Spectroscopic Techniques for Pharmaceutical and Biopharmaceutical Industries
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Lecture No. 11
Vibrational, Rotational-Vibrational, Raman Spectroscopy - III

Hello students, today I am going to discuss about application part of vibrational, rotational vibrational and Raman spectroscopy. In last 2 lecture, we have looked at theory part IR spectroscopy. During the last lecture, I started with application, but a lot of application needs to be discussed again and I will be doing that in this lecture. So, this lecture will focus on application part of IR spectroscopy.

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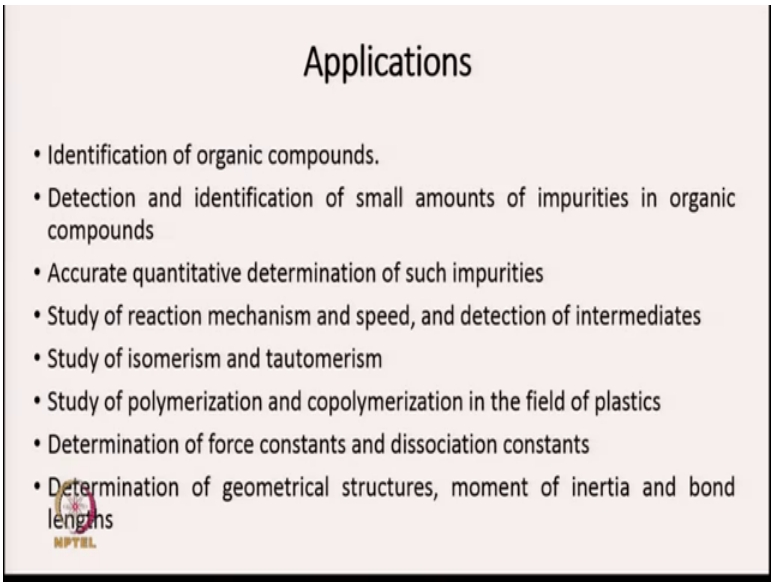

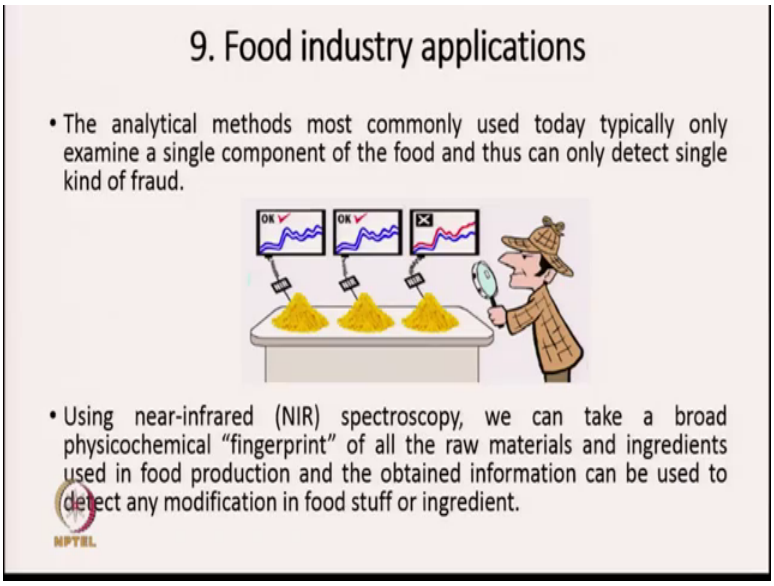

During last lecture, I discussed about how IR spectroscopy can be used to identify organic compounds, how it can be used to detect and identify small amounts of impurities in a organic compounds. Apart from detection, we also looked at how this impurity, how the amount of impurity can be determined. Apart from that, we can use IR spectroscopy in the study of reaction mechanism and speed it can be used for detection of intermediates.

It has been used to look at isomerism and tautomerism, it has been utilized to for a study of polymerization and co-polymerization in the field of plastics. It can be used to determine the force constant and dissociation constant of a bond in a molecule and it can be used for the determination of geometrical structures, moment of inertia and bond length. Apart from these applications IR spectroscopy can also be used in the food industry for quality control.

Most of the analytical methods which are used to know a particular ingredient in a food sample typically examines a single component of the food and thus can only detect single kind of fraud. So, food sample is generally mixed with different unwanted material and we need to know whether our food sample is good or not, or mixed with your unwanted materials.

So, other analytical methods can only detect a single component whereas, near infra IR spectroscopy can be used to get (physio) physico, physicochemical fingerprint of all the raw materials, ingredients used in food production. And thus in one go we can detect any modifications in food stuff or ingredient.

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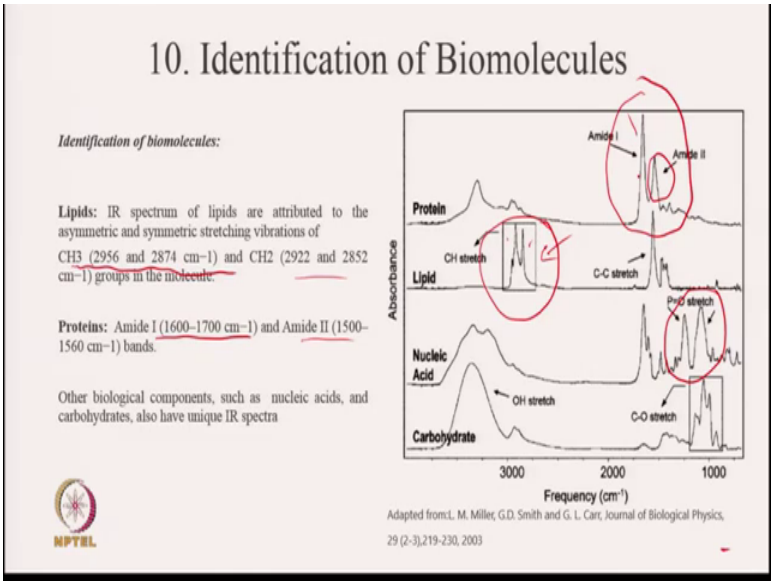

FTIR can also be used for identification of bio molecules, for example, lipids, proteins and DNA molecule, each 1 of them has a specific band in IR spectroscopy. For example, protein has band around this, whereas, liquid has band around this frequency, nucleic acid has PO stretching band around this frequency and carbohydrate has CO stretching around this frequency. So, lipids, IR spectrum of lipids are attributed to the asymmetric and symmetric stretching vibration of CH3 which is in between 2956 and 2874 centimeter inverse which is here and CH2, which is between 2922 and 2852.

So, these are the true asymmetric and symmetric stretching vibration of lipid and they can be utilized to know the presence of a lipid in a sample. Similarly, proteins has 2 bands amide I band between 1600 to 1700 centimeter inverse and amide II band between 1500 to 1560 centimeter inverse. So, this is your amide band this is between 1600 to 1700 centimeter inverse and this is your amide II band, which is between 1500 to 1560 cm inverse bands. Similarly, other biological components such as nucleic acids and carbohydrates have their unique IR spectra. So, based on the IR spectrum of sample you can tell whether a protein are lipid or a nucleic acid or carbohydrate is present in the sample.

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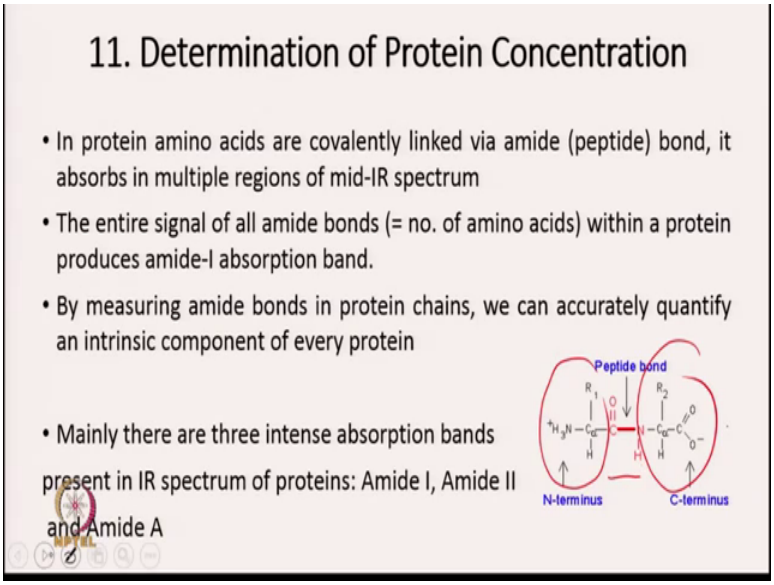

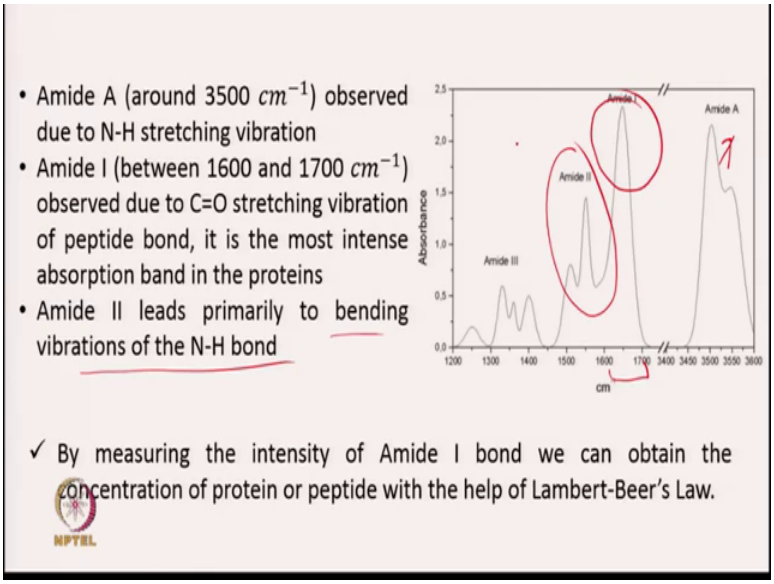

IR spectroscopy can also be used for the determination of protein concentration. In protein amino acids are covalently linked to amide bond and it absorbs in multiple region of mid-IR spectrum. The entire signal of all the amide bonds, which is basically generally equal to number of amino acids within a protein produces amide I absorption band. So, by measuring amide bonds in protein chains, we can accurately quantify an intrinsic component of every protein.

So, you can see that 2 amino acid is linked by amide bond and this amide bond has characteristic spectrum and by measuring the signal of amide bond, you can know the concentration of protein. There are 3 intense absorption bands in the IR spectrum of proteins and that is called and amide I

band, amide II band and amide A band. Let us see what is these 3 bands, amide A band is around 3500 centimeter inverse.

So, this is your amide A band and this is because of NH stretching vibration, this is because of NH stretching vibration. There is another band which is known as amide I band, it is between 1600 to 1700 centimeter inverse and you can see here 1600 to 1700 centimeter inverse and this is due to stretching vibration of peptide bond and it is the most intense absorption band in the protein and the third band is amide II band and that is because of bending vibration of NH bond and this is your amide II band. So, by measuring the intensity of amide 1 band we can obtain the concentration of protein or peptide with the help of Lambert Beer's law.

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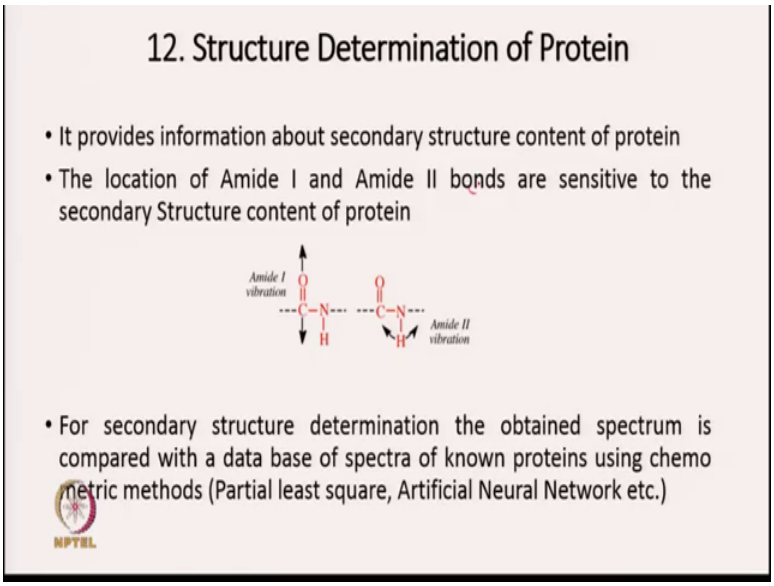

IR spectroscopy not only helps you in determining the concentration of protein, it can be used to determine the structure of protein. IR spectroscopy provides information about secondary structural content of protein, the location of amide I and amide II bands are basically dependent on are sensitive to secondary structural content of the protein. So, location of amide I and amide II bands, so let us make it bands, bands are sensitive to secondary structure content of protein.

So, alpha helical protein will have different location of amide I band compared to the location of amide I band for beta sheet protein and based on the location of a amide I and amide II band you can tell whether your protein alpha helical or beta sheet or in between. So, for secondary structure determination, what we do is we compare the obtained spectrum with a data base of

spectra of known proteins using chemo metric methods. And by doing that, we can know what is the secondary structure contain of a protein. The chemo metric methods which are used for doing that analysis is based on partial least square or artificial neural network.

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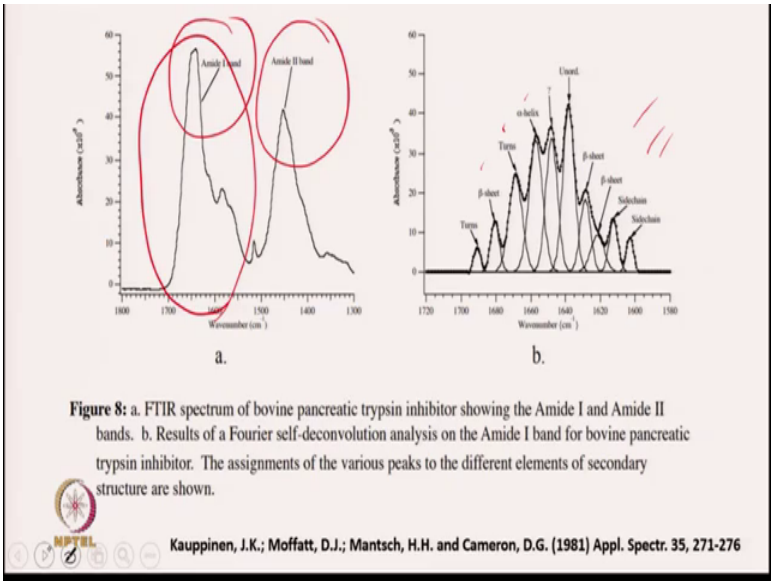

Figure 8: a. FTIR spectrum of bovine pancreatic trypsin inhibitor showing the Amide I and Amide II bands. b. Results of a Fourier self-deconvolution analysis on the Amide I band for bovine pancreatic trypsin inhibitor. The assignments of the various peaks to the different elements of secondary structure are shown.

Kauppinen, J.K.; Moffatt, D.J.; Mantsch, H.H. and Cameron, D.G. (1981) Appl. Spectr. 35, 271-276

So, here is 1 example. So, FTIR a spectrum of bovine pancreatic trypsin inhibitor has been shown here and this is your amide I band and this is your amide II band of FTIR spectrum of bovine pancreatic trypsin inhibitor. Now, you can deconvolute this amide I band and know what is the amount of different secondary structure in the protein and here is your deconvolution of the amide I band. Now, based on that, you can tell what is the amount of alpha helix turns beta sheets in the given protein, in the given protein. So, assignment of various peaks to the different element of secondary a structure is shown in this figure and that helps you in the determination of secondary structure of protein.

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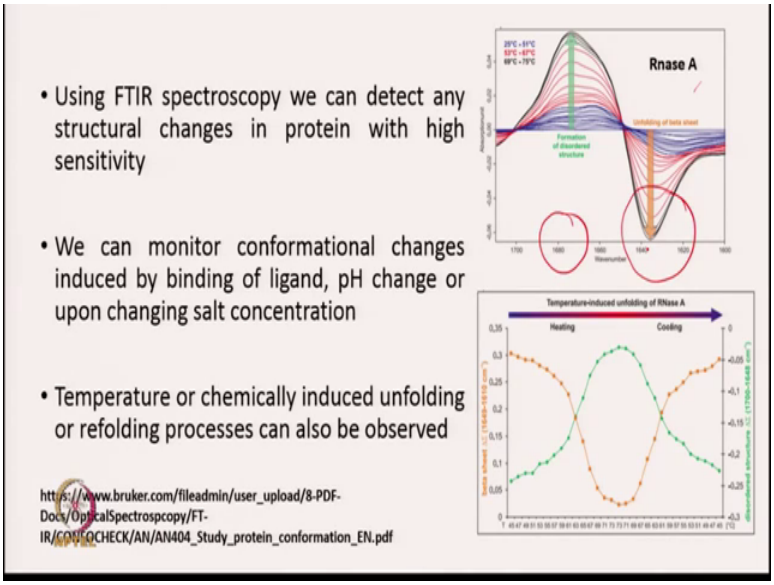

Now, you can also use FTIR spectroscopy to detect any structural changes in protein with high sensitivity. So, you can change the condition and look at how confirmation of protein is changing with change in condition. So, this is an example of ribonuclease A protein and what you are looking at is what happens to confirmation of protein with change in temperature, what is happening at the secondary a structure level with the change in temperature. So, now, you can see here that this peak is increasing, which is around 1680 centimeter inverse is decreasing, this peak is decreasing, this peak is decreasing.

So, what does that mean is your beta sheet RNA's is basically a beta sheet protein. So, beta sheet is decreasing and your beta sheet is decreasing and there is increase in the formation of increasing the formation of this ordered structure with increase in temperature and that is also shown here. So, this is your how beta sheet changes with increase in temperature. So, initially there is a heating, so, till this point, so, you are going from 45 to 73 and then the sample is cooled.

So, what you can see is that beta sheet is decreasing the extent of beta sheet in the protein is decreasing with increase in temperature, but when you cool it back to 45 degrees Celsius, it is again regaining its beta sheet structure which tells you that ribonuclease A is reversible protein you can see the desaturation of ribonuclease A with respect to temperature is a reversible process. So, we can monitor conformational changes in a protein either induced by temperature, or pH

change our binding of ligand, our salt concentration, salt concentration. Temperature are chemically induced unfolding or refolding process of a protein can also be observed using IR spectroscopy.

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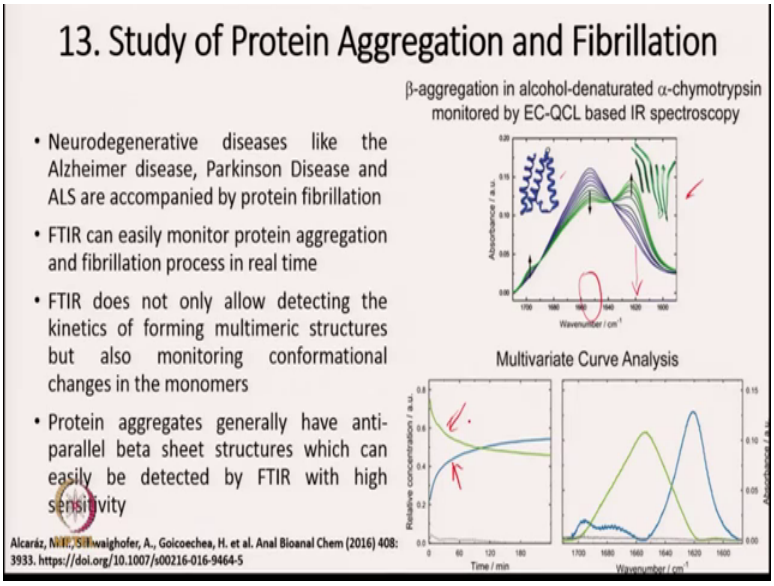

Not only that, FTIR can be used to study protein aggregation and fibrillation. Protein aggregation and fibrillation is important in context of neurodegenerative disease. So, protein aggregation and fibrillation is important in context of neurodegenerative disease like Alzheimer disease, Parkinson disease and ALS. These diseases are accompanied by protein fibrillation and FTIR can be used to monitor protein aggregation and fibrillation process in real time.

So, you see here, this is basically your FTIR spectrum of alpha chymotrypsin when it is aggregated in presence of alcohol and this blue 1, so, as you alpha helix, so, this is around 1660 centimeter inverse and this green 1, so, as your beta sheet, this is around 1620 centimeter inverse, and what you can see is that with the addition of alcohol, what is happening is your alpha helix is decreasing, you can see there is a decrease in the alpha helix, whereas, there is increase in beta sheet contain, which is due to aggregation.

FTIR does not only allow detecting the kinetics of forming multimeric structure, but also monitors conformational changes in the monomers, protein aggregates generally have anti parallel beta sheet structure which can easily be detected by FTIR with higher sensitivity. So, in this case of alcohol denatured alpha chymotrypsin you can see that this gain which shows you

your beta sheet, it is increasing. So, this one is your beta sheet and this is increasing, whereas, this is your decreasing. So, concentration of beta sheet it is increasing and concentration of alpha helix is decreasing, decreasing with time.

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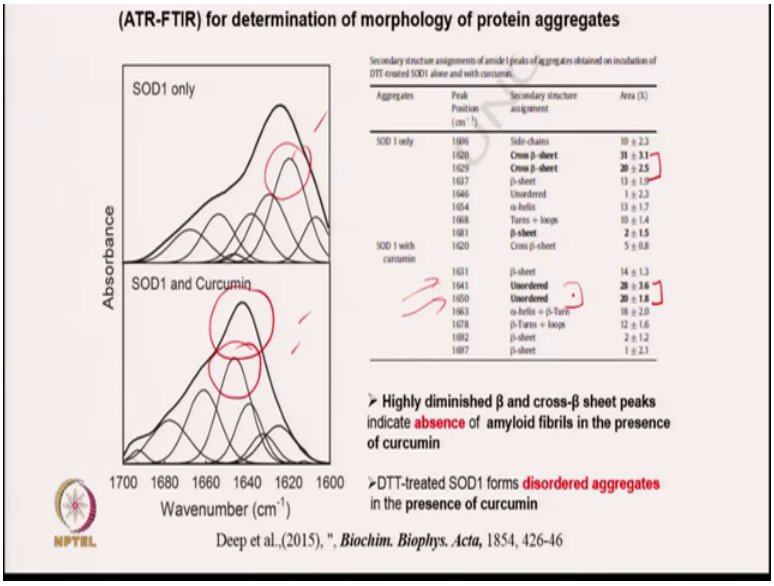

Apart from the amount of aggregate formed, we can also see the morphology of protein aggregates, morphology of protein aggregates. So, not only you can look at the aggregation process, but you can also discriminate between different morphology of protein aggregates using FTIR spectroscopy. So, these are the FTIR spectrum of aggregates of SOD 1, so, upper 1 shows you the FTIR spectrum of SOD 1 or SOD 1 aggregated in presence of DDT.

So, upper one shows you aggregation of SOD 1 in presence of DDT. The lower panel shows you the aggregation of SOD 1, in presence of DTT when carried out with curcumin. So, what you can look at the spectrum of aggregate formed in absence of curcumin is different than the one obtained in presence of curriculum. If you do deconvolution of this upper and lower panel, you can see that the upper panel has peaks or have bands of higher intensity and region1620 to 1629 centimeter inverse, centimeter inverse and that corresponds to beta sheet structure.

Whereas in the lower panel, the aggregate formed in the presence of curcumin shows peaks with maximum intensity in this region between 1640 to 1650 centimeter inverse, and that tells you that this aggregates had different morphology, then the aggregates form in absence of curcumin, and here aggregates have different morphology than the aggregates formed in absence of

curcumin and here aggregates are disordered, aggregates are disordered. So, what does that mean is that highly diminished beta and cross beta sheet peaks indicate absence of amyloid fibrils, amyloid fibrils are basically rich in beta sheet.

So, what you have seen is that highly diminished beta and cross beta sheet peaks indicate absence of amyloid fibrils in the presence of curriculum. DTT treated SOD 1 forms this ordered aggregate in the presence of curcumin whereas DTT treated SOD 1 forms amyloids in the absence of curcumin.

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14. Monitoring Protein Folding using H-isotope exchange

- It is a powerful tool for monitoring protein folding process.
- When a protein is dissolved in water the hydrogens attached to the amide nitrogen can readily exchange for hydrogens attached to water molecules.
- For unfolded polypeptides this exchange can occur at much higher rate as compared to the folded ones.
- To observe the rate of hydrogen exchange, a different isotope of hydrogen is used for the solvent water than that initially present on the protein e.g. deuterium (D).
- The exchange of the H isotope for the D isotope affects the vibrational frequencies of the amide bond and therefore affect the Amide I and Amide II bands of the infrared spectrum of a protein.

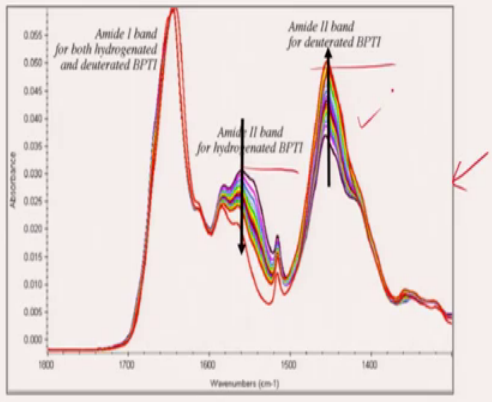

Figure 9: Time course for hydrogen/deuterium from BPTI, from 3 minutes to 374 minutes after initiating the exchange. The Amide II band at 1450 cm^{-1} is for deuterated BPTI and is increasing with time.

https://www.chem.uwec.edu/chem455_s05/pages/manuals/FTIR_of_proteins.pdf

Protein folding can also be monitored using hydrogen isotope exchange. FTIR is a powerful tool for monitoring protein folding process. So, suppose a protein a dissolved in water what you can think of the hydrogen attached to amide nitrogen can readily exchange for hydrogen attached to water molecule. So, what do you expect that for unfolded polypeptide this exchange can occur at much higher rate as compared to folded one.

To observe weight of hydrogen exchange what we can do is a different isotope of hydrogen is used for solvent water than that initially present on protein. For example, can choose deuterium. The exchange of the hydrogen isotope for the deuterium isotope affects the vibrational frequency of the amide bond. Since your reduce mass will change and therefore, they will affect now isotopes substitution will affect the amide bond and amide II bands of the IR spectrum.

So, here is you can see this is the IR spectrum of BPTI. And what we are looking at time course for hydrogen deuterium from BPTI from 3 minutes to 374. So BPTI was taken hydrogen, deuterium, were exchanged, and what we are looking at is time course for this deuterium exchange from 3 minutes to 374 minutes. So, you can look at that amide II band for hydrogen BPTI is decreasing in intensity whereas, amide II band for deuterated BPTI is increasing, increasing. So, you can know the rate of exchange, you can know the rate of exchange and that can tell you about folding process.

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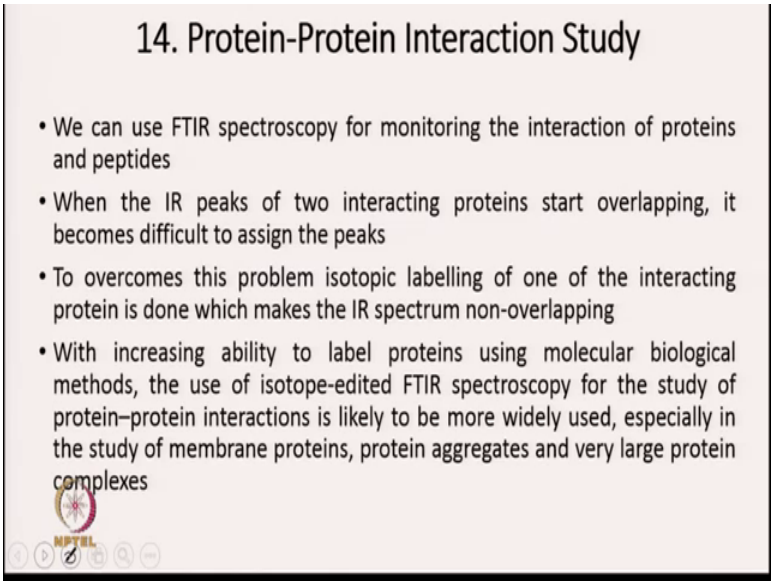

Similarly, we can also use FTIR to study protein-protein interaction. Sometime or most of the time IR peaks of interacting proteins start overlapping, and then it is difficult to assign the peaks. To overcome this problem, we can go for isotope labelling. So, what can be done is label 1 of the interacting proteins and what it does is it makes the IR spectrum non overlapping, non-overlapping. So, with increasing your ability to label proteins using molecular biological method, the use of isotope edited FTIR spectroscopy for the study of protein-protein interaction is gaining importance, is gaining importance, particularly to a study membrane protein, protein aggregates a very large protein complexes.

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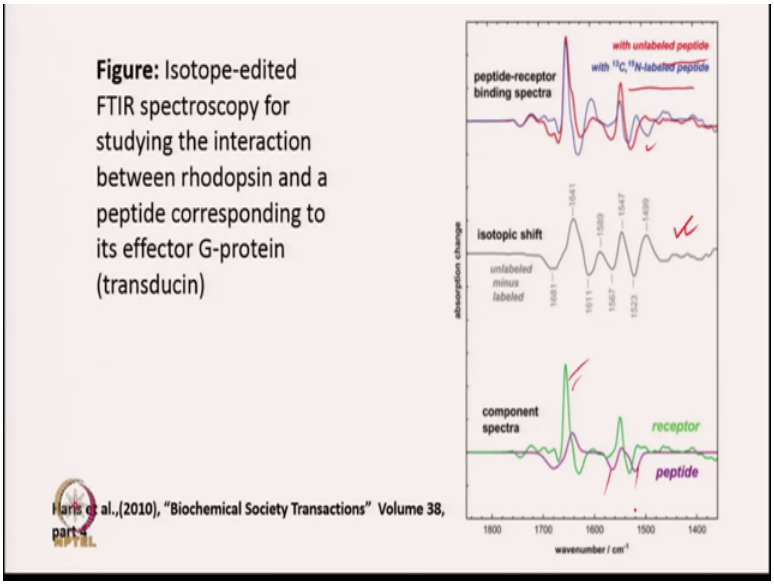

So, this is an example of isotope edited FTIR spectroscopy for study of interaction between rhodopsin and peptide. This peptide corresponds to its effector G-protein. So, you can see here, so, what is done is your peptide is label and a spectrum is obtained a spectrum of interaction between rhodopsin and peptide is obtained with labelled peptide and unlabeled peptide. So, this red one is with unlabeled peptide, where is blue 1 is with 13 C 15 N-labelled peptide.

Now, unlabeled minus labelled gives you this isotope itself and then you can do component analysis and what has been seen is this is FTIR spectrum the green 1 is FTIR spectrum of receptor in presence of peptide and this purple one is FTIR of peptide in presence of receptor. Now, you can compare this green one with the FTIR spectrum of original receptor and red one with FTIR of peptide alone. And then you can know where is the shift and then that gives you

idea about what are the regions in receptor which is involved in binding with the peptide and vice versa.

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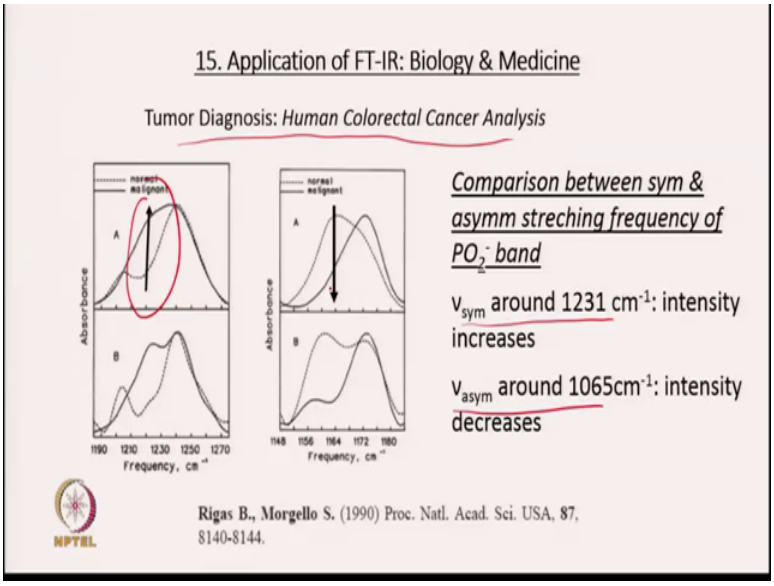

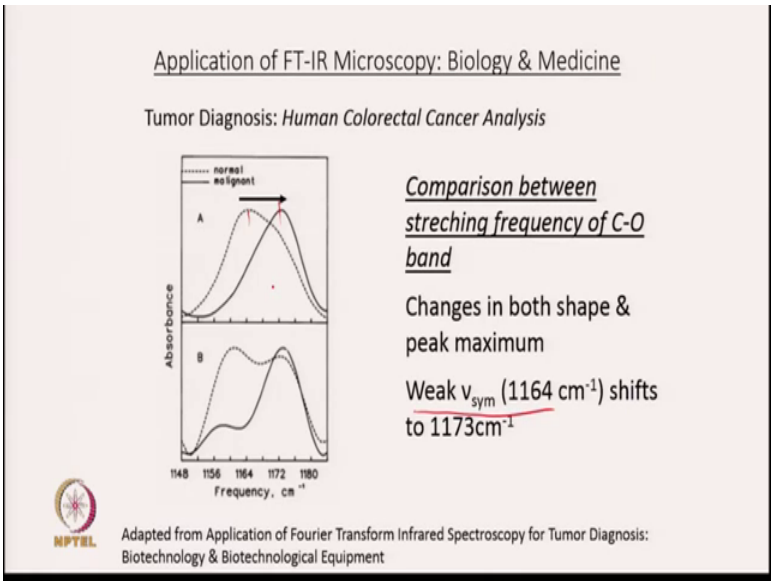

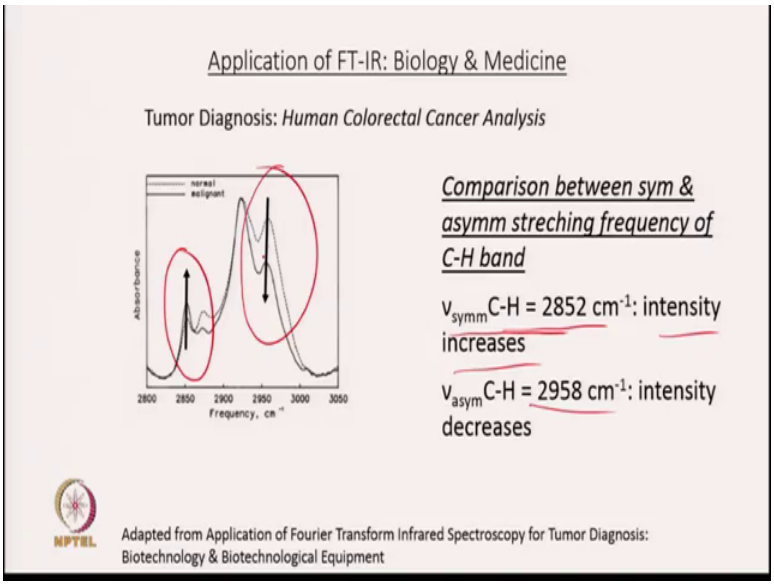

FTIR is quite often used in biology and medicine. So, here is the example of diagnosis using FTIR. For example, in tumor diagnosis, if suppose you are doing human colorectal cancer analysis, you can see this is the spectrum of normal person and a person with malignant, malignant tissue. And now, there is a comparison you can see that frequency around 1231 centimeter inverse, this is the symmetric stretching of PO2 minus band and what you can see is the intensity is increasing.

So, this is around 1231 centimeter inverse, this peak is around 1231 centimeter inverse. This is increasing in a person with a tumor. Whereas, the asymmetric stretching frequency around 1065 centimeter inverse, so, is decreased. So, just by looking at this to these 2 bands, you can tell whether a person has colorectal cancer or not, it can give you idea about were that particular patient is susceptible to colorectal cancer or not.

You can also compare the CO stretching frequency and what you will see is that in the tumor patient, there is a shift of this symmetrical stretching frequency of CO band from 1164, this is your 1164 to 1173 centimeter inverse. So, just by looking at FTIR spectrum you can diagnose whether a person is prone to colorectal cancer or not. You can also compare the symmetric and asymmetric stretching frequency of CH band of a normal person and your malignant tissue, so tissue from a normal person and tissue from a malignant person.

So, what you will see is that the symmetric CH is stretching around 2852 centimeter inverse the intensity of this band. So, if you look at the symmetric stretching frequency of CH band at 2852

centimeter 6inverse, intensity is increasing. And if you look at this band, which is at 2958 centimeters inverse, the intensity is decreasing. So, if you combine all 3 data, you will be able to tell whether a person is suffering from colorectal cancer or not.

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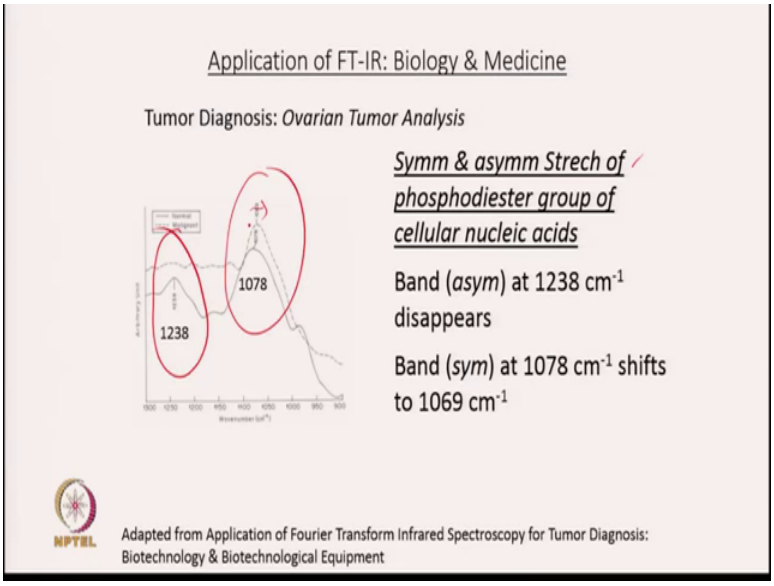

The second case will be, the second case which we are going to discuss is ovarian tumor. FTIR can also be used to diagnose ovarian tumor and what do you can look at is symmetric and asymmetric stretching of phosphodiester group of cellular nucleic acid, cellular nucleic acid and you can see that band at 1238 centimeter inverse, which is because of asymmetric stretching it disappearing and band at 1070 centimeter inverse, which is due to symmetric stretching is sifting to 1069 centimeters inverse, 1069 centimeter inverse so you see there is a shift like this.

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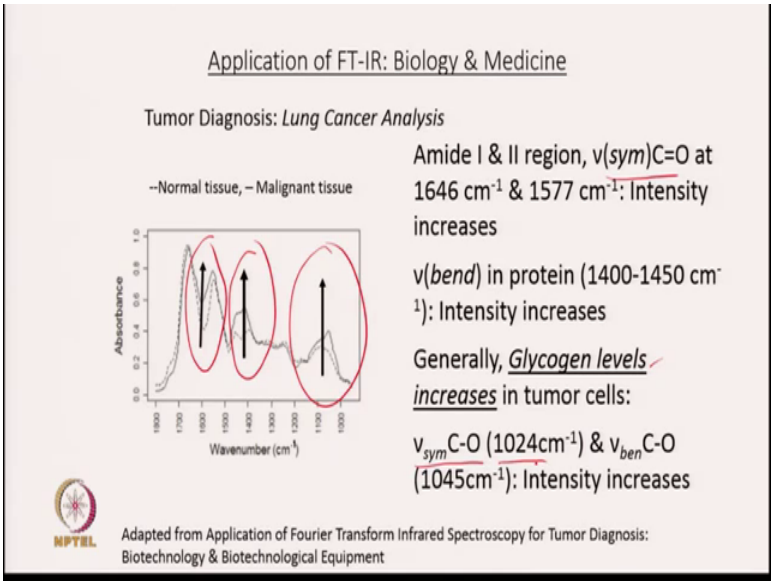

Apart from these 2 cancers, we can also use FTIR to diagnose lung cancer and here the spectrum of both normal tissue taken from a normal person and malignant tissue taken from a cancer patient and what you can see is that there is a difference in amide I and II region and this is because of symmetrical stretching of C double bond O, here intensity is increasing, intensity is increasing.

Similarly, you can see this frequency due to bending, between 1400 to 1450 centimeter inverse intensity is again increasing and glycogen level also increases the tumor cell and that can be seen from the symmetric stretching of CO band around 1024 centimeter inverse. So, just by looking at the spectrum of a malignant tissue from a patient to and compare that the FTIR spectrum of normal tissue you can know or you can diagnose either a person as lung cancer or not.

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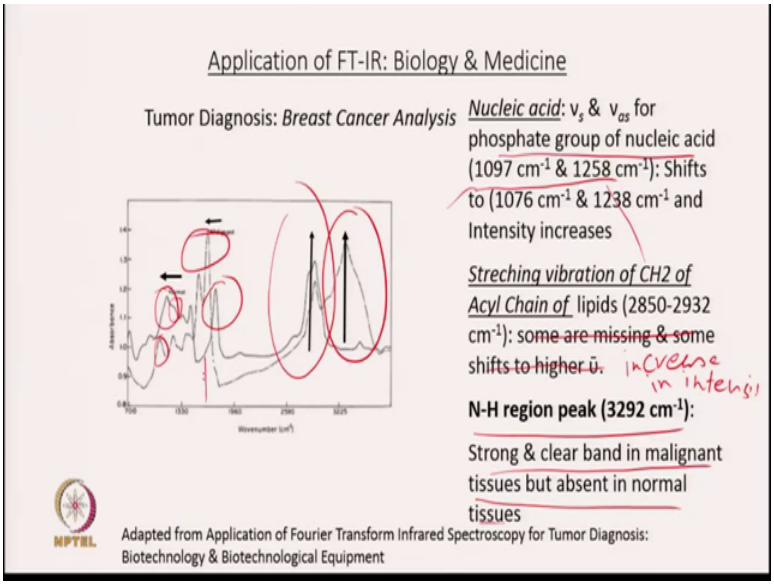

FTIR can also be used for breast cancer analysis, you can look at nucleic acid. So, phosphate group of nucleic acid comes around 1097 centimeter inverse and 1258 centimeter inverse. The first one is due to a symmetrical stretching and the second one is due to asymmetrical stretching and you can see that they shift to, they shift to 1076 centimeter inverse, 1076. So, here you can see that this 1097. So, 1097 is this one, this one and this is going to 1076 centimeter inverse. So, this is normal. So, this is for 1097 centimeter inverse and that is going to 1076 centimeters inverse.

Similarly, 1258 going to, 1258 going to 12308 1258 going to 1238. So, shift is 2 hours, lower wave number and intensity decreases, intensity decreases, intensity decreases, here you can see intensity decrease, the second one you can look at stretching vibration of CH2 of acyl chain of lipids 2850 and 2930 centimeter inverse. So, this is your 2850 and 2932 centimeter inverse. So, this we are talking about this peak and there is a certainly increase, so there is a increase. So this is increase in intensity, increase in intensity, intensity.

And if you look at NH region peak around 3292 centimeter inverse, there is strong and clear band in malignant tissue but absent in normal tissue. So, first is your nucleic acid symmetrical, symmetrical stretching frequency of phosphate group of nucleic acid and this is around 1097 centimeter, it goes to 1076 centimeter inverse. And here you see this is your at 1258, so this is

your this is a 1258, this goes to 1238 centimeter inverse. 1238 centimeter inverse and there is increase in intensity, there is increase in intensity.

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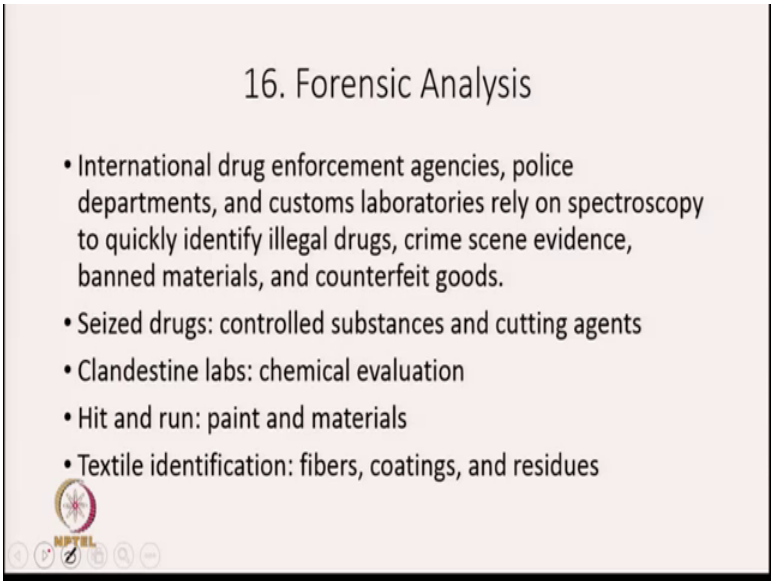

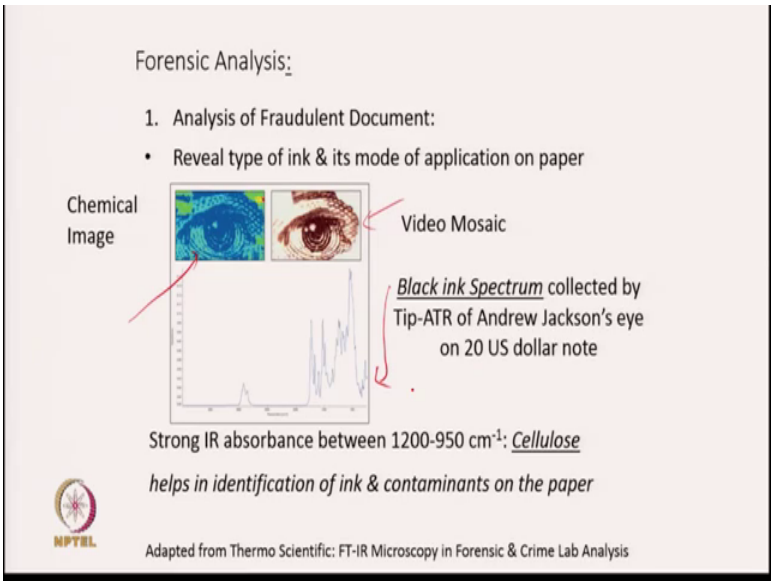

So, apart from biochemistry and diagnosis of cancers, FTIR can also be used for forensic analysis. International drug enforcement agency, police department and custom laboratories rely on a spectroscopy, IR spectroscopy to quickly identify legal drugs, crime scene evidence, band material and counterfeit goods. Seized drugs can be of 2 types controlled substances and cutting agents. The clandestine labs will use chemical evolution and they can manipulate the data.

For hit and run cases we can look at paints and materials and for textile identification, we can look at fibers coating and residues. By doing analysis, of these components we can get an idea about a suspect, idea about suspect or give some examples. For example, if suppose you want to know whether the document which is opt in is fraudulent or original, FTIR can help you in that what you can look at is that difference between type of ink on original paper and on a fraud paper. So, here is your Andrew Jackson's eye on 20 US dollar note. This is the chemical image of eye and here is video mosaic of that eye.

And the lower panel is shown black ink spectrum collected by tip-ATR for your Andrew Jackson's eye. Now, you can compare this spectrum with the, the sample paper which you have and if there is a difference in the IR spectrum, you can tell that the sample paper is a fake paper. So, apart from that, you can also look at a strong IR observance between 1200 to 950 centimeter inverse, which corresponds to cellulose and that can tell you about the kind of paper which is used for that document. And that helps you in identification of ink and contaminants which is there on a fraud paper.

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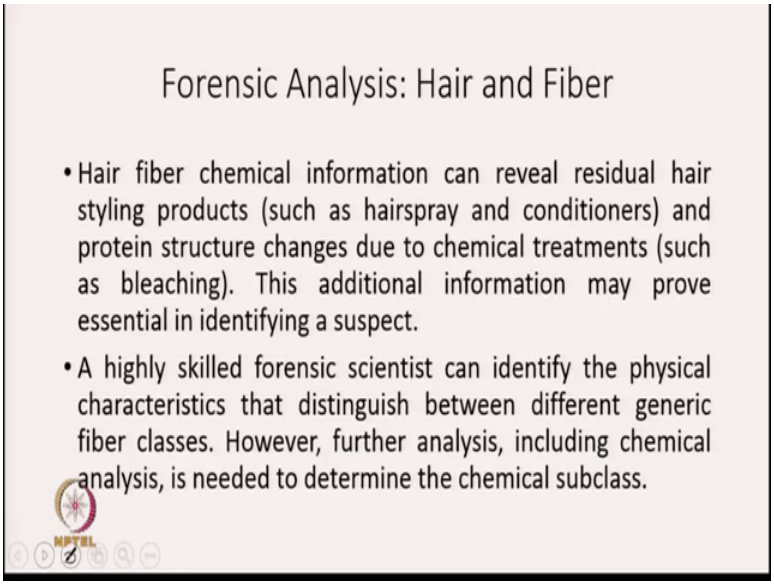

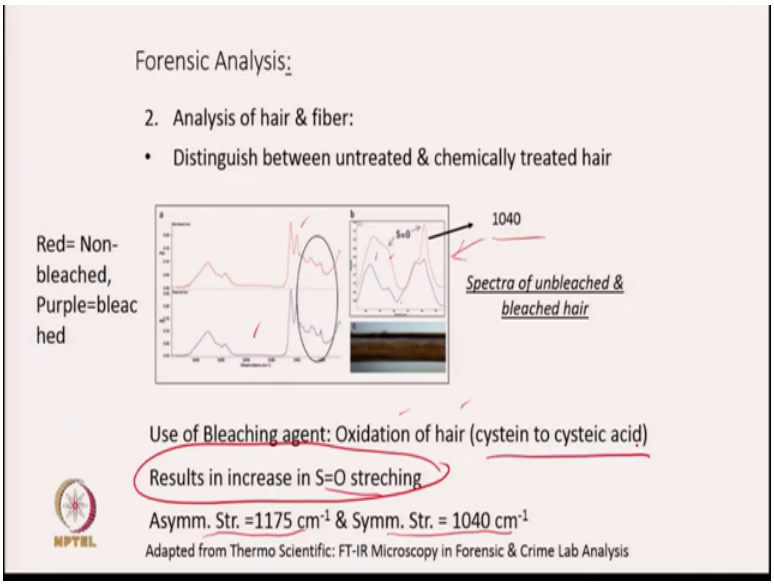

Analysis of hair and fiber is an important component to find to a suspect. So, chemical information of hair and fiber can tell you about hair styling products used by a suspect. And this additional information can prove to be essential in identifying a suspect. A highly skilled forensic scientist can identify the physical characteristics that distinguish between different generic fiber class.

However, further analysis including chemical analysis is needed to determine the chemical subclass of the fiber, but by analysis of hair and fiber, you can identify a possible suspect. So, here is the FTIR spectrum of untreated and chemically treated hair. So, the red one is non bleached here and purple one is bleached here and there is a difference between this which is junked at this place. A spectra of unbleached is the blue one in this case and a spectra of bleached is the red one. And so, you can see that this peak at 1040 centimeter inverse, which is due to symmetrical stretching of the SO bond, it is increasing and this asymmetrical stretching at 1175 centimeter is also increasing.

So, there is increase in SO stretching because of oxidation of hair involves oxidation of cystein to cysteic acid, cystein to cysteic acid. So, a particular suspect can use a particular bleaching agent and that can oxidize hair to different extent. And by looking at the normal hair spectrum and bleached hair spectrum you can distinguish between whether there is a bleached spectrum, bleached hair or unbleached hair. And that can that can point to a suspect.

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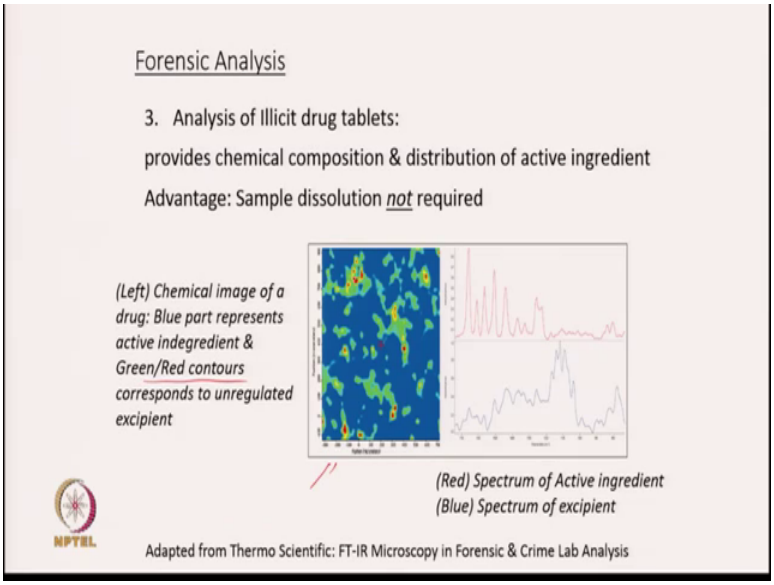

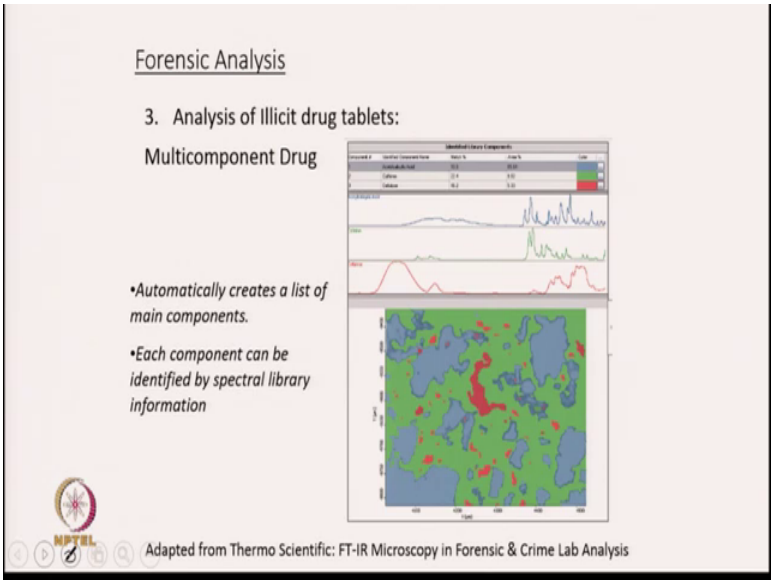

FTIR can also be used for analysis of illicit drug tablets, provides chemical composition and distribution of active ingredient and here sample dissolution not required. So, this is your chemical image of a drug, blue part tells you about active ingredient and the green/ red contours corresponds to unregulated. So, this is green/ red contours correspond to unregulated excipients.

So, just by doing this chemical imaging of a drug through IR microscopy, you can know what kind of unregulated excipient is present in the sample. FTIR is also used for analysis of illicit drug tablet again you can do multi component drug analysis, and the spectra can be analyzed to

get a list of main components. And each component can be identified by a spectral library of information, the information of any illicit component can be obtained through FTIR imaging.

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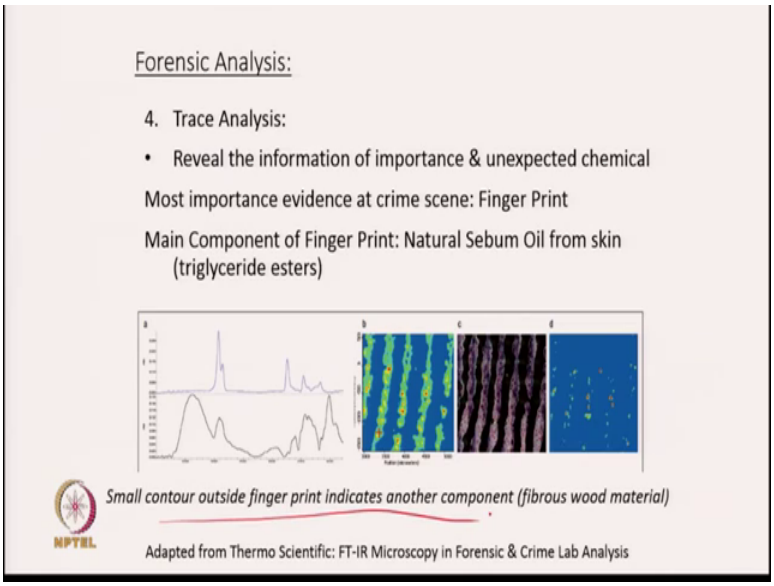

Trace analysis can also reveal the information of important and unexpected chemicals. So, most important evidence at crime scene is fingerprint and when component of fingerprint has natural sebum oil from skin which it basically triglyceride esters and by looking at different contours you can know whether fingerprint corresponds to a particular suspect or not.

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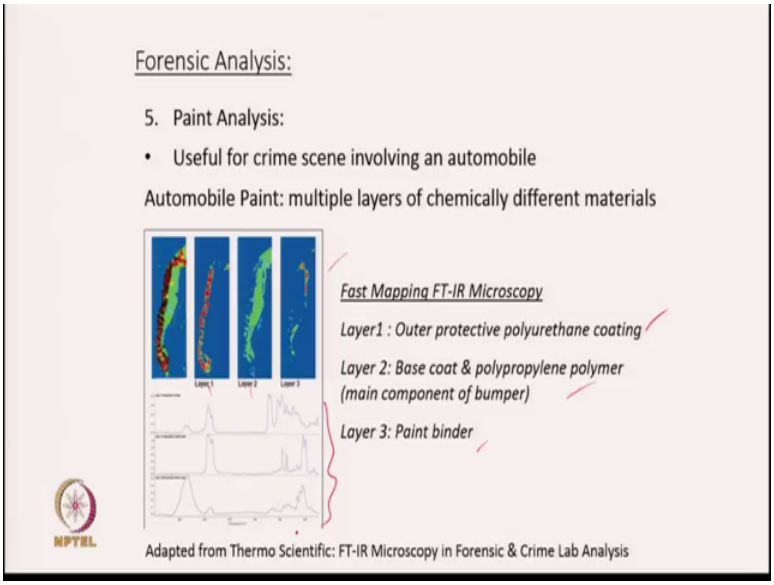

Similarly, in the hit and run cases paint analysis is very important and FTIR can be use to FTIR can be useful for crime scene involving an automobile. Automobile paints has multiple layers of chemically different materials and just by looking at FTIR spectrum of the paint of automobile involved, you can tell which kind of brand that automobile has and here is your mapping using FTIR spectroscopy, mapping of paint using FTIR microscopy.

And you can see the layer 1 is polyurethane coating, layer 2 is base coat and poly propylene polymer and third is paint dimer. So, this is overall and this is layer 1, layer 2, layer 3, and here is the IR spectrum of these 3 layers. And that can be compared with the paints used by different make a model of automobile and just by looking at the difference looking at the difference you can tell by looking at the similarly you can tell whether the automobile involved in hit and run case is a particular brand or not. So, analysis of pain is important evidence for hit and crime cases.

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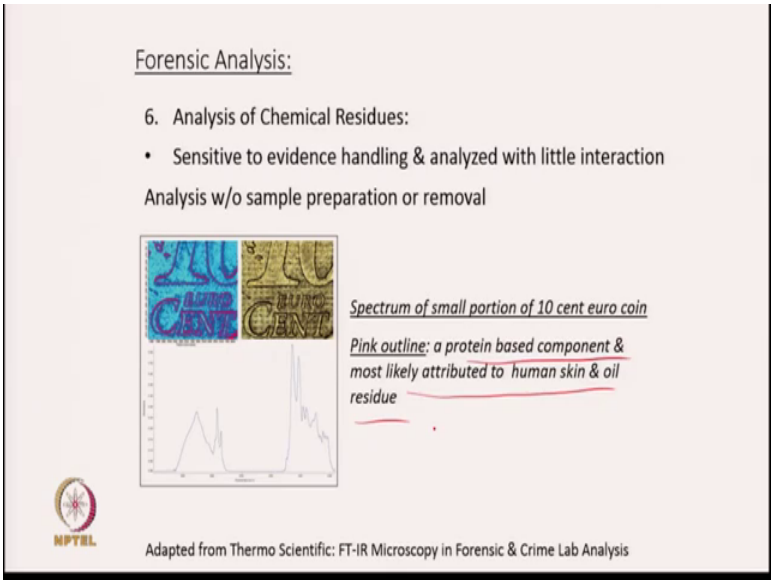

Forensic analysis, analysis of chemical residues is also very important. And these are very sensitive to evidence handling and analyzed with little interaction, little interaction. Here you can see that spectrum of a small portion of 10 cent euro coin. Pink outline tells you a protein based component and most likely attributed to human skin and oil residue. And just by looking at this pink outline, you can tell about the identity of a suspect.

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So, apart from the, apart from forensic analysis, FTIR can also be used for pharmaceutical industry and applications include basic drug research and structural elucidation, formulation development and validation, quality control processes for incoming and outgoing materials, packaging testing. This we have already discussed how to use FTIR for a structural elucidation, formulation development, quality control. So, I am not going to discuss this in detail, but pharmaceutical industry utilizes FTIR to a very great extent, you can say that FTIR is very useful instrument and pharmaceutical industry.

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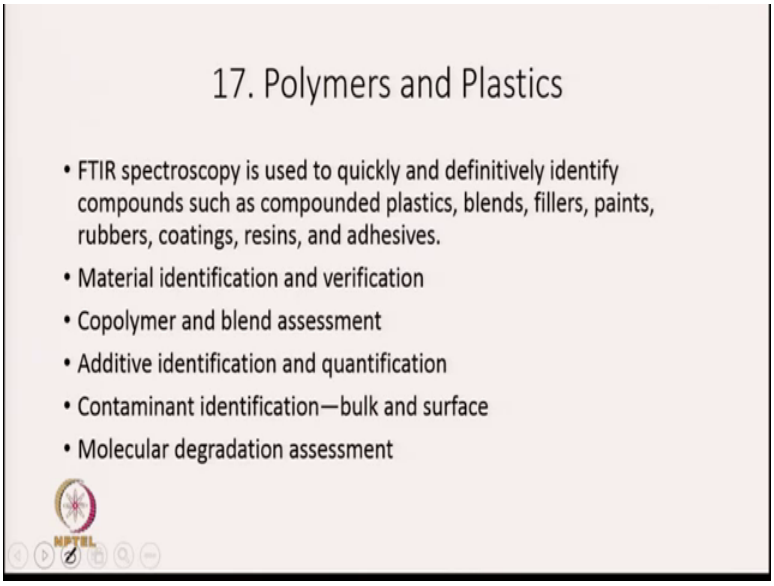

FTIR can also be used in the polymers and plastic industry. It can quickly and definitively identify compounds such as compounded plastics, blends, fillers, paints, rubbers, coatings, resins and adhesives. It can be used for material identification and verification. Tt can be used for copolymer and blend assessment, it can be used for additive identification and quantification.

It is used for contaminant identification, contaminant can be of different types, it can be a bulk contaminate or surface contaminate. All polymers are prone to degradation to different extent, and FTIR can be used to look at molecular degradation.

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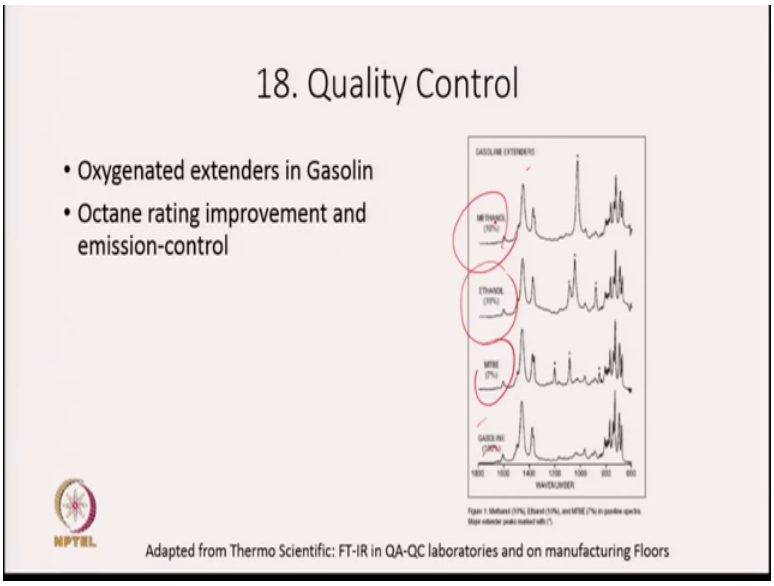

FTIR is quite often used for quality control, for example, for looking at oxygenated extenders and gasoline or to improve octane rating improvement and emission control. There you can see there is a gasoline extender and here you have methanol. So, this is your gasoline 100 percent this is 7 percent MTB, ethanol 10 percent and methanol 10 percent and just by looking at the gasoline you can tell what is the amount of these extender present in the gasoline and that can tell you about quality, quality of that gasoline sample.

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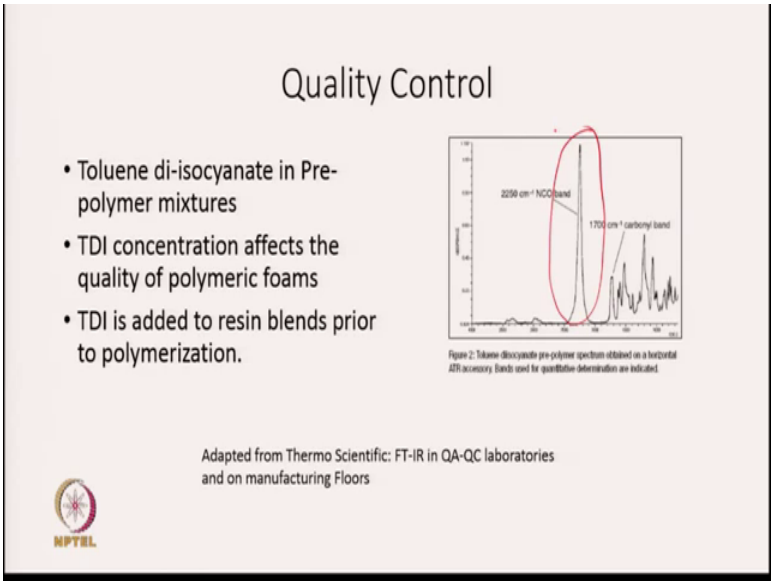

FTIR can also be used for TDI and to know the TDI concentration which is basically very important for quality of polymeric forms. TDI is basically your Toulene di-isocyanate and that is generally mixed in pre polymer mixtures and their concentration affect the quality of polymeric forms. So, FTIR can be used to check the quality by checking the TDI concentration in the sample, TDI is generally added to resin blends prior to polymerization. So, here is your 2250 centimeter inverse that corresponds to NCO band of Toulene di-isocyanate. So, just by looking at this you can tell what is the concentration of TDI and that tells you about quality of polymeric form.

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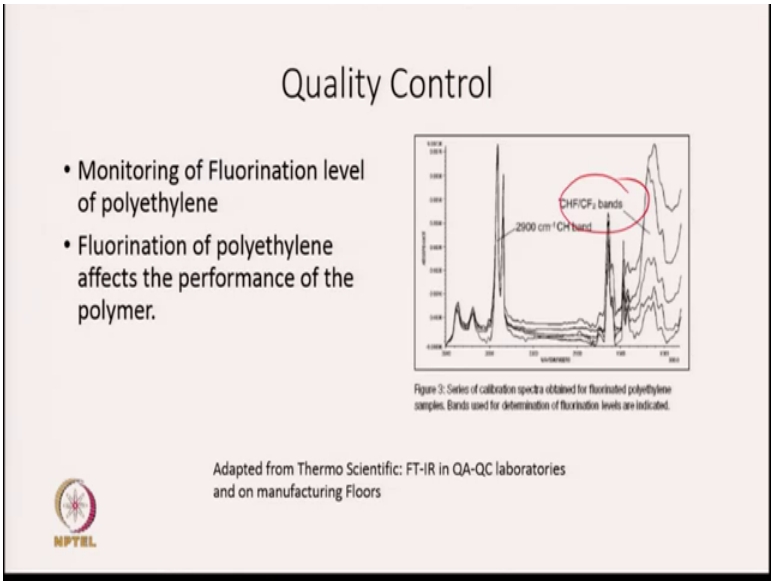

You can also monitor fluorination level of polyethylene. Fluorination of polyethylene affects the performance of polymer. So, just by looking at this band CHF and CF2 band, we can tell you about what is fluorination level of polyethylene, what is the fluorination level of polyethylene.

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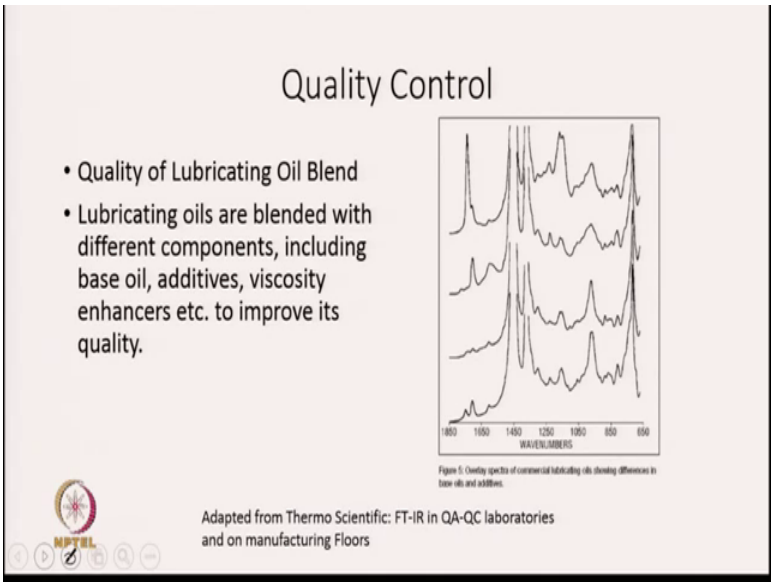

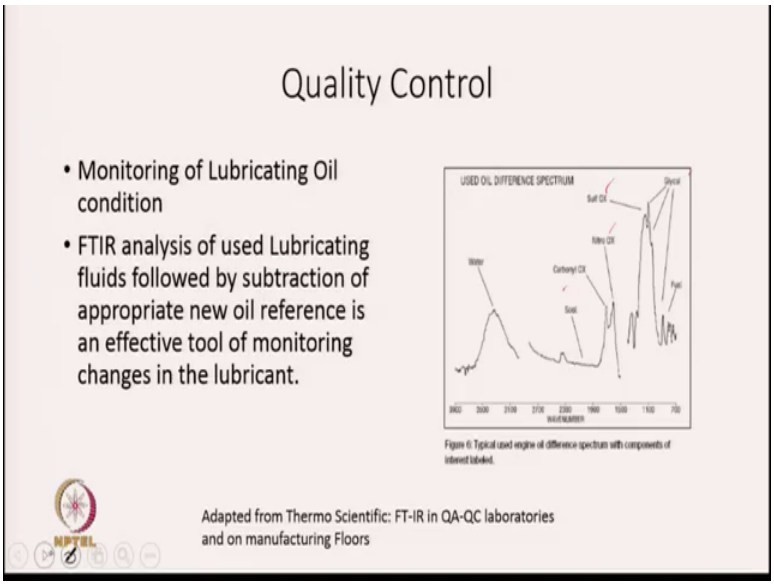

You can also know the quality of lubricating oil blend using FTIR spectroscopy. Lubricating oils are blended with different components including base oil, additives, viscosity enhancer and they are generally use to improve its quality. And just by looking at the spectrum, you can tell what is the amount of different adhesives and lubricating oil blend and that is important for quality control process.

You can also know the condition of lubricating oil, so you can monitor lubricating oil condition and that generally is done by taking the spectrum of the lubricant with the oil reference, with the oil reference and that tells you about how old is your lubricating oil, these are the different kind of stuff, you can look at different kind of components of lubricants you can look at and that tells you about quality of that lubricant.

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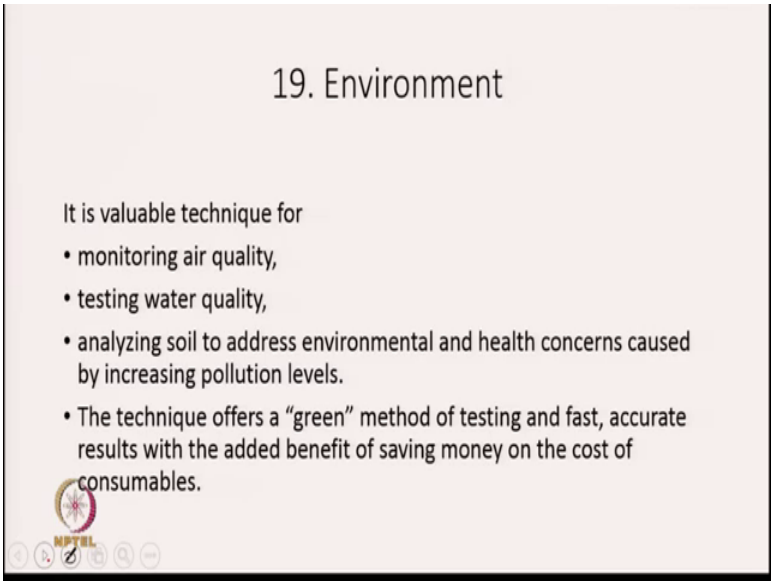

FTIR can also be used for environment, it is a very important technique to monitor air quality, it is very important technique to measure water quality, soils can be analyzed to know what is the pollutant level in the soil. And that is useful to address environmental and health concerns. This is also a green method of testing and fast accurate, you there is several advantages of using FTIR spectroscopy.

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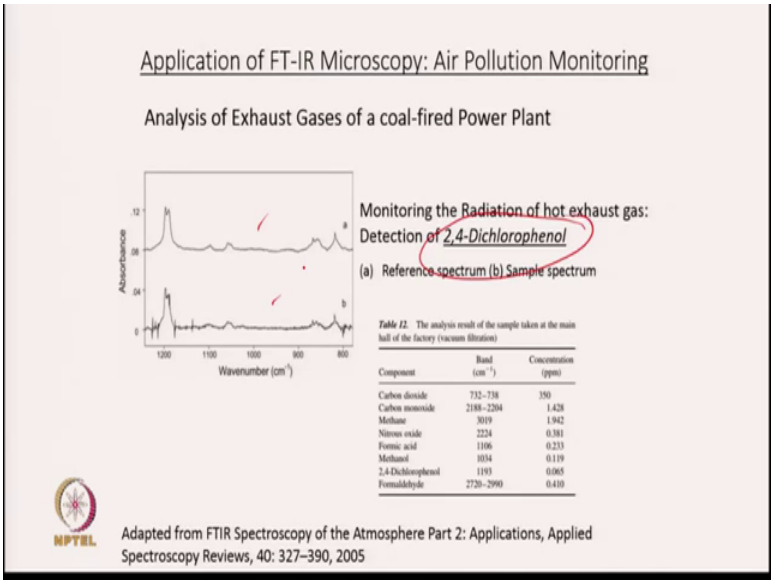

So, this is one example where FTIR is used for air pollution monitoring and here you can monitor 2, 4 dichlorophenol and A is your reference spectrum and B is your sample spectrum.

So, just by comparing the spectrum of reference 1 and the exhaust gas of coal fired power plant, you will be able to know the level of pollutants, the level of pollutants.

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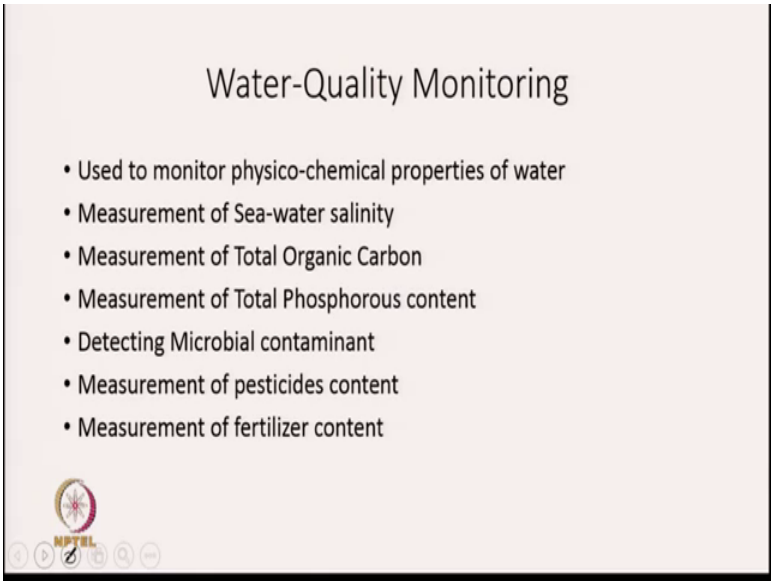

It can also be used for water quality monitoring. You can monitor physico chemical properties of water, it can be used for measurement of seawater salinity, it can be used for measurement of total organic carbon, it can be used for measurement of total phosphorus content, it can be used for detection of microbial contaminant, it can tell you about what is the amount of pesticides in the water sample and it can also be used for measurement of fertilizer content.

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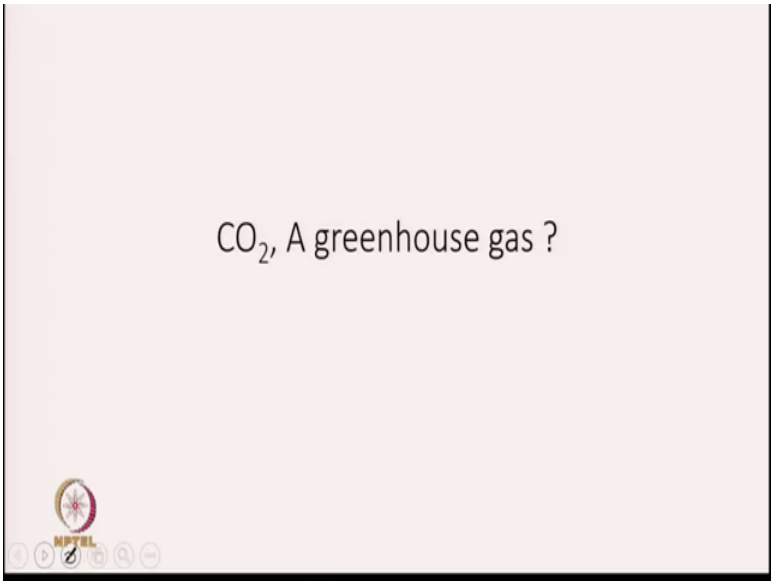

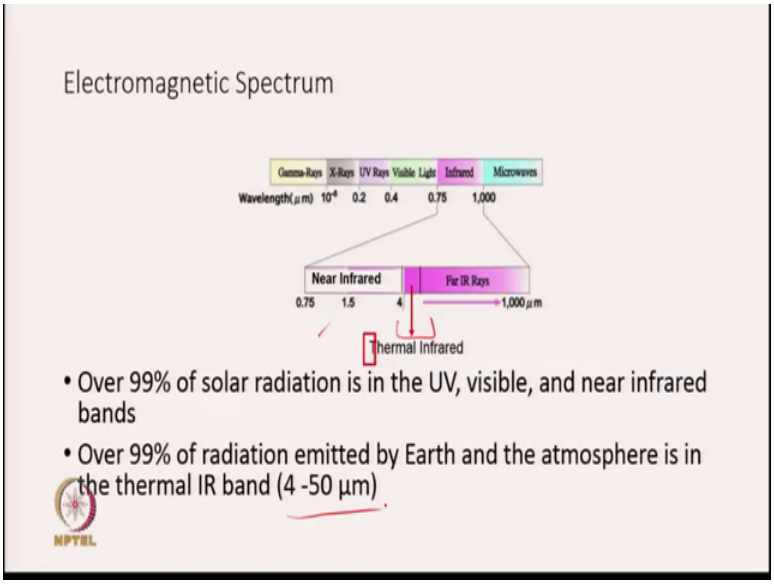

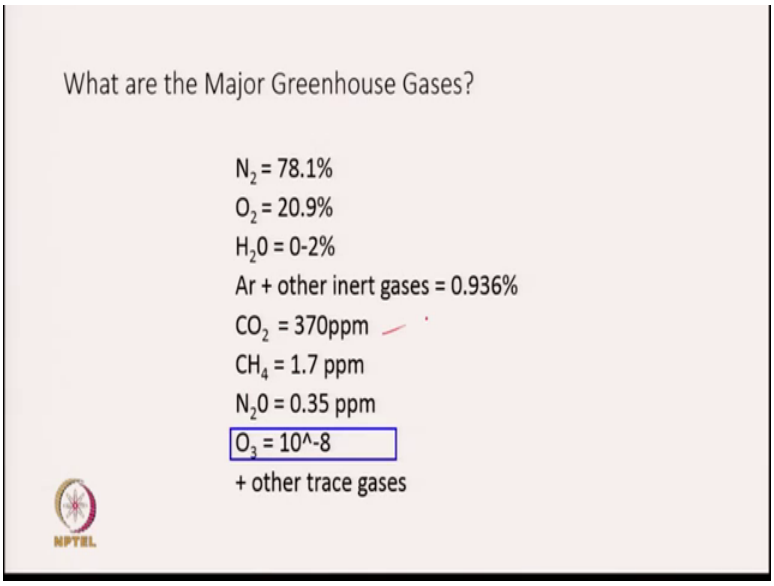

These are the examples where we can use FTIR spectrum and you can see that there is a wide range of application of FTIR. FTIR can also tell you, our principles of FTIR, if you know principles of FTIR, it can also tell you why CO2 is a greenhouse gas. So, infrared comes between 0.75 to 1000 micrometer. And you have 3 regions in infrared - near infrared, thermal infrared and far infrared region. 90 percent of solar radiation is in the UV visible and near infrared range. but the 90percent of radiation emitted by Earth and the atmosphere is in the thermal IR bands.

So, you are between so, 4 to 15 micrometers. That is what is the radiation emitted by the Earth. Now what happens is, if you look at the amount of gases in the atmosphere, N2 and O2 constitute major portion of the atmosphere, but they are not called greenhouse gases, because N2 O2 does not absorb IR radiation. It is CO2 which absorbs IR radiation and the global warming is due to absorption of IR spectrum by CO2 and that is why CO2 is called greenhouse gas, CO2 is called greenhouse gas.

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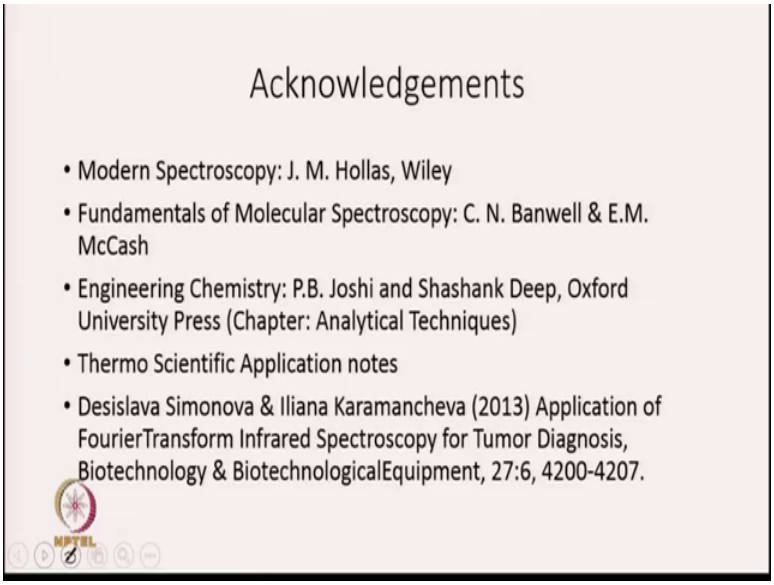

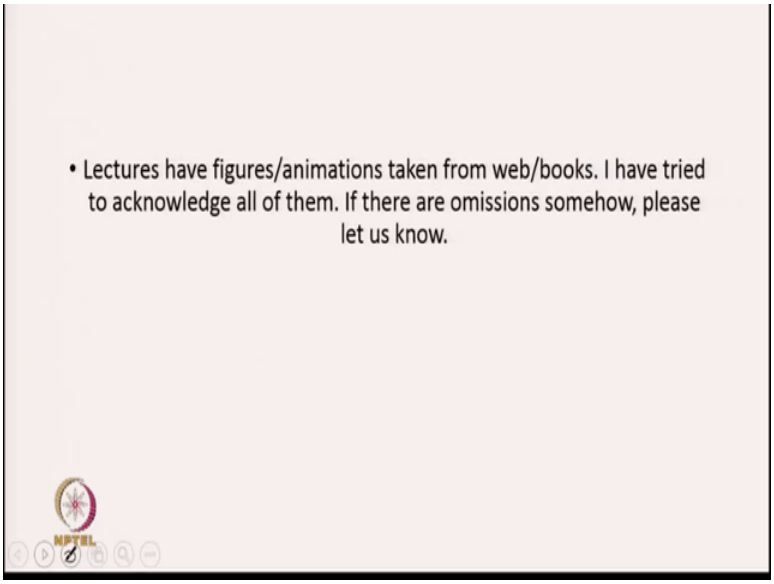

So, we have looked at the theory of IR spectroscopy, application part and we also asked some why kind of questions, I hope that you will be able to gain much from this chapter. For this, I would like to acknowledge this few books and papers which I have used for preparing the slides. I have also taken several figures from web or books, and I am thankful to them. And thank you very much for listening. See you, see you in the next lecture. Thank you. Bye.