what the working principle of FTIR spectroscopy is and

we are also going to learn about the parts of an FTIR spectroscope. At the end of the lecture, we will also have a practical demonstration to understand this technique in more detail. Let us see what infrared radiation is. Infrared radiation is a form of electromagnetic radiation with wavelengths longer than visible light but shorter than microwaves and this IR is categorized into three regions: near-infrared, mid-infrared, and far-infrared

and this mid-infrared region is very important in spectroscopy because it corresponds to the vibrational energies of molecular bonds and the mid-infrared region ranges between 400 to 4000. Let us briefly see the history of FTIR. Infrared light was discovered in the early 19th century.

And the first commercial FTIR spectrometer was introduced in 1967. So, due to advancements in technology, the DSP and ATR techniques significantly improved the speed and accuracy of FTIR analysis, and currently, this FTIR analysis is an important characterization technique across fields like pharmaceuticals, biotechnology, and material science.

Let us see what Fourier-transform infrared spectroscopy is. FTIR is a technique used to analyze the interaction of infrared light with a sample. It helps in identifying chemical bonds in a molecule by measuring their vibrational frequencies. The IR spectrum of a molecule acts like a molecular fingerprint.

How do we have different fingerprints? Similarly, the IR spectrum of molecules acts like molecular fingerprints, and it provides detailed information about the sample's chemical structure and composition. FTIR is commonly used in material science, pharmaceuticals, and environmental analysis for identifying functional groups.

Studying the molecular structures and analyzing the complex mixtures. Let us see the principle. Here, the molecules absorb IR radiation when the frequency of the radiation matches the natural vibration frequency of their bonds. The vibration occurs in two primary ways. One is stretching, and the other is bending.

Stretching means changes in the bond length, and the stretching can be divided into two types. One is symmetric stretching, which means bonds stretch or compress simultaneously. The other one is asymmetric stretching, where one bond stretches while another compresses. Under bending, there are several types. Including scissoring, rocking, wagging, and twisting, bending means changes in the bond angle.

For a molecule to absorb IR radiation, it must be IR active. The vibration must cause a change in the molecule's dipole moment. So, only then can we measure the spectrum. Each molecular bond absorbs IR at characteristic frequencies, creating a distinct fingerprint spectrum for the sample. From this table column, you can get a clear idea about the molecular vibrations of various molecules and their corresponding peak positions.

For example, if it is an amine, you get an NH stretch. And the peak portion will be between 3550 to 3250. Similarly, for other molecules, we can understand from this table what kind of vibration we get and where the peak portion will be in the FTIR. Let us learn about the parts of an FTIR instrument.

The first one is the IR source. We can use silicon carbide or zirconium oxide. The next one is the interferometer. The interferometer is the main component of an FTIR spectrophotometer; it includes three key parts. One is the beam splitter, another is a stationary mirror, and the next one is a movable mirror.

The beam splitter divides the incoming light beam into two parts. One part goes to the moving mirror. And the other part reflects to the stationary mirror. Both mirrors reflect the light back to the beam splitter. Then, the beam splitter recombines the two beams and sends them through the sample chamber.

Then, from the sample chamber, it goes to the detector. So, here the sample holder, it holds the sample for analysis. The sample holder can be for liquid cells or solid holders if you

are using KBr pellets. And we can also use ATR crystals if you are using ATR FTIR. And we have the detector.

The detector converts the transmitted or reflected IR radiation into electrical signals. And the electrical signals are processed by the computer for analysis. Let us see the working of FTIR spectroscopy in detail. The FTIR instrument emits infrared radiation from a broadband source. Then, the IR beam is directed to a beam splitter.

which divides the beam into two paths. One beam is directed to a moving mirror, while the other is directed to a stationary mirror, as I mentioned in the previous slide. Then, the beams recombine at the beam splitter to create an interferogram. Then, the combined IR beam passes through or reflects off the sample, where it interacts with the sample's molecules, causing them to absorb specific IR frequencies.

Then the transmitted or reflected IR light is detected by a detector which converts it into an electrical signal. Then the interferogram is processed by a Fourier transform to convert the time domain data into frequency domain spectrum. Then the software generates the FTIR spectrum which displays the absorption of IR light at various wavelength corresponding to the molecular vibration in the sample. Then the restricting spectrum is analyzed to identify the functional groups, chemical bonds and molecular structures in the sample. When compared to the traditional FTIR, most of the labs nowadays they are using the ATR FTIR.

Let us see what is ATR FTIR. ATR means attenuated total reflectance and this is the most commonly used sampling technique in FTIR spectroscopy. The ATR is based on total internal reflection and Evanescent wave. So, the TIR means when a light travels from a denser medium to a less dense medium at an angle greater than the critical angle , it undergoes total internal reflection

and let us see what is evanescent wave while undergoing the total internal reflection a portion of the electromagnetic wave penetrates slightly into the second medium that is your sample this wave is evanescent wave and the evanescent wave does not propagate And it also decays exponentially with distance from the interface. From this animation, you can understand when you apply the IR light to the sample, evanescent wave is created and that can be detected by the detector.

In ATR-FTIR, evanescent waves are crucial. The IR beam undergoes total internal reflection within an ATR crystal. The ATR crystal can be diamond or zinc selenide. At

each reflection point, an evanescent wave penetrates a short distance into the sample in contact with the crystal. The sample absorbs specific IR frequencies based on its molecular vibrations, modifying the evanescent wave.

The altered wave returns to the detector, where it is processed to generate the IR spectrum. ATR-FTIR gained popularity due to its ability to quickly and easily measure a broad range of samples, including liquids, solids, powders, semisolids, and pastes. In traditional FTIR, we have to use potassium bromide for sample preparation. Let us see the importance of potassium bromide in FTIR.

Potassium bromide is an essential part of sample preparation for FTIR. It serves as a carrier for powdered samples. It is highly transparent to infrared light, which is crucial for FTIR analysis, and it does not absorb IR radiation in the mid-IR range. Due to homogeneous mixing of the sample with KBr, the chances of heterogeneous absorption are minimized.

And the KBr pellet also helps to protect the sample from oxidation, contamination, and moisture absorption during the FTIR analysis. It also helps to get consistent and accurate FTIR measurements. Let us see the difference between traditional FTIR and ATR-FTIR. The first one is sample preparation. Traditional FTIR often requires extensive sample preparation.

We have to use KBr pellets, whereas in ATR-FTIR, the sample preparation is very minimal. There is no need for KBr pellets, and in the case of IR interaction, the IR beam passes through the sample directly. In ATR, the IR beam interacts with the sample through an ATR crystal. with the evanescent wave penetrating the sample surface as I told earlier. And in case of sample thickness, we have to use thin sample in the traditional FTIR to allow the IR light to pass through.

But in the case of ATR-FTIR, it works for samples of various thicknesses. The measurement principle for the traditional FTR is it measures the absorption of IR radiation as it passes through the sample. Whereas in the case of ATR-FTIR, it measures the absorption of IR radiation by the evanescent wave at the sample surface. And the traditional FTR measures bulk properties of the sample and ATR-FTIR primarily sensitive to the surface of the sample. So that is why it is make it ideal for thin films and coatings.

And in the case of traditional FTR and ATR FTIR, the data quality is almost similar. The traditional FTR provides high-quality data for bulk material analysis. In ATR FTIR, it provides the accurate surface level data and that may not reflect the bulk properties. Let us

see some of the important applications of FTIR. So, we can use FTR for understanding the surface functionalization of nanoparticles.

For example, we can identify the functional groups on the nanoparticle surface. For example, if you coat the nanoparticle with PEG (polyethylene glycol), it will have functional groups like OH. If you coat the nanoparticle with polyethylene amine, you will get the amino group. So, we can use FTIR to understand the functional groups present on the nanoparticle surface. We can also use it for the characterization of carbon-based nanomaterials.

For example, analyzing the functionalization and structural changes in graphene, carbon nanotubes, and their derivatives. For example, when functionalizing graphene or carbon nanotubes, we can compare the carbon nanotubes with the functionalized carbon nanotubes. We can identify the changes in the carbon nanotubes after adding the functional group. This can also be useful for evaluating cross-linking and chemical modifications in hydrogels or nanogels. So, before and after cross-linking, what kind of chemical modification occurred in the hydrogels?

So that can be analyzed using the FTIR. And this FTIR can also be useful for understanding thin films and nanocoatings. So it will determine the chemical composition and bonding in the nanostructure coatings. Which we are mainly using for electronics and optics applications. And this can also be useful for nanocarrier drug delivery systems.

So, it will be useful to confirm drug encapsulation, bonding interactions, and release mechanisms in the nanocarriers. For example, if the drug is encapsulated inside the nanoparticle, we can measure the chemical modification and structural modification using the FTIR. So, this will give a different kind of peak. And in some cases, if the drug is attached to the surface, that will give a different kind of FTIR peak compared to when the drug is inside the nanoparticle. So, based on that we can understand whether the drug is encapsulated or it is attached on the surface by using this FTR spectroscopy.

And this can also be useful for photocatalysts and energy nanomaterials for studying the surface chemistry of nanomaterials in photocatalysis, fuel cells, and batteries. We can also use it for environmental applications. For example, we can use it for analyzing functionalized nanomaterials for pollutant removal and environmental monitoring. So, these are the various applications of FTIR.

Let us see some of the common issues which we face when we do the FTIR spectroscopy and how to overcome that. The first issue is low signal intensity. It may be due to weak sample concentration. Or the sample is too thick or not properly aligned. Ensure that you have sufficient sample concentration and use thinner samples.

Also, check the alignment of the sample in the beam path. The next issue is poor resolution or noisy spectra. It may be due to insufficient signal-to-noise ratio, poor detector performance, or a noisy environment. This can be overcome by increasing the number of scans to reduce the noise ratio, minimizing environmental vibration, and ensuring proper calibration.

The third issue may be interference from water or carbon dioxide. To overcome this, use a desiccator to remove moisture from the sample before measurement. Another issue is baseline drift or fluctuations, which may be due to temperature instability or misalignment of optics. This can be overcome by calibrating the instrument before measurement. If you are getting incorrect or overlapping peaks, it may be due to sample contamination.

Or it may be due to inappropriate sample preparation. So always make sure that you are using clean equipment and adjust the sample amount to avoid saturation. And the last one is absorption by the KBR pellet. So the possible cause may be due to contamination or moisture in the KBR pellet. So this can be overcome by using a dry KBR pellet by drying the KBR pellet before use,

and properly mixing the sample and always preparing fresh pellets. I hope you got the overall idea about FTIR spectroscopy. Let us go to the lab and learn this technique in more detail. Today we are going to perform FTIR analysis for L-cysteine. First, we have to make a KBR pellet.

So, we will take around 2.5 grams of KBR powder and 2 milligrams of L-cysteine powder to maintain a ratio of 1:100 of sample to KBR. We have to grind these two powders together to make a fine, homogeneous powder. Now, to make the KBR pellet, first clean the parts of the KBR pelletizer using tissue paper. Arrange the parts of the pelletizer as shown in this video. After the assembly of the pelletizer,

Add a few scoops of the prepared sample mixture. Tap the assembly a few times to settle all the powder on the base of the pelletizer. Now, insert the other parts of the pelletizer from the top. Keep the pelletizer in the machine to apply pressure on the pelletizer. The

pressure is currently zero, so we will first close the pressure valve and then use the handle to increase the pressure in the pelletizer, which will convert the powder into a pellet.

Apply pressure on the pelletizer. Once it is done, First, release the pressure using the pressure valve. You can see that the pressure has gone down to zero. Now, loosen the screw and take out the pelletizer.

Take out the rod first and then remove the lower part of the pelletizer carefully. Take out the pellet carefully using forceps with a sharp, pointed end. Now, you can see the pellet. Store the pellet in tissue paper to avoid moisture. Now, moving on to the software part of the FTIR analysis.

The machine has been set to take transmittance data in the wavenumber range from 4000 to 400. First, we have to collect the background without the KBr pellet. So, ensure that the sample holder is clean and then take the background. It will start collecting the background. After it has collected the background, place the pellet in the pellet holder carefully without breaking it, as shown in this video.

Place the sample holder in the machine carefully. Close the cover. Now, press Next to take the FTIR measurement. The sampling is in progress. On the screen, you can see the FTIR measurement for the L-cysteine sample with all its characteristic peaks.

Now, save the file with the desired name and location. Click Done and exit the software. Remove the sample from the FTIR machine and turn off the machine. This FTIR spectrum of L-cysteine shows the characteristic vibrational modes of functional groups present in the molecule. Amine stretching is highlighted in pink color.

So this peak corresponds to the stretching vibration of the primary amine group in the L-cysteine. It indicates the presence of an amino group. Then, the thiol stretching is highlighted in green. So this peak represents the stretching vibration of the thiol group, a unique feature of cysteine. The intensity and the peak position of this may vary depending on the environment of the thiol group.

So the next one is the carboxyl group highlighted in purple. So this peak corresponds to the symmetric and asymmetric stretching vibrations of the carboxyl group. It confirms the carboxylic acid functionality deprotonated to form the carboxyl group. The high transmittance values indicate strong absorption in specific regions. where functional groups vibrate at characteristic frequencies.

The presence of distinct peaks in the pink, green, and purple areas confirms the characteristic groups amine, thiol, and carboxyl, essential for understanding the structural integrity of L-cysteine. As a summary, in today's lecture, we learnt what is infrared radiation and what is the working principle of FTIR spectroscopy and also what are the various parts of FTIR spectroscopy

and through practical demonstration, we also learnt this FTIR spectroscopy in detail. Thank you, everyone, for your kind attention. I will see you in another interesting lecture.