Application of Spectroscopic Methods in Molecular Structure Determination

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Lecture - 16
Mass Spectrometry

Hello, welcome to the course on Application of Spectroscopic Methods in Molecular Structure Determination. We are in module 16 now and with this module onwards for another 3 modules, we will discuss Mass Spectrometry.

Mass Spectrometry is a very powerful and sensitive technique and very widely used in the molecular structure determination of organic, inorganic as well as an organometallic compounds. It is not only used in the area of chemistry; it is widely applied in the area of forensic sciences and biological sciences. In biological sciences, the mass spectrometry has contributed enormously recently, after the discovery of Electrospray Ionization Mass Spectrometry and MALDI Mass Spectrometry. We will have a look at the basic information on mass spectrometry in this particular module.

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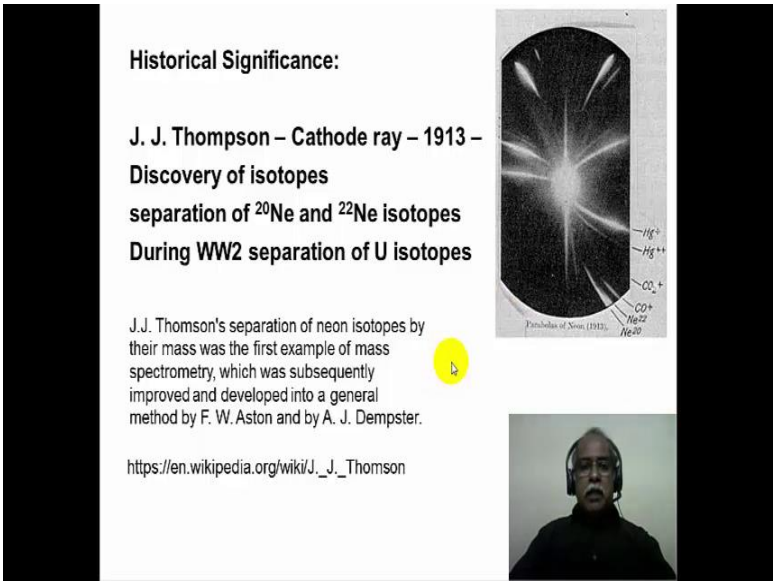

J. J. Thompson during his investigation of cathode ray tubes and discovery of electron, discovered the isotopes of neon 20 and neon 22. In fact, he separated thumb using a mass spectrometry technique in 1913. During World War II, separation of uranium isotope for the enrichment of radio active uranium isotope was undertaken. Now, this picture here shows the photographic picture of the mass spectrum that was recorded by J. J. Thompson. You can see here the 2 lines corresponding to the neon 22 and neon 20, in addition to carbon monoxide, carbon dioxide and peaks corresponding to mercury 1 and mercury 2. Now, the experiment that was done by J. J. Thompson probably was the first ever experiment on mass spectrometry in which the neon isotopes were separated based on their mass. This technique was further subsequently improved and developed by his co-workers Aston and Dempster.

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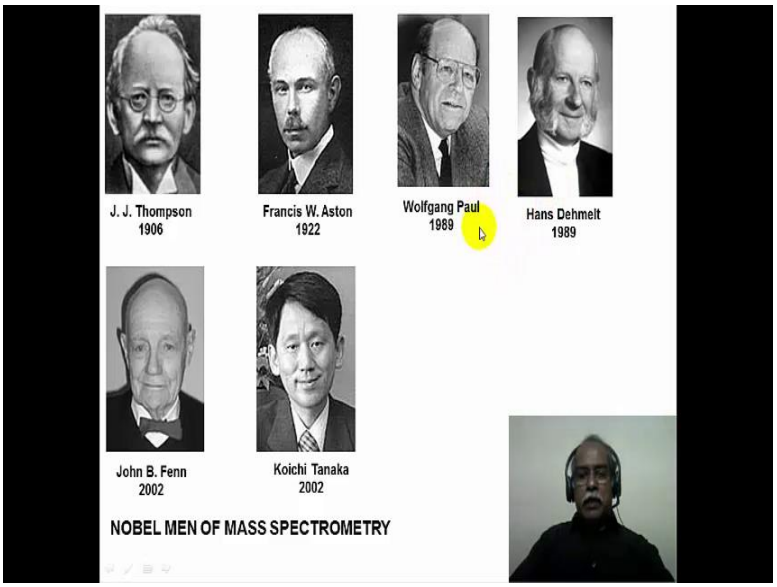

Now, J. J. Thompson received the Nobel Prize in 1906 for his contribution on cathode ray tubes and the discovery of electron and so on. Francis Aston continued the mass spectrometry that was developed by J. J. Thompson. In fact, Aston had discovered several isotopes of various elements in the periodic table and he received Nobel Prize in 1922. Wolfgang Paul and Hans Dehmelt they shared the Nobel prize in 1989, both of them worked on ion trap method of mass spectrometry which is known as ion cyclotron resonance mass spectrometry and recently in 2002 John Fenn and Koichi Tanaka shared

the Nobel prize for their discovery respectively of electrospray ionization mass spectrometry by Fenn and MALDI mass spectrometry by Tanaka. So, mass spectrometry had it is own share of a number of Nobel Laureates, who were made seminal contribution in this particular area.

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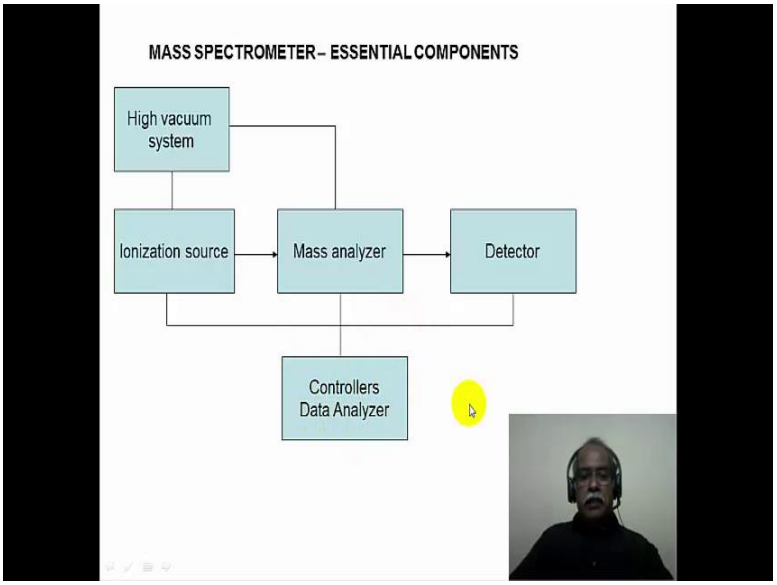

A mass spectrometry essentially consists of the following components. In other words, the 5 components that are mentioned in this particular figure, a high vacuum system, because mass spectrometry deals with ions that are generated in the gas phase; So, in order to generate molecular ions in the gas phase a high vacuum system is essential, you are talking about vacuum of the order of 10 to the power minus 6 to 10 to the power minus 9 tor pressure is what we are taking about. And then, you have ionization source depending on the kind of mass spectrometry technique different ionization sources are involved in the mass spectrometry and the ions are generated in the ionization source and the ions that are generated is fed into the mass analyzer. There are several different types of mass analyzers are also available in doing mass spectrometry. The mass analyzer essentially separates the ions of different masses and segregates them into based on their massed to charge ratio.

And this massed charge ratio separation takes place in the mass analyzer and the ion thus

separated or fed into the detector, the detector detects and puts out the signal which is recorded as a mass spectrum and the whole thing is controlled by computer, some microprocessors and the data analyzer is part of the mass spectrometer. The basic steps involved in mass spectrometry are as follows.

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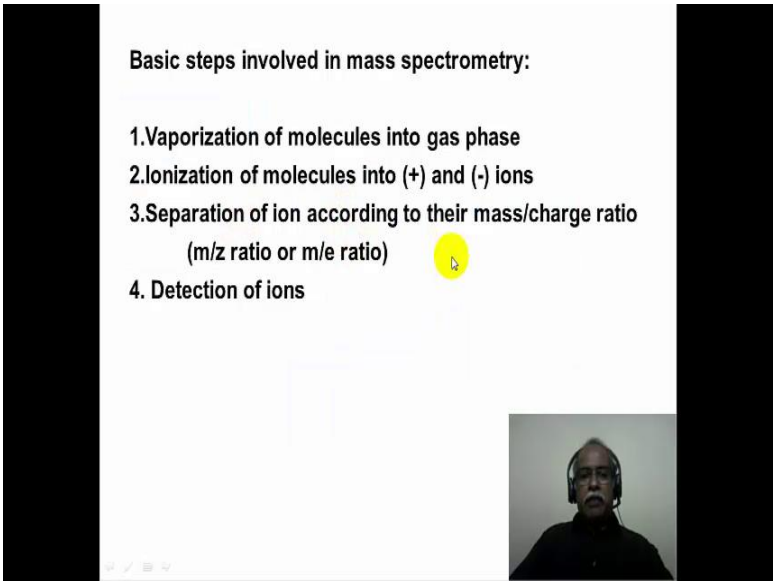

The sample has be vaporized and brought into the gas phase and the we are talking the gas phase technique in mass spectrometry. Ionization of molecules into positively charged ions and negatively charged ions is part of mass spectrometry technique. In other words one can say that mass spectrometry is all about the ions that are produced in the gas phase and to the chemistry of the ions that we deal within the gas phase. Then, the separation of ions according to their massed charge ratio is the second part of this particular mass analyzer does this separation part of it and the massed charge ratio is expresses as m by z in modern times, where z is a charge or m by e, where e is a charge in olden times text books you will see m by e, in the modern text book one sees m by z. Finally, the ions are detected and a current is produced in the detector corresponding to a signal that is produced in the mass spectrometer.

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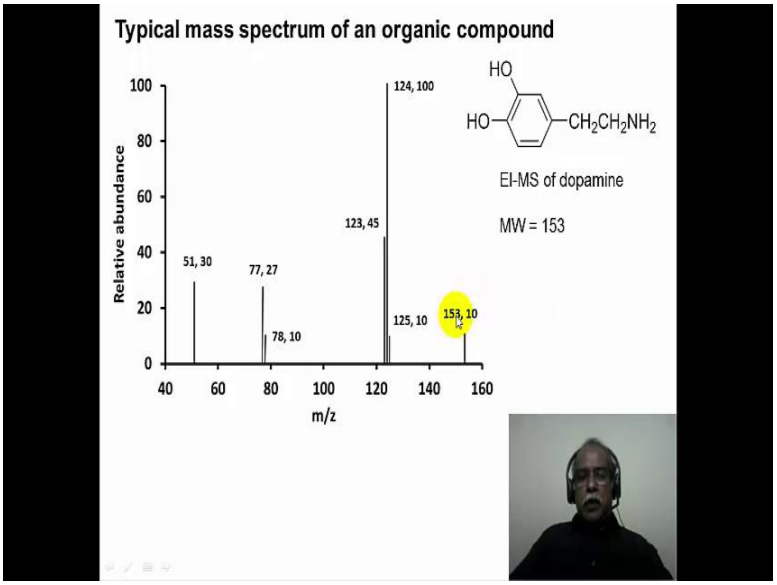

It is how where typical mass spectrum looks like, unlike other spectroscopic techniques where this x axis is a energy scale. In mass spectrometry, x axis is always m by z. In other words, massed charge ratio is what we are plotting against the relative abundance of the various ions that are produced during the mass spectrometry. The relative ion abundance is always expressed in percentage in terms of, the most intense ion in this particular case corresponding to a molecular weight of 124, corresponds to 100 percent abundance with reference to that all the ions are calibrated with their respective intensities.

Now, the 124 mass and 100 percent abundance is what is mentioned by these 2 numbers with each of these peaks that are mentioned here. Now, the molecular weight of this particular compound, this is called dopamine; this is one of the new neurotransmitters and it has a molecular weight of 153. We can see here, molecular ion is registered at the highest m by z value at 153, but never the less; it is not the most intense ion that is produced. The reason being the ion that is produced as a molecular ion, further under goes decomposition and fragmentation to give various other ions, in fact, one of the fragment ion is the most abundant ion of 100 percent intensity in the mass spectrum. So, we will see some more examples of the mass spectrum.

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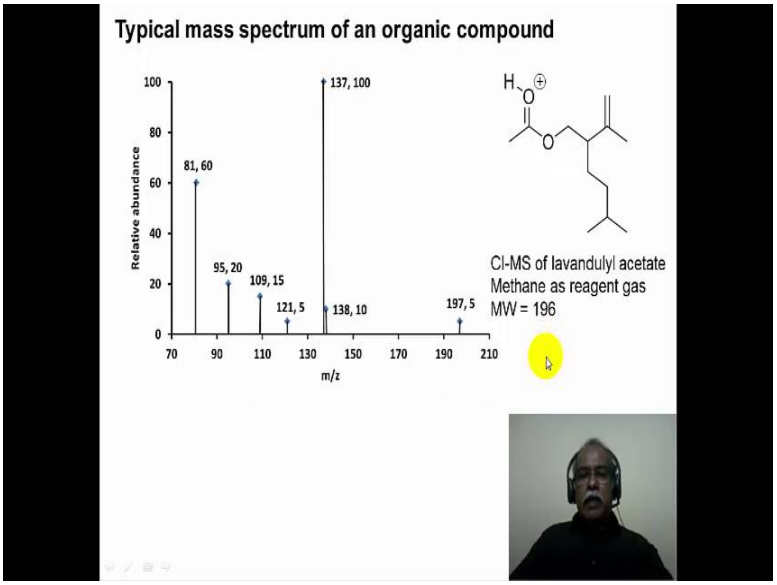

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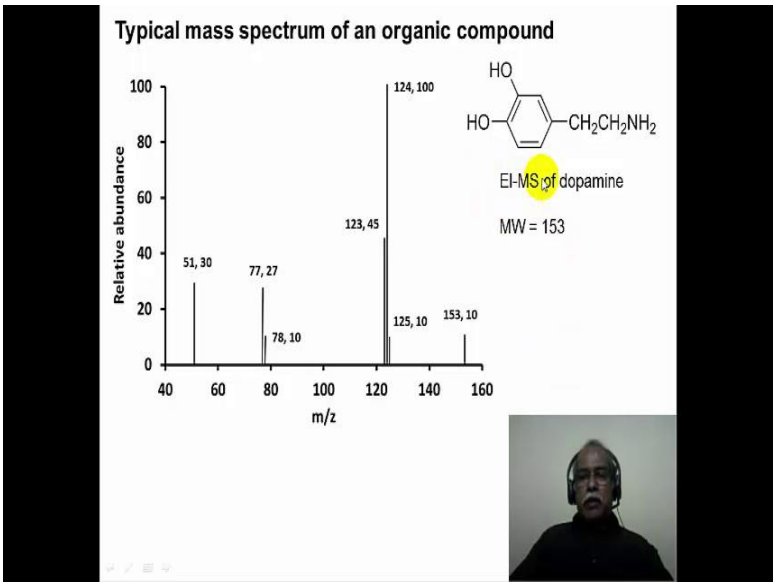

In this particular example, the ion is generated by removing an electron from this molecule. In other words, by the ionization of this molecule and the cat ion radical thus produced is the one that is responsible for all the other ions that are produced. So, essentially, the removal of an electron is what we are calling as ionization process in this particular technique. It is not always necessary to remove an electron, one can always

add a proton to the molecule, there by generating a positively charged ion and in this particular case, this is a lavandulyl acetate which is a fragrance agent isolated from lavender flower and this particular molecule has a molecular weight of 196, but the highest peak that is registered in the mass spectrum is 197, because this corresponds to the protonated species of this particular molecule. So, one can also generate instead of knocking of an electron from the molecule, add a proton and there by generate the charged species in the system and subsequently the molecular ion under goes fragmentation to produce all the other ions that are registered here. We will deal with the fragmentation pattern in a while.

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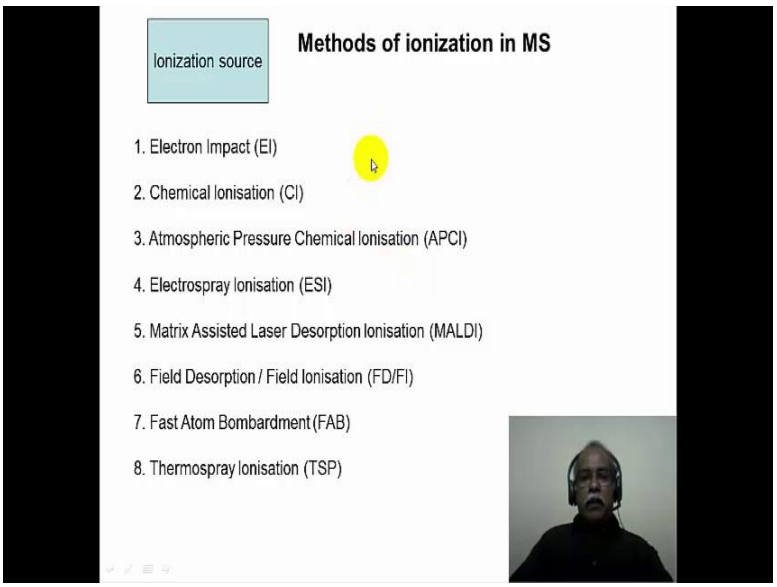

Now, the different methods of ionization in a mass spectrometry technique are as follows. Electron impact ionization is the oldest technique which J. J. Thompson discovered, this particular technique electron stream or a flow of electron under a current of electron in bump bonding on the sample. The electrons typically have 70 electron holes or so, in terms of their energy and this high energy electrons impinge upon the substrate, there by removing an electron from the substrate to produce the cat ion radical.

In Chemical Ionization certain reagent gasses are first ionized and then they are made to react with the substrate molecule. In other words, the substrate itself the molecule that

needs to be analyzed itself is not directly ionized as in the case of electron impact. The substrate is indirectly ionized, but first ionizing the reagent gas typically methane, ammonia, isobutene are used as reagent gasses. The reagent gas is first ionized and the ions produced by the reagent gas subsequently react with the substrate, producing the substrate ion and that technique is called the Chemical Ionization technique.

One can do the chemical ionization technique under vacuum or under atmospheric pressure. When it is done under atmospheric pressure it is called APCI or Atmospheric Pressure Chemical Ionization. Electrospray Ionization, ESI; Matrix Assisted Laser Desorption Ionization, MALDI; these are the new techniques which are very recently discovered and these are the techniques which we will deal with little later in a different module in detail, because these are extremely important techniques in modern day of mass spectrometry. The other less often used techniques are the Field Desorption or a Field Ionization, Fast Atom Bombardment where xenon atoms are bombarded for example; on to the substrate and they substrate then ionizes to produce the ions and gives a mass spectrum. Thermospray Ionization is also related to the electrospray ionization that is also not very widely used, but it is a known technique for the ionization.

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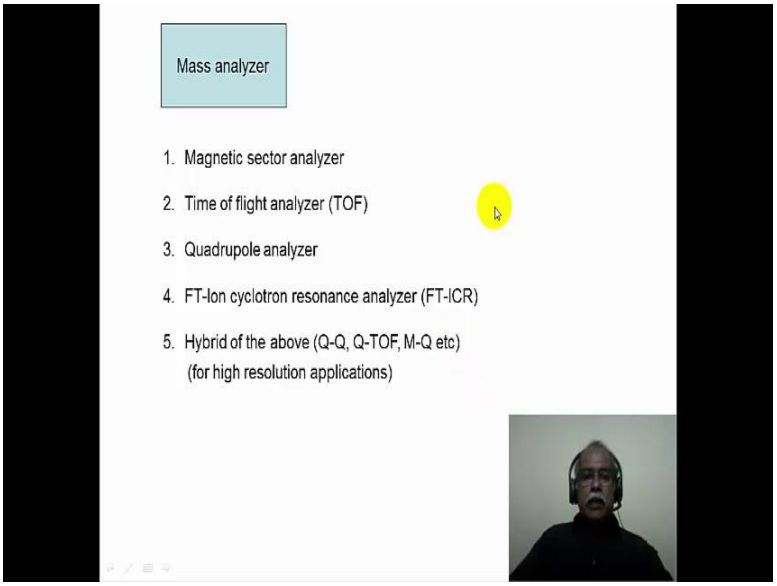

Once the ions are produce, they are analyzed either in an electric sector or a magnetic

sector analyzer. Magnetic sector analyzers are very common and the ions are separated based on their massed charge ratio in the magnetic sector analyzer or one can use the Time of flight analyzer. Here also the ions are allowed to travel certain distance, the heavier ions move slower and the smaller ions move faster that is a principle that is used in the time of flight analyzer. One can have a Quadrupole mass spectrometer. Also, Ion cyclotron resonance analyzer is also commonly used. This is a very sensitive technique, it is a Fourier transform techniques and then one can use a combination of any of these analyzers in terms of the hyphenated hybrid varieties of mass spectrometer. For example, one can have a quadrupole quadrupole or a tripple quadrupole, quadrupole time of flight analyzer, a combination of this analyzers are generally used when in high resolution is necessary in the mass spectrometry.

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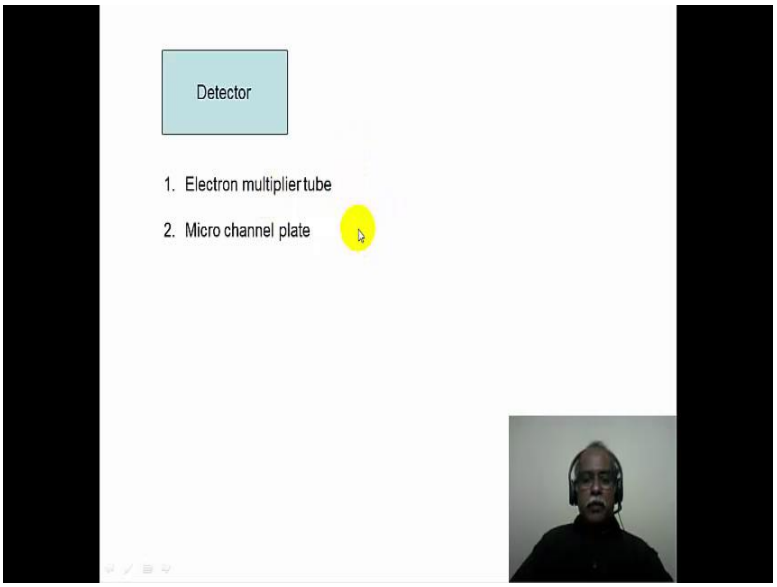

Now, the detectors are essentially electron multiplier tubes or micro channel plate. The ions impinge on these electron multiplier tubes and produce a current and the current is what is registered as a signal corresponding to the mass spectrum that one records.

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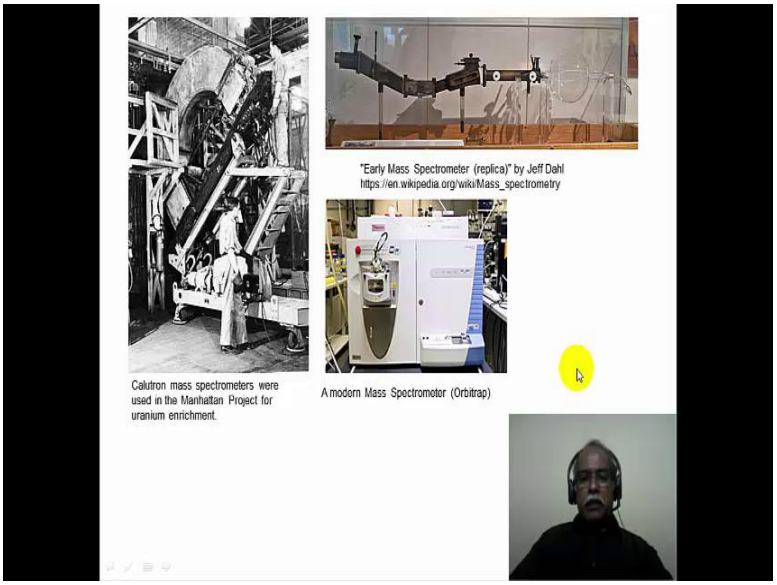

These are some pictures of mass spectrometers. The very early mass spectrometer is shown here. The one that is developed by J. J. Thompson, this is a replica of that. Here you have the ionization chamber where the substrate is reduced and ionized. It is passed on to an accelerator chamber where they are given a certain kinetic energy to travel and then, they enter the magnetic sector analyzer. This is the magnetic sector analyzer.

Finally, they get segregated in the magnetic sector analyzer and one by one they come out of this, exit here to the detector that is detecting the mass spectrum, the ions that are produced in the mass spectrometer. Now, this large unit that you see here is a magnetic field it is a magnetic sector mass spectrometer, part of the magnetic sector mass spectrometer. This is called the calutron mass spectrometer that was used in the Manhattan project for the enrichment of uranium to isolate the radioactive uranium from the uranium ore for example. This is the modern day mass spectrometer, it is the small equipment which fits on a table top and it has the capacity to analyze m by z values up to 6000 to 8000 and it has a resolution of nearly 1 million or so. It is a highly sensitive instrument, typically the current day mass spectrometers use anywhere between picomolar to femtomolar substrate concentration of analyte for analysis.

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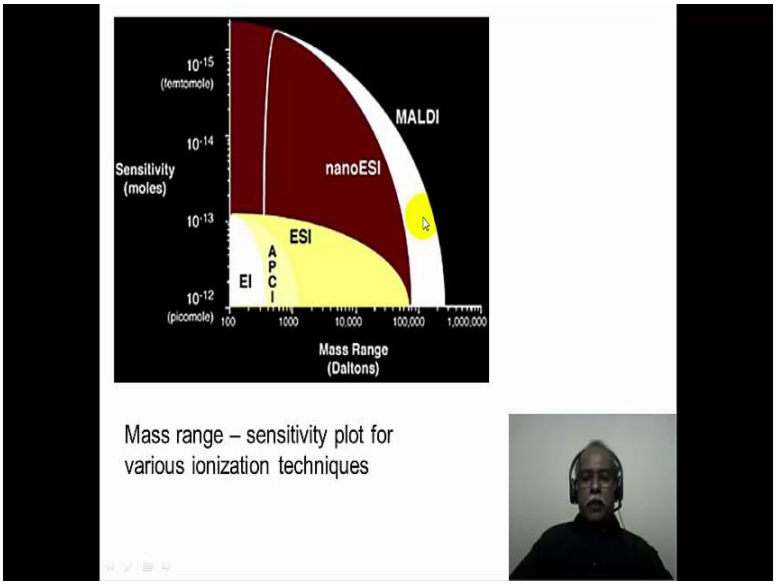

This picture essentially tells us the mass range and the sensitivity of the various ionization techniques that one has today. To start with the oldest technique namely the Electron Impact Ionization Mass Spectrometry is shown here. This has a limited mass range of about 800 or so, or about 800 to 900 or so. But, it has a good sensitivity of the order of picomole one can detect using this mass spectrometer. The electrospray ionization mass spectrometer, which is typically used for macro molecular systems has a fairly large mass range, it is not use full for small molecular weight compounds, but it is very useful for large molecular weight compounds of the order of 100,000 which are typically protein, DNA kind of bio macro molecule is what we are taking about.

The sensitivity is essentially good, it has picomolar to sub picomolar sensitivity. The Nano Electrospray Ionization Mass Spectrometer has a huge sensitivity it can go up to femtomolar concentration of the analyte. Finally, the MALDI spectrometer although less sensitive than the electrospray ionization mass spectrometer, it has a much wider mass range and this is essentially used in the analysis of polymers, whether it be it bio polymers or synthetic polymers they can be analyzed using the MALDI mass spectrometer.

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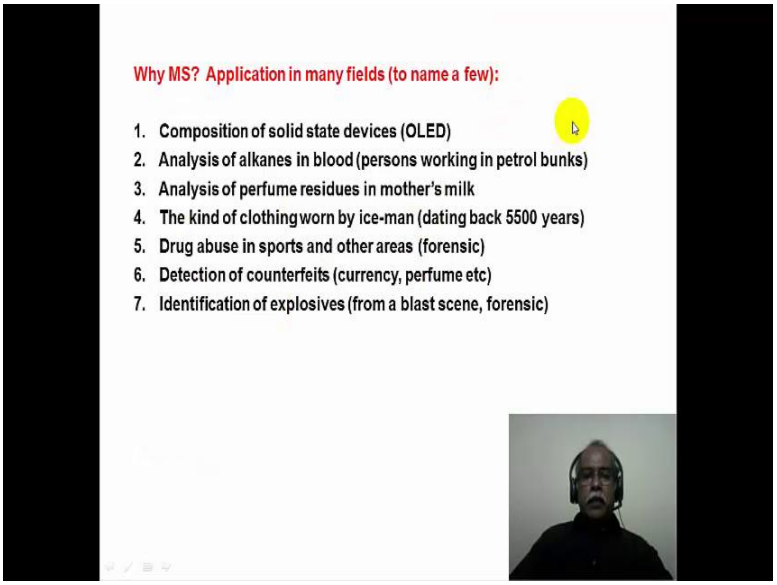

Why is mass spectrometer such a popular technique? First of all, it is a very sensitive technique, one needs only a femtomolar or picomolar quantities of analyte to be analyze to get a mass spectrum. It is very widely applied in many areas of research; some of the areas are listed here. For example, the devices that are fabricated for organic light emitting diodes, they have a very thin layer of various substrates which are coated on top of each other to produce the device, and we are talking about micro gram or picogram levels of substrate being deposited on this kind of a material which is of the order of micro meter or sub micro meter thickness of material is what we are talking about.

One can directly introduce this in the mass spectrometer and analyze the various compositions or components of the organic light emitting diode materials. Now, persons who are working in petrol bunks for example, is possible that they inhale a large amount of the alkanes, which enters their blood stream. In order to analyze this alkanes in the blood stream of such persons, mass spectrometry is used. This is from the health point of view, one needs to know how much a person inhales in terms of the working conditions that is prevailing in the petrol bunks and so on.

Now, Mother, when she wears a perfume on her skin it can get absorbed under the skin and it can enter the body and blood stream and so on, eventually, it can end up in

mother's milk, if mother is a nursing mother for example and such analysis is extremely important again from the health risk point of view. The kind of cloth worned by ice man, whether he was wearing a cellulose based material or animal skin based material can be easily analyzed using a mass spectrometer. This ice man's cloth is preserved over a period of about 5500 or so, it is a frozen condition. So, one can use the pieces that are available to do the mass spectrometry investigation.

In forensic science, one uses the GCMS or HPLCMS kind of a combination hyphenated technique. And, one can analyze urine samples or blood samples to detect the drug abused problem in sports and other areas for example. Currency notes have different colors, different kind of chemical substances which are used for printing, one can easily detect the counterfeit currencies using the mass spectrometer because the colors and the dice that are used for printing the currency or very unique in nature they have a very unique signature in the mass spectrometer. The mass spectrum of such compounds and they can be easily detected by means of mass spectrometry technique. Again, in the area of forensic analysis, one can use mass spectrometry to analyze very small amount of residues that is left behind in a blast seen for example, of explosives can be easily analyzed by mass spectrometry technique.

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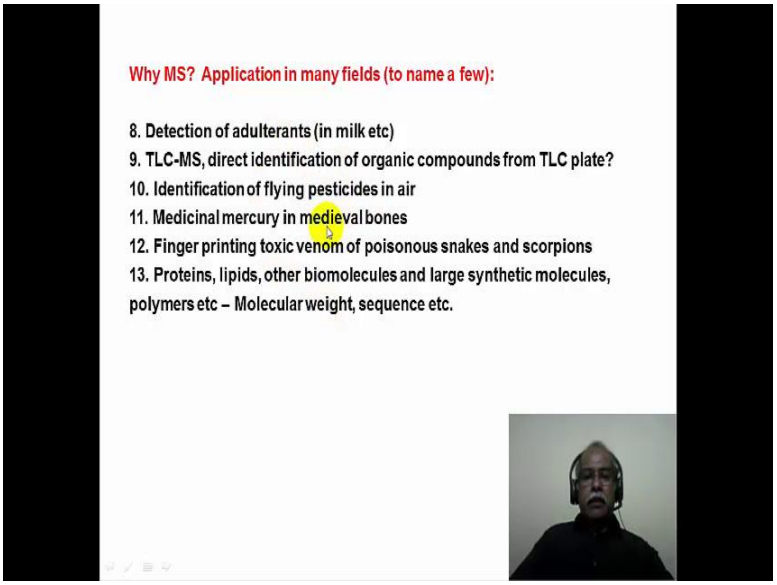

These are some other applications of the mass spectrometry technique in various areas. Let me go to point number 13, this is a very important area. Structures of proteins, lipids, and other large bio molecules whether it is bio synthetic molecules or a bio molecules with large molecular weight they can be determined by the recent mass spectrometry techniques like electrospray ionization and MALDI mass spectrometry techniques. We will see some examples of this techniques, when we talk about the electrospray ESI or the MALDI technique.

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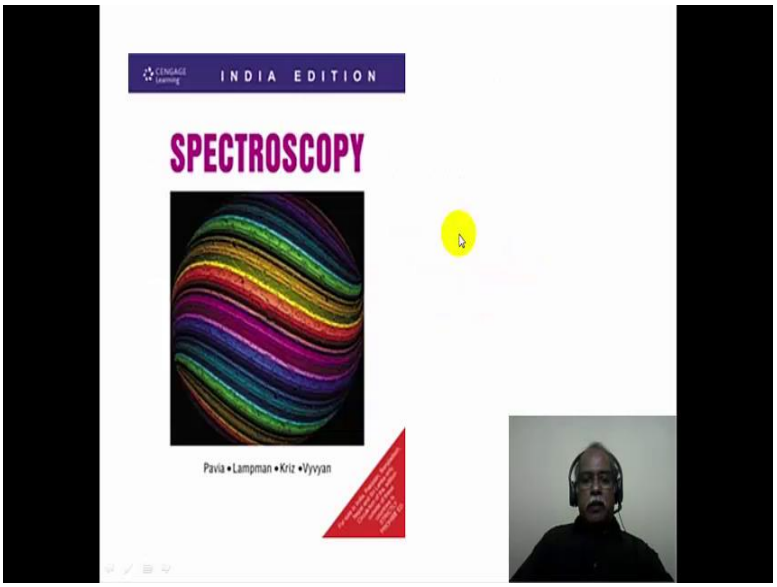

Now, the resource material for the mass spectrometry topic is available in this particular book, this is a very nice book which has a chapter on mass spectrometry and it is a spectroscopy by Pavia is the book that I am referring to. So, it is recommended that you read this book for mass spectrometry chapter.

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If you want a little specialized book on mass spectrometry that is also available, this is a text book on mass spectrometry by Jurgen Gross. This is also a nice source of information for mass spectrometry. I would likely thank you for your attention, we will continue in the next session or the next module about more details of mass spectrometry technique.

Thank you very much.