Advance Analytical Course
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Lecture No # 29

Determination of caffeine content in coffee using FT-IR; now, this is a very interesting case, that I thought of talking about, because how do we determine in day-to-day life, the main chemical constituents and how does FT-IR play a role, both in our domestic sample analysis, as well as in other kind of samples, which are available or are sent to the laboratories, from environment, from biological labs and so on?

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Determination of Caffeine content in coffee using FT-IR

Specialized use of FT-IT

So, there must be a way of analyzing them and interpreting them. And this was one very interesting case, where I thought determination of caffeine content in coffee, and how is FT-IR responsible for its analysis?

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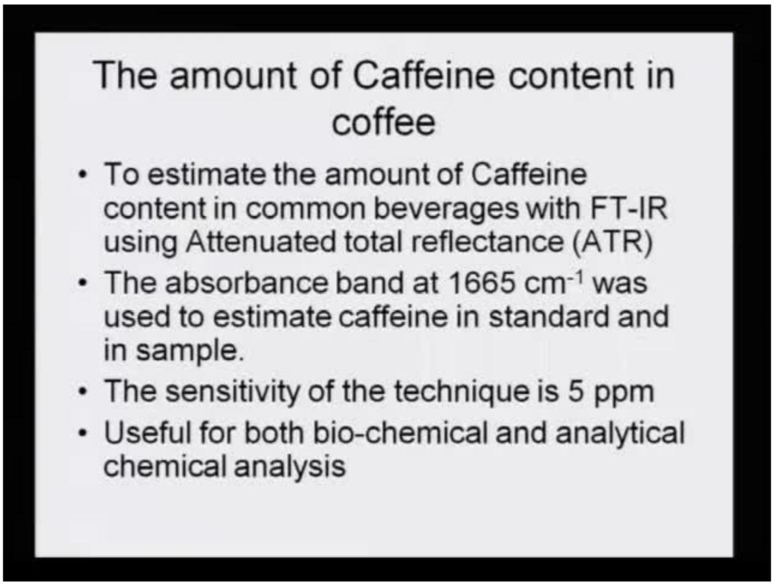

The amount of Caffeine content in coffee

- To estimate the amount of Caffeine content in common beverages with FT-IR using Attenuated total reflectance (ATR)
- The absorbance band at 1665 cm^{-1} was used to estimate caffeine in standard and in sample.
- The sensitivity of the technique is 5 ppm
- Useful for both bio-chemical and analytical chemical analysis

The amount of caffeine content in coffee has to be estimated because in coffee manufacturing units, it is important for them to evaluate the caffeine content in the seeds that they obtain from various sources.

So, to estimate the amount of caffeine content in common beverages with FT-IR, using attenuated total reflectance, now you see, that FT-IR and ATR both, are being used in this particular analysis of caffeine content.

The absorbance band at 1665 centimeter inverse was used to estimate the caffeine in standard and in the sample. So, it was, at the most identifiable or the caffeine related functional group was identified as a band at 1665 centimeter inverse and that was what was analyzed, both in the standard and in the sample. Again, here we have taken into consideration, that it is a calibration method.

So, first a pure caffeine sample was analyzed and the peak was identified as 1665 centimeter inverse and then, various samples from various beverages containing caffeine, were analyzed. So, they were taken as sample.

Always, when there is a standard, we make a calibration curve to see the linearity response of the machine as well as the method. We have to find out, whether this method fits the Beer-Lambert’s law fundamentals or not; if it fits, so then it will give, that absorbance is directly proportional to the concentration.

The sensitivity of technique is almost up to 5 ppm, up to 5 parts per million. One can say that yes, this system or this particular method of FT-IR ATR works for caffeine analysis, useful for both, biochemical and analytical chemical analysis. So, this FT-IR ATR combination is very good, not only for caffeine analysis, but it is also very well practiced for biochemical and analytical chemical analysis. Now, FT-IR and ATR, when they are combined, that means, it is an IR technique with an ATR kind of arrangement.

This is a special device; it is not that FT-IR machine has an ATR, why? Because this is a special separate attachment; if the analyzer feels, that there is a necessity to do polymeric substances, to do film analysis, to do surface analysis, then this ATR attachment or device is required, which can be connected to the main FT-IR machine, otherwise a simple integrated machine does not have an ATR attachment, to begin with.

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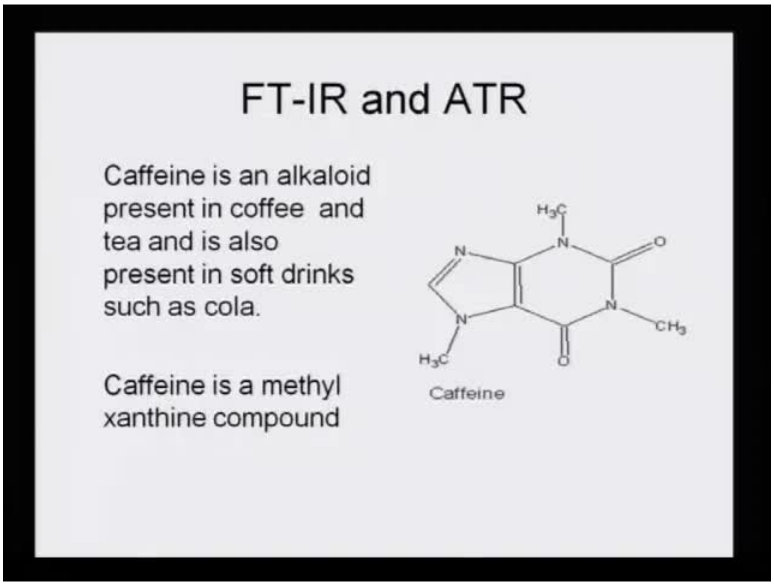

FT-IR and ATR

Caffeine is an alkaloid present in coffee and tea and is also present in soft drinks such as cola.

Caffeine is a methyl xanthine compound

Caffeine is an alkaloid; here is the structure, of that alkaloid present in coffee and tea and also present in many soft drinks, such as cola. So, this caffeine molecule is a very important molecule and it has some structural details - it has carbonyl groups, it has carbon double bond, it has carbon-nitrogen double bond, and so on and so forth, as you can see, from the molecular structure. And caffeine is a methyl xanthine compound; so, this moiety is called xanthine and it has a methyl group on the nitrogen, so it is called methyl xanthine.

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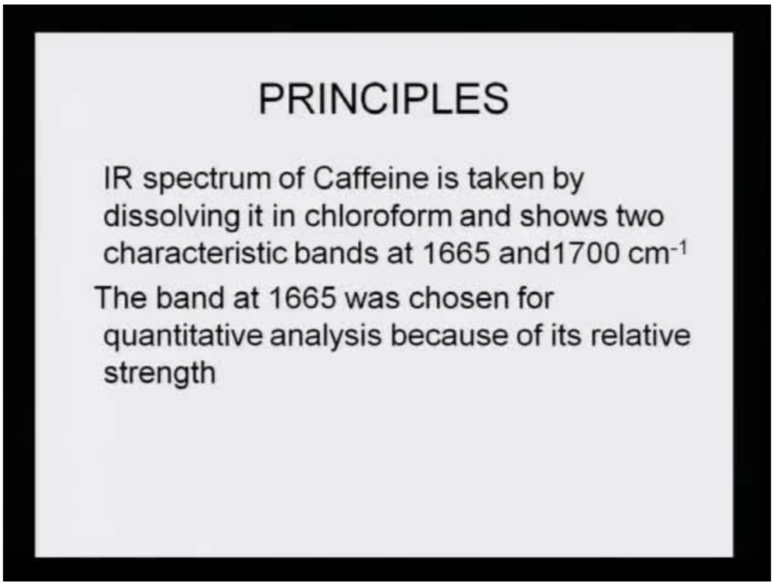

PRINCIPLES

IR spectrum of Caffeine is taken by dissolving it in chloroform and shows two characteristic bands at 1665 and1700 cm^{-1}

The band at 1665 was chosen for quantitative analysis because of its relative strength

The IR spectrum of caffeine is taken by dissolving it in chloroform and shows 2 characteristic bands - one at 1665 and the other one at 17 centimeter inverse.

Now, obviously, the 1700 centimeter inverse peak corresponds to the carbonyl and the band at 1665 centimeter inverse was chosen for the quantitative analysis because of its relative strength. So, the peak at 1665 was more intense as compared to the 1700 centimeter inverse and that is why, that was the characteristic peak for identification of caffeine in various drinks.

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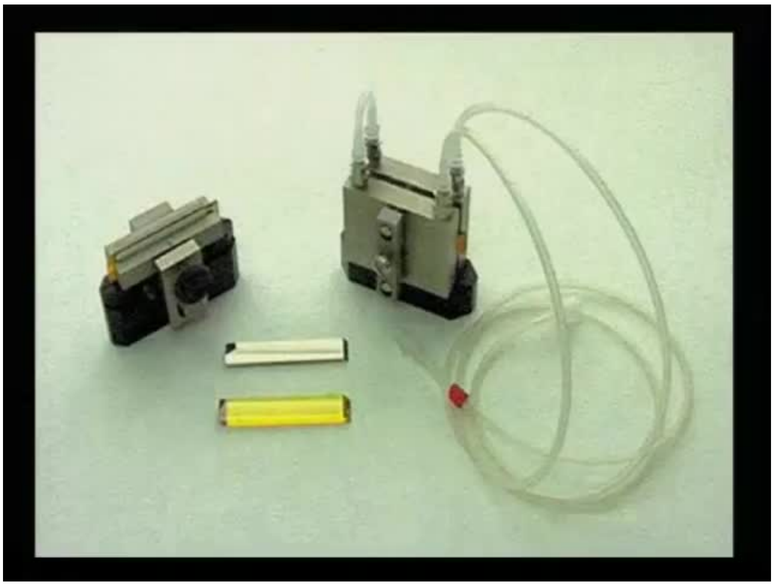

Now, this is an ATR attachment. So, it, if it is required, it can be connected and this yellow plate, you can see, or white plate is the IRE material, that is, the internal reflection element.

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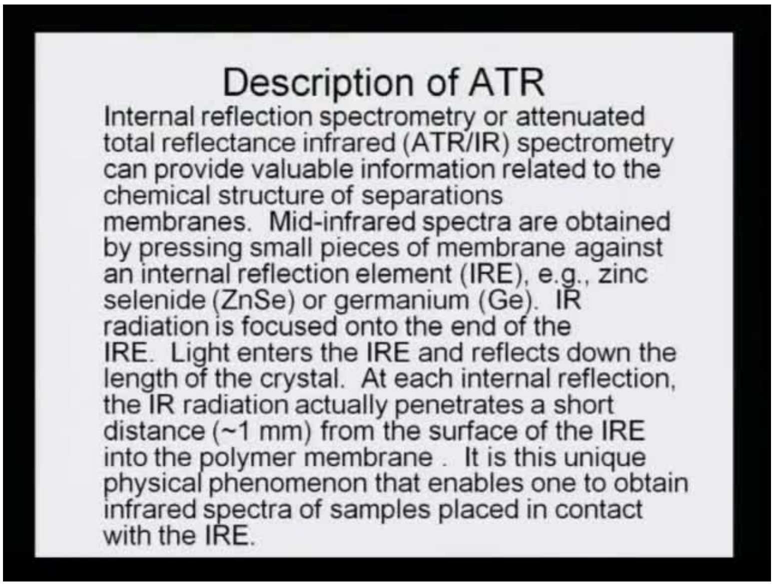

Description of ATR

Internal reflection spectrometry or attenuated total reflectance infrared (ATR/IR) spectrometry can provide valuable information related to the chemical structure of separations membranes. Mid-infrared spectra are obtained by pressing small pieces of membrane against an internal reflection element (IRE), e.g., zinc selenide (ZnSe) or germanium (Ge). IR radiation is focused onto the end of the IRE. Light enters the IRE and reflects down the length of the crystal. At each internal reflection, the IR radiation actually penetrates a short distance (~1 mm) from the surface of the IRE into the polymer membrane . It is this unique physical phenomenon that enables one to obtain infrared spectra of samples placed in contact with the IRE.

Description of an ATR - because here also, I would like to make you remind it because this is a special device, which is attached to the FT-IR or IR simple machine, but IR machines are now obsolete, only FT-IR’s are being used.

The internal reflection spectrometry or attenuated total reflectance infrared spectrometry (ATR/IR) can provide valuable information related to the chemical structure of separation membrane. Mid-IR spectra are obtained by pressing small pieces of membrane against an internal reflection element, that is, IRE, which is made up of zinc selenide or germanium, not both at a time. It will have only one type of coating and that is zinc, either zinc selenide or germanium.

IR radiation is focused on to the end of the IRE. Light enters the IRE, that is, the internal reflection element and reflects down several times, during, through the length of the crystal. At each internal reflection, the IR radiation actually penetrates a short distance, that is, 1 mm from the surface of the IRE into the polymeric membrane or whatever is the surface material that has been kept on top of that.

It is this unique physical phenomena, that enables one to obtain infrared spectra of sample placed in contact with this internal reflection element. So, because of this, in little penetrations and penetrations occurring at several intervals and finally, the incident light coming out, it produces enough vibration in the bonds of the materials that has been kept on top of the IRE.

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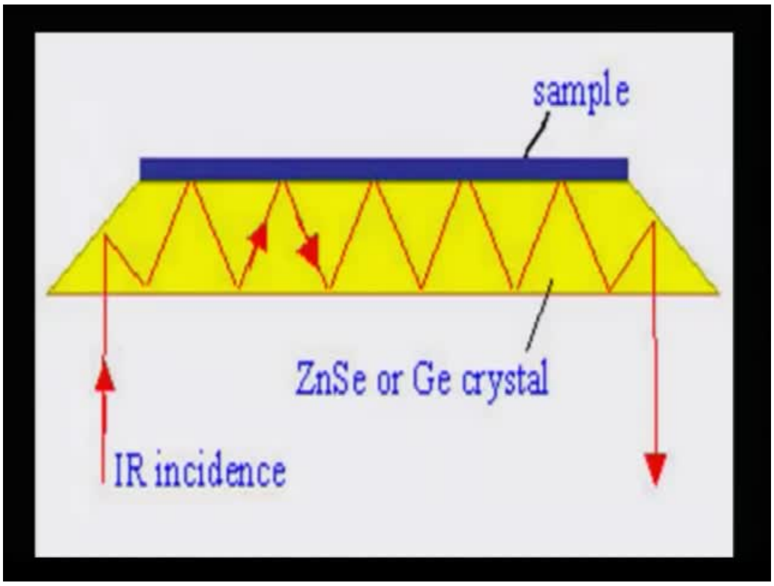

So, this is what I had shown earlier, because of this internal reflection, as the name also suggests, it creates an IR spectrum.

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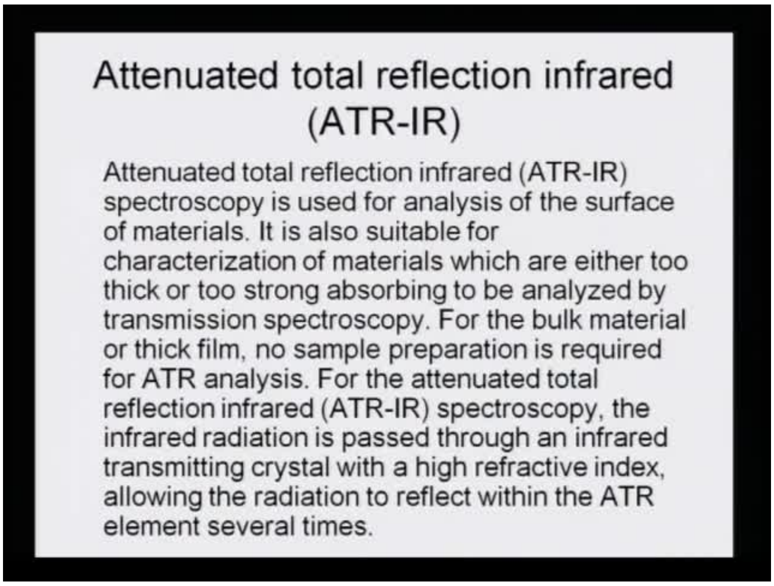

Attenuated total reflection infrared (ATR-IR)

Attenuated total reflection infrared (ATR-IR) spectroscopy is used for analysis of the surface of materials. It is also suitable for characterization of materials which are either too thick or too strong absorbing to be analyzed by transmission spectroscopy. For the bulk material or thick film, no sample preparation is required for ATR analysis. For the attenuated total reflection infrared (ATR-IR) spectroscopy, the infrared radiation is passed through an infrared transmitting crystal with a high refractive index, allowing the radiation to reflect within the ATR element several times.

Attenuated total reflection or the infrared (IR) - attenuated total reflection infrared (IR) spectroscopy is used for analysis of surface of materials. I have told this time and again because it should be clear, that fugacious samples, liquid samples, solid samples, we have the sample region, where we needs need to put it in the q at or we need to have thin film or we need to have a pallet which holds the pallet and so on, but this is a much simplified machine, where no sample preparation is required, no kbr is required, no solvent is required. It is just a small piece of film of 0.2 to 0.3 micrometer, which is kept on this selenide or germanium surfaces and simply, the analysis is carried out.

It is also suitable for characterization of materials, which are either too thick or too strong absorbing to the analyzed spectroscopy. For the bulk material or thick film, no sample preparation is required for ATR. So, you see, there sample just needs to be cut into small piece and kept it on top of that, and it should be in direct contact with the IRE material. For the attenuated total reflection infrared IR spectroscopy, the infrared radiation is passed through an infrared transmitting crystal with a high, reflect reflective, refractive index, allowing the radiation to reflect within the ATR elements several times.

So, we saw in that diagram, that how several times, there are penetrations into the material and that is what actually causes to extract the information from the material about the different types of functionalities, that are present in that particular material.

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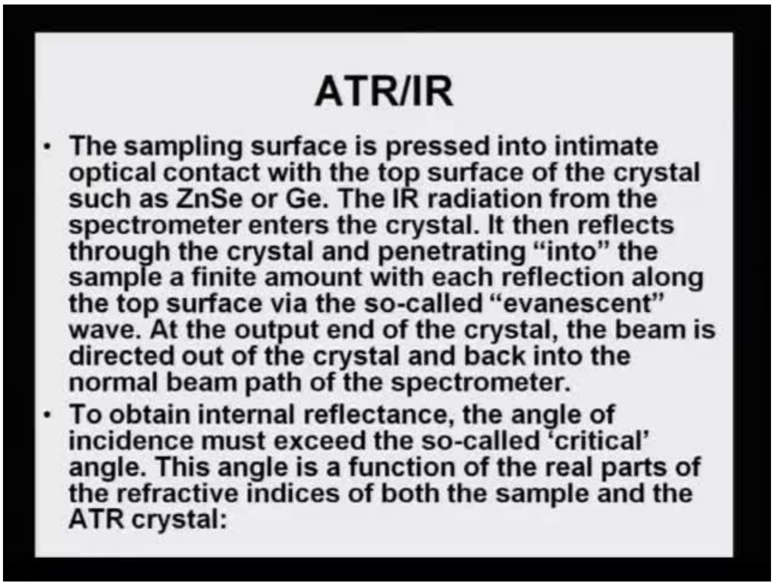

ATR/IR

- The sampling surface is pressed into intimate optical contact with the top surface of the crystal such as ZnSe or Ge. The IR radiation from the spectrometer enters the crystal. It then reflects through the crystal and penetrating “into” the sample a finite amount with each reflection along the top surface via the so-called “evanescent” wave. At the output end of the crystal, the beam is directed out of the crystal and back into the normal beam path of the spectrometer.
- To obtain internal reflectance, the angle of incidence must exceed the so-called ‘critical’ angle. This angle is a function of the real parts of the refractive indices of both the sample and the ATR crystal:

The sampling surface is pressed into intimate optical contact with the top surface of the crystal, such as the zinc selenide or germanium. So, it is made to simply sit on top of it, must be in optical, intimate optical contact; that means, the light should be able to penetrate, otherwise it has no consequence. If there is an air gap or something like that, and the light is not able to go into the surface, it is of no consequence.

The IR radiation from the spectrometer enters the crystal. It then reflects through the crystal and penetrating into the sample, a finite amount of each reflection, along the top surface via the so-called evanescent wave. At the output end of the crystal, the beam is directed out of the crystal and back into the normal beam path of the spectrometer.

So, it is this evanescent, which is the penetrated light, which has come, come, come out of each of these penetrations, is what is finally sent into the spectrometer for analysis, to obtain internal reflection. The angle of incidence must exceed the so-called critical angle; that means, it must be able to penetrate into the material. This angle is a function of real parts of refractive indices of both, the sample and the ATR crystal.

So, because of the refractive change in refractive index of the ATR crystal, that is, the zinc selenide or germanium and the sample, there is a change in the light that is being observed. If both were the same material, do you think, an, any change, which has, would have taken place? No. Why, because the materials are different.

ATR crystal is different, that is, the IRE material is different, and the refractive index of this material and the refractive index of the light in the penetrating, into the sample is different. And because of these difference in their refractive indices, this, thus the ATR spectrum is possible to be analyzed.

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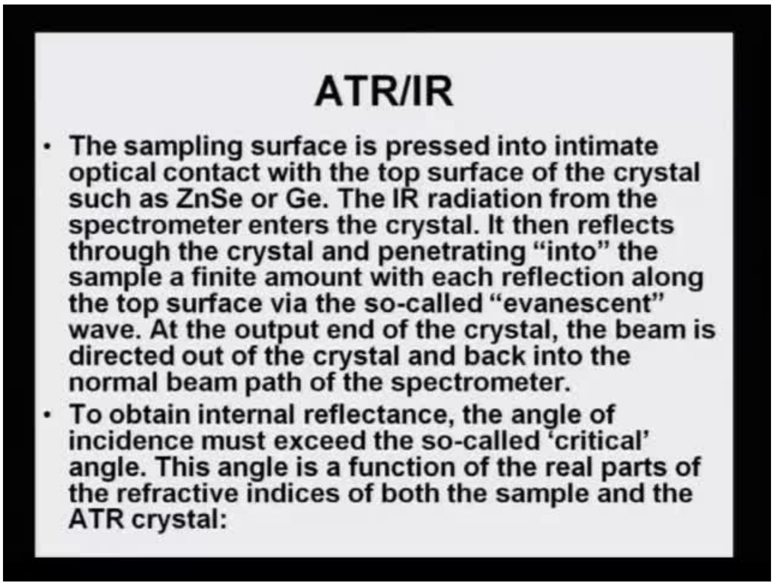

ATR/IR

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- To obtain internal reflectance, the angle of incidence must exceed the so-called 'critical' angle. This angle is a function of the real parts of the refractive indices of both the sample and the ATR crystal:

ATR accessories - the ATR accessories as was I told you a little while ago, are especially useful for obtaining IR spectra of difficult samples that cannot be readily examined by normal transmission mode.

Samples, which cannot be analyzed on FT-IR need to be analyzed on ATR, otherwise it, there is no necessity to use this, they are suitable for thick or highly absorbing solids and liquids, including films, coatings, powders, threads, adhesives, polymers and aqueous solutions.

Now, as I told you, aqueous solutions cannot be analyzed on a simple FT-IR machine because the moisture will either destroy the sodium bromide or potassium bromide pallets or sodium chloride pallets or calcium fluoride pallets, and they will simply corrode it. So, for aqueous samples, for powder samples, for film samples, for coatings, various types of paintings, paints and coatings, adhesive polymers, they can be very easily analyzed on ATR accessory.

The ATR requires no sample preparation for most of the samples, so it is only, the sample piece needs to be cut and placed on the IRE material.

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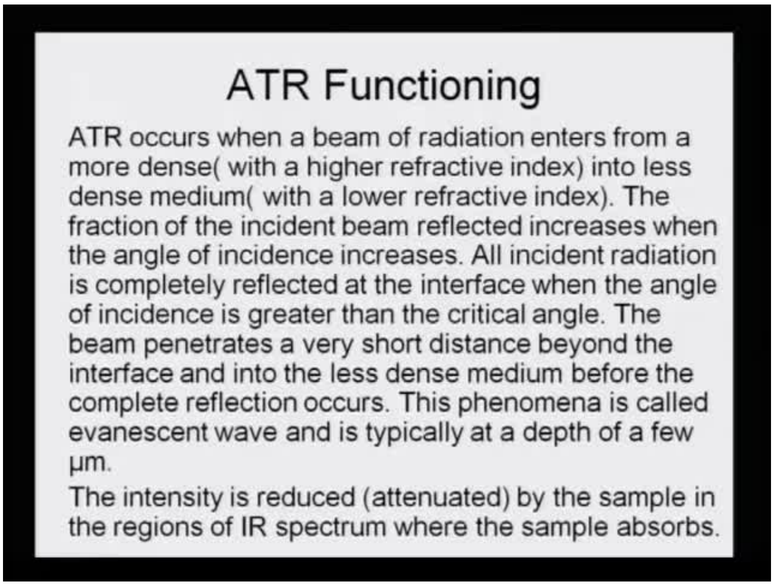

ATR Functioning

ATR occurs when a beam of radiation enters from a more dense(with a higher refractive index) into less dense medium(with a lower refractive index). The fraction of the incident beam reflected increases when the angle of incidence increases. All incident radiation is completely reflected at the interface when the angle of incidence is greater than the critical angle. The beam penetrates a very short distance beyond the interface and into the less dense medium before the complete reflection occurs. This phenomena is called evanescent wave and is typically at a depth of a few µm.

The intensity is reduced (attenuated) by the sample in the regions of IR spectrum where the sample absorbs.

The ATR functionality or how does it function. ATR occurs, when a beam of radiation enters from a more dense with a higher refractive index, to a less dense medium with a lower refractive index. The fraction of incident beam reflected increases, when the angle of incidence increases. All incident radiation is completely, refracted, reflected at the interface, when the angle of incident is greater than the critical angle.

So, if we try to look at the physics of what actually happens in an ATR machine or accessory, it is just a question of difference in refractive indices that we saw. And if the sample and the refracting material, that is, the zinc selenide or germanium, have any kind of difference, only then, it will show some kind of, you know, light being changed into different…, the incident light and the critical angle, and all those will show some changes, otherwise the incident radiation and the transmitted radiation will remain the same. So, it is at the interface, where the sample and the IRE material is contacting there, they should be, the angle of incident should be different and should create, be greater than the critical angle.

The beam penetrates a short distance, a very short distance, beyond the interface into the less dense medium of the sample, before the complete reflection occurs. So, it just penetrates, comes out, penetrates, comes out, penetrates and comes out, and these penetrations are of a very short duration; that is why, they are just entering and coming out and this gives information. This phenomena is called the evanescent wave and is typically at a depth of a few micro meters only.

The intensity is reduced, that is, attenuated by the sample in the regions of IR spectrum where the sample absorbs.

So, as what, the rest part remains the same, this attenuation is only taking place and is related to the bonds, that are present in that material and whatever the material is made up of molecular structure.

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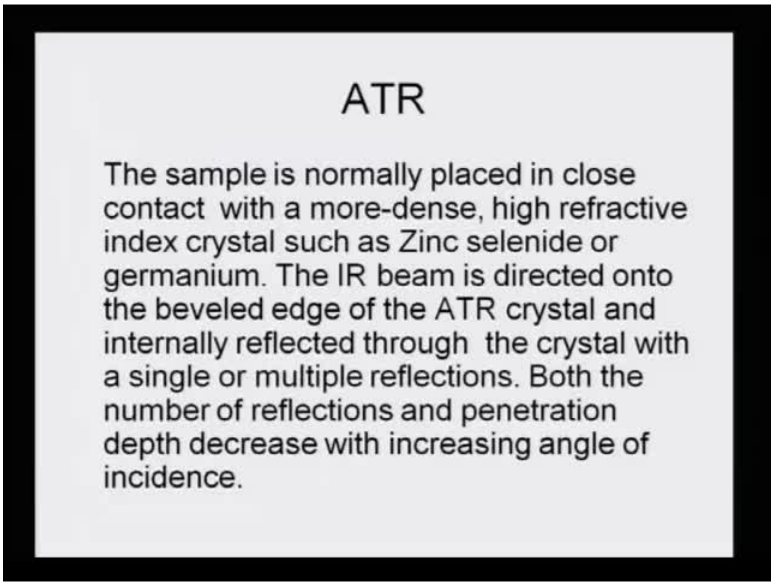

ATR

The sample is normally placed in close contact with a more-dense, high refractive index crystal such as Zinc selenide or germanium. The IR beam is directed onto the beveled edge of the ATR crystal and internally reflected through the crystal with a single or multiple reflections. Both the number of reflections and penetration depth decrease with increasing angle of incidence.

Thus, ATR - the sample is normally placed in a closed contact with more dense, high refractive index crystal, such as zinc selenide or germanium. The IR beam is directed onto the beveled edge of the ATR crystal and internally reflected through the crystal with a single or multiple reflections. Both the number of reflections and penetration depth decrease with increasing angle of incidence.

So, it is all correlated. If the number of reflections and penetrations will go on decreasing as the angle of incident will go on decreasing and eventually, it is, kind of, sent into the detector part of the machine spectrophotometer, so that, it, the information can be then interpreted in terms of peaks or signals.

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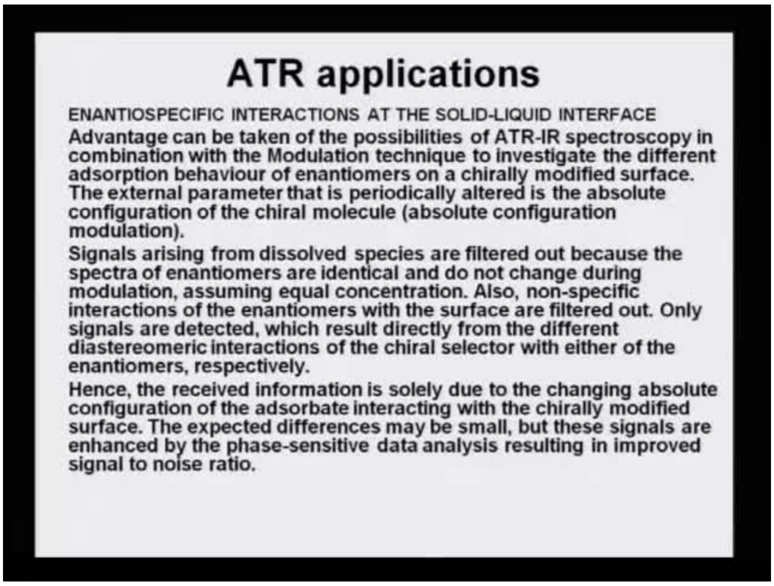

ATR applications

ENANTIOSPECIFIC INTERACTIONS AT THE SOLID-LIQUID INTERFACE

Advantage can be taken of the possibilities of ATR-IR spectroscopy in combination with the Modulation technique to investigate the different adsorption behaviour of enantiomers on a chirally modified surface. The external parameter that is periodically altered is the absolute configuration of the chiral molecule (absolute configuration modulation).

Signals arising from dissolved species are filtered out because the spectra of enantiomers are identical and do not change during modulation, assuming equal concentration. Also, non-specific interactions of the enantiomers with the surface are filtered out. Only signals are detected, which result directly from the different diastereomeric interactions of the chiral selector with either of the enantiomers, respectively.

Hence, the received information is solely due to the changing absolute configuration of the adsorbate interacting with the chirally modified surface. The expected differences may be small, but these signals are enhanced by the phase-sensitive data analysis resulting in improved signal to noise ratio.

ATR applications - enantiomeric interactions at the solid-liquid interface can take place. Advantage can be taken of the possibilities of ATR-IR spectroscopy in combination with modulation technique, to investigate the different absorption behavior of enantiomers on a chirally modified surface. The internal, the external parameter, that is periodically altered is the absolute configuration of the chiral molecule or the absolute configuration modulation.

So, if we try to analyze this ATR application, it is not only related to all the polymeric substances or only the coatings and the films, but even when it comes to chemical analysis, the enantio specific interaction of solid-liquid interface can also be studied through this, and even chiral molecules can be identified.

Signals arising from dissolving dissolved species are filtered out because the spectra of enantiomers are identical and do not change during modulation, assuming equal concentration. Also, non-specific interactions of enantiomers with the surface are filtered out. Only the signals are detected, which result directly from the different diastereomeric interactions of the chiral selector with either of the enantiomers, respectively.

So, there are chiral selectors and with the help of that only one of them will be identified, and the other one will be remaining unidentified.

Hence, the received information is solely due to the changing absolute configuration of the absorbate interacting with the chirally modified surface. So, the surface has been modified chirally and only 1 enantiomer will be identified, the others will not be. So, it is possible to do this kind of enantio-specific interactions and then only, it can be identified, the chiral or the enantiomers can be identified.

The expected difference may be small, but these signals are enhanced by the phase-sensitive data analysis relating to improve signal to noise ratio.

So, now, this has become a very high tech analysis where chiral molecules can also be identified. Normally, the enantiomers will not show any difference in their IR. But because of this surface activity, where only one an enantiomer is selectively connected and the other one is not, and keeping in mind the diastereomeric interactions, it was possible to use chiral selectors, which could identify one type of enantiomer and the other type was rejected out.

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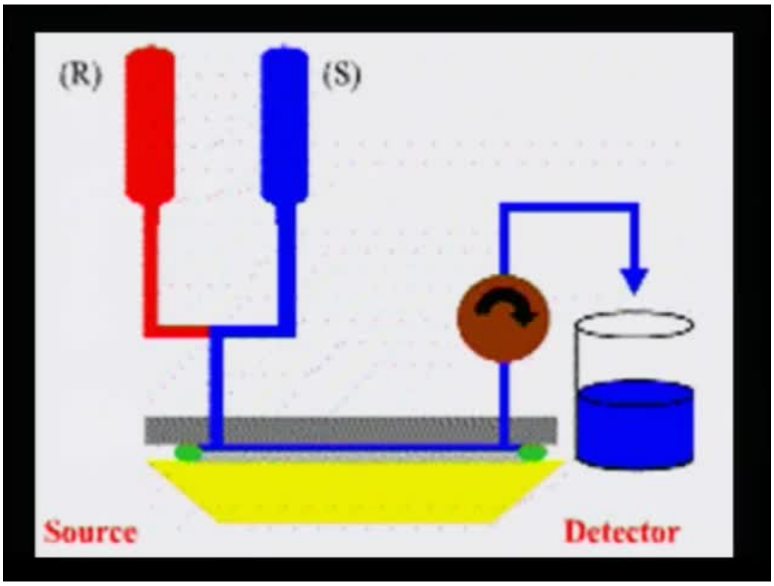

Now, if you take this kind of an example, here is the ATR element and this is how the R and S are connected. So, when R is going into the solution, only the red one is getting connected, when S is being used on the surface.

This is a very, very nice pictorial diagram. R and S can be separately identified using a material at the surface of the ATR, when different chiral selectors are used.

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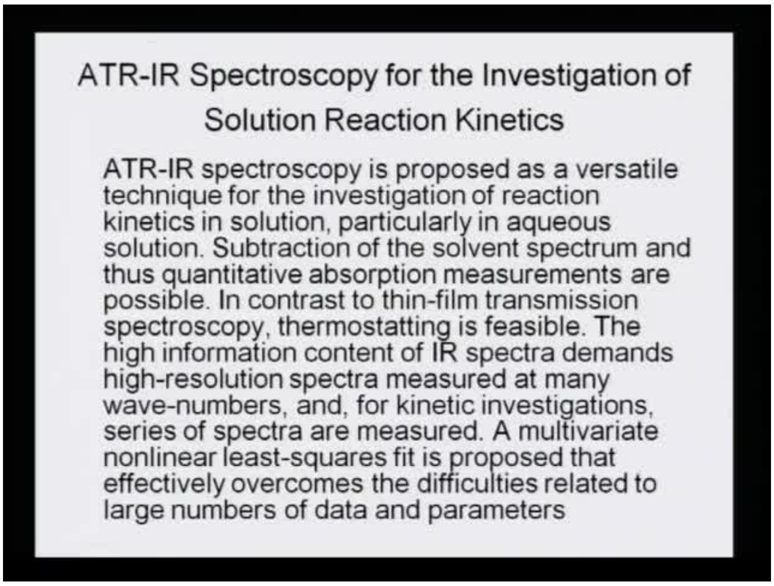

ATR-IR Spectroscopy for the Investigation of Solution Reaction Kinetics

ATR-IR spectroscopy is proposed as a versatile technique for the investigation of reaction kinetics in solution, particularly in aqueous solution. Subtraction of the solvent spectrum and thus quantitative absorption measurements are possible. In contrast to thin-film transmission spectroscopy, thermostatting is feasible. The high information content of IR spectra demands high-resolution spectra measured at many wave-numbers, and, for kinetic investigations, series of spectra are measured. A multivariate nonlinear least-squares fit is proposed that effectively overcomes the difficulties related to large numbers of data and parameters

ATR-IR spectroscopy is also used for the investigation of solution reaction kinetics. I just mentioned a while ago, that even kinetic studies can be done by the help of ATR-IR. Thus ATR-IR spectroscopy is proposed as a versatile technique for the investigation of reaction kinetics in solution, particularly in aqueous solution. Subtracting, subtraction of solvent spectrum and thus quantitative absorption measurements are possible.

So, what is done? It is used to study the kinetics in a solution. Kinetic means, what is the rate of reaction, whether the reaction is taking place at a fast rate, whether the reaction is taking place at a slow rate? That can be studied and in order to do this kind of study, what are the different parameters that have to be taken into account.

First, the solvent spectrum should be subtracted from the quantitative absorption measurements and only then, we can go ahead. In contrast to thin-film transmission spectroscopy, thermostatting is feasible. That means, temperature needs to be kept static and that is why, if the temperature is kept static, the rate of reaction can be studied.

The high information content of IR spectra demands high-resolution spectra measured at many wave-numbers, and, for kinetic investigations, series of spectra are measured. A multivariate nonlinear least-square fit is proposed, that effectively overcomes the difficulties related to large number data and parameters.

So, there are certain different software that need to be used for this kind of kinetic studies. However, it is used for solution reaction kinetics very effectively, keeping in mind the various adaptations that are required.

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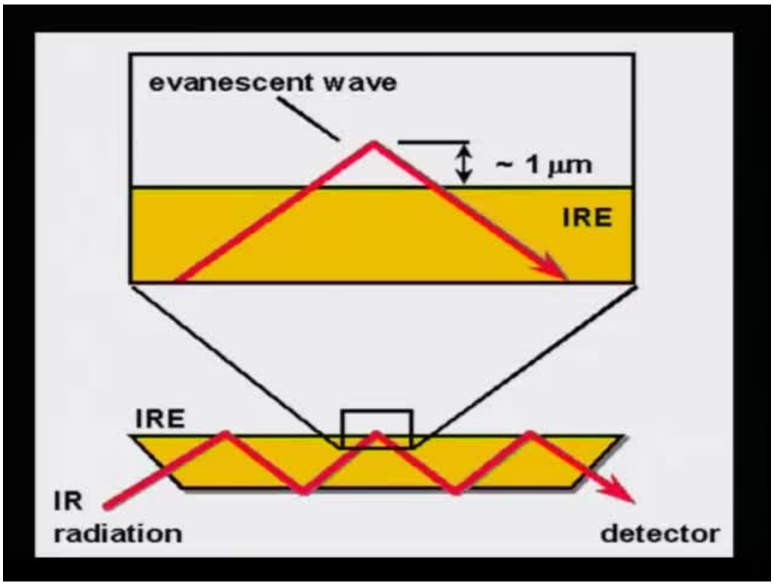

So, you see, we were talking about the evanescent waves, how they penetrate into the material of 1 micrometer and this, how the radiation, the sample is just made to sit here, and if we try to blow it up and see, then we will see, that the incident light is actually penetrating and coming back and again penetrating, but if the whole surface is occupied with the sample, it will penetrate several times before it goes to the detector.

Now, a part of this IR radiation will be absorbed in molecular excitations or bond excitations and that will be then, detected at the end of the experiment.

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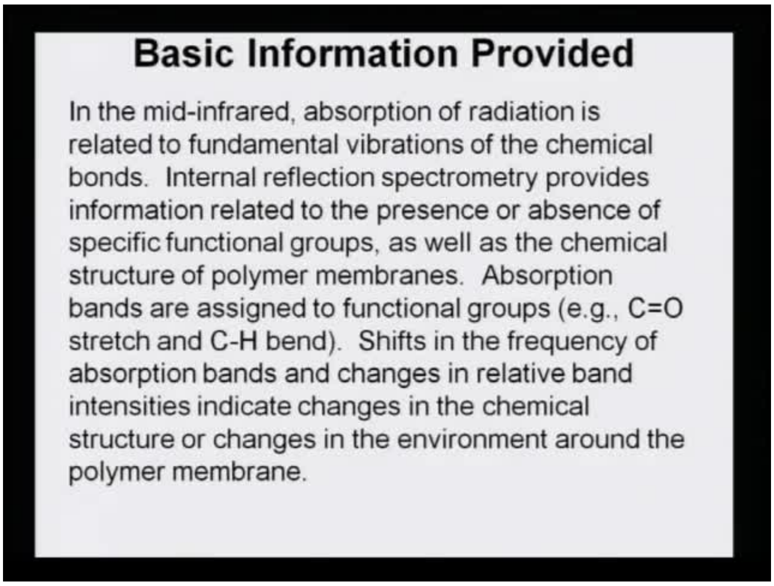

Basic Information Provided

In the mid-infrared, absorption of radiation is related to fundamental vibrations of the chemical bonds. Internal reflection spectrometry provides information related to the presence or absence of specific functional groups, as well as the chemical structure of polymer membranes. Absorption bands are assigned to functional groups (e.g., C=O stretch and C-H bend). Shifts in the frequency of absorption bands and changes in relative band intensities indicate changes in the chemical structure or changes in the environment around the polymer membrane.

Basic information that is provided in the mid-infrared, absorption of radiation is related to fundamental vibrations of the chemical bonds. Internal reflection spectrometry provides information related to the presence or absence of specific functional groups, as well as chemical structure of polymeric membranes. So, it not only gives us an idea about the functional group, whether it is present or absent, but it also gives us an idea about the various chemical structures, that are in the polymeric membrane.

Absorption bands are assigned to functional groups, that means whether, the, it has a carbonyl group, carbon-oxygen double bond, stretching or carbon-hydrogen bending, bonds shift in the frequency of absorption band and changes in relative band intensities indicate changes in chemical structure or changes in environment around the polymeric membrane.

So, it gives an entire overview of what are the different functional groups, not only carbon-carbon stretching, bending, carbon-hydrogen bending, carbon-oxygen stretching, but there are other kind of information related to chemical structure, that can be ascertained for polymeric membranes.

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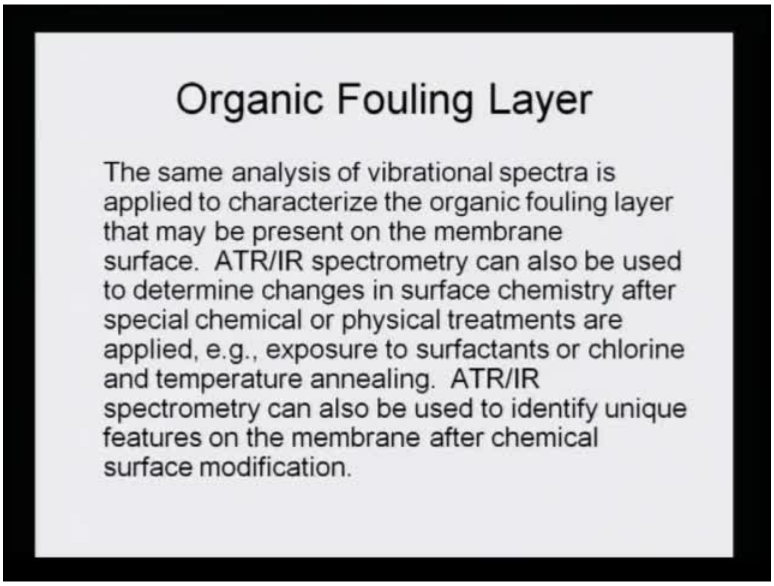

Organic Fouling Layer

The same analysis of vibrational spectra is applied to characterize the organic fouling layer that may be present on the membrane surface. ATR/IR spectrometry can also be used to determine changes in surface chemistry after special chemical or physical treatments are applied, e.g., exposure to surfactants or chlorine and temperature annealing. ATR/IR spectrometry can also be used to identify unique features on the membrane after chemical surface modification.

Even organic fouling of the layers can be analyzed. Now, you have known that membranes are being used for ultra-filtration, in RO's, in might membrane filtration system. Now, how do we understand, that the membrane is now completely used up and it needs to be changed? The organic fouling, if it is causing, how is that analyzed? The same analysis of vibrational spectra is applied to characterize the organic fouling layer and that may be present on the membrane surface.

ATR, if there are organic substances, which are creating the membrane to get blocked, then they can be analyzed by simply ATR/IR spectrometry, and it can be used to determine changes on the surface chemistry after special chemical or physical treatments and are applied, that is, exposed to surfactants or chlorines and temperature annealing. ATR/IR spectrometry can also be used to identify unique features on the membrane after chemical surface modification.

So, you see, that because these films can be directly kept in touch with the IRE material, that is, the zinc selenide and the germanium or the germanium crystal, they can be identified to understand the surface chemistry after special chemical and physical treatments, that means, if it has been used for certain removal of organic compounds, like surfactants.

Whether it has got completely choked up or not or what is the kind of substance that has been actually trapped by these membranes can be studied, because they are sitting on the surface of the membrane and they can be identified.

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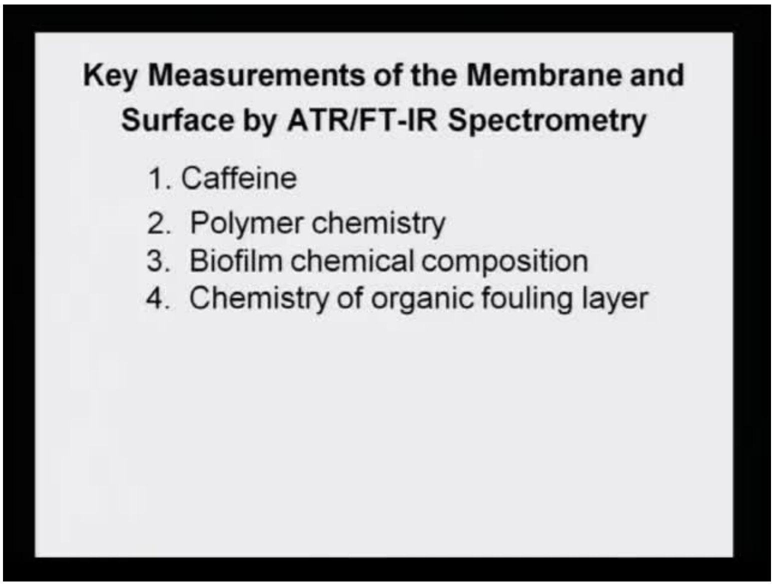

Key Measurements of the Membrane and Surface by ATR/FT-IR Spectrometry

1. Caffeine
2. Polymer chemistry
3. Biofilm chemical composition
4. Chemistry of organic fouling layer

Key measurements of the membrane and surface by ATR/FT-IR spectrometry - can be not only analysis of caffeine, where we use the typical 1665 centimeter inverse peak identification, it can also be used for polymer chemistry. So, a vast number of polymers, which could not have been analyzed by, I, FT-IR could be analyzed by these adapted or devices, that is, the ATR/FT-IR because the film could be simply kept on the surface of that crystal and could be analyzed.

The biofilm chemical composition could also be analyzed. Various types of membranes, that are used in the filtration processes, what is the kind of material, that has adhered to the film after it is been used, can also be analyzed.

The chemistry of organic fouling layer can be analyzed with the help of ATR/FT-IR. So, you see that it is such a diverse field, that it can be used for several, several purposes.

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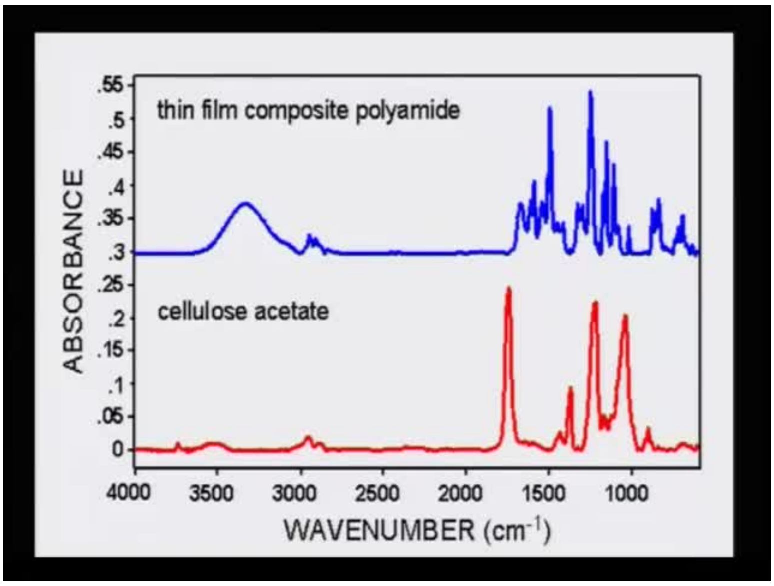

Now, in this particular slide, I have shown, that cellulose acetate, which is a membrane material and a thin film composite polyamide, I have been compared and shown in red and blue peaks and you can see, that they show different kind of peak separations because different materials have been adhering to it, so they can show absorbance. And you see, on the x-axis is the wave number expressed in centimeter inverse, whereas on the y-axis is the absorbance. So, one can find out, what are the kind of peaks, what is the region and these regions show very clearly, that in the case of thin film composite, there is an o edge group, there are other functionalities, which are appearing, whereas in cellulose acetate case, there is an intense acetate peak, which is coming between 2000 to 1500 or at the peak, which is at 1700 or so, which is a typical peak of an ester.

So, one can see the different materials can be analyzed, these IRs have been taken by the machine ATR-IR and have come out with such beautiful IR spectrum, that one can, may use them for diagnostic purposes.

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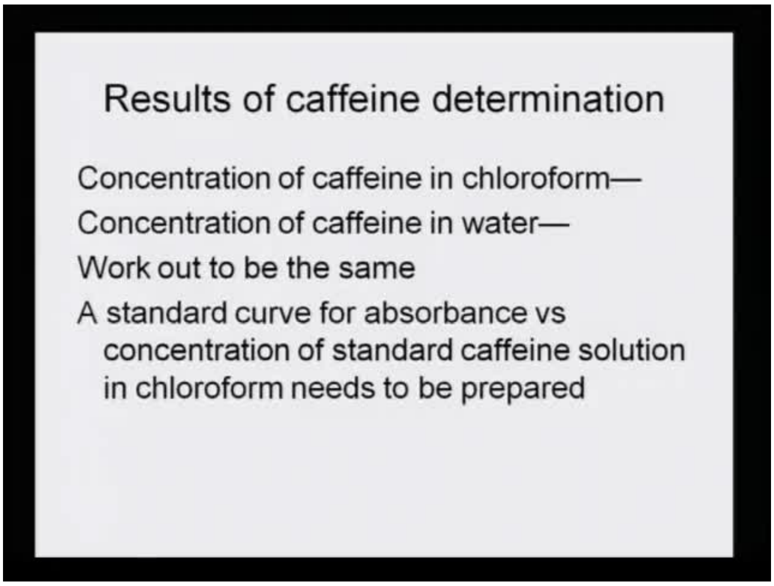

Results of caffeine determination

Concentration of caffeine in chloroform—

Concentration of caffeine in water—

Work out to be the same

A standard curve for absorbance vs concentration of standard caffeine solution in chloroform needs to be prepared

Now, coming back to caffeine analysis. The concentration of caffeine in chloroform, the concentration of caffeine in water is first, figured out. How much can caffeine, it, dissolved in chloroform? How much caffeine is dissolved in water? What is the solubility effect?

And both, when they are worked out, then a standard curve for absorbance versus concentration of standard caffeine solution in chloroform, needs to be prepared. I said that it is a validation or a calibration method. That means, there has to be standards made up of 1, 2, 3, 4 ppm and then, the absorbances of each must be taken and that would give, if it gives a linear response, means, the machine is working alright, the method is working alright and now, new samples, unknown samples of caffeine can be analyzed on this calibration curve.

Coming back to the analysis; the analysis completely shows one effective, that this calibration method can be used for analyzing the caffeine content in various kinds of beverages. It is not just, that only one type of beverage, like it can be the caffeine content in cola, in, caffeine content in tea, caffeine content in coffee, all can be determined by making because when, see I gave you an example, why caffeine needs to be dissolved in chloroform? Why needs to be dissolved in water?

Because the solubility of caffeine was much better in chloroform. Obviously, the more the compound, the better will be the absorbance and so that is why, the choice of standard curve preparation or calibration curve preparation for caffeine solutions, was the use of chloroform instead of water. It was found out that water was not the ideal solution for it and therefore, the best method was to use chloroform because that would surely, be the ideal choice of solvent.

And when these calibration curves were made, the standard solutions were prepared in different concentrations and the concentrations were taken as 1, 2, 3, 4 and 5 ppm and it was then found out that, what are the absorbances?

Then a linear graph against the absorbance and concentration was drawn and if it goes, shows a very linear line passing through the axis, it means, that the method is validated. It is, the calibration method is correctly chosen for the analysis of caffeine in different samples.

So, with this, we have come to an end of the very interesting chapter related to IR. We saw, that how IR can be used for functional group determination and how this particular IR region of the electromagnetic spectrum can be utilized for, get, extracting information from any organic molecule or any inorganic molecule or any kind of polymeric substance, adhesives, any type of substance, even industrial samples, fouling, organic fouling layers, and so on and so forth.

And with the advancement of FT-IR and ATR-IR, we could now cover the whole range of samples for the analysis and it definitely, adds on to information related to molecules for the structure, required for the structural determination.