Application of Spectroscopic Methods in Molecular Structure Determination

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Lecture – 23
ESI and MALDI Mass Spectrometry

Hello, welcome to module 23 of the course on Application of Spectroscopic Methods in Molecular Structure Determination. In this module, we will consider MALDI spectroscopy, but before we go to the MALDI spectroscopy I want to demonstrate an important application of Electrospray Ionization Mass Spectrometry in a while.

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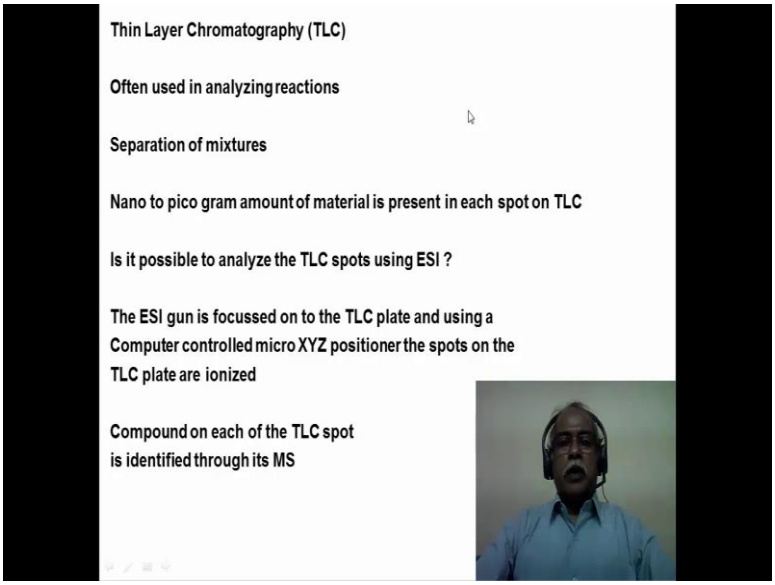

Now, we all know Thin Layer Chromatography is used for analyzing reaction mixtures, however complex the reaction mixture can be it can be analyzed by TLC separation. Essentially, TLC is used for separation of mixtures and nanogram to picogram amount of materiel is present in each spot on the TLC. Now the question is, is it possible to analyze the TLC spots using electrospray ionization mass spectrometry? It is possible because the electrospray ionization gun can be focused on to the TLC plate, and using a computer controlled XYZ positioner's the spots on the TLC plates can be ionized. Remember, TLC

plate is coated with the non volatile silica or alumina as a supporting material, so they do not get disturbed doing the process of the electrospray ionization mass spectrometry done on a TLC plate. The compound on each TLC spots is identified through a mass spectrum.

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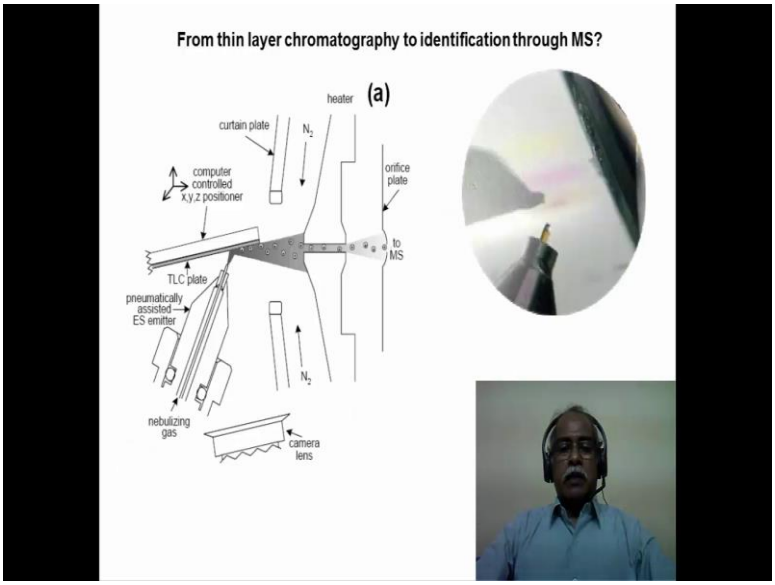

This is a diagrammatic representation of how TLC plate is analyzed using electrospray ionization mass spectrometry. This is a TLC plates which is mounted on to the XYZ positioner which is computer control, so there is a possibility of moving this in the XYZ direction with the resolution of a micrometer kind of a resolution on movement can be carried out. The electrospray ionization (Refer Time: 01:50) brought very close to the particular spot on the TLC plate and the solvent is sprayed on to the TLC plate and this droplets that hit the solvent takes the sample, that is spotted on the particular sample on that spot and they get ionized and they are injected into the mass spectrometer which is shown schematically. This is the actual picture of the TLC plate where there is a colored substance on the TLC plate and the electrospray ionization injector is focused on to that particular spot here.

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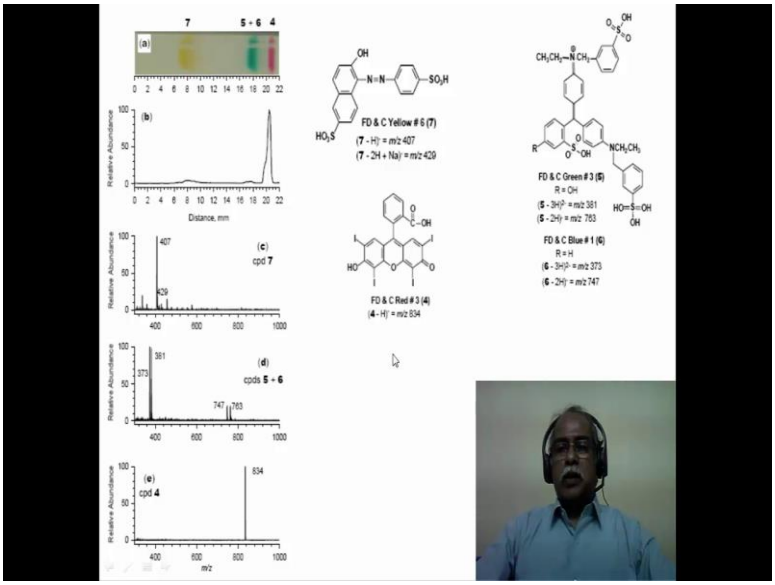

Now, here is an example of analysis of 3 different dyes, we have dye which is yellow in color which is labeled as 7 here that is another dye which is greenish blue in color which is. There are 2 kinds of dyes in this particular spot and there is another dye which is magenta color or of slightly purplish color in this particular region of the TLC plate. Now, if you select the mass of this dyes and run chromatogram kind of a thing, you will see when the mass is selected to compound number 4 which is this 1 for example, 834 that gives a maximum peak around the distance of about 20 millimeter of the TLC plate which corresponds to this. You can see here from 0 to 22 millimeter is the TLC plate length and around 21 millimeter or so, this spot is there which corresponding to molecular weight of 834 here.

And, now as the distance of the TLC plate is moved from 1 spot to another spot, each one of the spot is analyzed by the electrospray ionization mass spectrometer. For example, this is electrospray ionization mass spectrum of compound 7 which is this yellow color substance which in Azo dye is a sulfonic acid derivative and you can see for example, the molecular weight is 407 there is a peak which is a base peak at 407, then 407 plus sodium ion which is 23 corresponding to 429 which is this, 429 peak is also seen in the electrospray ionization mass spectrum. Now, spot number, blue spot is essentially a combination of 2 dyes.

The dye structures are shown here for example, there is a green dye which is called dye number 5 and there is another blue dye which is called dye number 5, essential difference between these 2 dyes is the R group is either phenolic OH group or hydrogen. These are the difference in the molecular formula of these 2 dyes. And, one can see for example, the molecular formula corresponding to R is equal to OH has a peak around 381 for tripely protonated compound and that corresponds to a molecular weight of M by Z 763 which is 2 hydrogen. In other words, this is a mono cationic species and this is dye cationic species that is why molecular weight is exactly half of the molecular weight of 763. You see 763 as well as 381 corresponding to the monocationically charged and dye cationically charged compound of this particular dye. Dye number 6, which is a blue color dye has a molecular weight of 373 and 747 the molecular weight is actually 747, the doubly charged ion comes at 373. So, one can see if 373 as well as 747 which is the molecular weight of this particular peak.

Lastly, the red color dye which is this particular dye, this has 4 iodine atoms, it is a fairly large molecular weight compound 834 and that is essentially seen as a 1 single peak at 834 corresponding to the molecular ion of this particular compound.

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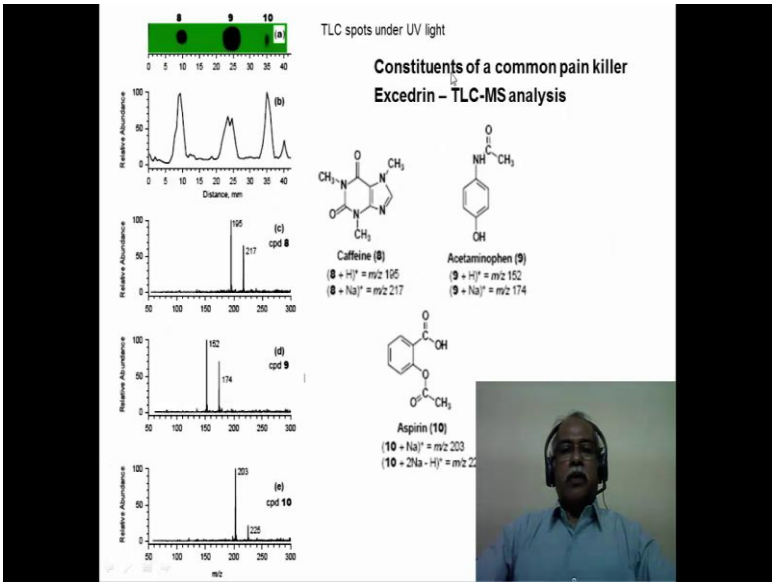

So, this is analysis of a mixture of dyes. Here, the constituent of a common painkiller

called Excedrin, this is available in the market. And the excedrin has 3 different molecules which are caffeine, acetaminophen and aspirin. Aspirin and acetaminophen are analgesic in nature, caffeine is essentially a stimulant. So this is a mixture that is present in Excedrin. A TLC analysis essentially shows 3 spots, spot number 8, spot number 9 and spot number 10 corresponding to compound number 8, 9 and 10, which are marked here for example.

This is the total chromatogram of this particular compound detected with certain molecular weight, so around 9 millimeter from the base you have the compound number 8 which is this caffeine. So, this correspond to the molecular weight of caffeine for example, this corresponds to the molecular weight of acetaminophen and this corresponds to the molecular weight of aspirin in this particular case. Now, the individual mass spectra are shown here corresponding to compound number 8 with a molecular weight of 195 and the sodium addict is 217, so 195 is the base peak, 217 also fairly intense peak. On the other hand, the acetaminophen has the molecular weight of 152 and the sodium addict is about 174, so 152 and 174 are clearly seen here. And the aspirin has a molecular weight of 203 and sodium addict plus sorry, minus 2 sodiums and a protons corresponds to 225. So, 225 and the molecular (Refer Time: 07:00) at 203 are also very clearly seen.

These examples, essentially demonstrate the versatility of thin layer chromatography combination with electrospray ionization mass spectrometry. How effectively this combination can be used for direct identification of organic compound that are absorb on to a TLC plate, which are separated by allocating the TLC plate.

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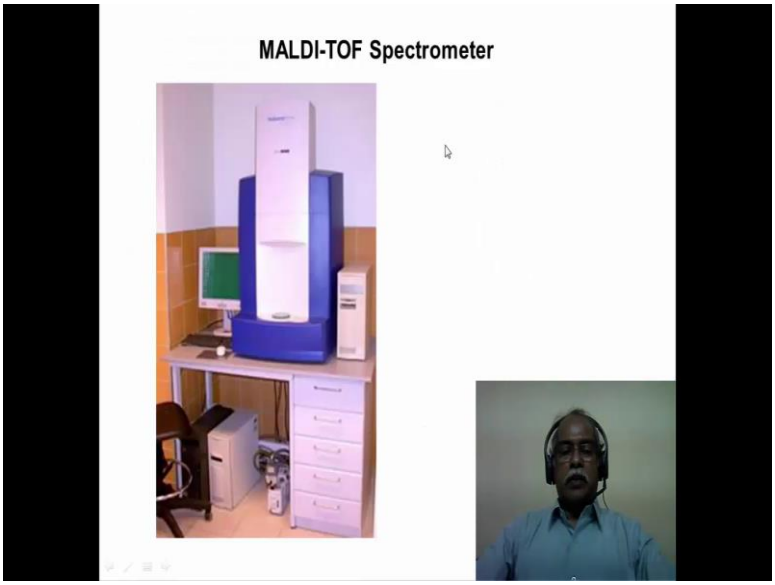

Now, we will go to the other most important mass spectrometry technique namely the Matrix Assisted Laser Desorption Ionization Mass Spectrometry as Ionization source and Time Of Light as an Analyzers in this particular mass spectral ionization analyzer combination of mass spectrometer.

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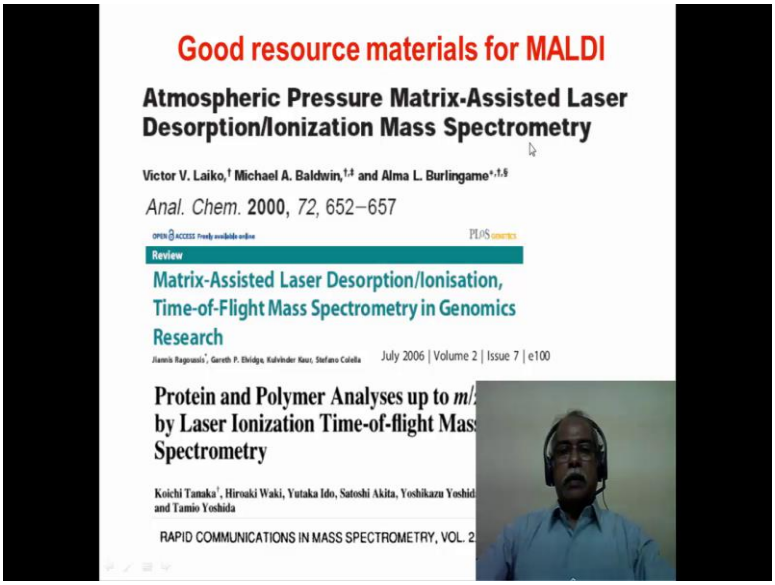

The good resource for materiel for the MALDI spectrometry is the 2 reviews that are given here, 1 is published in analytical chemistry in 2000 and other is published in rapid communication mass spectrometry in 1988. Both of them are good sources of information as per as the matrix assisted laser desorption ionization mass spectrometry in combination with the time of light analyzer for the mass spectrometry.

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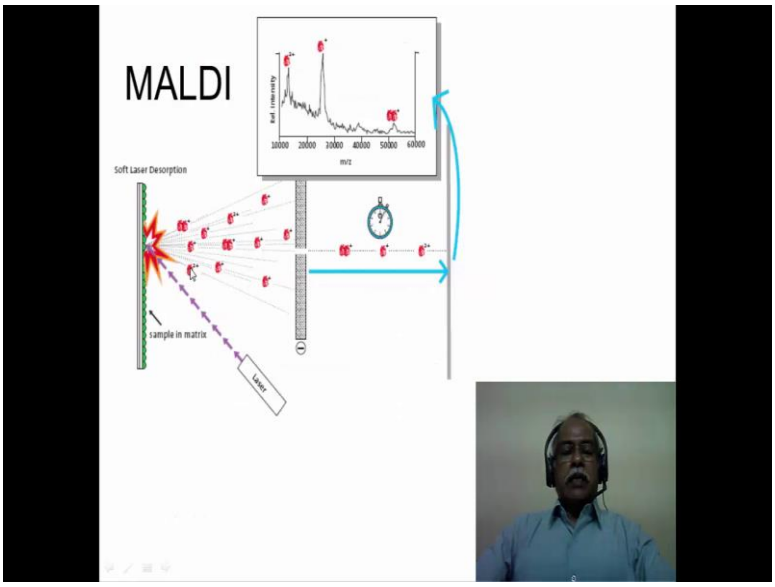

Now, the basic principle is very simple, the sample is mixed with the matrix and it is kept on a plate and laser light is shine on the sample, upon which the sample actually gets desorped and the matrix actually gets desorped along with the sample and during the process of desorption the ionization takes place, typically by a proton transfer from the matrix to the substrate that is investigated and differently protonated substrate molecules are flying as a result of that they enter the time of light analyzer. In the time of light analyzer, the basic principle is that smaller the ion it moves faster, larger the ion it moves slower. So, the ions get separated in a field free drift zone in the time of light analyzer and one by one the ions get detected in the mass spectrometer.

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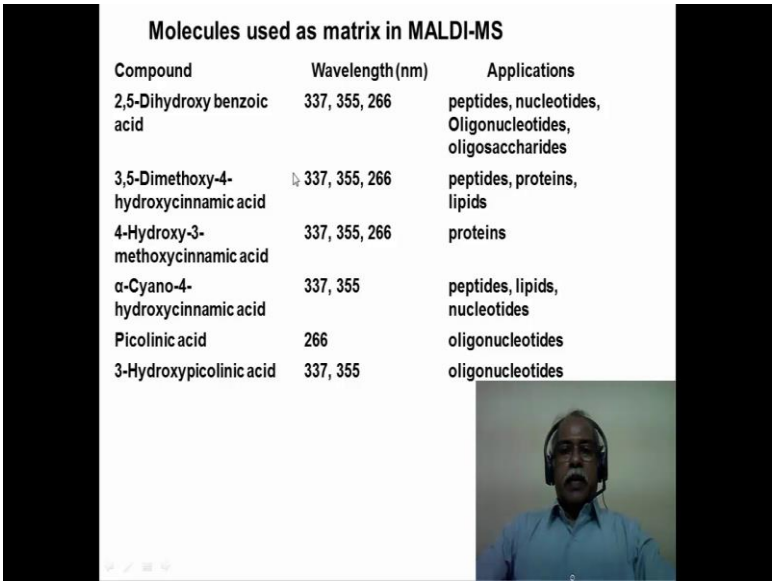

Now, the molecules that are used as matrix in MALDI spectrometry the list is given here, most of them are acids in nature and they have UV visible absorption and they are essentially used for the analysis of peptides, nucleotides, protein, lipids and so on; different application are shown here. The important aspect is that, these molecule they absorb UV light which is the laser light they get excited, in the excited state actually these are much stronger acid than in the ground state. Is one of the reasons from the excited state of this molecule, a proton can be easily transferred on to the substrate there by ionizing the substrate resulting in the formation of the ions that are produced in the gas trace for analysis.

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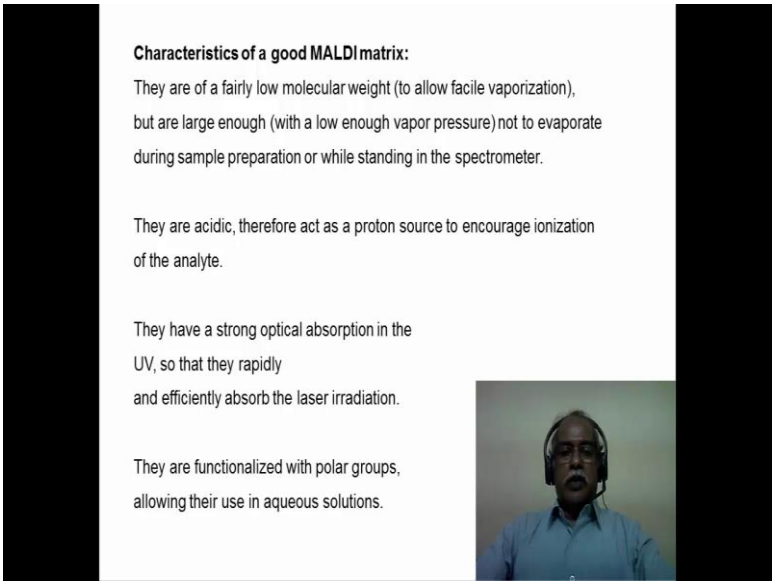

This is an important aspect of a MALDI mass spectrometry. The characteristics features of a good MALDI matrix are given in this particular slide, they are fairly low molecular weight compound so that it allows fissile vaporization upon impeachment with the laser light. But they are large enough not to have a very large vapor pressure, so that they do not evaporate during the sample preparation or while standing the spectrometer. This is extremely important. There should be low molecular weight so that they do not interfere with the mass analysis of other compound, at the same time they should have sufficient large, they should not extremely low vapor pressure to evaporate at room temperature and so on. They are generally acidic in nature therefore, as a proton source they encourage ionization of the analyte.

In other words, proton transfer takes place from the excited matrix to the sub analyte or the substrate that needs to be analyze as a result of that the charged ions produced in the MALDI spectrometry. They have a strong optical absorption in the UV, so that the rapidly and efficiently absorb the laser light and get disrobed into the gas phase. This is also extremely important they have to observe the UV light of the laser. Their functionalized with the polar groups allowing them to be used as aqueous solution, in other words during the sample preparation they can be dispersed either in a polar solvent like acetonitrile or polyethylene glycol kind of a sample or they can be simply dissolved

in water and eco solutions can be plated on to the MALDI spectrometer.

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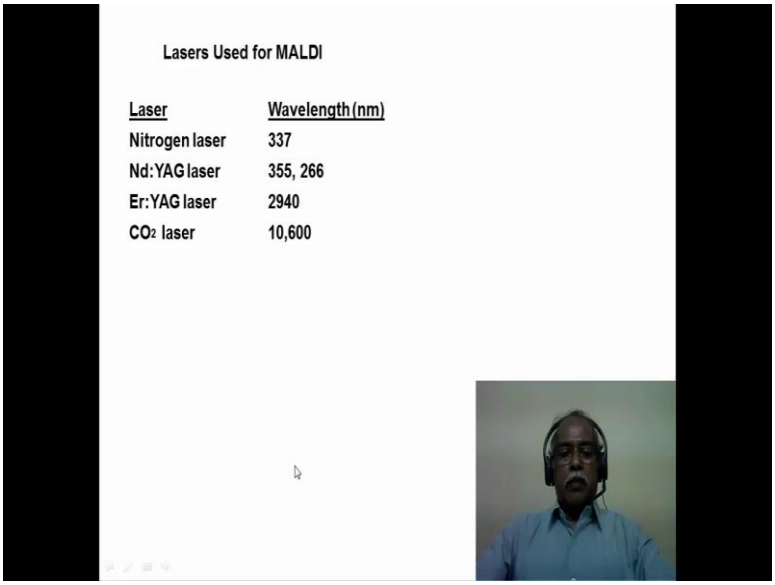

The lasers that are normally used are shown here, as we can see here they can be either ultraviolet lasers, they can be near infrared laser or far infrared lasers also can be used for this purpose. Typically, nitrogen laser is what is used in most of the MALDI spectrometer sometime YAG laser is also used to the second harmonic and the forth harmonic generation of the YAG laser are used for this particular purpose.

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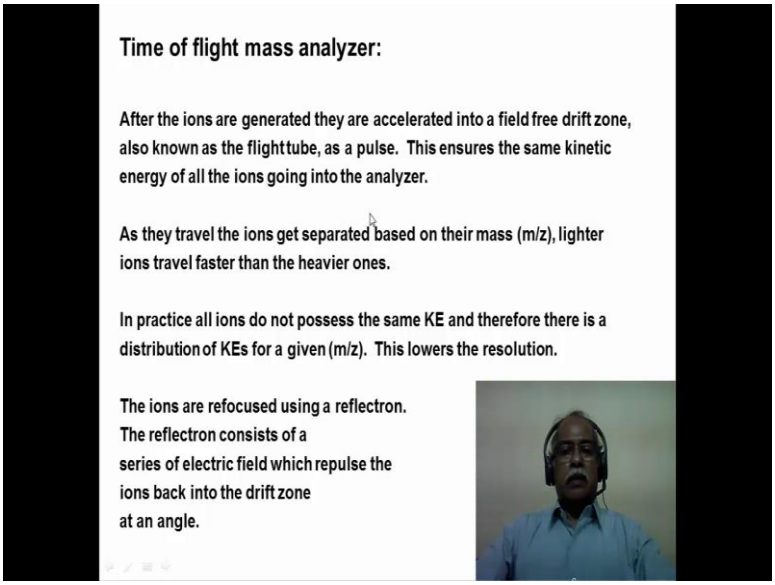

Let us look into the basic principle of the time of flight mass analyzer. After the ions are generated they are generally accelerated into the field free drifts zone which is known as the Flight Tube or the Time of Flight tube is what is given. And the samples are sent in the form of a pulse, why is sent as pulse? Because, that ensures the same kinetic energy for all the ions that goes in to the analyzer because in the analyzer there is no electric field or anything that is controlling the movement of the ions.

The ions are essentially drifting on their own kinetic energy such that the lighter ions move faster than the heavier ones, this is the basic principle of the separation. As they travel the ions get separated based on their master charged ratio, lighter ions travel faster than the heavier ions. In practice, all ions do not possess same kinetic energy this could be a limitation in terms of lowering the resolution of this spectrum that you see.

It is better to make sure that the all the ions have sufficiently the same kinetic energy, so that during field free drift region they use the mass charge ratio as a means of separation rather than the differential in the kinetic energy for means of separation. When there is a lower resolution, there is an option available to have the ions refocused using a reflectron, this will be explained in a next slide using a diagram Reflectron, essentially consist of series electric field which repulse the ions back in the drift zone at an angle

and thereby they have same kinetic energy during the course of that drift.

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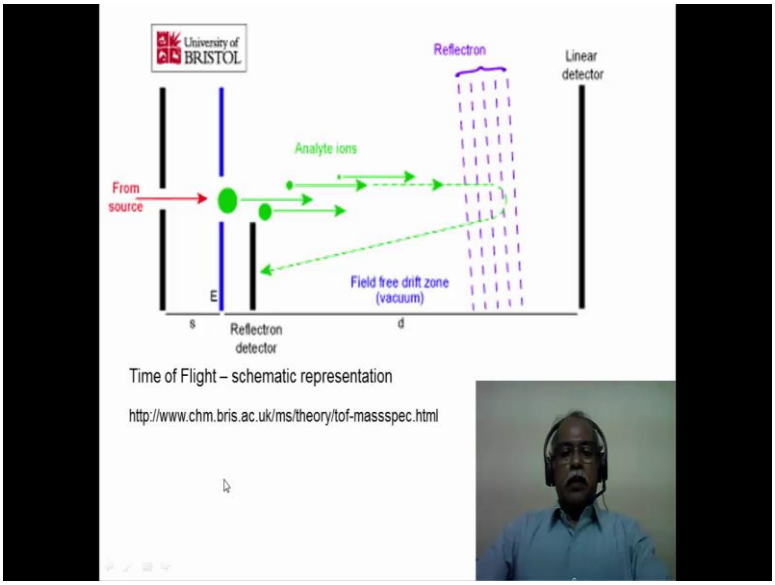

Here, is a source of ions which is produced by the shooting the laser on to the matrix and the samples get ionized for example, and the samples get into the gas phase and you can see here, this is the field free drifting zone which is the time of light tube for example. So the lighter ions travel faster because they have the same kinetic energy, but the mass by charge ratio is small so they move faster compare to heavier ions which although have the same kinetic energy because of the mass they have a lower velocity and as a result of that they do not move fast in this particular field free zone. If they do not have a reflectron essentially, they will be going to a linear detector and get detected and under this condition maybe a low resolution spectrum can be obtained. However, in a reflectron mode you have the electric field applied here, so that these get deflected and their send back in another field free zone under vacuum, so that in the reflectron detector it is detected.

In other words, to ensure same kinetic energy of the various ions this particular methodology is adopted, this is called the Reflectron technology and this particular methodology ensures the higher resolution of the spectrum that you see in certain MALDI spectrometers.

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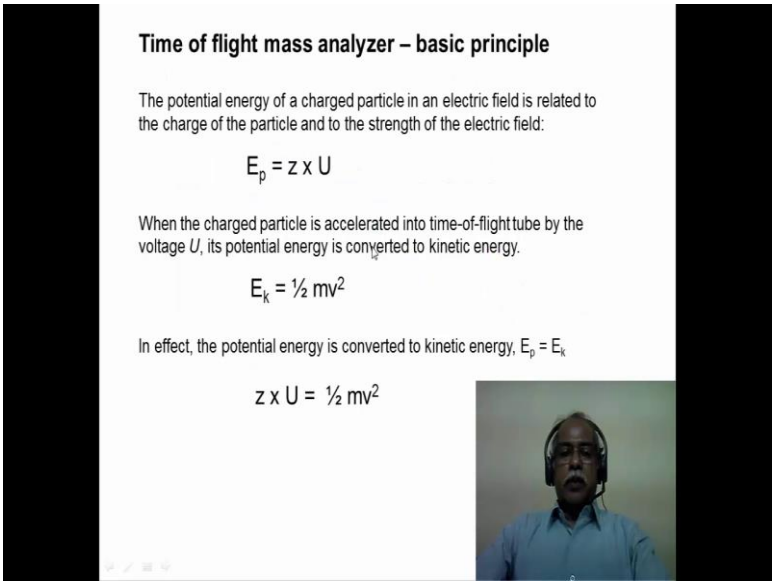

Now, in the time of flight analyzer of the basic principles like this, the potential energy of a charged particle in a electric field is related to the charge of the particle times the strength of the electric field. In other word, this is a potential energy which corresponds to E Z which is charge of that particular ion and the electric field which is U in this particular case. When the charged particle is accelerated in the time of flight tube, the potential energy gets converted into the kinetic energy. Essentially, kinetic energy is half mv square, so if we equate the z U into the half mv square this is the expression that one gets by equating the potential energy to be E same as the kinetic energy.

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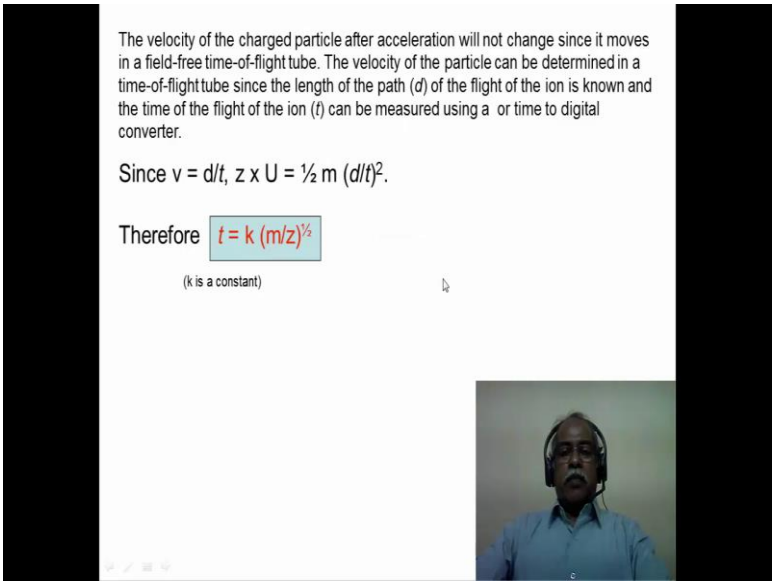

Now, the velocity is essentially distance by time. So, 1 can solve this equation by substituting velocity to be distance by time you have this particular expression where mv square the v is replaced by d by t here, so d by t square. If you rearrange this particular equation, essentially we get the time that is the flight time corresponding to the m by z square route of m by z times some constant.

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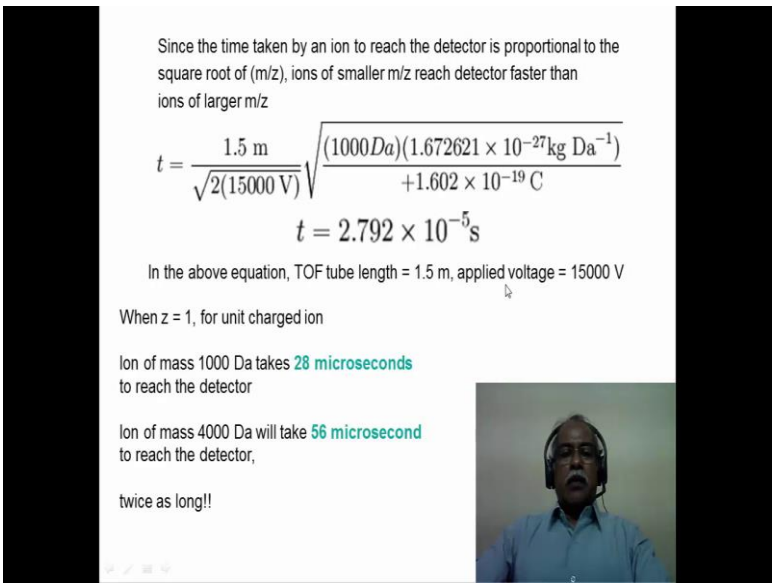

What is this constant? We will see in the moment.

Since, the time taken by each ion to reach the detector proportional to the square root of m by z of the ion, smaller ions reach faster compared to larger ion. This is a basic principle of time of life mass spectrometry and this is the expression that you saw with certain constant times m by z the whole power half (Refer Time: 15:51) square root of m by z is what you see. Let us say for example, a mass of 1000 Dalton is given this is the atomic mass unit in kilogram, in other words kilogram per Dalton 1.67 10 to the power minus 27 kilogram per Dalton is the atomic mass unit and this is the columbic charge 1.602 10 to the power minus 19 is the charge on in electron that corresponds to a mono positive charge corresponding to that particular ion. And, this is in a voltage application of above 15000 volt is what is used for the acceleration of the ions in a kinetic energy producing voltages is what is given here and the flight tube has a length of about 1.5 meters.

So, these are essentially constant as well as the measurement is concerned which correspond to the k. If you solve this equation, then for a 1000 Dalton molecular weight the ion takes about 28 micro seconds to travel a distance of 1.5 meter of that drift free zone of the time of light tube 1.5 meter it takes about 28 micro second. If you substitute 4000 and solve this you will get 56 microsecond, in other words a molecular ion with the molecular weight of 1000 Dalton, in other words 1000 m by z value will reach in about 28 microseconds the detector. Whereas, 4 times more molecular weight will take twice as long as the 1000 molecular weight for example, 56 microseconds will be taken by a 4000 molecular weight compound, so it is twice as long and so this ion will reach the detector faster and this will reach about twice as long it takes for this ion to reach the mass spectrometry detector.

This essentially established, is the basic principle which is stated here, that is ions with the smaller m by z value reach the detector faster this is 28 microsecond for 1000 Dalton compared to a larger ion, let us say, 4000 Dalton takes as twice as long to reach the that particular detector of the same tube of 1.5 meters.

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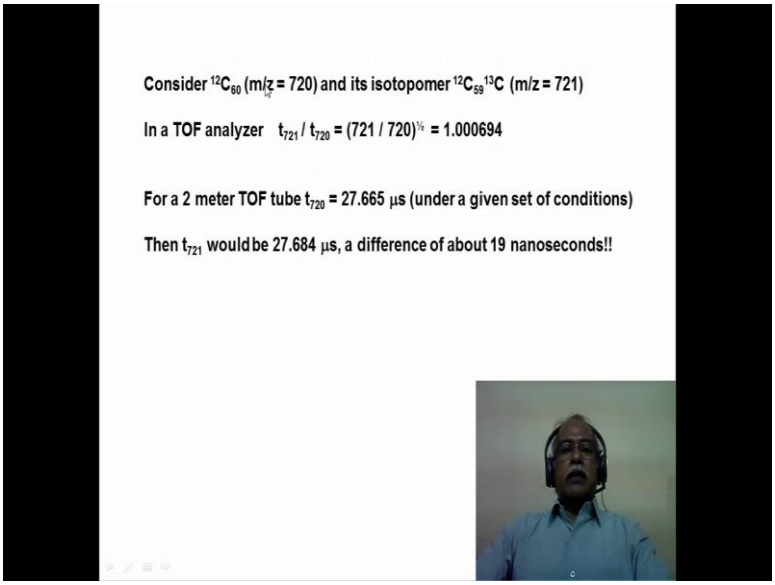

If we consider C 60, C 60 molecular ion with the carbon 12 isotope 720 and it is isotopomor will be having C 59 of 12 and 1 C 13 that will be 739 mass units. In a time of flight analyzer there is 1 mass unit difference here, so that would essentially correspond to 721 by 720 to the square root which is corresponding to 1.000694. So, for a 2 meter tube if you take this 720 molecular ion peak will take about 27 micro seconds to reach the detector and there given set of condition and 721 mass unit that is the isotopemor will take 27.684 microsecond. A difference of about 19 nanosecond is what is needed for the separation of this 2 ions in a flight tube of about 2 meter length, and this essentially, illustrates the resolution of the mass spectrometry in terms of being able to separate masses of 1 unit, 1 mass unit difference by about 19 nanosecond difference in their time of flight time.

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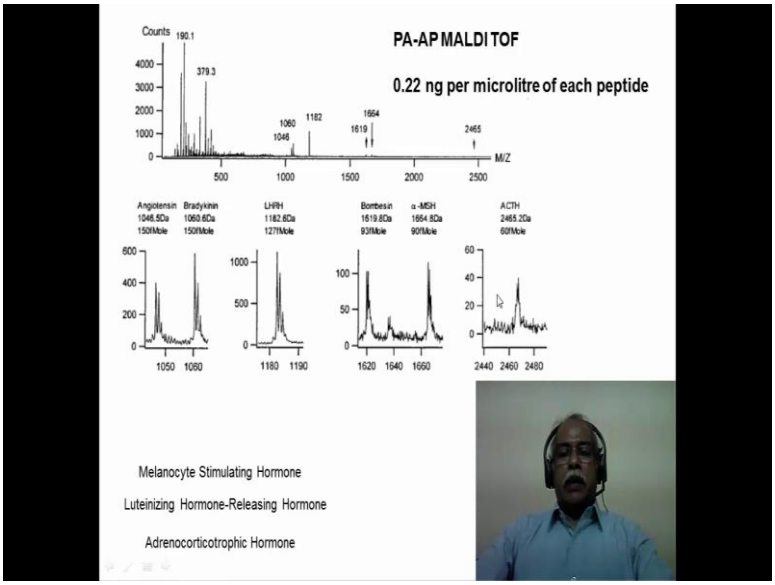

Now, let us look into some application of the MALDI spectrometry, here some simulating hormones and several hormones are given as the mass spectromer several hormones are given. These are all recorded under MALDI condition for example, Angiotensin, Bradykinin this are molecular weight of half the order of 1046 and 1060 and 150 femtomolar concentration is what is injected and the molecular weight 1046 is recorded here and 1060 is recorded here for this particular compound. And this is Luteinizing Hormone-Releasing Hormone. This is another hormone with the molecular weight of 1182 and about 127 femtomolar concentration is what is injected in the MALDI spectrometer to obtain this particular spectrum.

Similarly, for Bombazine and Melanocyte stimulating hormone, the spectrum is given here and this is a adrenocorticotrophic hormone that is a another hormone with the molecular weight of 2465 that spectrum is also shown. So, essentially indicating that medium size molecules are fairly readily analyzed by the MALDI spectrometry using the time of flight analyzer. This is a mixture of this derivatives that is injected together indicating that there is a possibility to form molecular complexes of 2, in other words, the protein ligand complex can also be formed under this condition resulting in the formation of certain molecular adducts which could be detected here.

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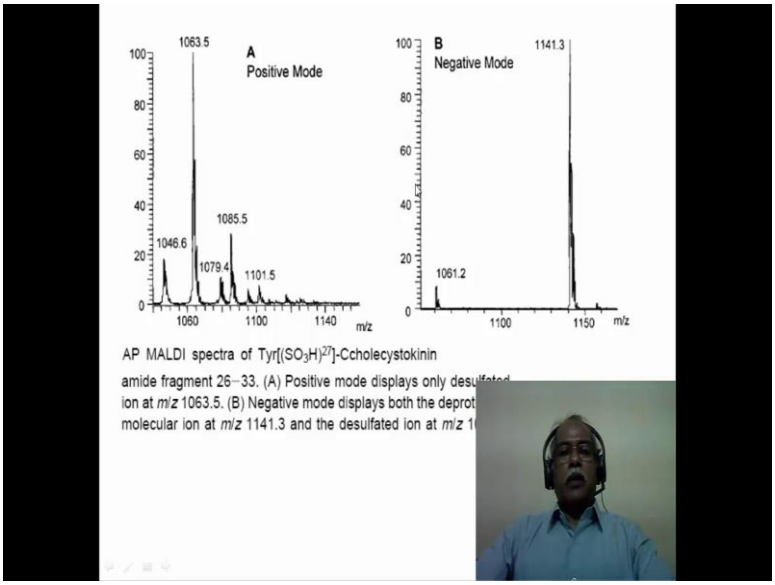

If you take the Tyrosine Ccholecystokinin, this is a particular bio molecular system and this has a molecular weight of 1141 or 1061 if it is a desulfated molecule it is 1061 if it is a protonated deprotonated system it is 1141. So, if you look at the positive ion mass spectrum it is a desulfated ion that has the prominence in the positive ion mass spectrometry. Whereas, in the case of the negative ion mass spectrometry the deprotonated species is what you seen has 1114. This essentially illustrate the use of MALDI spectrometry, atmospheric pressure MALDI spectrometry in the positive ion mode as well as in the negative ion mode. In the positive ion mode, you get the desulfated system as the major peak, whereas in the negative ion mode the deprotonated molecule is what you seen as the base peak in this case.

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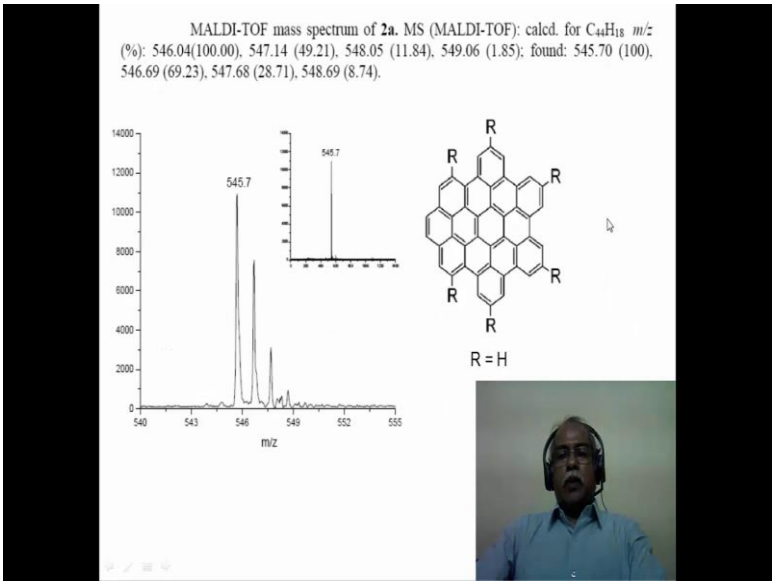

These are molecules which are considered to be graphene analogues. In other words, graphene is the mono layer of graphite for example. These are all model compounds which is supposed to mimic the property of the graphene, so the synthesis and the characterization of this class of molecule has been undertaken by a number of groups particularly by the group of class Mullen at the max Planck Institute for polymer portion, the large number of this class molecule have been synthesized. One of the ways to analyze this molecule to use to record the MALDI top mass spectrum of this compound. This particular sample has a molecular formula of C 44, H 18, it is not a very large molecule. Nevertheless, the molecular ion peak can be seen as 545 here and the isotope abundance peak can also be seen as 546, 547 and so on.

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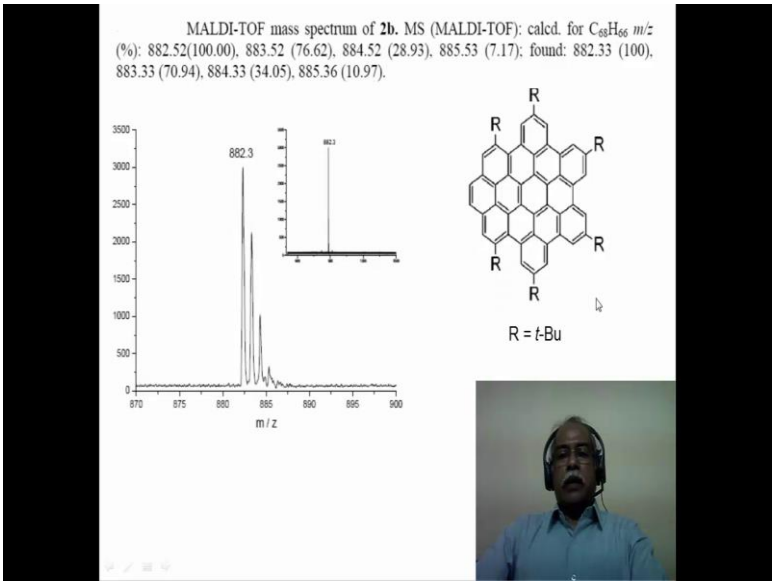

As a cluster of peaks in the molecular ion region, where R is equal to hydrogen. The same molecule when R is equal to tertiary butyl is shown here. The isotope abundance peaks are shown for the molecular ion region, so essentially the cluster of peaks that you see in the molecular ion region corresponds to the M plus M plus 1 M plus 2 and so on. This is a unit resolution mass spectrum of the same compound is shown here.

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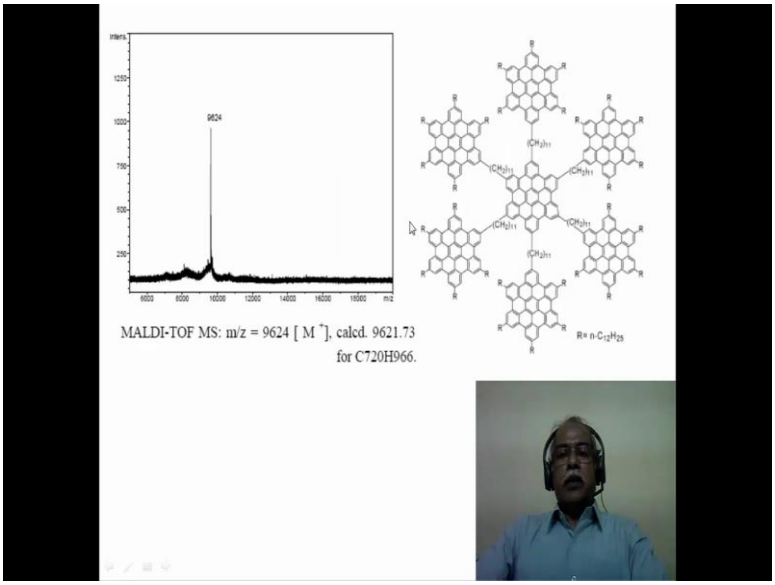

This is a fairly large molecule. Molecular weight is fairly high 9624 in this particular case. The molecular formula is C 720, H 966 it is basically a hydro carbon molecule containing only carbon and hydrogen with this molecular formula with the molecular weight of 9624. MALDI spectrometry clearly tells a peak around 9624 indicating the formation of this particular molecule which is a large molecular systems, synthetic molecule where hexabenzo coronene has been connected to the central, this is like a star burst kind of a molecular system is what is investigated here.

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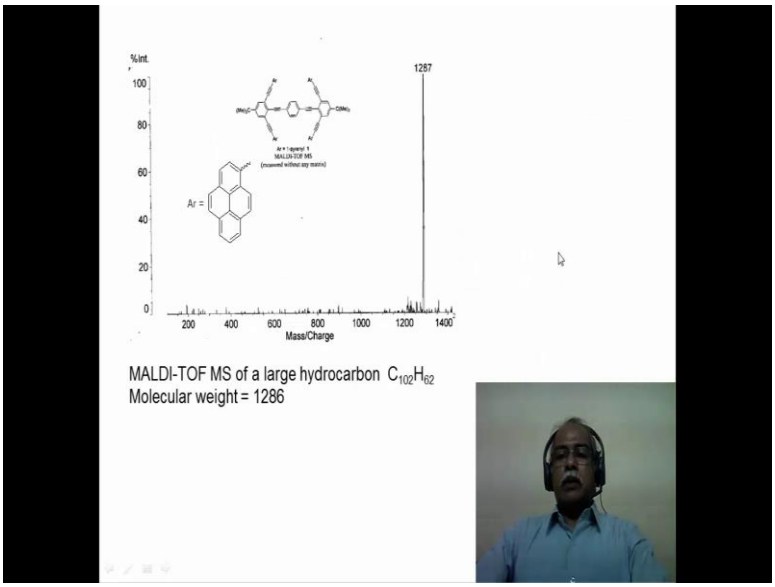

This is a molecule that was synthesized in our own laboratory. This has a large hydro carbon with the molecular formula of C 10, H 62, because it has a large number of acetylic units the number of hydrogen is much less than earlier example for this reason. Molecular weight is 1286 what you see is a protonated species, so M plus, H plus is what you seen as the 1289 peak of this molecule with 4 (Refer Time: 24:00) units attached to the spindle kind of a molecule that is shown here.

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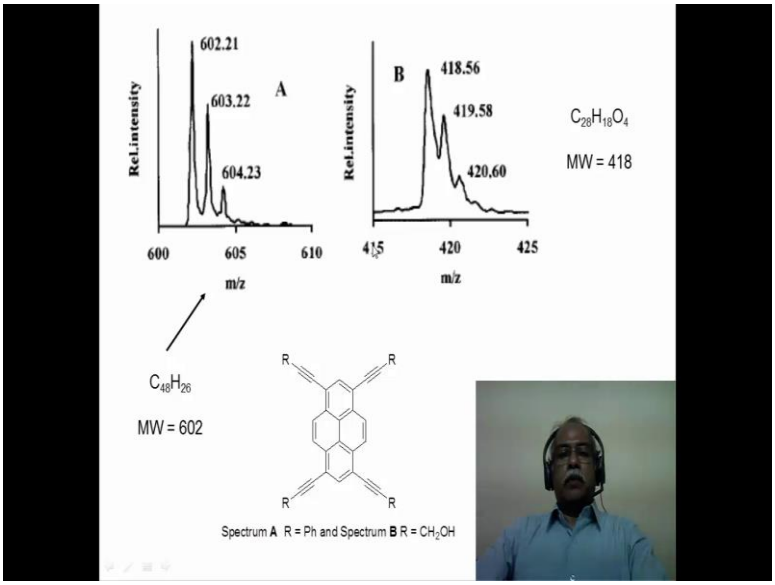

These are tetra ethylene pionene derivatives and these derivatives are analyzed by MALDI spectrometry. MALDI spectrometry not only the molecular ion peak is seen, resolution is good enough to see the M plus 1 ion as well as the M plus 2 ion in this cases. The molecular weight of this compound is 602, so you see 602, 603 and 604 when there is a hydroxy functional group in the form of a CH 2, OH is attached here the molecular weight is 418, so you see 418, 419 and 420. This is R is equal to phenol derivative and this R equals to CH 2, OH derivative the R groups are mentioned in this diagram here.

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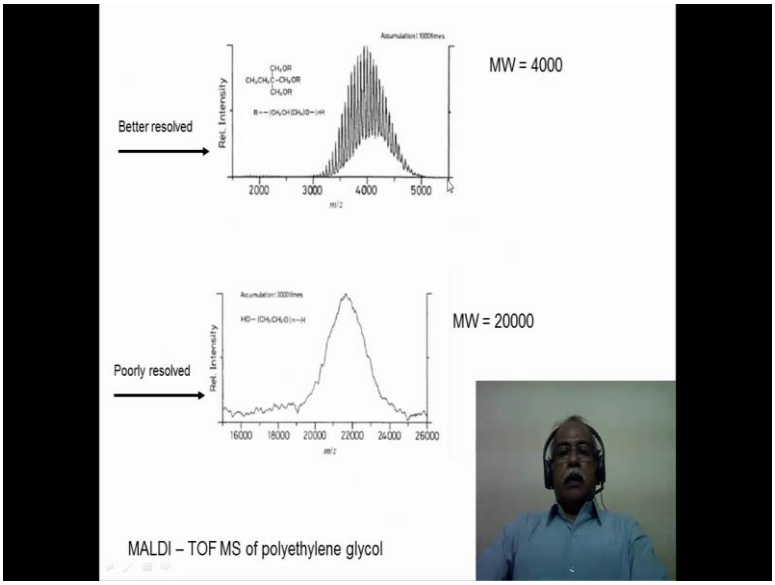
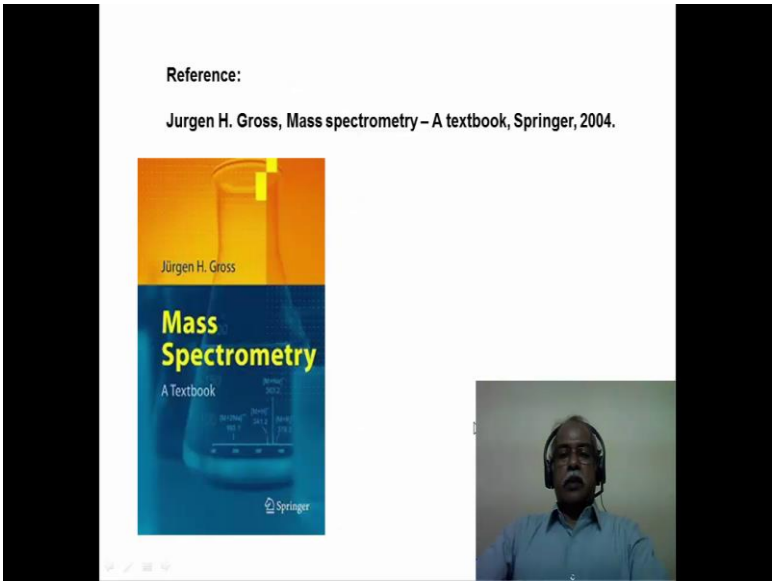


The earlier in the electro spray ionization mass spectrometry we saw some examples of analysis of polyethylene glycol; this can also be done by MALDI mass spectrometry. This is a polyethylene glycol with the molecular weight of 4000. Now, remember when we analyze the molecular ion peak of the 4000 molecular weight polyethylene glycol, in the case of electrospray ionization mass spectrometry several protonated species with the charge 1, 2, 3, 4, 5, 6 up to 6 charges have been recorded.

However, in the MALDI spectrum a singly protonated system is what you see here, corresponding to a molecular weight of about 4000 nominal molecular weight which corresponds to the molecular weight of this polyethylene glycol. With higher molecular weight of the polyethylene glycol with a 20000 molecular weight for example, the resolution is fairly poor in this particular case, this is a fairly well resolved spectrum where you can see the individual molecular weights of the oligomers, whereas, here the individual oligomers are not resolved, you just a essentially get a Gaussian type of an envelop for the entire molecular weight distribution of the polyethylene glycol of this sample.

(Refer Slide Time: 25:51)



Now, this book is a good source of information for generally for the mass spectrometry particularly, for electrospray ionization mass spectrometry as well as MALDI mass spectrometry by Jurgen Gross Mass Spectrometry text book published in 2004. So, what we have seen in this module, is the application of electrospray ionization mass spectrometry for the analysis of TLC plates, then introduction to MALDI Mass Spectrometry the basic principle. The principle behind the time of a flight analyzer of the MALDI Mass Spectrometry and some examples of various compounds being analyzed by MALDI Spectrometry.

Thank you for your attention.