Spectroscopic Techniques for Pharmaceutical & Biopharmaceutical Industries
Professor .Shashank Deep
Department of Chemistry
Indian Institute of Technology, Delhi
Lecture 1
Introduction to Spectroscopy-I

Dear students, welcome this NPTEL course on Spectroscopic Techniques for Pharmaceutical and Bio Pharmaceutical Industry. I am Shashank Deep a professor in the department of chemistry of Indian Institute of Technology, Delhi.

I have opportunity to teach a physical chemistry courses on spectroscopy, thermodynamics and kinetics, I have also taught bio physical chemistry and taken bio chemistry practical, our research area is based on application of spectroscopic and microscopic to look at different processes associated with protiens.

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In this course three assistance will be helping me, Komal Singh Khatri who has done PHD under my guidance, Shilpa Sharma and Vishaka Goswami who are doing PHD currently under me.

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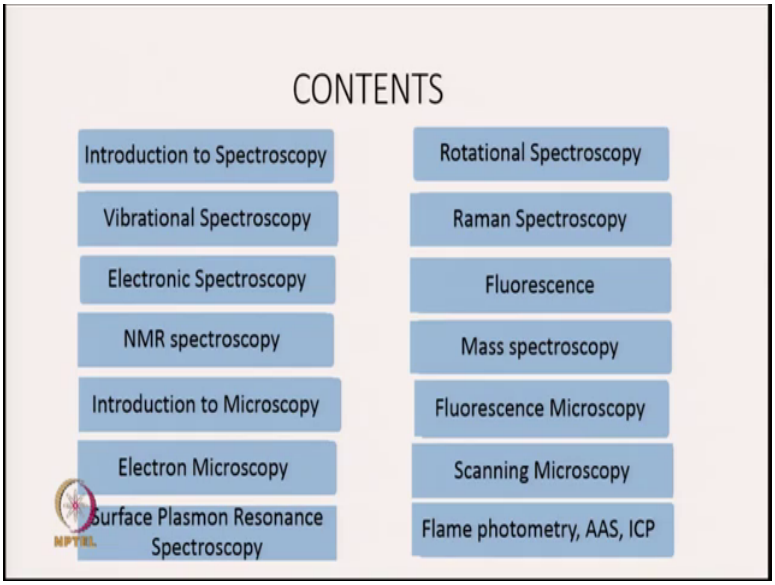

First we will start with introduction to Spectroscopic, we will talk about interaction of electromagnetic radiations with different materials and then we will go to your different kind of spectroscopic, vibration spectroscopic, rotational spectroscopy, Raman spectroscopy, electronic spectroscopy and we will go to fluorescence.

We will study NMR spectroscopy and mass spectroscopy, we will also discuss microscopy, basics of microscopy and then we will one by one we will discuss fluorescence microscopy, electron microscopy and scanning microscopy. We will also discuss surface Plasmon resonance spectroscopy and flame photometry.

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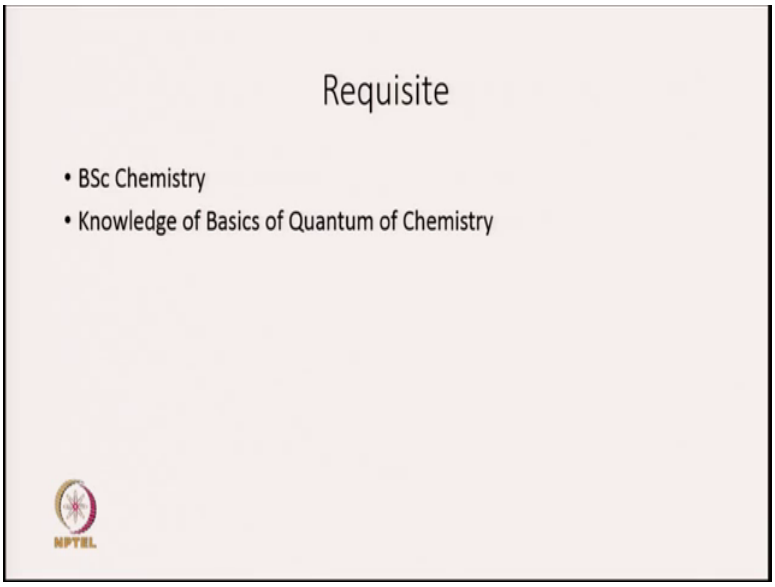

The requisite for this course is you must have done BSc in Chemistry, you should have knowledge of basics of phantom chemistry,

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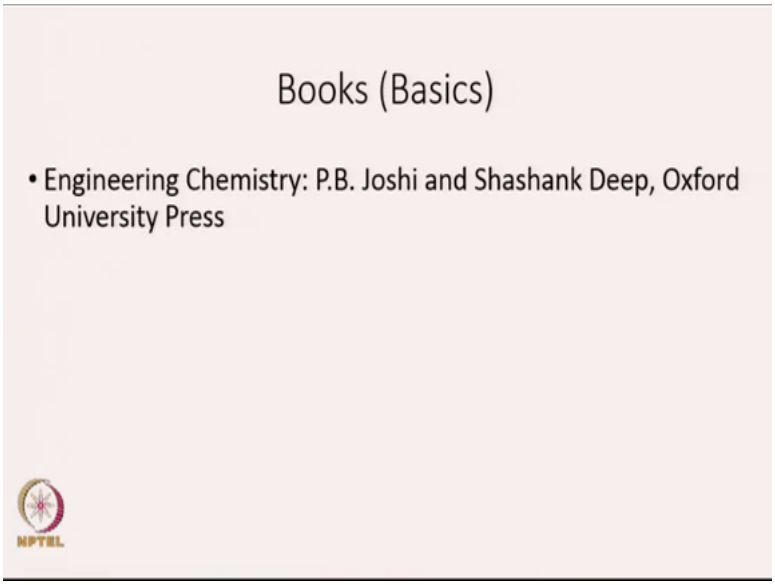

The books which I am going to refer is your one basic book which is engineering chemistry by P.B Joshi and myself, so this is a book which is coauthored by me and this has come from Oxford university press.

Good thing about that is it gives you about basic of physical chemistry and other chemistry and also it discuss the applied part of the chemistry, so utilization of chemistry in different kind of applications.

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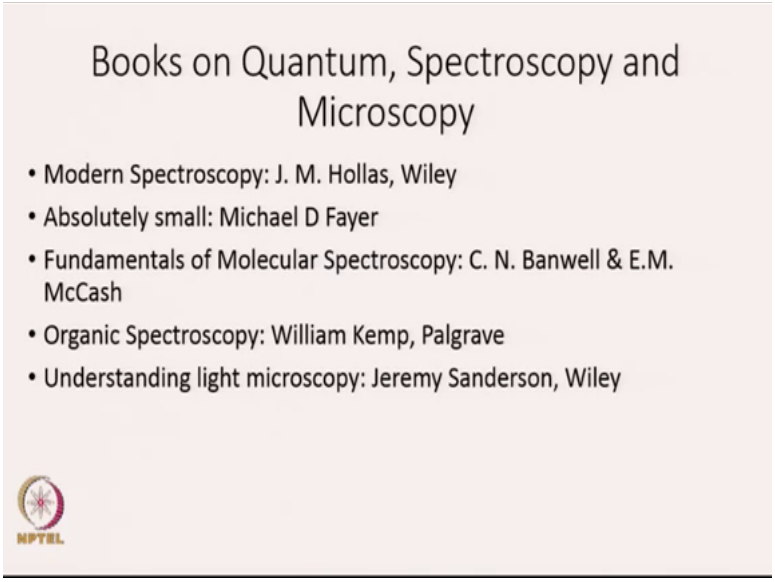

Then we will refer to books on Quantum, spectroscopy and microscopy a book which we are going to refer quite often is modern spectroscopy by Hollas and this is from Wiley publication, absolutely small by Michael D Fayer is a very nice book to read and I will be referring to this quite often and then fundamentals of molecular spectroscopy by Banwell and Mccash.

These are all about physical part of spectroscopy, then I will also go to organic spectroscopy and the book which I am going to refer is a book by William Kemp from Palgrave publication and for microscopy I will be referring to understanding light microscopy by Jeremy Sanderson which is from Wiley publications.

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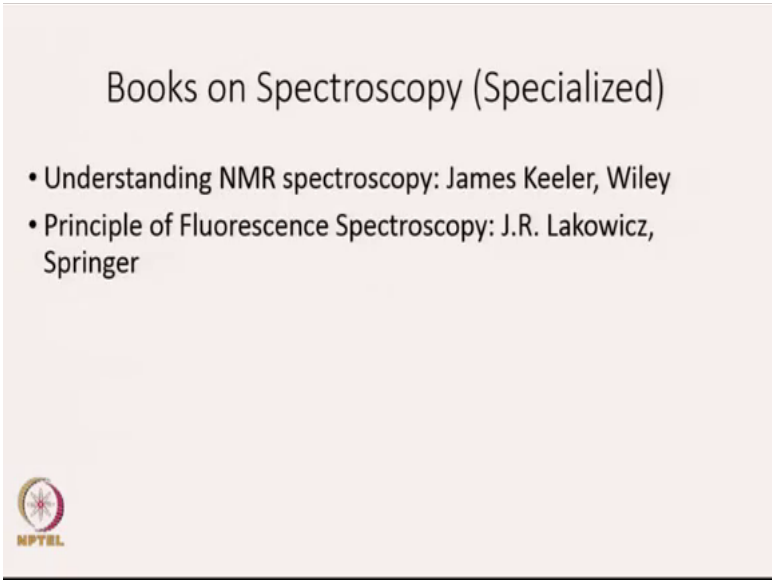

We will also look on some specialized book on spectroscopy for example for NMR spectroscopy particularly when we are discussing principles of NMR spectroscopy I will be referring to understanding NMR spectroscopy by James Keller this is also from Wiley publications and then a fluorescence I will be referring to principles of fluorescence spectroscopy by Lakowicz which is from Springer.

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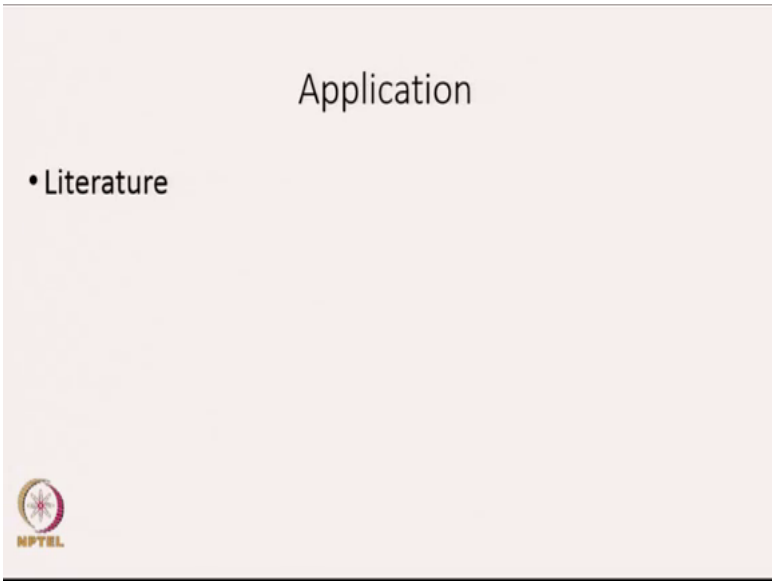

For applications part I will be going to different literatures and I will be taking one paper out of it and I will discuss you and this is particularly important when I am discussing applications in different areas in different areas which utilizes the spectroscopic techniques.

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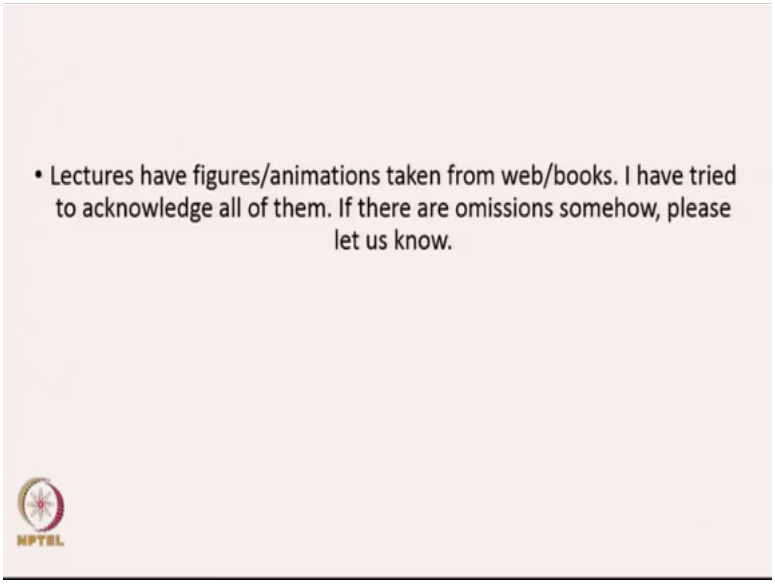

We have taken lot of figures, animations from web, books. I have tried to acknowledge all them, if there are omissions then please let us know.

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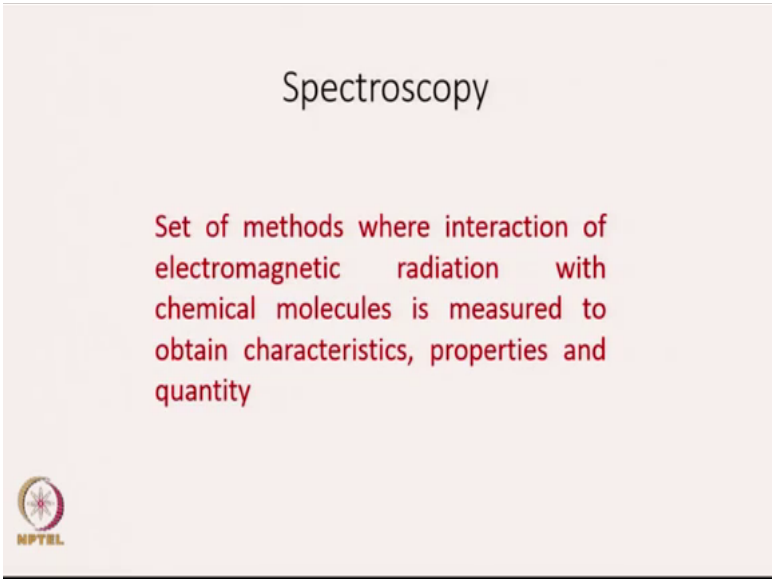

So what is spectroscopy? Spectroscopy is set of methods where interaction of electromagnetic radiation with chemical molecules is measured to obtain characteristics, properties and quantity.

So there are two part of this, one is your interaction of electromagnetic radiations with chemical molecules and here we will look at how does electromagnetic magazine radiations interact and what is the principle behind there interactions and then the second part is your characteristics, properties and quantity that is your application part. So in applications we will

discuss how to use spectroscopy to obtain characteristics, properties and quantity of different chemical molecules or bio-molecules.

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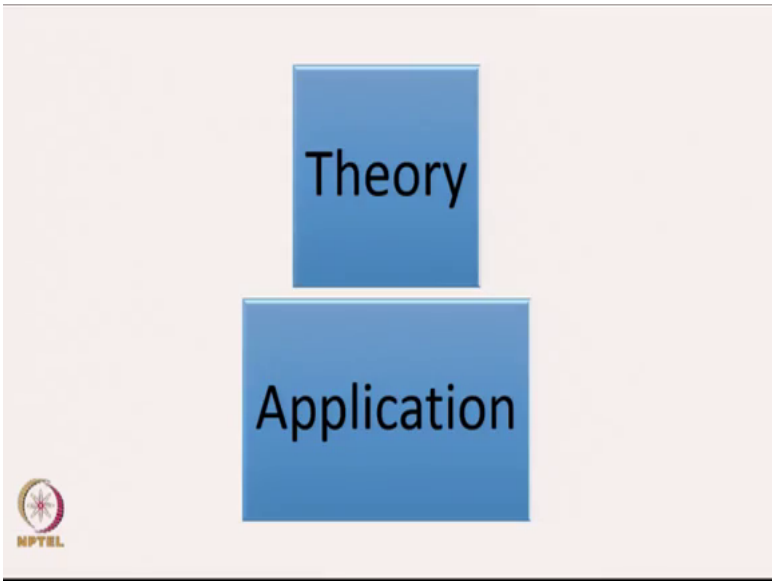

So this course has two more very important part theory and application. So in theory part I will be discussing the physical chemistry of the spectroscopy, in application part I will be going to organic molecules, bio-molecules and medicines to show you how spectroscopy can apply.

So this the combination of both physical chemistry part of spectroscopy, organic chemistry part of spectroscopy and then spectroscopy application in medicine spectroscopy application in medicines.

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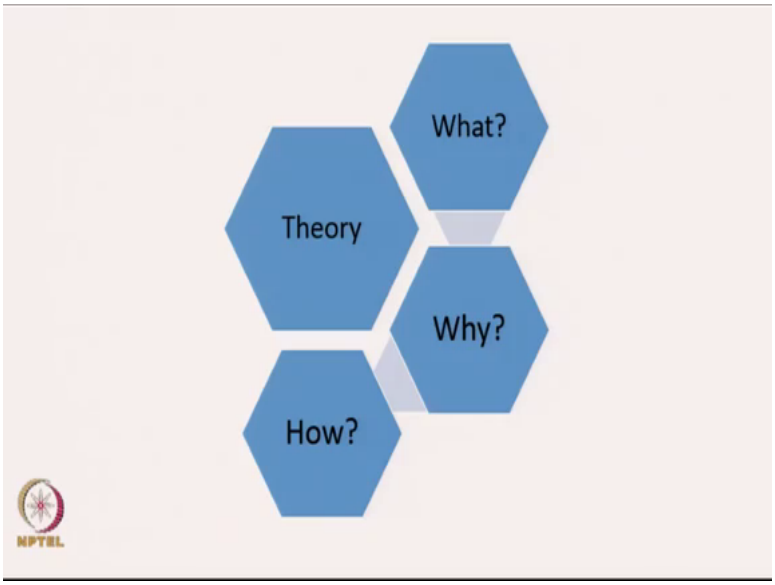

So in theory part we will discuss three different kind of questions. What? What happens? The second is why? And the third is how? We will go one by one and discuss what I mean by this three type of questions and how a spectroscopy can help in knowing the answers of this question knowing the answers of this questions.

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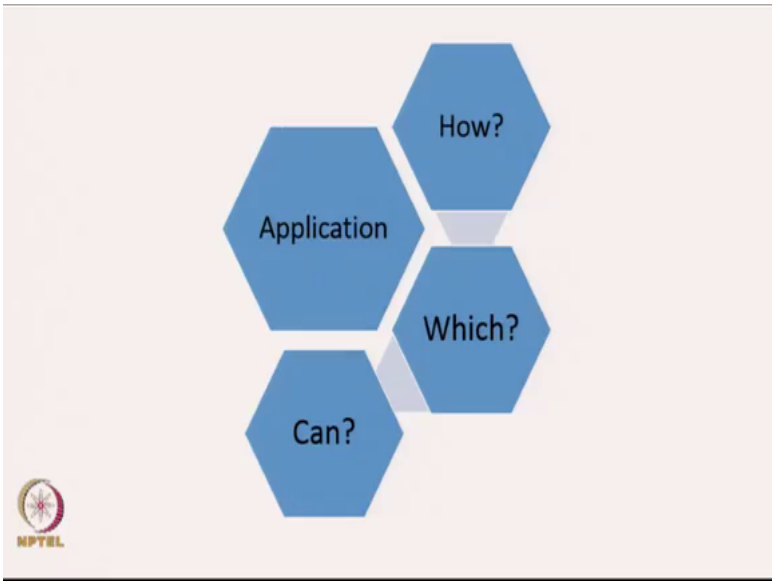

Now second part applications again we will discuss three different kind of questions How? Which? And can? How? Which? And can?

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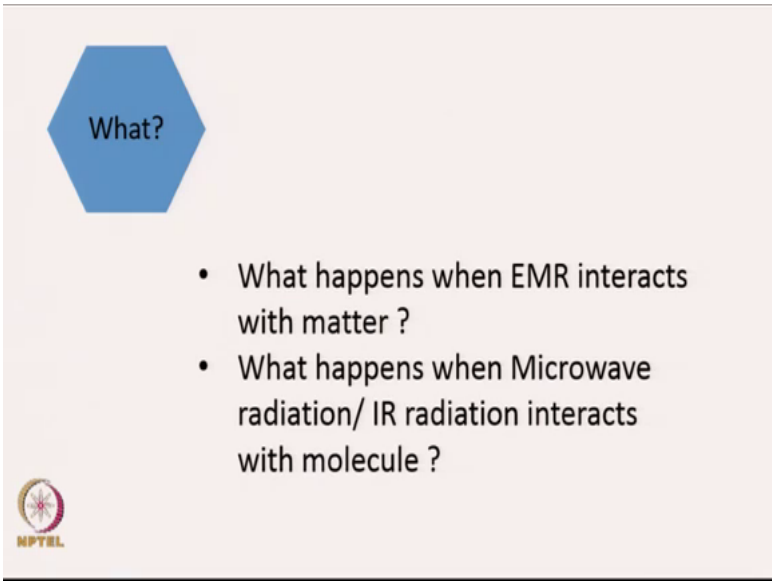

So for example if are discussing what then we will discuss what happens when electromagnetic radiations interact with matter. So in this course we will going to discuss

what happens when electromagnetic radiations interact with matter, what happens when microwave radiations or IR radiations interact with the molecule . What is the difference in the event which takes place when a molecule interacts with microwave radiation or molecule interact with IR radiation?

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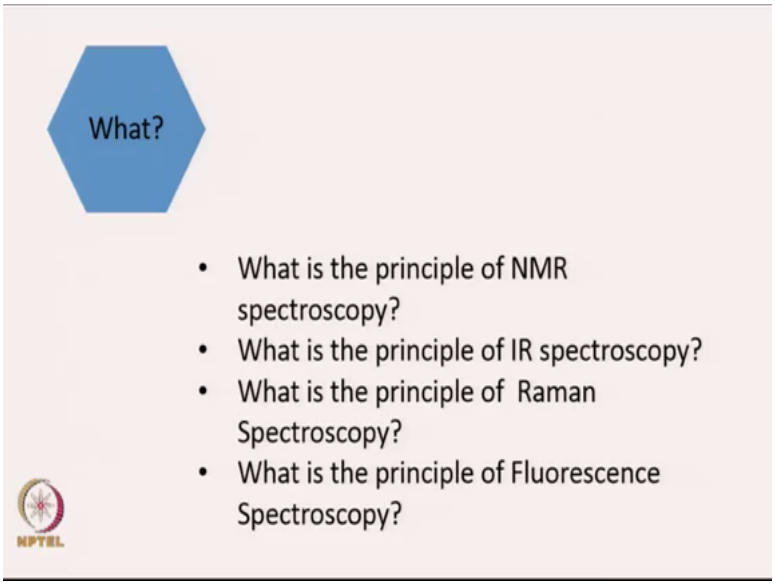

We are going to discuss what is the principle of NMR spectroscopy what is the principle of IR spectroscopy? What is the principle of Raman spectroscopy? And what is the principle of fluorescence spectroscopy?

So we will go one by one to different kind of spectroscopy, and we will discuss the priorities behind it.

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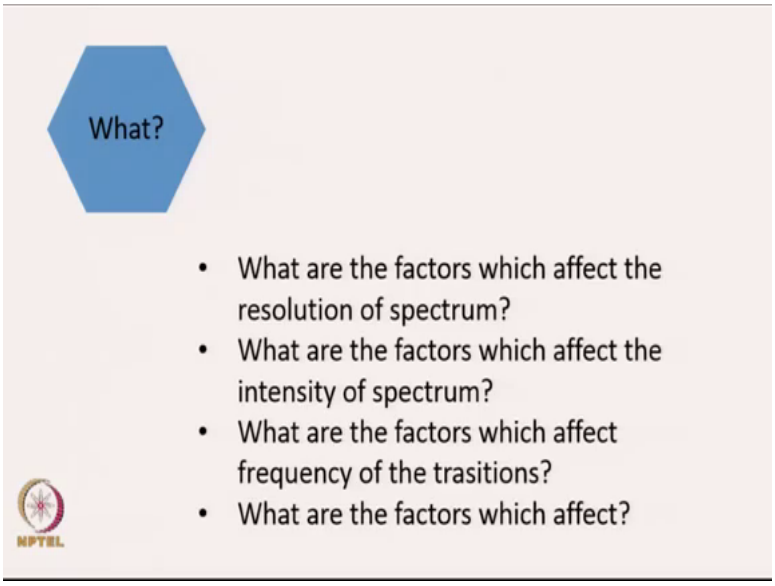

Then we are going to ask some questions like this what are the factors which can effect resolution of spectrum? So resolution of spectrum of broadness of spectrum depends on how many factors? What are the factors which can effect intensity of spectrum and what are the factors which effect frequency of transitions? What are the factor different kind of spectroscopy? Different kind of spectroscopy?

So we will discuss the factors which effects the resolution, factors which effects the intensity the factors which effects the frequency of transition and factors which effects whether a particular molecule is IR active or not? Microwave active or not? These are the things we are going to discuss in this course.

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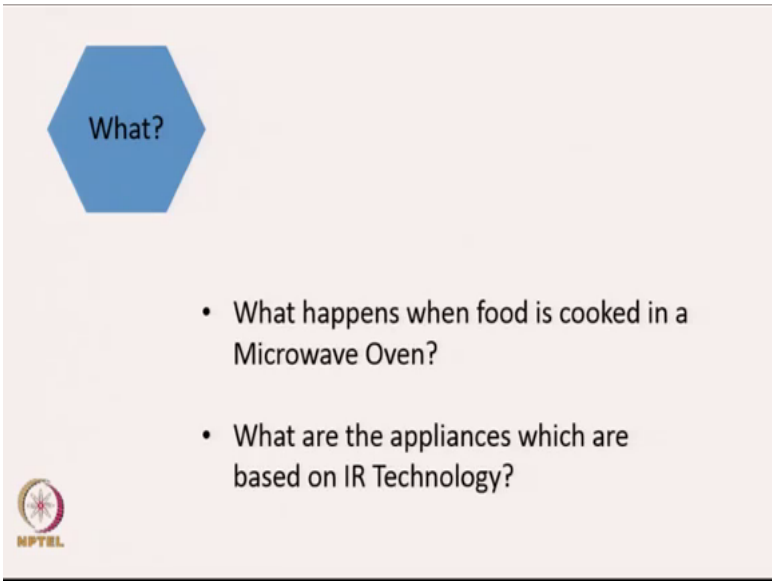

We will also discuss what happens when food is cooked in a microwave oven? What are appliance which are based on IR technology? What are the appliance which are based on NMR technology? What are the appliance which are based on X-ray spectroscopy?

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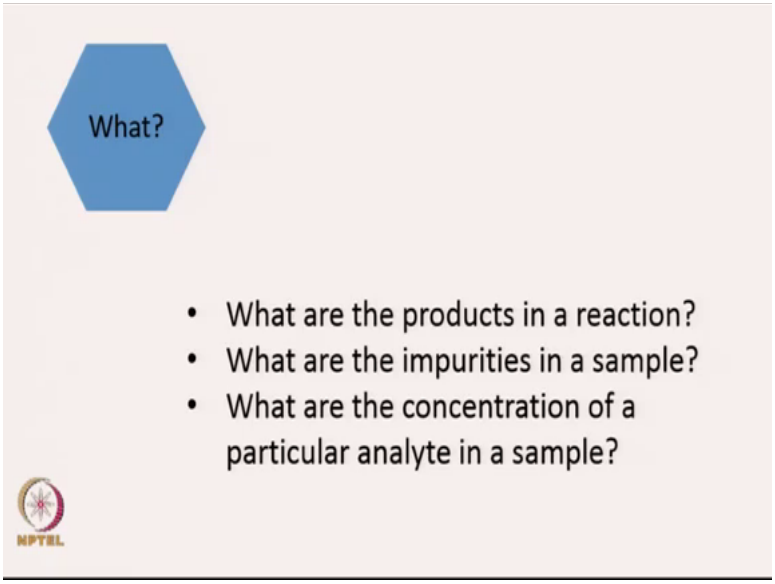

We will also discuss what are products in the reaction? What are the impurities in a sample? What are the concentration of a particular analyte in sample? So this kind of questions we are going to ask. Then come to second part of the question why?

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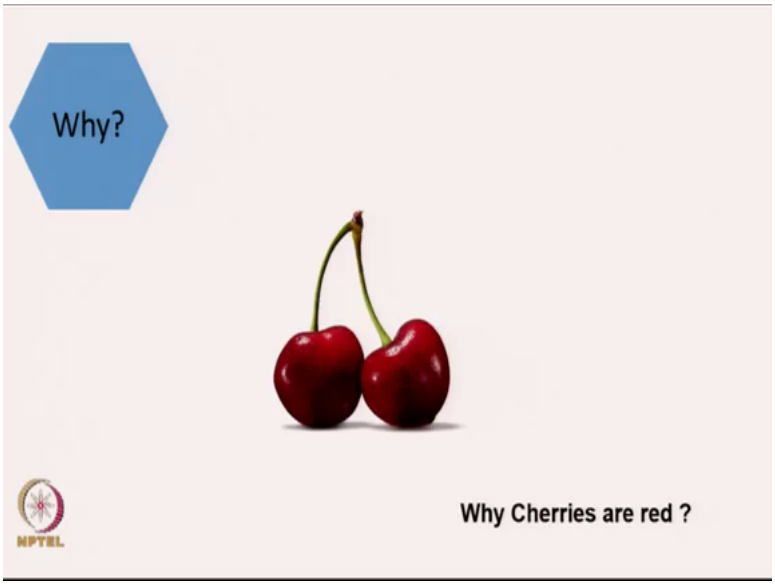

So in this course we are going to discuss why cherries are red?

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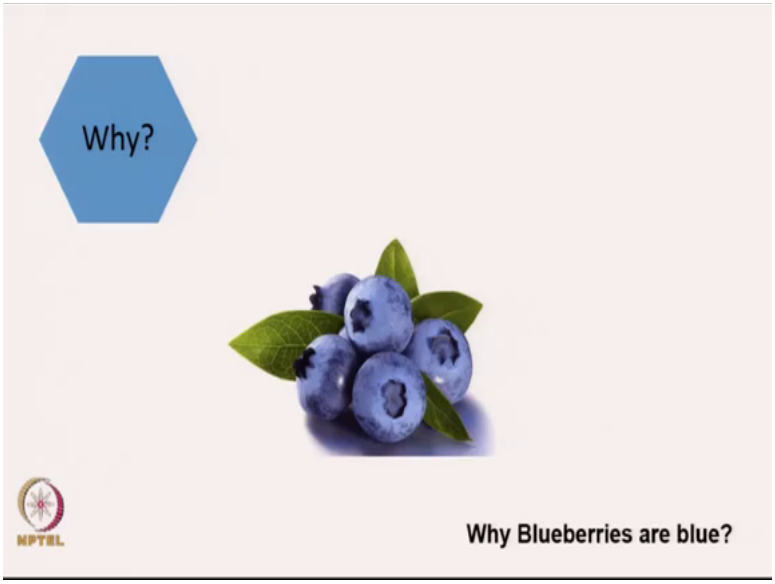

Why blue berries are blue? Why olive is green? This kind of questions we are going to discuss. Our world is colorful and different objects have different color, we are going to discuss why the particular object has that particular color.

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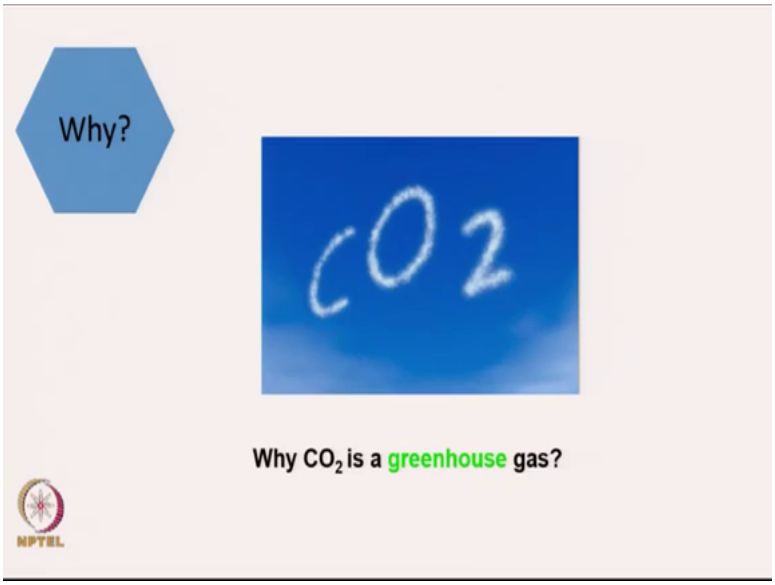

Then we are going to discuss why Co2 is greenhouse gas? So our atmosphere has different kind of gases and some are in greater amount in comparison to Co2 still Co2 is known as greenhouse gas, so what is the reason for that.

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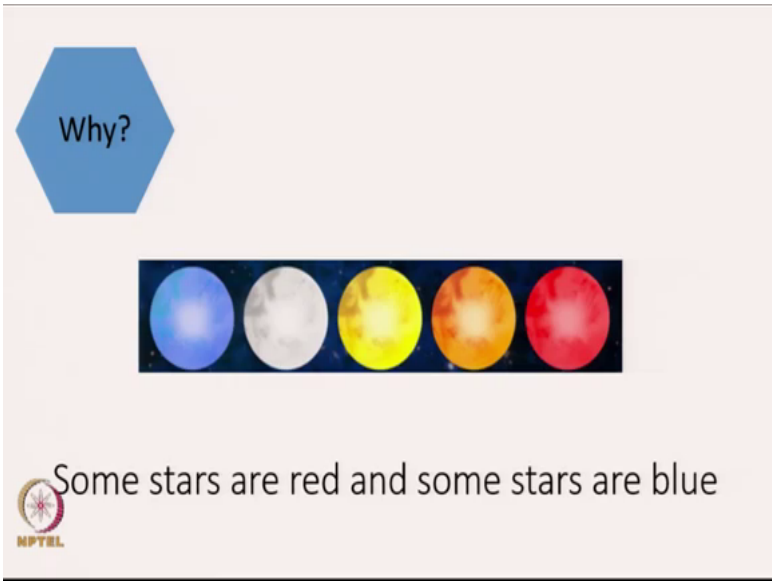

We are going to discuss why some stars are red and some stars are blue so when you look in the sky in the night time you can see some stars looks like red some stars looks like blue and some are in between them, we are going to discuss why their color is different? Why their color is different?

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If you look at sky during different times of the day for example during the day time the sky will look blue while when sun is sitting the sky will look red, so we will discuss why sky looks blue or red during different part of the day, Now we are also going so this is about why part.

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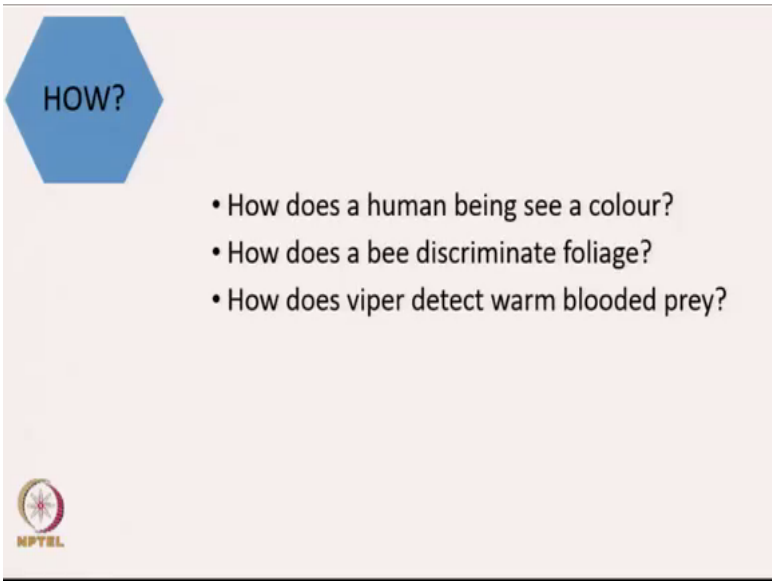

Now let's come to the next question. How? So first question we will ask that how does a human being see a color? Why to ask a particular color looks like that color? So apple look red? Or why blue berries look blue? So we are going to ask this question that how does we see a color?

We will also ask how does bee a discriminate foliage? How does bee discriminate foliage? We are going to discuss how does viper detect warm blooded prey? These are the questions which are related to spectroscopy and so we will discuss that how does a particular animal discriminate between two objects?

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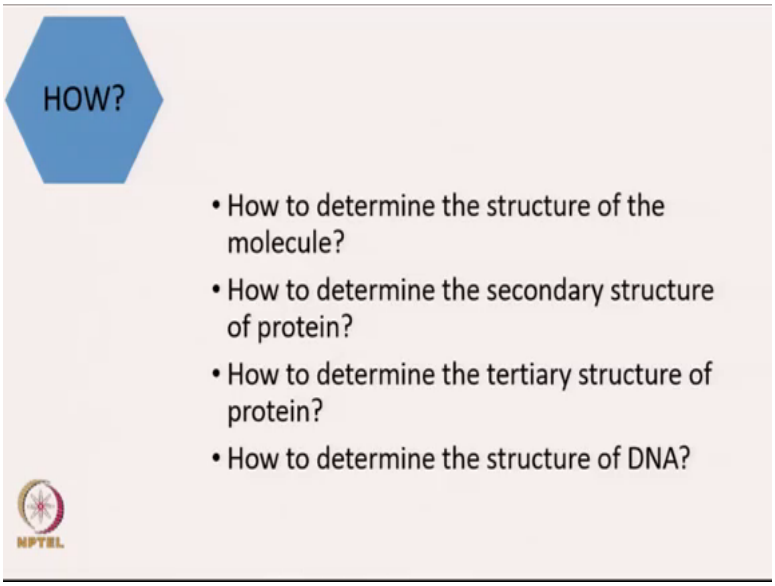

We are going to discuss how determine the structure of the molecules, and this is very big field not only molecule we are going to even discuss how to determine the secondary structure of protein? How to determine the tertiary structure?

So for example how to determine whether a protein is alpha helical protein or beta seed protein? Similarly we are going to discuss whether a protein is folded or unfolded can be use as spectroscopy to know the confirmation of the protein, we are also going to discuss how to determine the structure of DNA molecule? Not only about a structure, we are also going to discuss the concentration.

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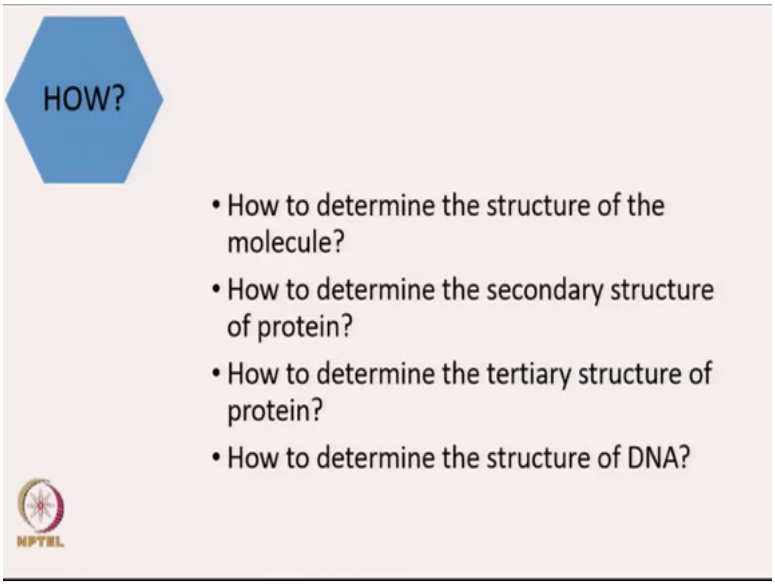

How to determine the concentration of a simple molecule? How to determine the concentration of protein in a particular sample? How to determine the concentration of DNA or RNA in a particular sample? And how to determine the amount of viable cells? So when cells are dying then how can we know how many cells have died and how many are viable?

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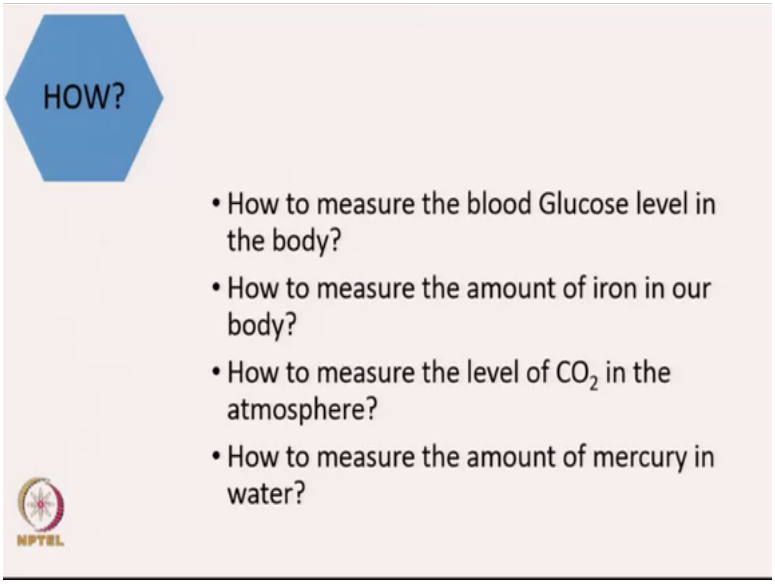

Then we are going to discuss how to measure the blood glucose level in the body? Since this is related this is very important if you consider a diabetic patient, we need to measure his blood glucose level so we are going to see how we can measure the blood glucose level in the body we want to know how to measure the amount of iron in the body.

Then if you look at the atmosphere and try to measure the amount of pollutants, how we are going to measure the amount of pollutants in the atmosphere. For example Co2 in the atmosphere, so we are going to discuss that.

We are going to discuss how to measure the amount of mercury in the water or amount of any toxic material in the water so this is related to your pollution. Then there are different sets of questions,

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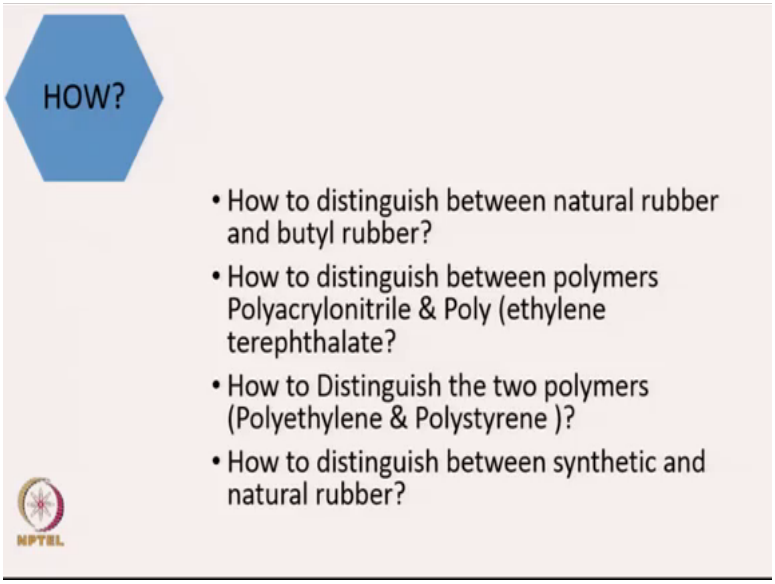

For example how to distinguish between natural rubber and butyl rubber? So this question is how to distinguish? So how to distinguish between polymers, for example poly acrylonitrile and poly ethylene terephthalate? How to distinguish between polyethylene and polystyrene? This are two different polymers which looks same so how we will distinguish between this two polymers? And then we are going to discuss difference between the synthetic and natural rubber.

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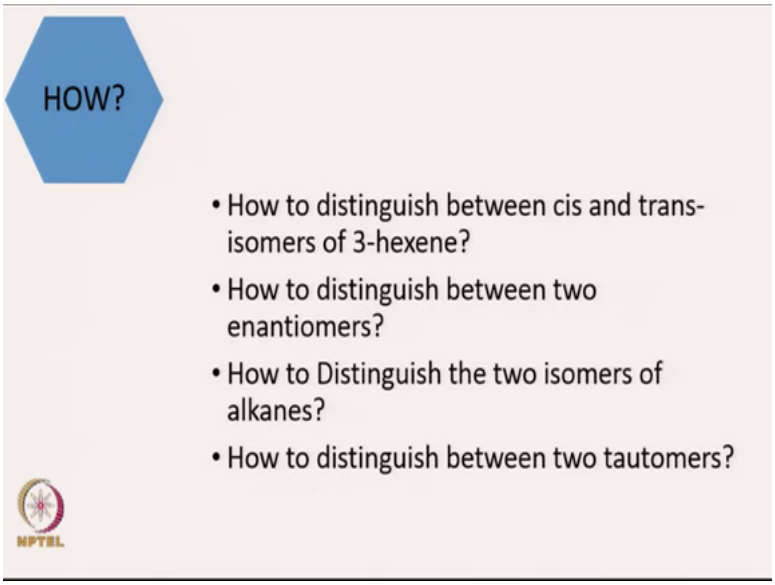

We can also ask question based on the distinction two different isomers for example we will ask question such as how to distinguish between cis and trans isomers of 3-hexene? How to

distinguish between two enantiomers? And How to distinguish between two isomers of alkanes? How to distinguish between two isomers?

So what are the ways to distinguish between two different confirmers? Two different isomers? Two different enantiomers? And two different tautomer's? We are going to discuss that.

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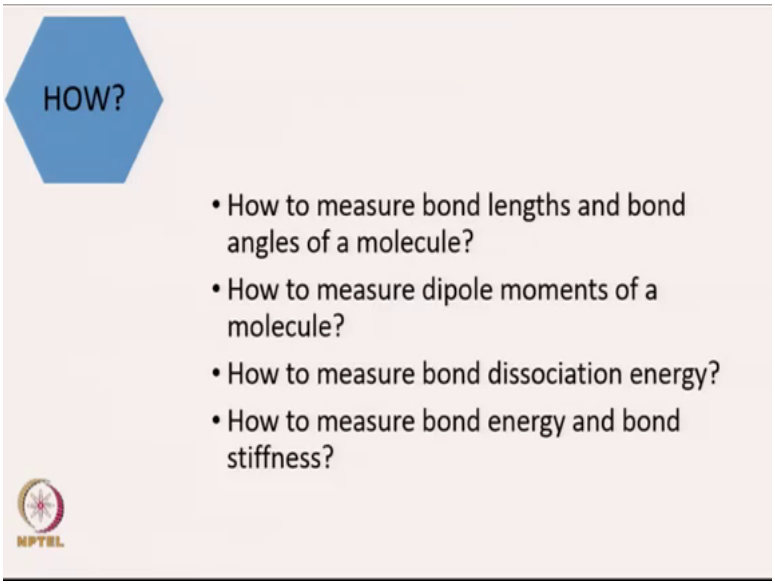

We are also going to discuss how to discuss bond lengths and bond angle of a molecule? We are going to discuss how to measure different physical properties? Different physical parameters of a molecules, for example bond length, bond angles. We are going to discuss how to measure dipole moments? Bond dissociation energy? And how to measure bond energy and bond stiffness? So this are the few of the physical parameters we are going to see how to measure them.

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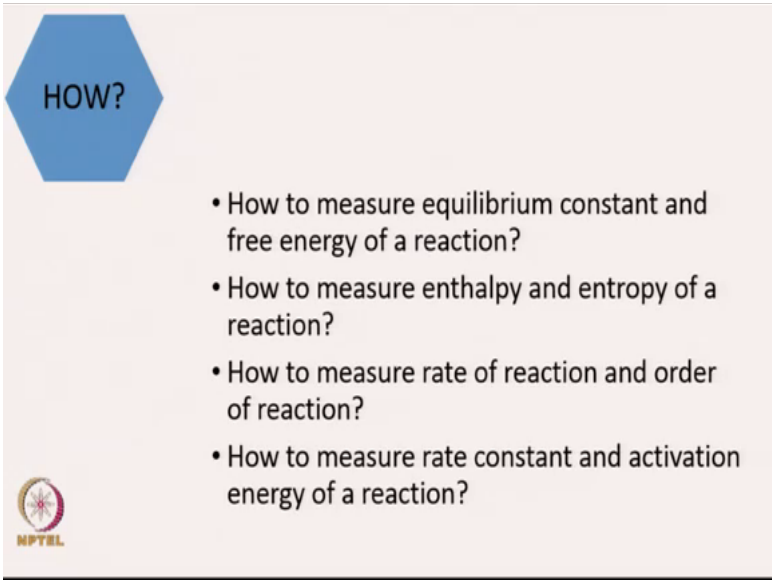

For a reaction we will discuss how to measure equilibrium constant of free energy we can also see that we can utilize spectroscopy to measure enthalpy and entropy of a reaction, we can also measure rate of reaction and order of reaction with the help of spectroscopy, and if you measure rate of reaction with respect to temperature we can also measure your activation energy, we can measure rate constant of the reaction and the activation energy of the reaction. Now this is about how we will do that?

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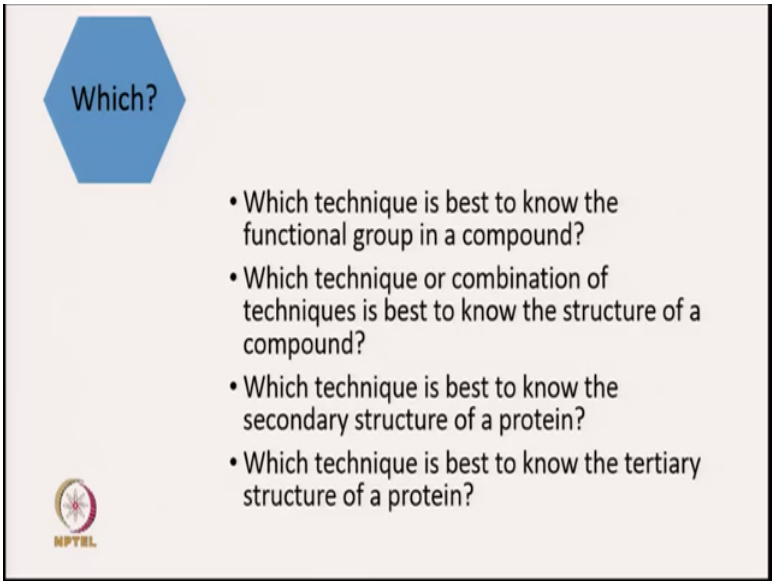

Now next question is which? So we are going to ask which technique is based to know the function group in a compound. So every technique every spectroscopy for example IR spectroscopy is very off native lies to functional group in a compound, and microwave or

rotational spectroscopy is used for different purpose. So which technique is best to know the functional group in a compound? Which technique or combination of techniques is best to know the structure of a compound? Which technique is best to know the secondary structure of the protein? And which technique is best to know the tertiary structure of a protein?

So every spectroscopy technique has their own advantages and their own limitations and after doing this course you will be understanding what the limitations associated with particular kind spectroscopy is, or what is the strength associated with particular kind of spectroscopy, and that will help you to decide which spectroscopy will be suitable for particular kind of application.

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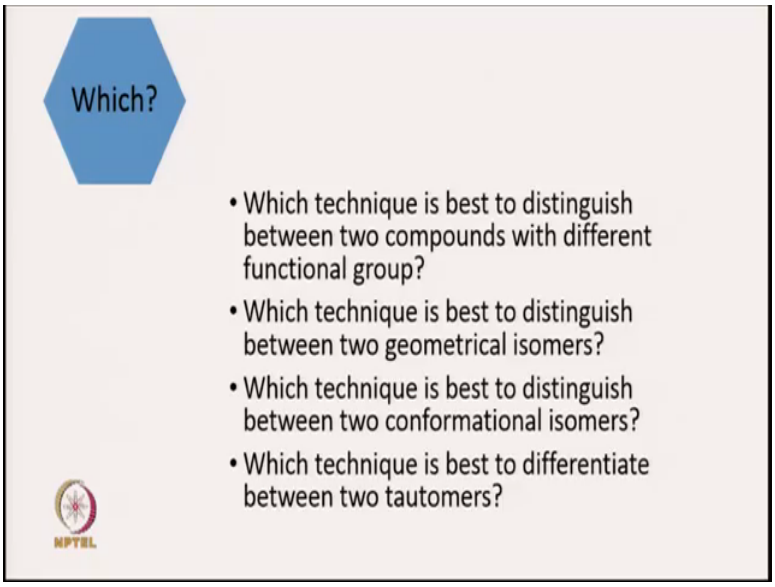

We are going to discuss which techniques is suitable to distinguish between two compounds with different functional group? Which techniques will be best between geometrical isomers? Which techniques is best in best to distinguish between two conformational isomers? And which techniques is best to differentiate between two tautomers?

So this structural features can be distinguished between two different molecule using one spectroscopy technique and we are going to discuss which spectroscopy technique is going to base for particular application for particular application.

So we just discuss spectroscopy can be use to calculate the different physical parameters

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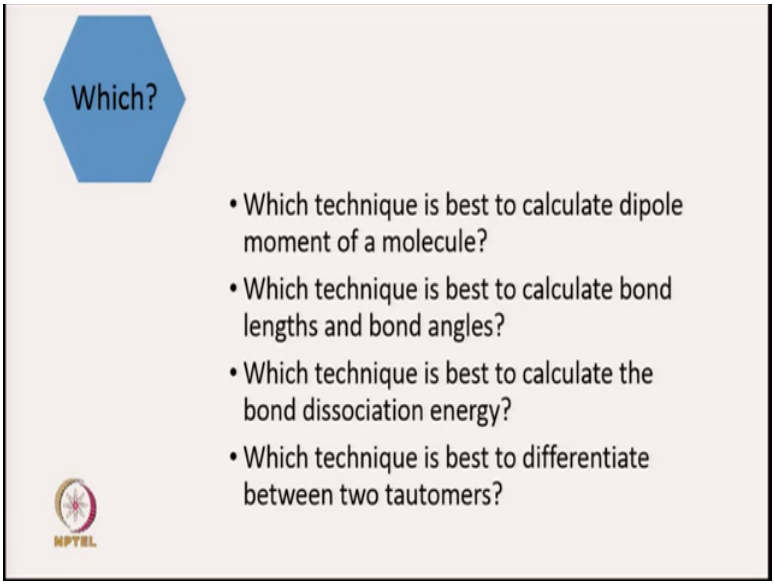

And so we are going to ask or we are going to see that which techniques will be best suited to calculate the dipole moment? Which techniques will be best suited to calculate the bond lengths and bond angles of a particular molecule? Which technique is best for calculating the bond dissociation energy? And which technique is best to differentiate between two tautomers?

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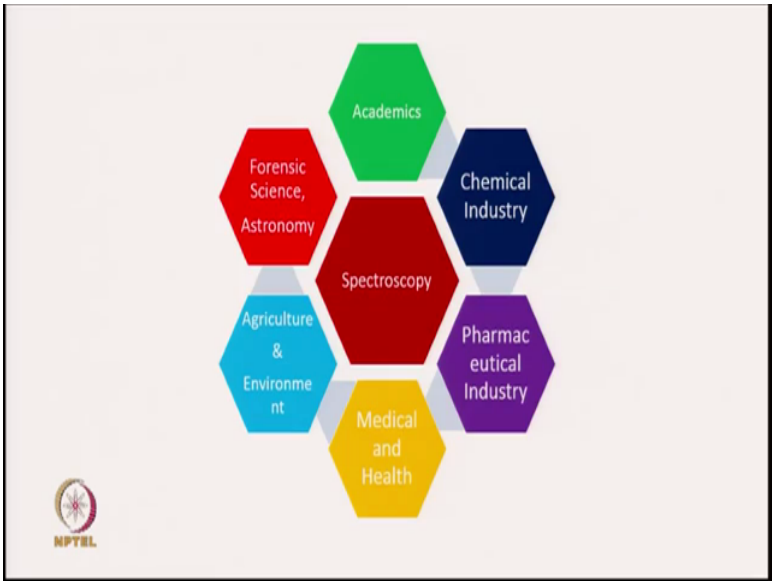

So spectroscopy is utilized by different industry apart from the academy of people, people from academy. So spectroscopy is used in chemical industry it is also used in pharmaceuticals, it has uses in medical and health, it is quite often used in agricultural and environment and it is also used in forensic science, in forensic science and astronomy. So we

will be discussing about how spectroscopy is going to be used in the different industry, where this industries uses the spectroscopy in which kind of application they utilized a spectroscopy.

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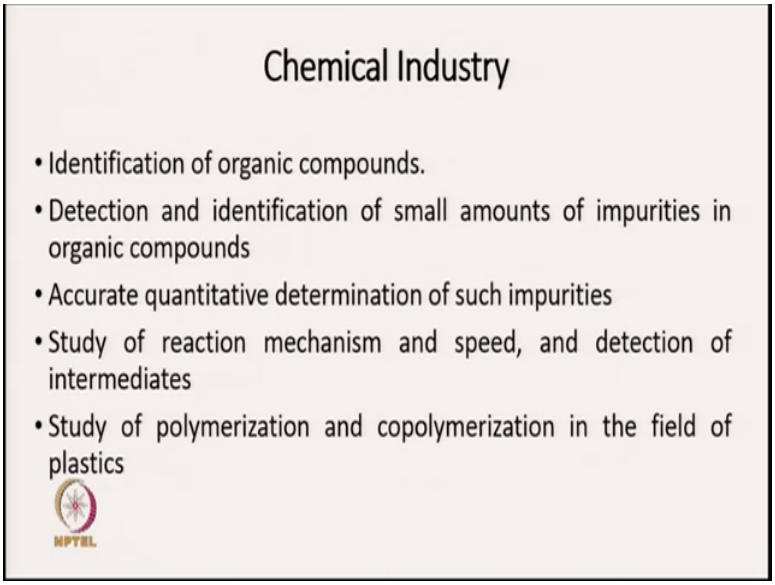

For example if you take a case of chemical industry the uses the spectroscopy in identification of organic compound, they also utilizes spectroscopy in detection and identification of well small amount of impurities in the synthesized organic compound, they also need the quantitative determination of such impurities so they often use spectroscopy to determine the amount of impurity, they also study reaction mechanism and rate of reaction, kinetics is very important and so spectroscopy can be use to study reaction, mechanism and speed and even In detection of intermediates. Spectroscopy, is quite often used for the study of polymerization and co polymerization which is related to the plastic industry.

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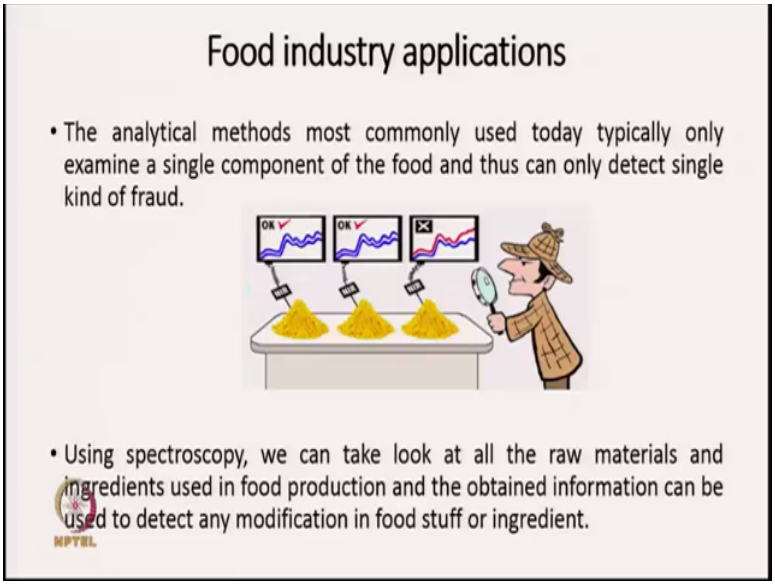

Spectroscopy is also used din food industry, it can tell you about every component of food, every component of food so if you have a food sample there are several different component you can look at each of the component and you can detect if the component the food is mixed with some unwanted material, so you can detect the kind of fraud if it has been with that food sample.

Using spectroscopy we can look at all the raw materials and ingredients used in food production and thus we can use that information to detect any modification in food stuff any modification in food stuff.

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Again spectroscopy is it will lies quite extensively in pharmaceutical industry and some of the applications are basic drug research and structural elucidation, so if you want to make a drug then you need to know the structure of the drug after formation and thus spectroscopy is essential tool in pharmaceutical industry not only in the synthesizing and characterizing the drug, spectroscopy is also used in formulation development and validation.

The another area where spectroscopy is quite often used in pharmaceutical industry is quality control processes for incoming and outgoing materials and finally all the drugs are packaged and so packaging is also tested in pharmaceutical industry using spectroscopy.

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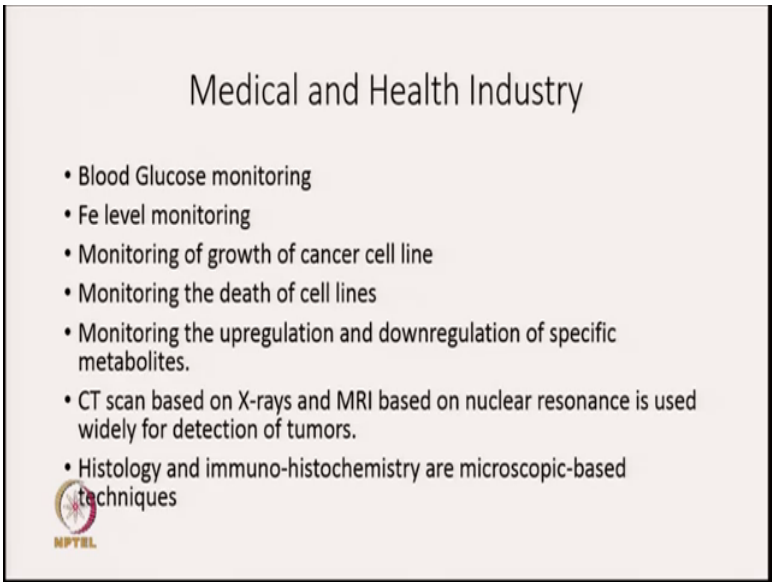

Apart from that spectroscopy is quite often used in medical and health industry for example if you want to blood glucose level spectroscopy can be an important tool you can monitor iron level of a person and tell whether he has anemia or not, spectroscopy is utilized in monitoring of growth of cancel cell lines, they also utilize to monitor death of cell lines.

You can regulate up monitor up regulation and down regulation of specific metabolites which will tell you have symptoms of a particular disease or not. CT scan based on X-rays and MRI based on nuclear resonance spectroscopy is widely used for detection of tumors and histology and immune-histochemistry are microscopic based techniques.

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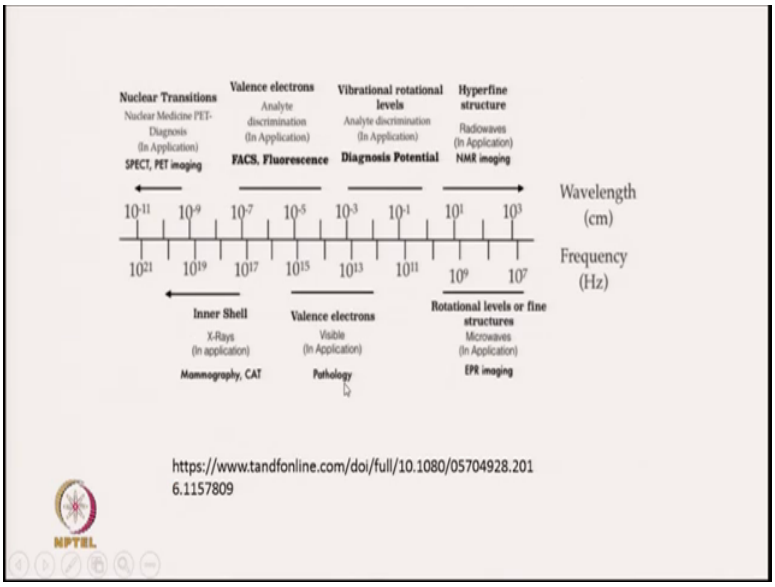

So here is your different spectroscopy and application for example nuclear transition is used in SPECT, PET imaging they are fluorescence is often used for analyte discrimination, vibrational rotational analytes is also used for analyte discrimination, in NMR is radio wave is quite often used for NMR imaging.

Your X-rays are used for CAT or mammography your visible spectroscopy is used in pathology and microwaves are used for EPR imaging. So nuclear transition is used for your SPECT and PET imaging, your fluorescence is used for analytical discrimination, vibrational rotational spectra is used for your analyte discrimination which has potential in diagnostic, hyper fine structure by using radio waves is used for NMR imaging, X-rays are used in mammography and CAT, visible spectroscopy is used in pathology and your microwaves are used for EPR imaging.

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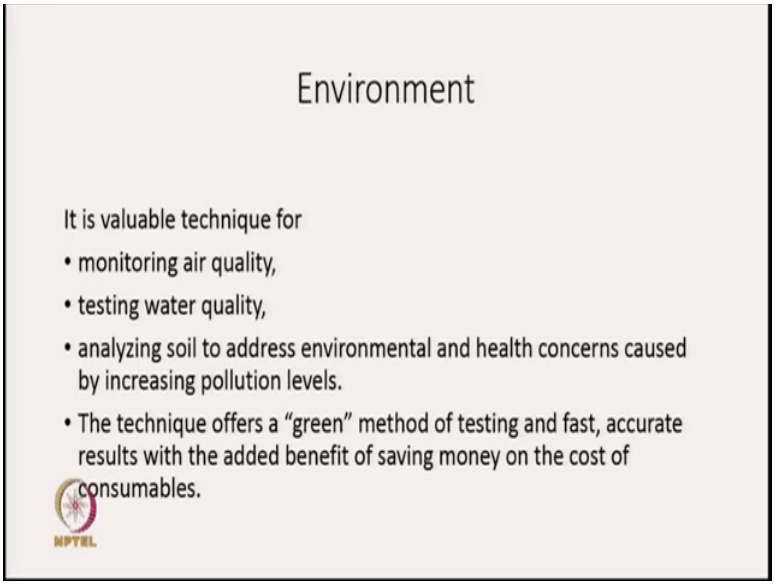

Apart from medicine spectroscopy is also used in environment, in environment it is a very important technique for monitoring air quality, it is quite often use to test the water quality. For example the level of mercury and iron in water can be monitor using spectroscopy, similarly the level of Co2 or other toxic molecules in air can be monitor using spectroscopy.

You can analyze soil to address any environmental or health concern caused by for different molecules, for example pesticides or herbicides, so this can be used to analyze to know the amount of this toxic compound in the soil.

This technique offers the green method of testing so this is quite a green method and it is it gives you fast accurate results with the added benefit of saving money on the cost of consumables.

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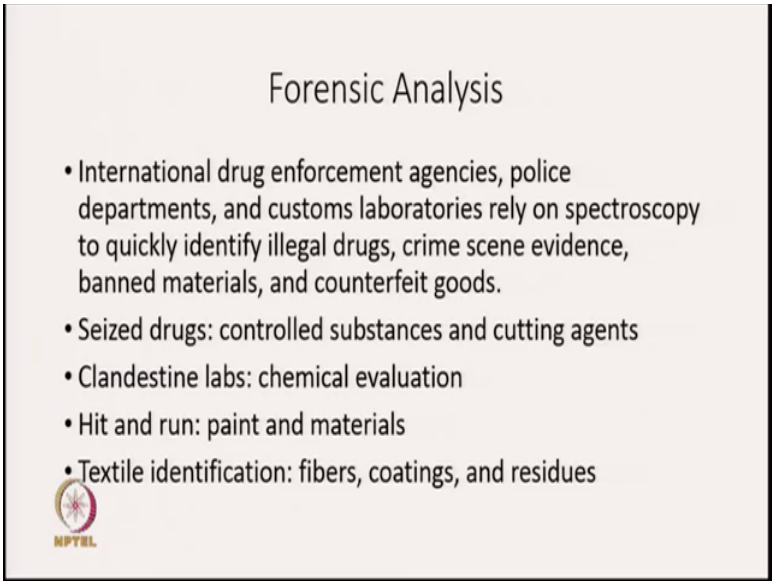

Spectroscopy has been quite often used for forensic analysis, so international drug enforcement agencies, police departments, custom laboratories, rely on spectroscopic technique, they can identify illegal drug, crime scene evidence can be analyze, banned materials can be identified and counterfeit goods can also be identified using spectroscopy.

So generally the seized drugs are basically are controlled substances and cutting agents they can be identified, clandestine labs also be identified in the case of heat and run paints and materials associated with the vehicle can be analyze to catch a suspect.

In the crime scene the identification of the textile is a very important part, so you can look at fibers, coatings and residues associated with textile and that can help you in identifying a particular suspect.

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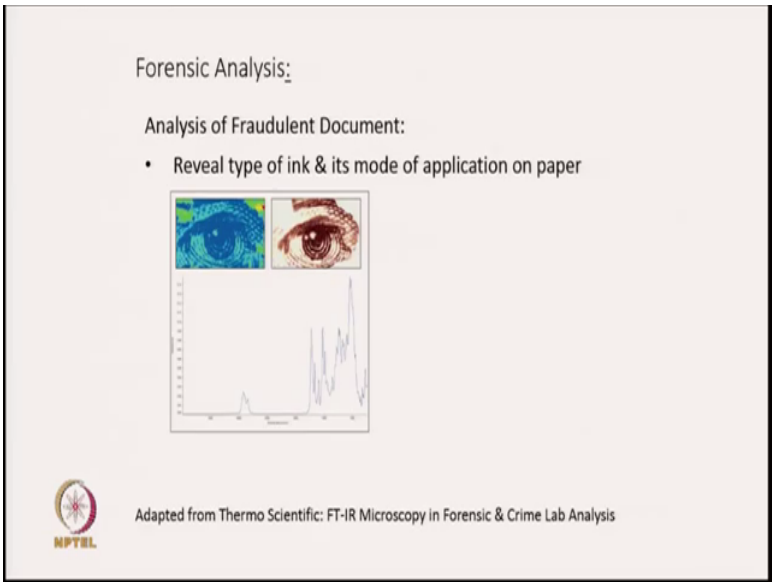

So in forensic analysis lot of time you have to analyze fraud documents, for example like when fraud currency fraud currency has a different kind of ink of different kind of paper but it looks like same if you compare with the original currency so by identifying the different type of ink and different type of paper you can tell whether the document is a valid document or a fraud document.

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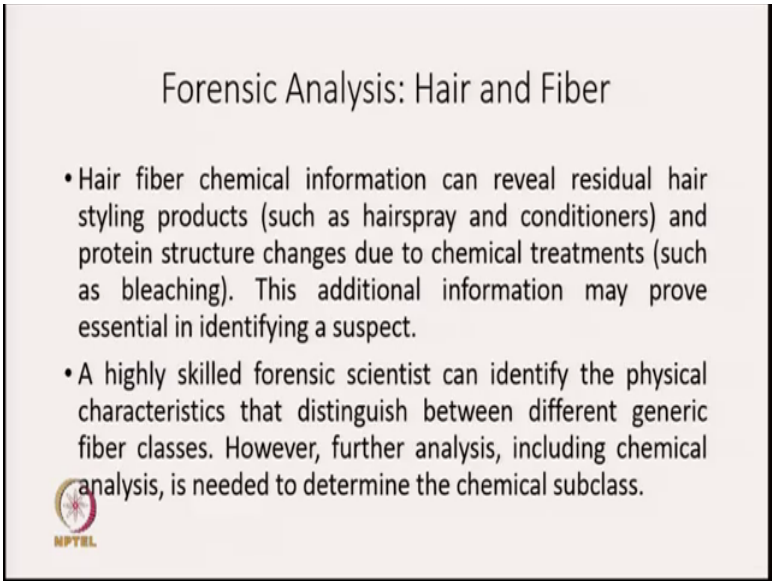

As is talk earlier hair and fiber are important source in identifying a suspect, so hair fiber chemical information can reveal residual hair styling product and protein structure changes due to chemical treatments such as bleaching, this additional information can prove to be very useful in identifying a suspect.

A highly skilled forensic scientist can identify the physical characteristics that distinguish between generic fiber classes. However the further analysis including the chemical analysis is needed to determine the chemical subclass.

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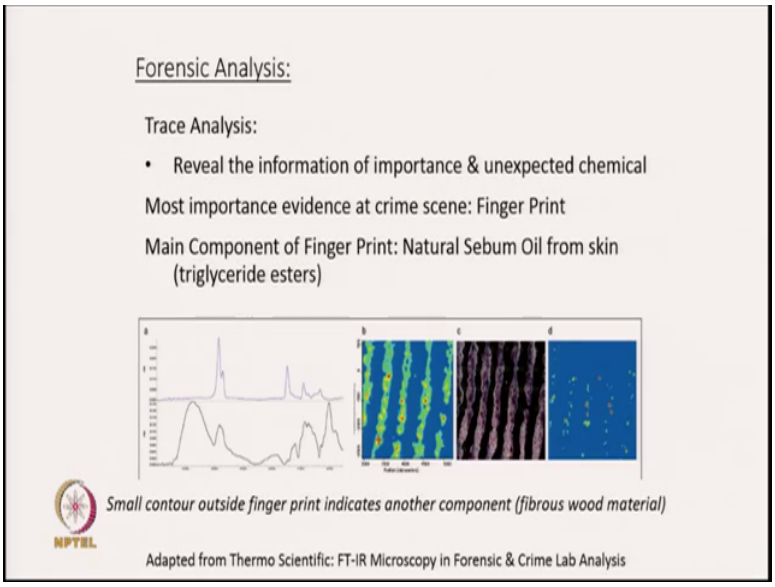

Trace analyses is also very important, most important evidence in the crime scene is your finger print and finger print has different chemical component, for example natural sebum oil

from skin which is basically a triglyceride ester, that can provide analysis of that can provide information about a particular suspect.

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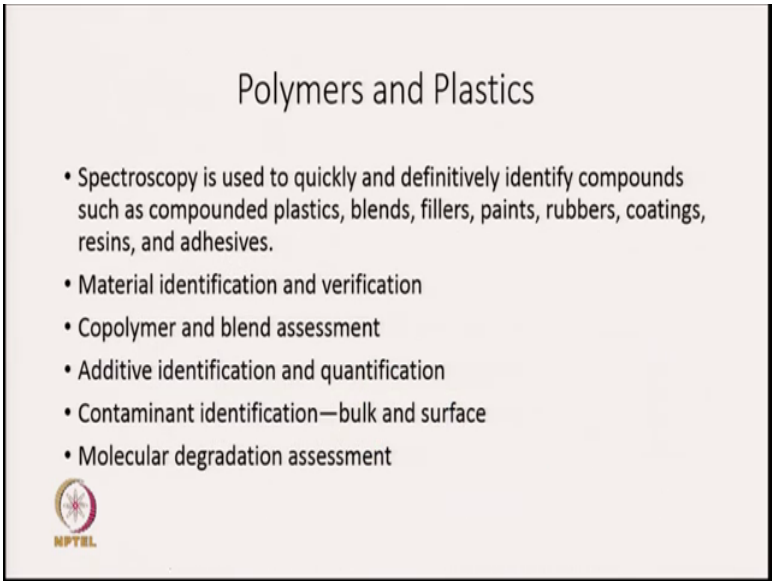

Apart from this applications spectroscopy also has applications in polymers and plastics, it can be used to quickly and definitively identify compound such as compounded plastics, blends, fillers, paints, rubbers, coatings, resins, and adhesives.

It can be used for material identification and verification, it can also be used for copolymer and blend assessment, it can tell you what are the additive in those polymers and what is the amount of those additives in that polymer.

It can also identify contaminants associated with the that polymer and this contaminants can of different types, bulk or surface contaminant and different kind of spectroscopy can be used to identify whether a particular contaminant is bulk contaminant or surface contaminant.

All the molecules degrade with time some are very prone to degradation and so spectroscopy is quite often used for molecular degradation assessment. So spectroscopy has wide range of applications spectroscopy has wide range of applications.

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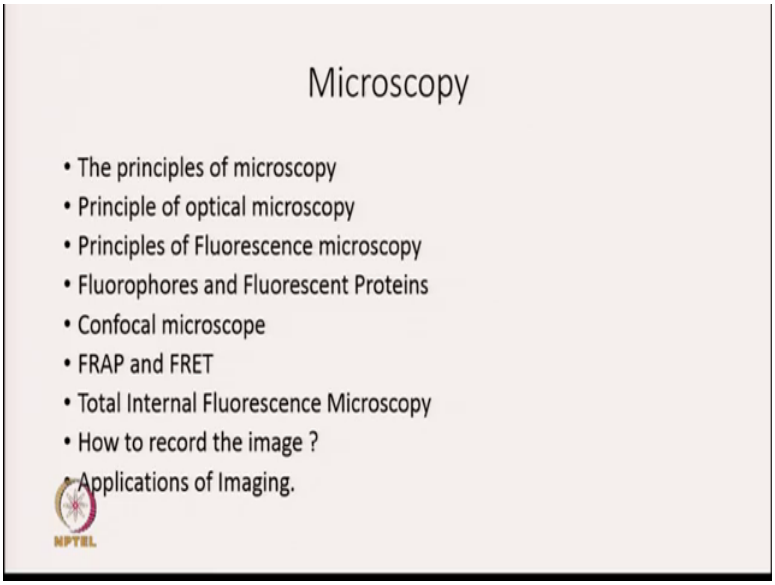

So apart from spectroscopy we will be discussing microscopy, we will discuss the principles of microscopy and I will go to principles of optical microscopy, I will also discuss principles of fluorescence microscopy, what are the different fluorophores and fluorescent proteins and how to make use of them to study the different phenomenon associated with proteins.

I will also discuss confocal microscopy and I will be discussing fat and fate. Then I will go to total internal fluorescence microscopy then we will discuss application of all this microscopic techniques.

I will also let you know how to record the image and if what kind of information we can get from the image and how imaging can be applied to look at different processes at several level.

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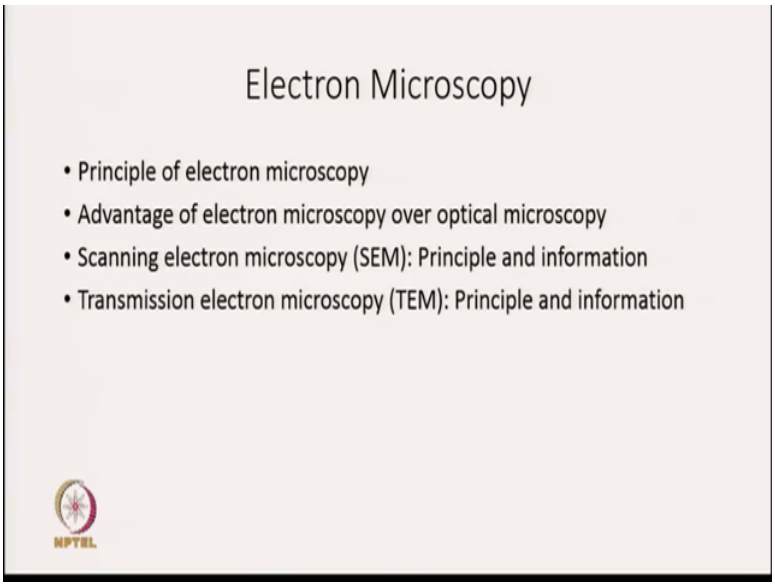

Apart from optical spectroscopy I will also be discussing electron microscopy, first I will start with principle of electron microscopy then I will discuss advantage of electrons microscopy over optical microscopy.

So what are the advantages of electron microscopy? I will also discuss scanning electron microscopy popularly known as SEM technique. What is the principle of this microscopy and what kind of information's we can get from cells?

I will then go to transmission electron microscopy popularly called TEM and I will discuss principles and principles of electron microscopy and how it can be used to know the different information's about the cell or about the proteins.

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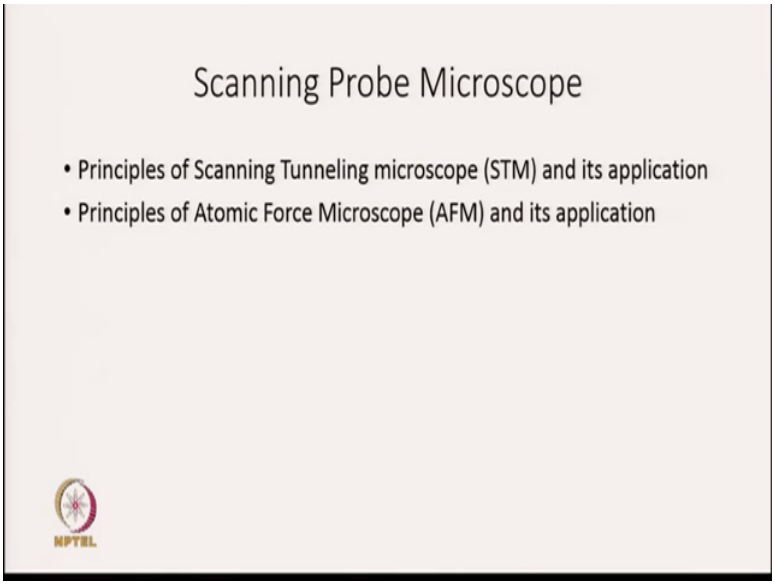

Apart from optical microscopy and electron microscopy I will also discuss scanning probe microscope, what is the principle of tunneling microscope known as STM and how it can be applied to study different processes? I will then go to atomic force microscopy and as I have done for other microscopy I will discuss principles and its applications,

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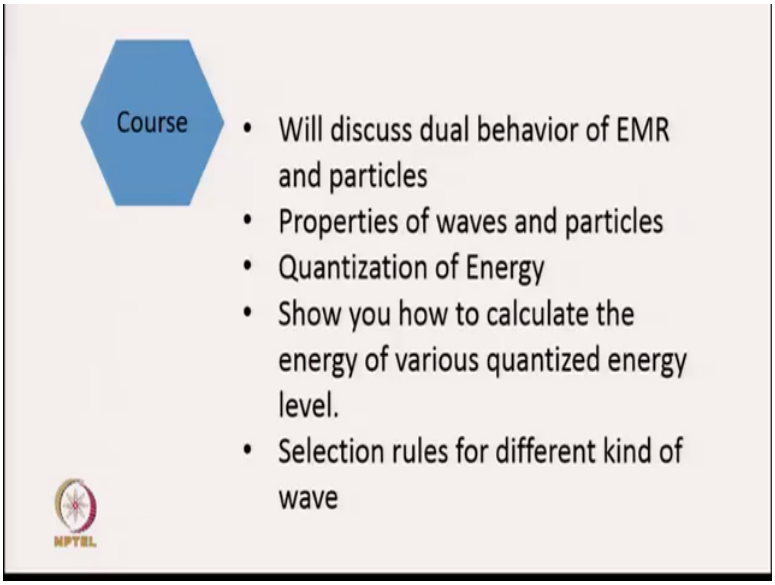

This is the I will start I will start with discussing dual behaviors and I will talk about what are the properties of waves and particles then I will discuss about the concept of quantization of energy came and what is mean by quantization of energy?

Once we understand the dual behavior of waves and particles and quantization of energy I will show you how to calculate energy of various quantize levels. And then finally I will be discussing about selection rules for different kind of spectroscopy.

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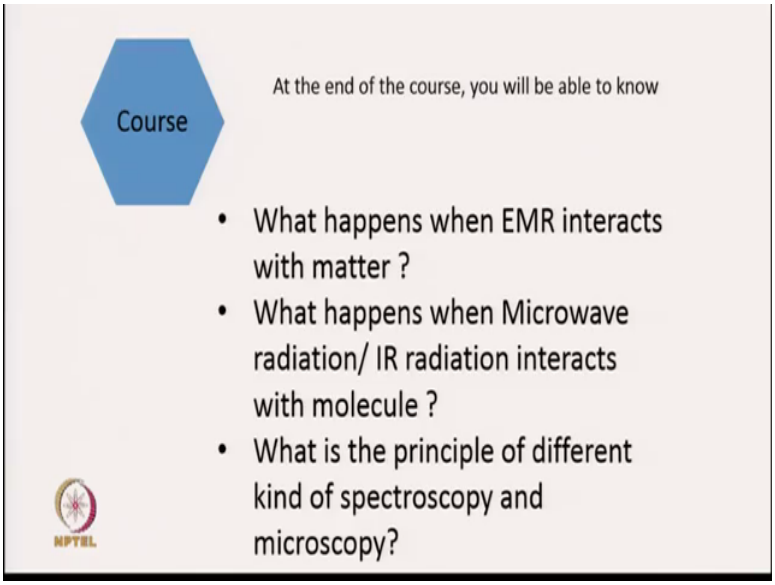

I will also let you know what happens when electromagnetic radiations interact with matter. What happens when microwaves radiation IR radiations interact with the molecule? So I will go to each of the spectroscopy one by one and I will tell you about the principles of this kind of spectroscopy and how to use it for different applications. Similarly I will go to each of the microscopy and I will tell you about the principle of that microscopy, technique and then applications.

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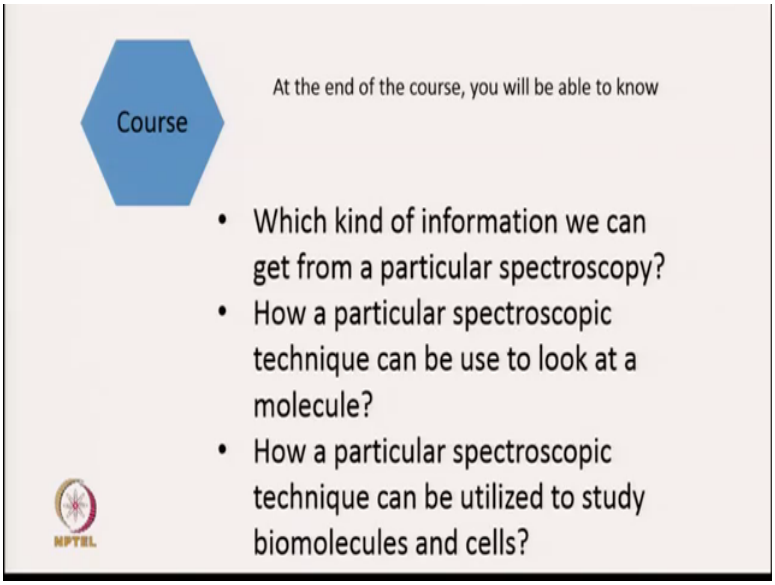

So after the course you will be able to know what kind of information we can get from a particular spectroscopy and how a particular spectroscopic technique can be used to look at

molecules and how a particular spectroscopic technique can be utilized to study biomolecules and cells?

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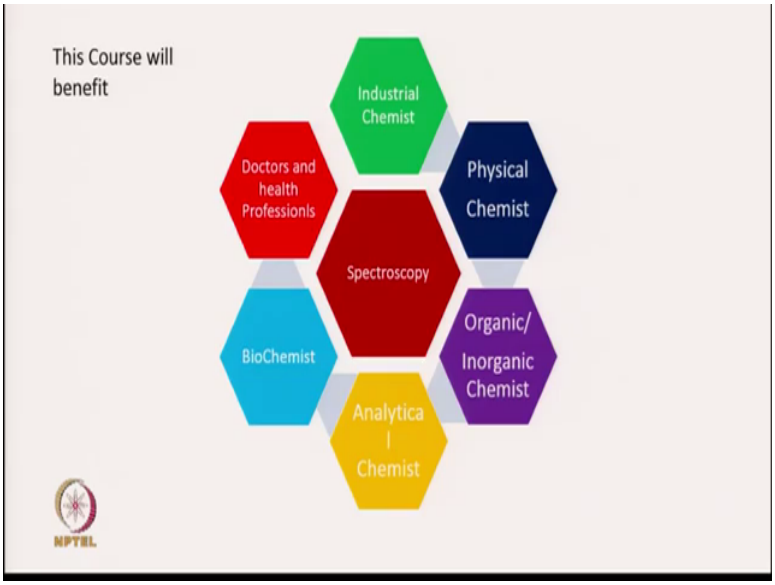

The course will benefit a physical chemist and organic or inorganic chemist, it will also benefit analytical chemist, biochemist, doctor and health professionals and industrial chemist. So basically this course is useful for different kind of chemist and biologist.

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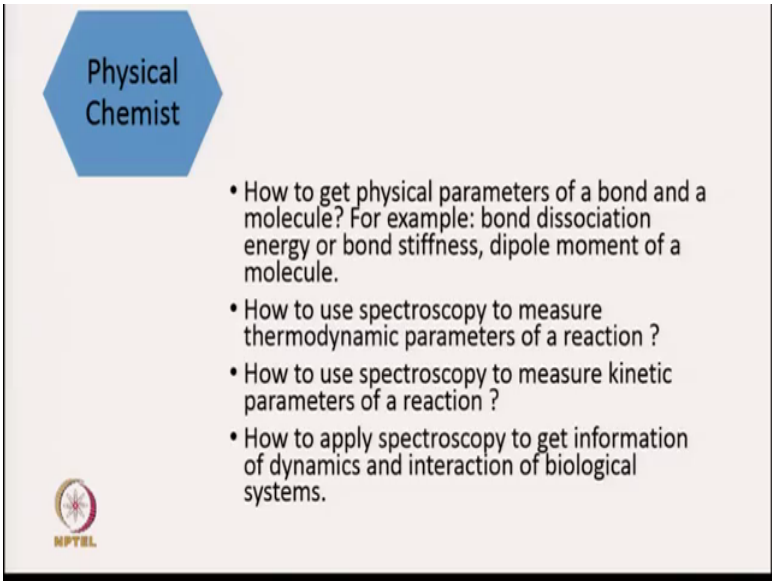

So for example if you take a physical chemist what he wants to get? He wants to get physical parameter of a bond of a molecule. So a physical chemist will know how to get bond dissociation energy, or a dipole moment of a molecule using spectroscopy.

After the course you will able to know how to use spectroscopy to measure different thermodynamic parameters of a reaction, for example enthalpy of a reaction, entropy of a reaction, free energy of a reaction, similarly he will be to use a spectroscopy to measure the kinetic parameters of a reaction.

For example activation energy, enthalpy of activation, entropy of activation. So spectroscopy can be used to get kinetic parameters and that is what physical chemistry is interested about. And physical chemist will also be able to know how to apply a spectroscopy to get information of dynamics or interaction of biological system.

So spectroscopy can be used to study the dynamics of the molecule or dynamics of a biomolecule and how two molecules interact, so you can look at the mechanism, you can get the parameters like binding constant, this every parameters can be measured using spectroscopy. so a physical chemist will utilize this course to understand physical chemistry of a reaction.

Now come to organic chemist, how an organic chemist or inorganic chemist can gain from this course, there will be knowing to use spectroscopy to get the structure of the molecular or product, so if you think of a reaction you need to know a product, what is the final product?

You can use spectroscopy to know the structure of the molecule and that is what an organic chemist will come to know after doing this course. Now organic can also use spectroscopy to know the mechanism of the reaction and everybody is familiar how mechanism reaction is useful.

For example substitution reaction which goes by SN1 mechanism requires different kind of conditions in comparison to a reaction which is going through a SN1 mechanism SN2 mechanism. So for example polar solvent will be useful if a reaction is going by SN1 mechanism whereas it will not be that useful if a reaction is going by SN2 mechanism.

We will also be I will also be telling you how to use spectroscopy to know the impurities in the cell, so an organic chemist can know what are the impurities in the sample by using spectroscopy and he will be able to apply a spectroscopy to differentiate between isomers of a molecules isomers of the molecules. So all of this aspects are important for organic chemist and organic chemist can use spectroscopy to further his research, his or her research.

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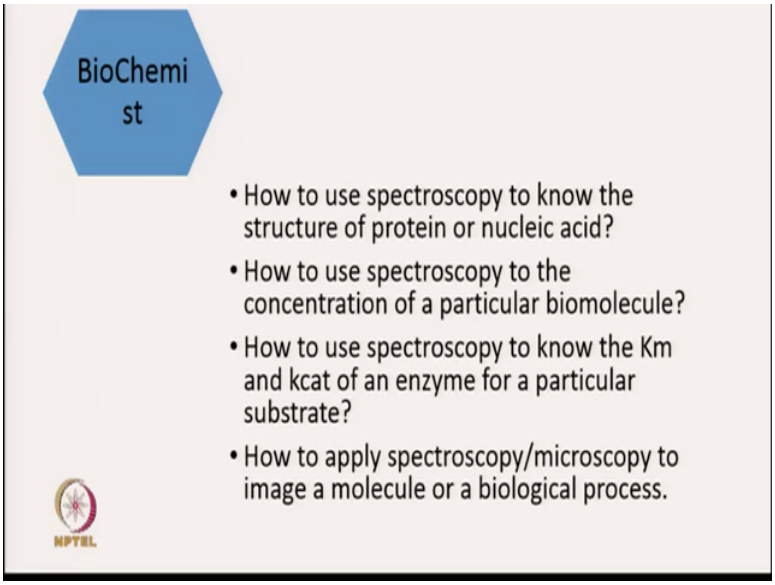

Similarly this course will also be useful for a biochemist, he can use spectroscopy to know the structure if the protein or nucleic acid, he can use a spectroscopy to know the concentration of a particular particle of biomolecule and he can use spectroscopy to know the Km and Kcat of an enzyme for particular substance.

Not only that a biochemist can use spectroscopy or microscopy to image a molecule on biological process and I will be discussing all of this applications in this course all of this applications in this course. So I hope that biochemist will also be able to gain from this course.

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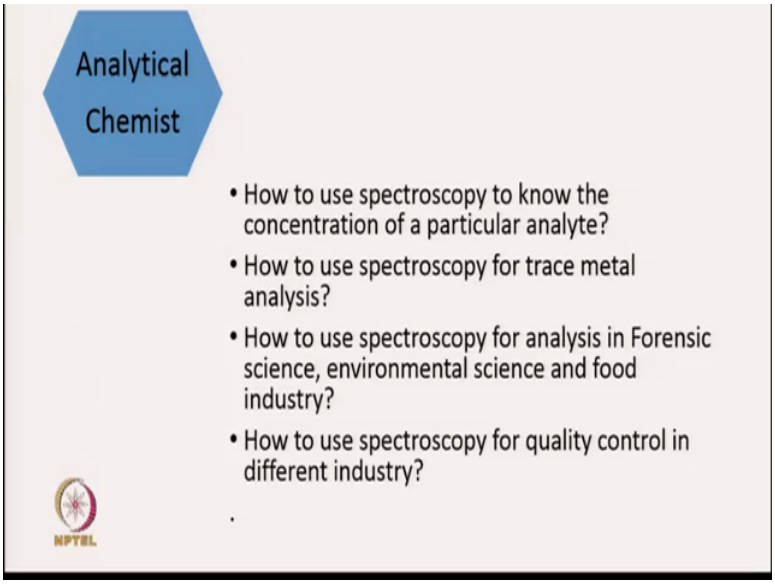

This course will also help an analytical chemist, he will be able to know how to use a spectroscopy to get the concentration of a particular analyte. He will be able to use spectroscopy for trace metal analysis for trace metal analysis and that is very important particularly in environmental science and different kind of industries.

He will be able to use spectroscopy for analysis in forensic science, environmental science and food industryand he will be able to use spectroscopy control in different industry. So analytic chemist can also benefit it from this course.

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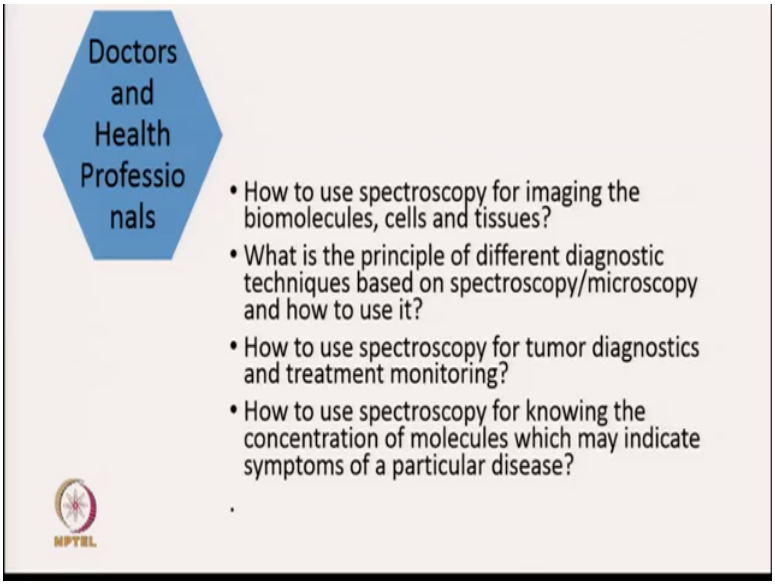

Similarly doctors and health professionals can also be able to use a spectroscopy to get help in their profession. They can use spectroscopy for imaging the biomolecules, cells and tissues after this course doctors and health professionals will be able to know principle of different diagnostic techniques based on spectroscopy and microscopy, and how to use it for different applications.

For example how to use a spectroscopy for tumor diagnostic and treatment monitoring, similarly they will be able to use spectroscopy and they will be able to know how to use spectroscopy for knowing the concentration of the molecules which may indicate the symptoms the a particular disease, for example they will be able to know the amount of glucose level and by looking at glucose level they will be able to tell whether a particular patient is diabetic or not.

So in summary I will be discussing spectroscopy as discussed by a physical chemist, organic spectroscopy has discussed by an organic chemist, spectroscopy has utilized by a biochemist,

spectroscopy has utilize by an industrial chemist or a biochemist and imaging has applied by a health professionals and doctors.

So I welcome all of you to this course and I am quite sure that you will be able to gain a lot from this course. Thank you and looking forward for next lecture, Thank you very much.