Application of Spectroscopic Methods in Molecular Structure Determination

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Lecture - 21
ESI and MALDI Mass Spectrometry

Hello, welcome to module 21 of the course on application of spectroscopic methods in molecular structure determination. Now, we have electrospray ionization mass spectrometry and a MALDI mass spectrometry to cover in these few modules. That we will be dealing with in a next couple of weeks.

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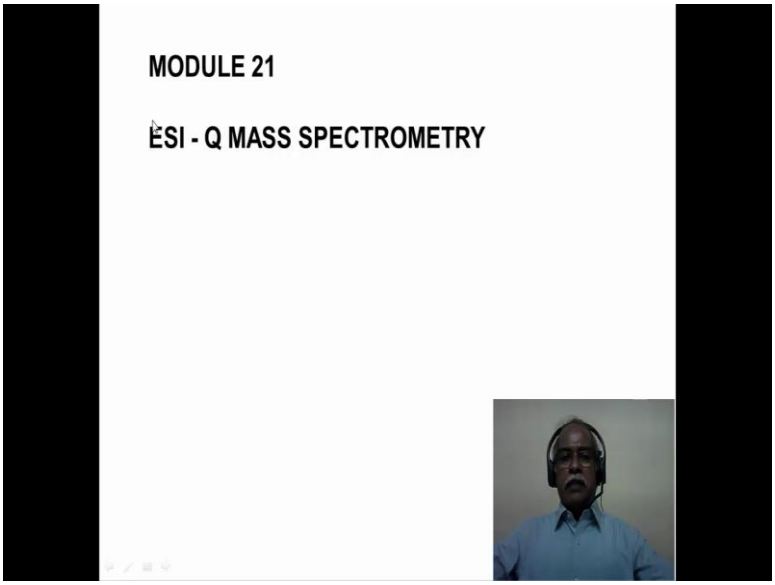

First, we will start with the electrospray ionization mass spectrometry. Electrospray ionization mass spectrometry as a ionization source and the quadrupolar mass analyser is a very powerful combination in mass spectrometry. Many modern spectrometers are equipped with this combination of ionization source and a mass analyser as a standard tool for mass spectrometry.

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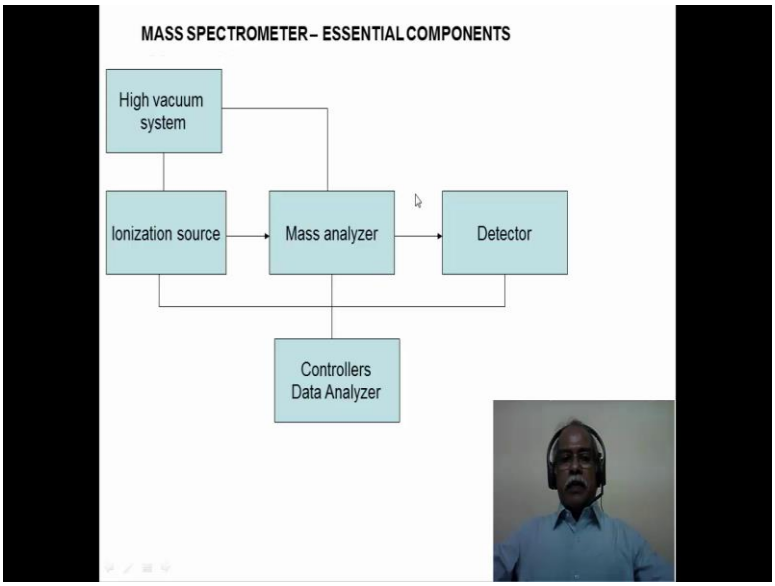

Now, in the mass spectrometry generally we have a very high vacuum system. And, the ionization source is also under a high vacuum of the order of 10 to the power minus 6 or 7 (Refer Time: 01:00).

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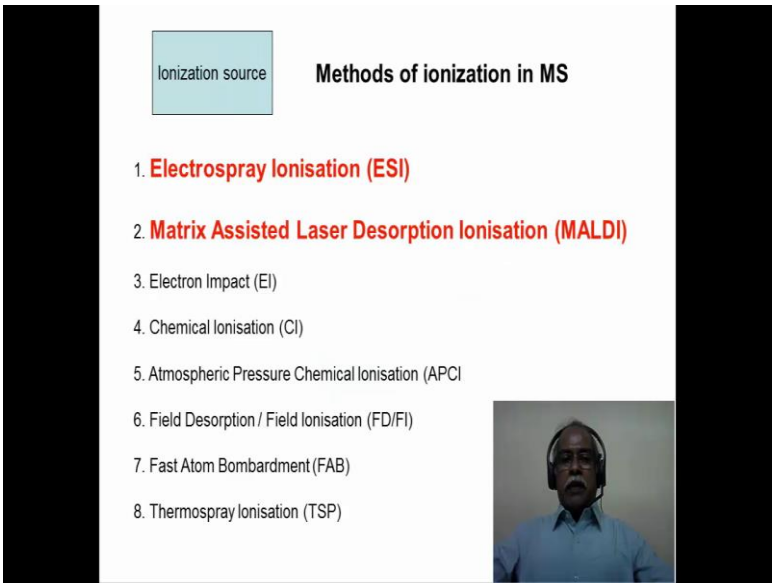

But, there is an exception in the case of the electrospray ionisation mass spectrometry and the matrix assisted laser desorption ionisation mass spectrometry; where the ionization actually can take place at the atmospheric conditions and then, the ionised samples taken into a vacuum chamber into the analyser and so on.

So, we will consider first, the electrospray ionisation mass spectrometry. The basic principle behind the electrospray ionisation mass spectrometry in combination with the quadrupole analyser.

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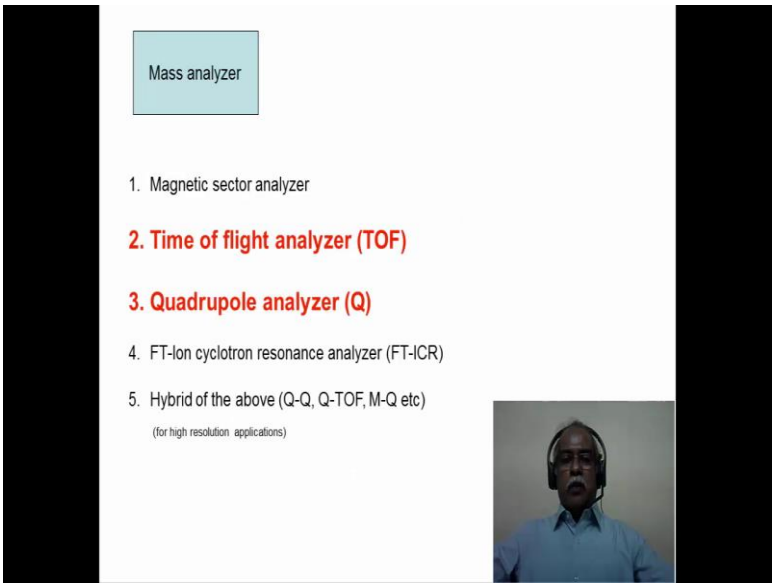

Typically, the electrospray ionisation mass spectrometry with the quadrupole analyser and the time of flight analyser, what we call as the Q-TOF ESI is a standard combination. Whereas, in the case of MALDI, MALDI-TOF is the standard combination; where MALDI is the ionization methodology and the time of flight analyser is the mass analyser as a standard combination.

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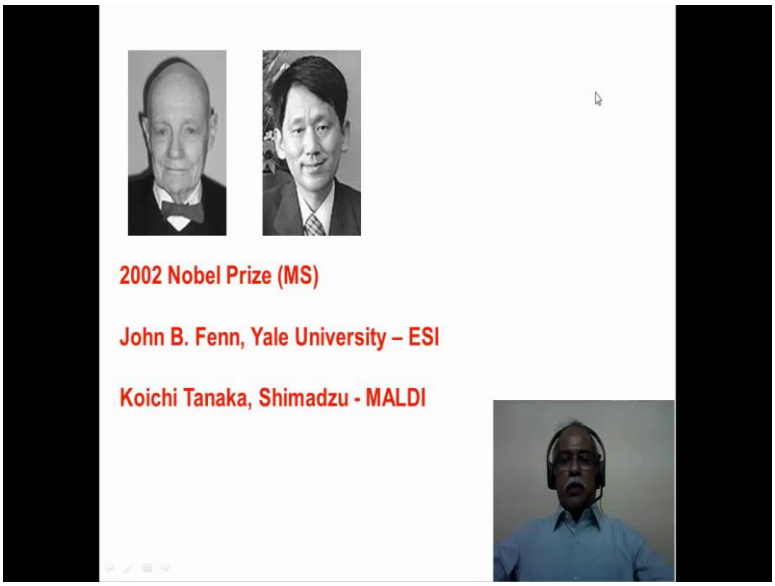

Now, John Fenn and Koichi Tanaka were the discoverers of the electrospray ionization mass spectrometry and MALDI mass spectrometry. John Fenn from Yale University and Koichi Tanaka from Shimadzu Company were the inventors of this modern technique. They were awarded the Noble Prize for the reason that these two techniques are very powerful techniques in the spectroscopic technique or the identification of large macro molecular system with the molecular weights of the order of hundreds or thousands and so on.

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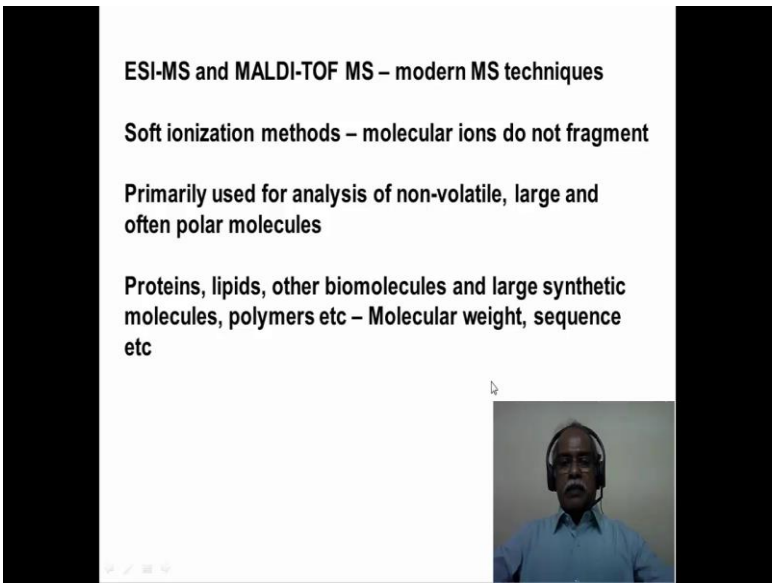

Now, the electrospray ionization mass spectrometry and the MALDI mass spectrometry; these are modern mass spectrometry techniques. These are also known as soft ionization methodologies because of the soft ionization technique; the molecular ions do not possess so much of energy like the electronic impact ionization mass spectrometry. So, the molecular ions do not fragment under this ionization condition. That is extremely important.

It is primarily used for the analysis of non-volatile, large and often polar molecules. Typical examples are proteins, lipids and other biomolecules like DNA and so on. Large synthetic polymeric materials also can be analysed using this mass spectrometry technique. One can do, for example, molecular weight analysis. As well as in the case of proteins and DNA, one can even do sequence analysis in terms of the primarily structure of this bio macro molecular systems.

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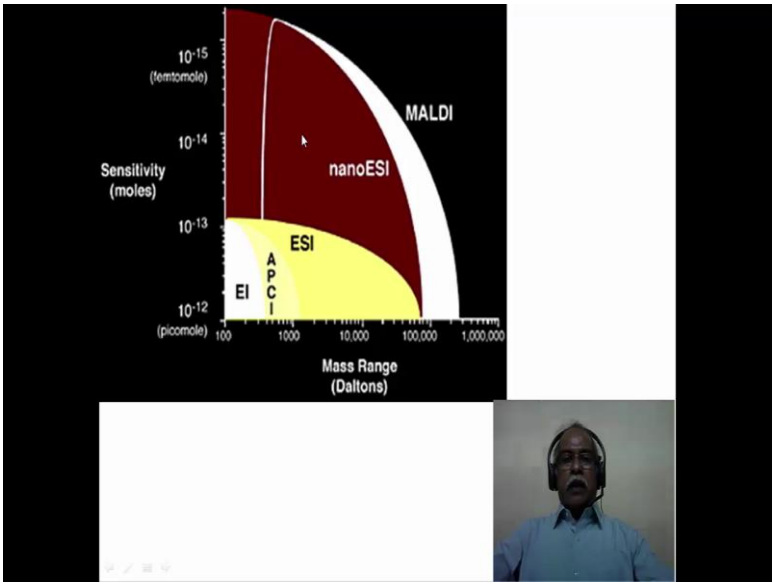

This is a familiar graph that we have already seen. Dealing with the mass range that is covered by the various ionization techniques and the sensitivity related to that particular technique. Let us focus on the electrospray ionization mass spectrometry. Now, the electron impact ionization mass spectrometry and the chemical ionization mass spectrometry typically go up to a mass value of 1000, 200 Daltons or so. Beyond that, it is not possible to use these two techniques. Whereas, the electrospray ionization goes

beyond 1000, all the way down to under 1000s or so with the sensitivity of the order of picomolar range of concentration is what we are talking about here.

The nano electrospray ionization is a much more sensitive technique, essentially covering the same molecular weight region up to 100, 1000 or so, whereas, the MALDI covers even much better. It can go up to 1 million; close to 1 million or 500, 1000, kind of molecular weights can be analysed using MALDI spectrometer. Also, the sensitivity is fairly high. It is of the order of femtomolar concentration, one can analyse these systems.

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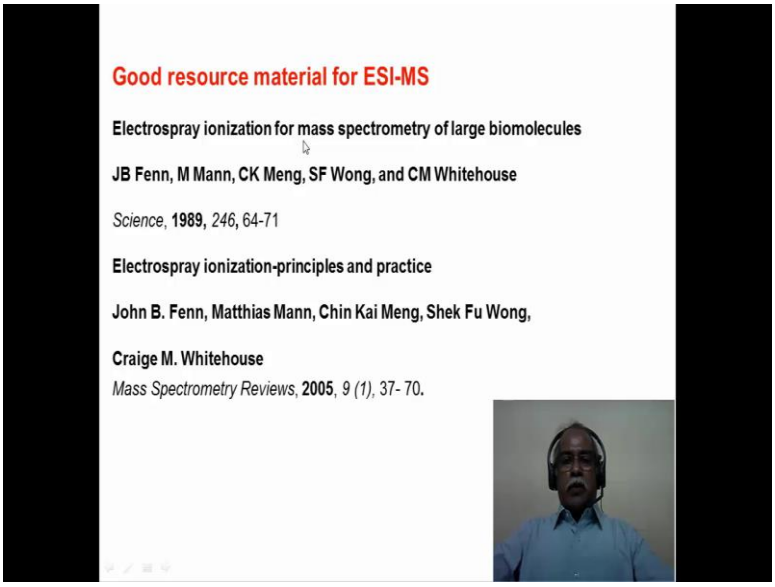

Now, a good resource material to read on electrospray ionization mass spectrometry is given here. There are 2 reviews written by John Fenn himself. One, in Science in 1989, this is the older review article. Whereas, the modern review article is available in mass spectrometry reviews, which is published in 2005. So, I recommend those of you are interested in learning more about the molecular electrospray ionization mass spectrometry for large bio molecular system can read these 2 articles. It will be very useful. Some of the materials are taken from these 2 articles, which are presented in the subsequent slides.

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Now, here is a electrospray ionization mass spectrometer, housed in the department of chemistry at IIT, Madras. You can see here. This is just a box that can fit on a table top. But, it can do wonders in terms of the mass analysis that it can do in terms of the molecular weight and the sensitivity and so on.

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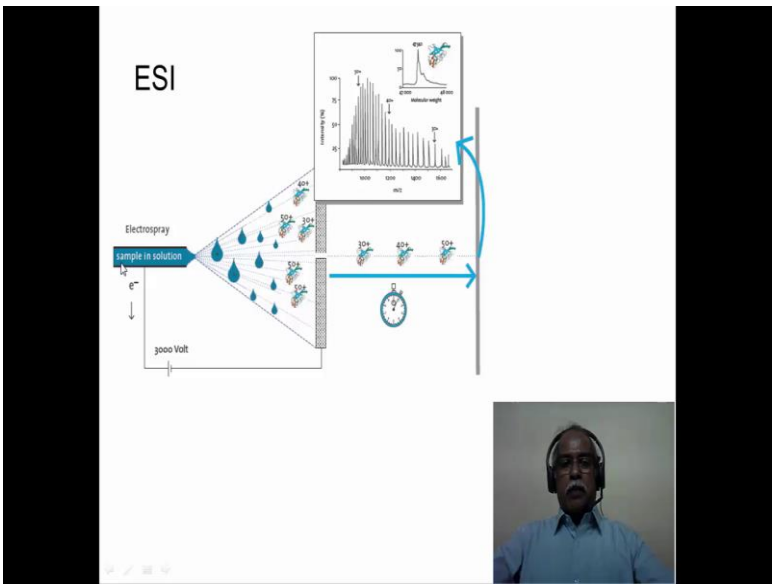

Now, in the electrospray ionization mass spectrometer, there is an injector which sprays the solution or the analyte to be analysed in the form of very fine droplets. And, it does so in a voltage region, where the sprayer itself is a one electrode and there is a counter

electrode which is kept here. And during the course of the fine spray, there is also a nebulising gas which is passed through the sample. This is better represented in the next slide.

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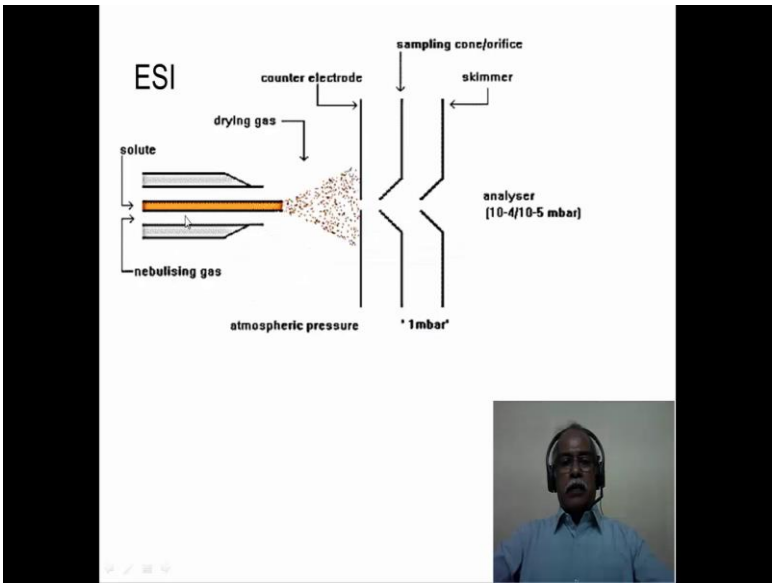

You can see here, there is a coaxial tubes that are placed. In the central tube, the analyte solution is actually injected. And, the surrounding tube; for example, the nebulising gas which is typically dry nitrogen gas is what is passed through. And, in the fine spray that is produced here, the droplets are formed and because of the nebulising gas, the droplets undergo slow evaporation of the solvent, until the system that the solvent molecules are completely evaporated and only the solute molecule in the gas phase is obtained. In doing so, the solute molecules also acquire charges of the order of, let us say, here that is typically represented as 30 positive charge, 40 positive charge, 50 positive charge and so on. Depending upon the kind of molecule that we are dealing with, how many basic sides are there that many protonation can take place. And, this kind of large charged molecular systems can be obtained in the electrospray ionization mass spectrometry.

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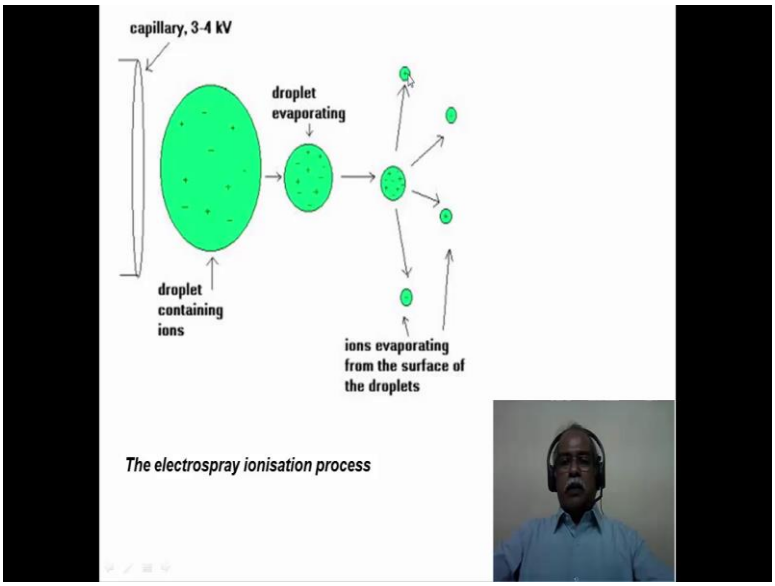

This (Refer Time: 06:42) representation essentially gives the idea that this is the sprayer head, which is kept at a voltage of about 3000 to 4000 volt or so. And, this is a dimension of a spray. This is actually a small droplet is what is being found consisting of the ions, which are already in the form of ionised sample, but in the solvent surrounding this one. As the droplet proceeds towards the other electrode, there is evaporation of the droplet and the droplets become smaller and smaller. In the process, the droplets burst into smaller droplets, which are called the daughter droplets. And, the ions get evaporated from the surface of the droplet in the process, eventually producing only the ions of interest without the solvent molecules being present in the system. By the time it reaches this dimension of droplet, all the solvent molecules would have evaporated and the substrate or the, analyte is actually in the form of charged species in the gas phase in this.

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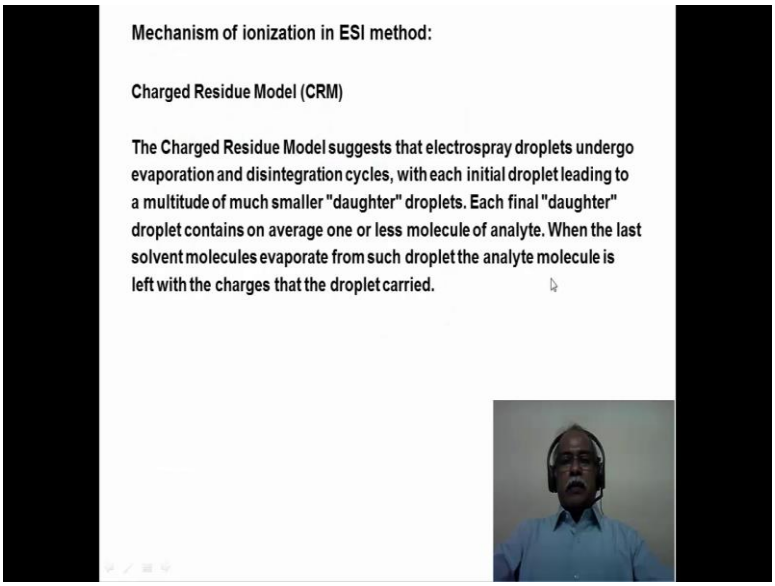

There are 2 mechanisms which are explaining the ionization process in an electrospray ionization methodology. Essentially, they say the same thing, but in a sort of slightly a different language. The charged residual model is shown here. The charged residual model suggests that electrospray droplets undergo evaporation and disintegration cycles, and each initial droplet leads to a multitude of smaller droplets. And, each final daughter droplets contains an average of one or less molecule of analyte. When the last solvent molecules evaporate from such droplet, the analyte molecule is left with the charge that was originally present in the droplet. So, this is essentially saying that the large droplet goes to smaller and smaller droplet, until the analyte alone is produced in the gas phase in the form of charged particle.

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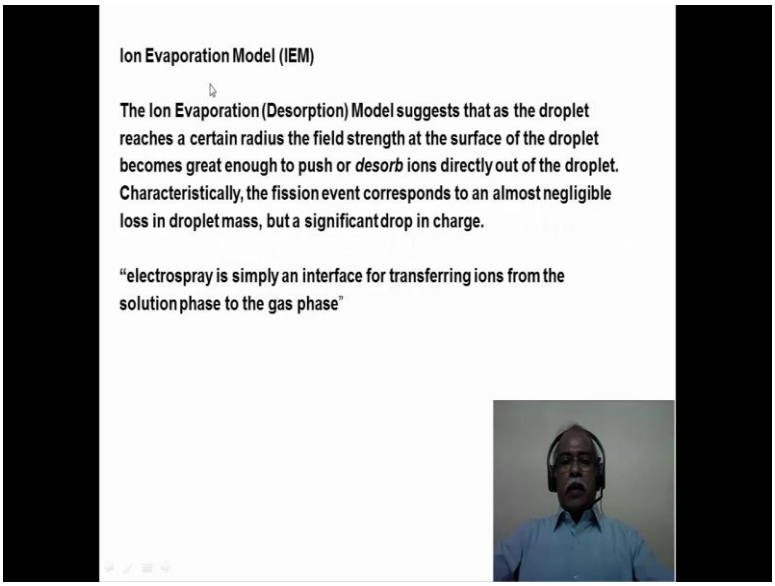

In the case of, the Ion Evaporation Model, as the radius of the droplets becomes smaller and smaller, the field strength at the surface of the droplet becomes enormous. And, that directly pushes the ions out of the droplets. So, this is ion evaporation model. It is slightly different from the earlier model. Essentially, the electrospray ionization is simply an interface for transferring ions from the solution phase into the gas phase. If you look at the mechanism by which they undergo this.

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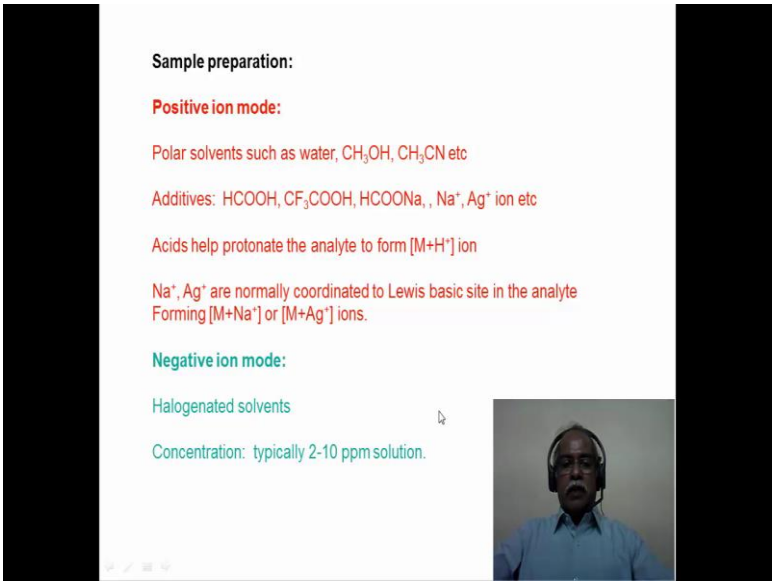

Sample preparation is important in the case of electrospray ionization mass spectrometry. Typically, polar solvents such as water, methanol, acetonitrile are used. Water and methanol are useful because they can also donate a proton to the analyte molecule, there by producing an M plus H ion. Protonated species can be produced in this particular solvent. And to dissolve the polar molecules, typically solvents like acetonitrile are very useful. Additives like, for example: formic acid, trifluoroacetic acid, sodium acetate, in other words sodium ion that is necessary for ionization process, sliver ions, etcetera can be added.

Essentially, the proton sources are added to protonate, the analyte substrate to get the protonated form of the ions or the sodium salts are added essentially to form a M plus sodium ion or M plus potassium ion as the case may be, in order to bring the analyte into the charged form of some kind. Now, the acid helps to protonate the analyte to form this ion. Sodium and sliver are normally coordinated to the Lewis basic site of the analyte, forming this kind of ion; M plus Na and M plus sliver plus and so on.

We will have a look at the M plus sliver ion ionization mass spectrometry. A few examples from our own research I will show you at a later stage. One can do the electrospray ionization also, either in the positive ion mode or in the negative ion mode. Typically, halogenated solvents are used in the negative ion mode. In both of this methodologies, the typical concentrations are of the order of 2 to 10 ppm solution is what is necessary for the analysis.

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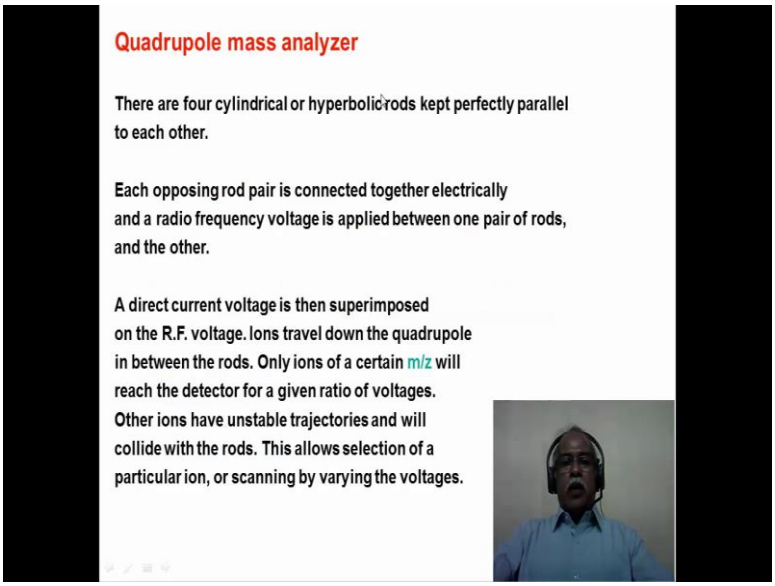

Now, let us have a look at the quadrupole analyser. A quadrupole is a quadruply charged system. And, there are 4 cylindrical or hyperbolic rod, which are kept in parallel; kept parallel to each other. Each opposing rod is connected to an electrically as well as radio frequency voltage that is applied between a pair of electrodes.

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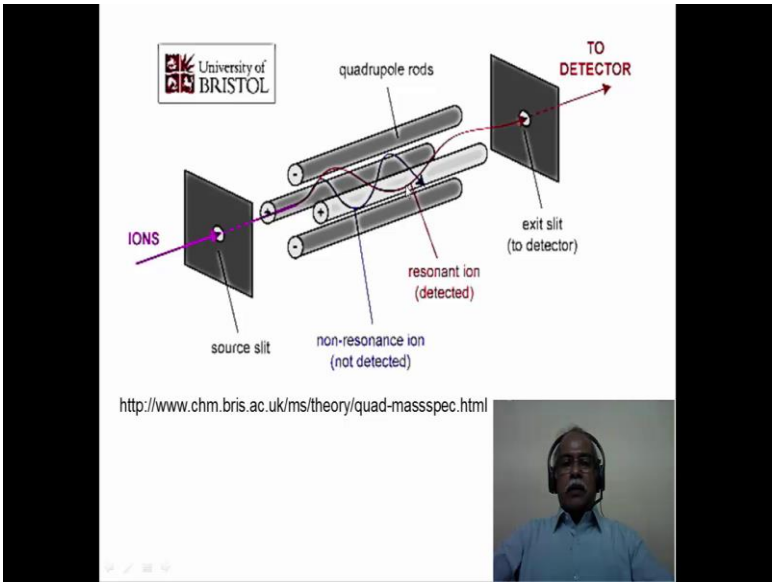

This is better explained using a diagram like this, for example. These 4 poles that are shown here are the quadrupoles of this type. And, the quadrupole has the opposite, the adjacent quadrupolar rods have the opposite polarity; plus minus, plus and minus. So,

essentially the ion that is produced in the ionization chamber is passed into this particular field. And, this is both; a dc voltage is supplied as well as a radio frequency voltage is applied. In other words, the radio frequency voltage will alternatively change the charge plus to minus and minus to plus. And, rapid change will be there. And, the ions has to resonate in a particular frequency in order to escape this intact. For example, only the resonant ions will pass through the analyzer to the detector. The non-resonant ions will essentially hit the walls of that quadrupolar analyzer and gets destroyed in the process. So, this is a mechanism by which the quadrupole analyzer works.

A direct current voltage, in other words, a dc voltage is applied and it is superimposed on a radio frequency voltage. Ion travels down the quadrupolar rods, between the rods. And they in doing so, they take a path which looks like a corkscrew kind of a passage is what it goes through. In other words, helical path is what it takes when it goes through the quadrupolar analyzer.

For example, a positively charged ion will come closer to the negatively charged electrode. But, then by the time it close approaches to the negatively charged electrode, you will be charged positive because the alternating radio frequency will alternate the charges along the electrode. So, it will get tripled. Then come closer to, let us say for example, another negatively charged electrode. By the time it comes close here, it will be positively charged. In other words, the charge will, the potential will change essentially.

And, in the process it will essentially take a helical pathway. Only certain m by z ratio mass will pass through this analyte for a given applied voltage and the radio frequency voltage. All the other m by z values will get destroyed by hitting the walls of this. So, by scanning the voltage one can take one mass at a time and pass it through the slit here into the detector and thereby detecting the mass of this ion.

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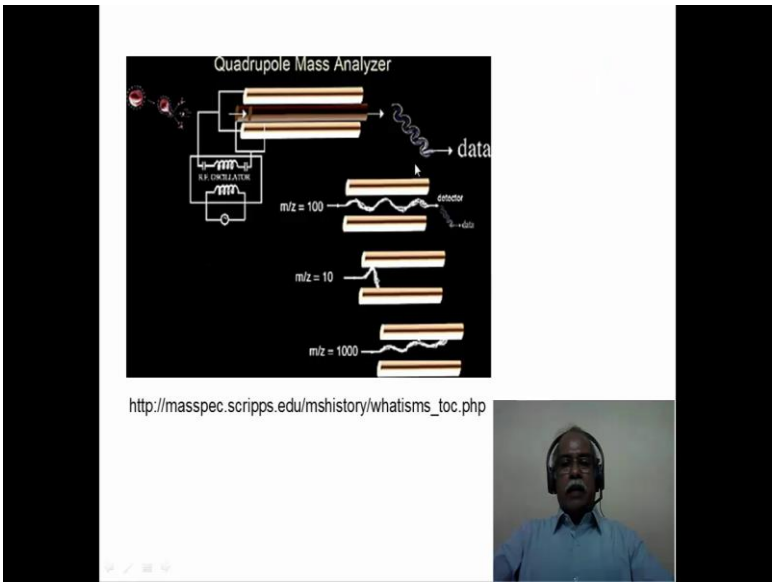

This is given in another diagrammatic representation here, for example, this is a quadrupolar analyzer. For example, in this particular case, there are 3 ions m by z of 10, m by z of 1000 and m by z of 100. The applied voltage is such that only the m by z value of 100 passes through the; this is called the resonant ion, which passes through the quadrupole analyzer to the detector.

On the other hand, the smaller ion which is m by z value of 10 has a non-resonant nature. And, it hits the walls of the quadrupole analyzer at a very earlier stage itself. Whereas, m by z of 1000 travels through certain distance, and finally hits the walls of the quadrupole analyzer before it comes out of the quadrupole analyzer. So, only a particular m by z value for a given voltage will pass through the quadrupole analyzer, and that is a basic principle of the quadrupole analyzer in this case.

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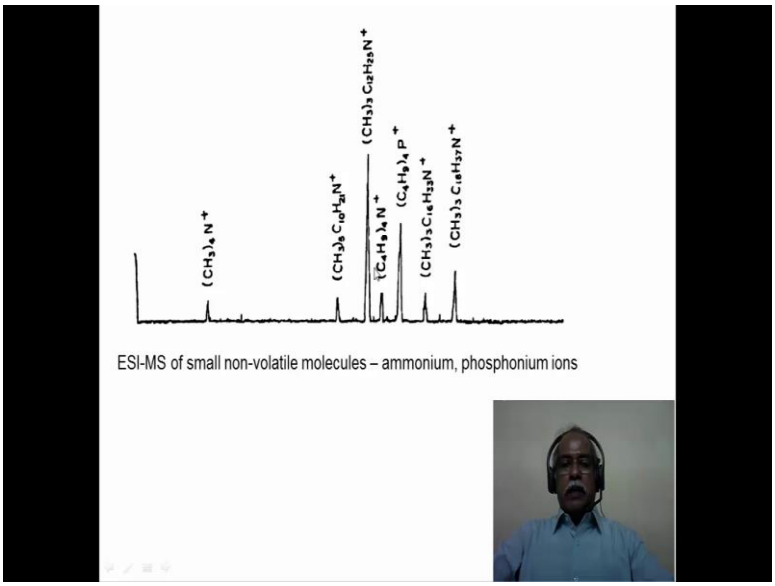

Now, this is a mass spectrum of certain non-volatile molecules. These are actually ammonium and phosphonium ions. This is tetrabutylammonium bromide or ammonium iodide. Please, remember that when you do a positive ion mass spectrometry, only the positive ions will be detected. The counter ion as it may be chloride or bromide; it is not going to be detected in the mass spectrum. So, this is a positive ion mass spectrum; is of the various ions is shown. These are all non-volatile ionic substances.

Normally, it would be difficult to ionise them or get them into the gaseous phase in the case of electron impact ionization mass spectrometry. It is not easy to do electron impact ionization mass spectrometry of such non-volatile material. However, in the case of electrospray ionization, since these are dissolved in polar solvents like water, methanol or acetonitrile and sprayed into the ionization chamber, they are already ionised; because they possess a positive charge by themselves.

So, all they have to do is bring them into the gas phase and get them into the analyzer to be analysed and so on. And, this is precisely what the electrospray ionization, quadrupole mass spectrometer does in analysing this kind of a non-volatile material.

You can see here, this is tetrabutyl ammonium ion, this is a trimethyl decyl ammonium ion, this is a trimethyl dodecyl ammonium ion, this is tetrabutylammonium ion, this is tetrabutyl phosphonium ion, for example. So, essentially these are all ionic substances which are easily analysed by the electrospray ionization mass spectrometry.

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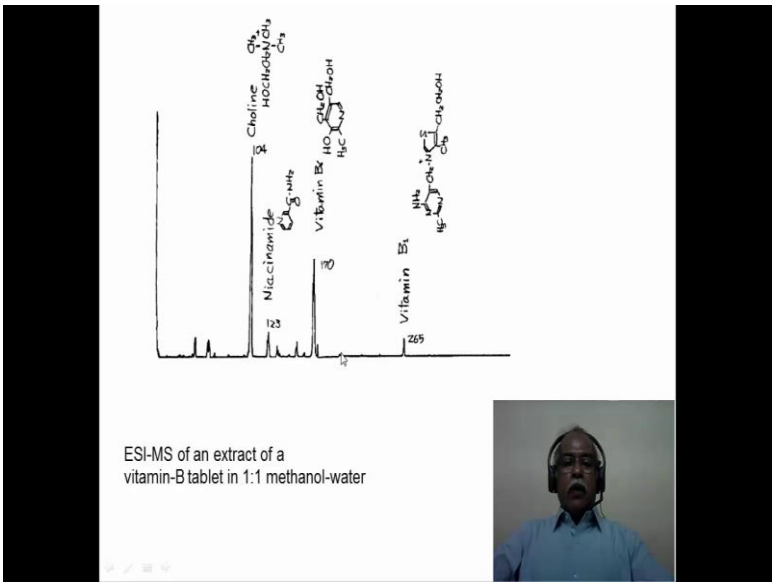

Now, vitamin-B 12 complex is fairly a complex mixture of various vitamin containing in system. And, this is analysed by dissolving it in a one is to one mixture of methanol and water. And, taking that solution into the electrospray ionization mass spectrometer, what you essentially see is choline which has a molecular weight of 104, niacinamide which has a molecular weight 123, vitamin-B 6 which has a molecular weight 170 and vitamin-B 1 which has a molecular weight of 265. These are also highly polar substances. It is not easy to bring them into the gas phase because they are non-volatile material.

And, hence the electrospray ionization mass spectrometry is extremely useful in this (Refer Time: 16:09). Although these are not very high molecular weight compounds, the power of electrospray ionization mass spectrometry is that it can bring non-volatile material into the gas phase, even if you have low molecular weight. For example, this is basically an ammonium salt and it is non-volatile in nature. For example, it is a high melting solid. And, as a result of that it is not possible to bring it into gas phase in an electrospray impact ionization mass spectrometer, however in an electrospray ionization mass spectrometer because it is going through a solution phase and then the ions are getting evaporated and so on. It is easy to detect them in the mass spectrometry condition.

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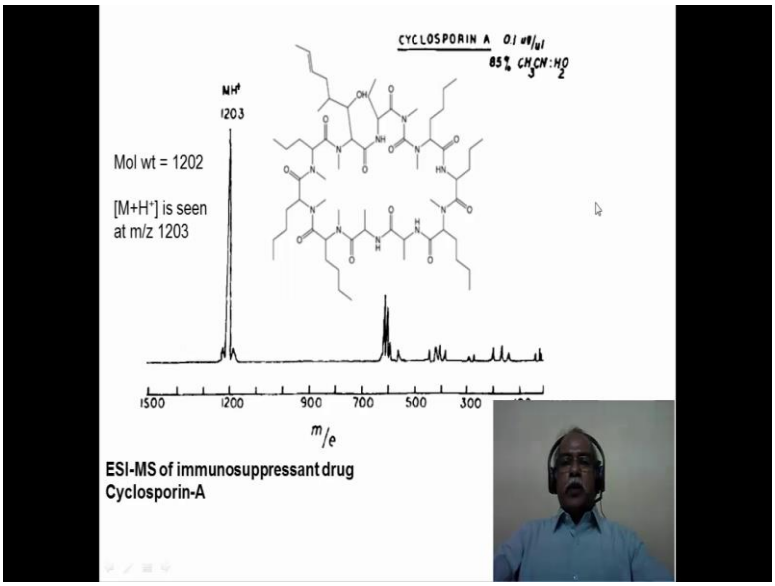

This is cyclosporine. This is a large cyclic peptide. This is an immunosuppressant drug that is used in surgery and so on. And, in organ transplant kind of a surgery this is used. The molecular weight of this macro cyclic system is 1202. And, this is essentially analysed by the electrospray ionization mass spectrometer. You can see here the molecular weight plus the proton; in other words, the protonated species is what is done. The concentration is something like point one micro gram per micro litre is what is used here. And, it is about 85 percent of acetonitrile water mixture. It is a highly polar solvent. Water itself can donate a proton and protonate this species.

So, what you see is essentially m by z of 1203, which corresponds to molecular ion plus a proton or a hydrogen, for example. And, there is a small extent of fragmentation process one can see in this particular case. This is about 1202 or so. This need not to be a fragment ion, these could be m by z; where z is equal to 2, for example. A doubly protonated ion could be seen for example. And, this could be a doubly protonated ion and this could be a triply protonated ion and so on, depending upon how many protons are added to the substrate to give the specific ion.

However, the most intense peak is the protonated species in this particular case. So, what we have seen in this module is essentially the basic principle behind the electrospray ionization mass spectrometry and the quadrupole analyzer, how it functions and some

examples of how non-volatile material can be analysed using electrospray ionization mass spectrometry.

In the subsequent modules, I will give you lot of examples of large bio molecular system which are analysed by the electrospray ionization mass spectrometry.

Thank you very much for your attention.