Analytical Technologies in Biotechnology
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Module - 6
Spectroscopic Techniques
Lecture - 6
Atomic Spectroscopy and Mass Spectrometry

In this lecture, we are going to discuss about two techniques; one is Atomic Spectroscopy and second one will be the Mass Spectrometry, both are very useful techniques for application in different areas of biotechnology.

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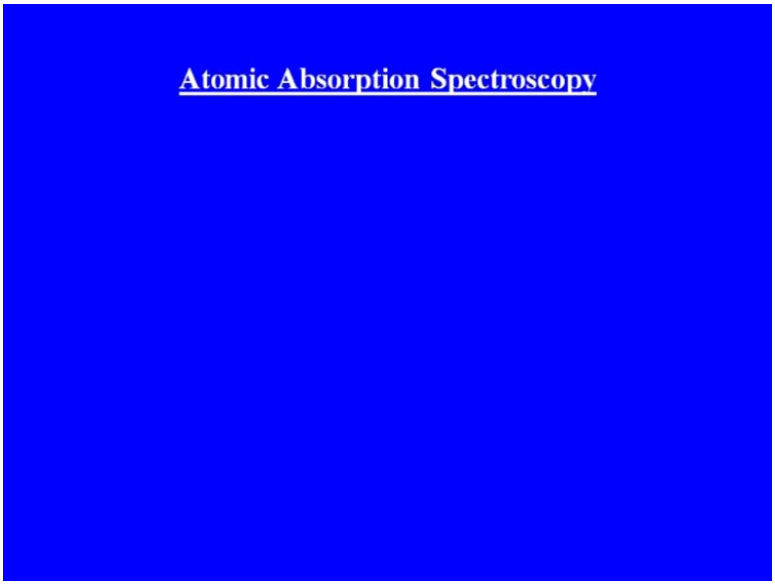

Now, let us first discuss about atomic spectroscopy, now absorption and emission of radiant energy by atoms is a powerful tool for lot of analytical and quantitative purpose. Now, it could be like flame emission or emission spectroscopy, which is atomic emission of flame emission spectroscopy is started long back in say 1900, and atomic absorption spectroscopy came around 1960. Both techniques complement each other, and both techniques are utilized for analysis of different types of atoms in gaseous state or you we can say atoms in free state.

Now, if we say there are certain limitations of these techniques; one is that they cannot distinguish between the oxidation states, different oxidation states and chemical

environment, second is they are incentive to nonmetallic atoms, but there are provisions, where by certain modification nonmetallic could also be identified. Second thing is that mostly here flame is utilized for both atomic absorption or atomic emission, but now new things like flameless atomic spectroscopy has also come in, where like electro thermal furnace are utilized.

Now, here essentially the main part is that every atom has a particular transition energy from one state to another state, that is when it is absorbed like we have seen in other spectroscopic techniques also. So, when it absorbs energy and get transit and it is transition from one energy state to another energy state, that will happen if it absorbs a particular energy that is the energy difference between the two states.

So, it will as the it has the discrete, these are discrete energy levels, they will have a particular spectra at particular wavelength, it is like the particular wavelength line lines will be absorbed or when it emits actually from when it comes back from higher state to lower state it will emit, also characteristic wavelength. So, these we can call spectral lines actually, so we can call it spectral lines, where atom either when absorbing it will absorb and given absorption spectrum with lines missing, that is black lines you can say and when these are characteristic spectral lines for particular atom.

So, that likewise when it is emission spectroscopy, then there will be characteristic lines will be seen or in the emission spectrum and these are both spectroscopic techniques the identification of atoms, and the quantitation can be performed, because of these characteristic patterns. Now, before we go on to these techniques where we are going to discuss about atomic absorption and emission spectroscopy, here the flame or combustion flame acts here as the main component to change or to convert atoms to free atoms.

Atoms in a particular molecule or in a particular liquid solution to free atoms, so first thing is introduction of atoms into the flame. So, different parts of absorption or different parts of atomic spectroscopy, one is introduction of the atoms into the flame, second is the conversion of atoms into free atoms by flame, and then analyzing it through particular spectroscopy technique.

So, what is what essentially is happening is the combustion flame, when an particular atom is introduced in a form that is liquid state form, then in the atoms are converted to

free atoms that is divide of their environment. And these free atoms can be analyzed in two ways, one is that an external source of radiation is there is present, and due to this the atom, which is free atom in the flame absorbs particular radiation from this external source and gets into or to higher energy state.

Now, when it gets into the higher energy state then that particular absorption is recorded or particular wavelength, which is there is recorded, where in a spectra you will find continuous spectrum except for those wavelength, which are absorbed. In flame emission spectroscopy rather than here, it after it is converted into free atoms the combustion flame acts as a sample cell, here combustion flame only provides energy for it is to get into higher excited state.

And free atoms when gets into higher excited state, then when they come back to the ground state they will emit certain specific radiations, and those spectral, the spectra will be where it is those lines which are emitted will be seen in the spectra, and characteristic spectra lines could be recorded. So, both here absorption spectroscopy and emission spectroscopy are done in a flame traditionally, now electro thermal furnaces are available, where the same temperature furnace would be provided, rather than flame, and it could be the both spectroscopy could be performed.

Another variation, which is very useful which has come in it is atomic fluorescence spectroscopy, where indictably coupled plasma atomic fluorescence spectroscopy instruments have come, and these also are very useful for different analysis. So, like as we said major part or the procedural part in both spectroscopic technique is one is that sample has to prepared, there should be lot of wet chemistry goes on in this particular technique.

Where, one has to know about, whether what solutions the particular atoms needs to be put in, if there are any interfering things and all those things has to be looked into and sample has to be prepared. Once, the sample is there this liquid sample in the form of droplets which is done through nebulization, and nebulizer is used here, and nebulizer are like kind of like if you have seen a spray things, those are like that were high velocity oxidant gas is passed through a capillary and through an orifice.

Because, of this pressure difference the liquid sample is drawn towards this high velocity jet and finally, it comes out as liquid is drawn in it then breaks into first it is in a column.

And then it breaks into droplets, and these droplets through aerosol modifier will be put into a particular size droplets will be put into the flame, at the right place which is utilized for this spectroscopy technique. And finally, the spectral lines like monochromators are used, so that only particular spectral lines are can be recorded, and spectra then could be analyzed for both qualitative and quantitative purpose.

So, let us start with first one that is atomic absorption spectroscopy, now atomic absorption spectroscopy uses the absorption of light to measure the concentration of gas phase atoms. And when using atomic absorption spectroscopy as analytical technique the absorption of electromagnetic radiation of, like as an analytical technique like absorption of light of by free atoms is measured. This is based on the first half of the excitation process, while atoms absorb light getting their electrons from the ground state to higher energy level, and in emission it will be when they come back to the ground state.

The technique basically realize on the fact that when the element is atomized at a very higher temperature, it will show a line spectrum and this employs that the atom absorbs energy at discrete energy levels. The excitation energy in this case are determined by the difference between the energy level of the ground, and state and one of the excitation states of the electron as we have discussed earlier.

So, only a light with discrete wavelength belongs to each of these excitation energies, and when this light is absorbed it is missing from the continuous spectrum of the electromagnetic radiation. And a black line appears in the absorption spectrum and using A S free atoms like here, monochromatic light called you can say resonance radiation, it belongs to one of one line of their spectrum and therefore, it has the suitable excitation energy. So, only here the atoms, which are being examined will absorb this light, so remember when you are doing this you have to know to for which atom you are doing it, so that suitable monochromators could be utilized.

As, a result of absorption the intensity of the light decreases which is also proportional to the numbers of atoms, so quantitation can also done as the intensity will differ as per the number of atoms present. So, this is like quite sensitive quantitative measurement possible here, now here as I said flame is utilized for this atomization, but electrothermal furnaces could also be utilized like such as graphite furnace could be utilized.

Now, there are three steps in here, which are involved into one is desolvation that is liquid solvent is evaporated and dry sample remains, then vaporization that is solid sample vaporizes to a gas. And finally volatilization that is the compounds that compose sample are broken into free atoms, you will get peaks which are certain like if you have a standards and then those peaks to be compared. Another important part of this whole thing is monochromator, which is used select the specific wavelength of light that is absorbed by the sample and to exclude other wavelengths.

So, the selection of the specific wavelength of light allows for the determination of the specific element of interest when it is in the presence of other elements. So, remember you have to no like which element you are looking for otherwise, a very complex spectra will be coming. The light selected by the monochromator is directed on to the detector, and typically photomultiplier tube, which is a photomultiplier tube whose function is to convert the light signal into an electrical signal proportional to the light intensity.

So, this the challenge of requiring the wave bandwidth of the absorbing species to be broader than that of the light source can be solved with radiation source with very narrow lines, so many times one has to look for this or solve this problem also. So, monochromator in essence will be separated the spectral line of particular element from other spectral lines and will allow only certain light to pass through. Now, basic instrumentation here every atomic absorption spectrometer have basic components, which are like radiation source, which is important as far as atomic absorption spectroscopy, is there lot of advancement though has taken place.

But, basically it is a radiation source is one may be continuous emitting from visible to infrared wavelengths, or from lines that emit discrete lines specifically from each chemical element. The modulator helps to differentiate radiation emitted by radiation lamp, coming from the environment and mainly from the atomization system. The atomic absorption spectrometric methods is based on measuring the amount of radiation, coming from the lamp before and after passing through the sample, there are other light sources as the environment and mainly the atomization system captured in the monochromator.

So, the other light sources interfere here in the measurement, so by reducing signal to noise ratio sensitivity and accurate accuracy of analysis. So, the solution for this problem

is the modulator drive signal of the lamp, and combined with the synchronization of the detector to amplify selectively the signal of the lamp actually, so these modulators are used for this specific purpose.

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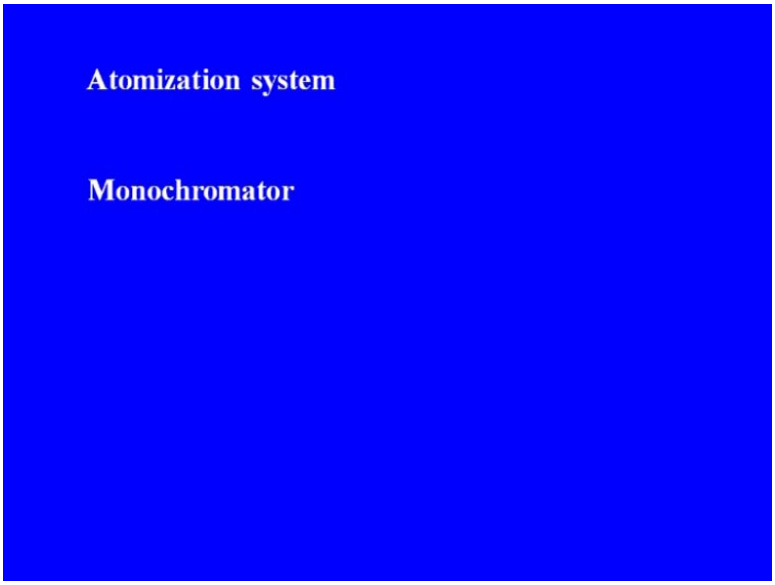

Then atomization system removes analyte atoms in the solution and generates atomic vapors composed of atoms in a ground state putting them between the source and the detector, and to observe the radiation emitted. Monochromator as we have discussed is responsible for selection of photons due to the wavelength that will reach the detector.

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Now, detector transforms the energy of photons into proportional electronic signal and amplifies it, the signal intensity obtained is treat treated by system for data acquisition and processing. There are lot of advantages of these systems these are expensive and high sample throughput can be done easy to use very high precision, disadvantages are only solutions can be analyzed, and relatively large sample quantities are required. There are certain for times there are less sensitivity for certain elements, and other problems could be there.

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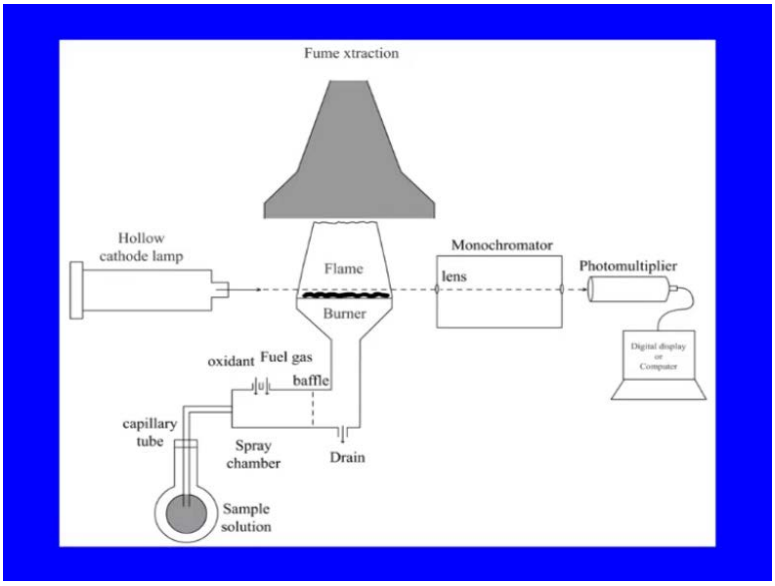

Very simple schematic of absorption spectroscopy instrument, now if you see there is a hollow cathode lamp as a radiation source, there is a flame here, and there is a oxidant and fuel there is these capillary tubes it is sucked in through a spray chamber. You can nebulizer and it is introduced into the frame the cathode lamp is a radiation source, to the particular radiation will be absorbed here and it goes through the monochromator. And finally, photomultiplier, and to the to a detector and to display on the computer for analysis.

There are lot of applications of atomic absorption spectroscopy for example, it is the sensitive quite sensitive method, it is more sensitive it is considered to be more sensitive then flame emission spectroscopy. It could be utilized for different things like analysis of various kinds of metals, in say water or food or animal feed stuffs, it could be like biological fluids like in for clinical purposes and….

So, it is utilized in lot of different fields like medical or clinical, diagnosis, environmental samples could be analyzed, agricultural samples for presence of metals to be analyzed, lot of biological fluids for metal presence could be analyzed. Except for alkali metals, atomic absorption spectroscopic is quite sensitive for lot of metals like for example, calcium, magnesium, iron, manganese, all these could be very can be analyzed very efficiently in atomic absorption spectroscopy.

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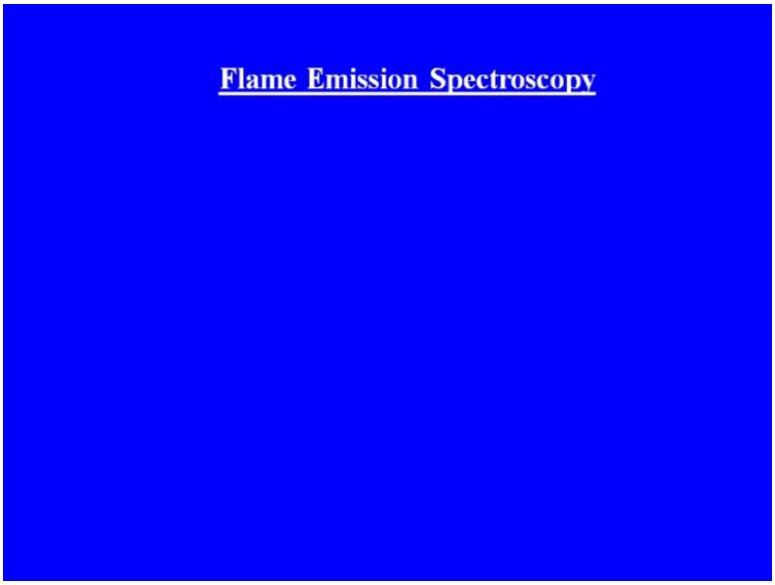

The next one is flame emission spectroscopy, and this is a method where light is emitted rather than absorb, the recorded and that is the emission pattern is recorded. Flame emission spectrometry, the sample solution is nebulized converted into fine aerosol, and introduced into the flame, where it is desolvated evaporated and atomized all in rapid succession subsequently, atoms and molecules are raised to excited sate by a thermal collisions within the constituents of partially burned flame gases.

Upon their return to a lower or ground state, the exited atoms and molecules emit radiations, and these are characteristic like I said of sample component, the emitted radiation passes through a monochromator that isolates the specific wavelength for the desired analysis. Photodetector measures the radiant power of the selected radiation, which is then amplified and then send to readout, device let me show you this a very simple schematic of flame emission spectroscopy.

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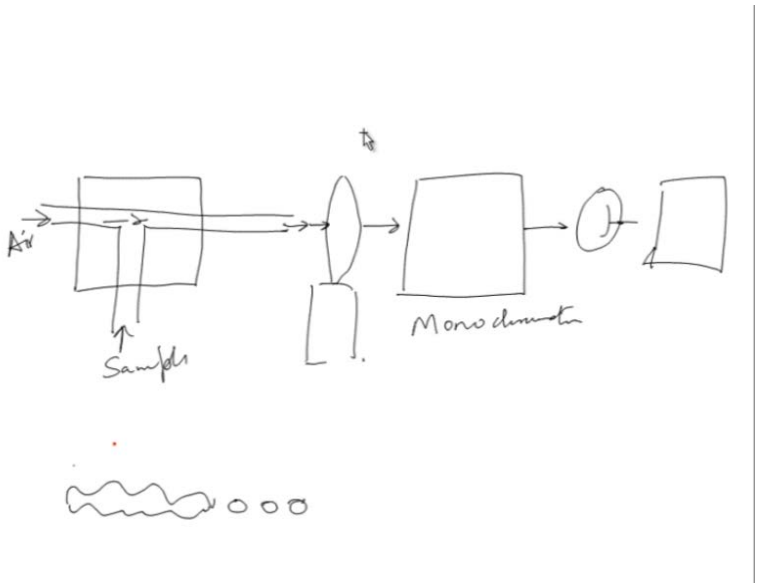

So, what you have is like as we said you have nebulizer, so you have compressed air or oxidant which comes from in this it is a compressed air or mixture with oxidant comes in here. And your sample is placed in here and, so when very high jet of air is passed through then the sample will oscillate and will be attracted or drawn towards this, and it will come in the form of droplets you can just to show you if it is a something like that, it will oscillate in this form.

And then, it will broken into the droplets here which will enter the flame, so once it is in here then there is a flame which is at particular right part of the flame, and the flame is put with laminar flow, so it is properly oriented. Then sample is introduced into the flamem where this excitation process, or and then emission occurs this is then recorded it is passed through a monochromator. Monochromator will allow only certain wavelengths to pass through, then from monochromator it will be a detector which will be placed in and from detector there will be a read out.

So, this is a very simple schematic of flame emission spectrometer instrumentation, so you have a nebulizer you have aerosol modifier, then small droplets comes into the flame emission occurs emission spectra is spectral lines which are of particular element are allowed to pass through the monochromator. And then there is a detector and finally, you get a read out, so this is the basic instrumentation and basic concepts of this emission spectroscopy.

So, combustion flame provides here in both cases a means of converting analytes into solutions to atoms, in the vapour phase field of their chemical surroundings. And these free atoms are then transformed into the excited electronic states by absorption, or by like additional thermal energy from flame or by absorption of radiant energy from the external source.

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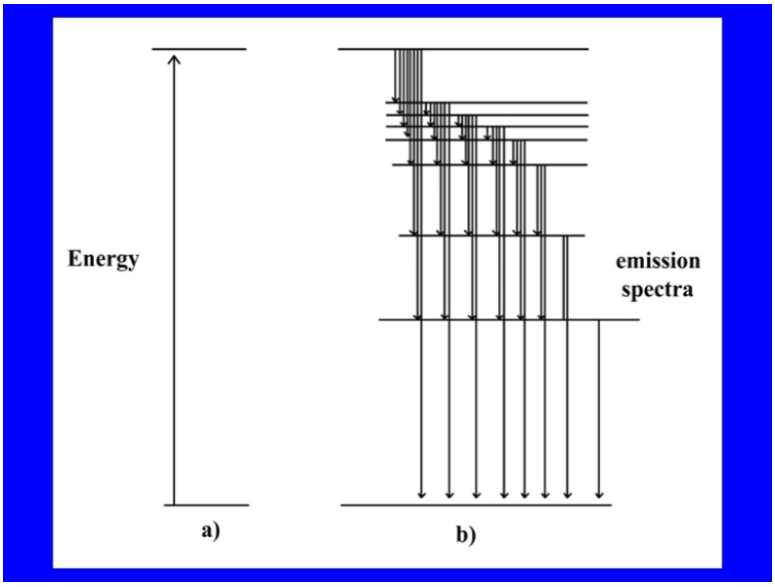

So, this is like somehow looks like emission spectra, where they come down to the ground state and lot of different types of spectral lines will be emitted, which will be recorded.

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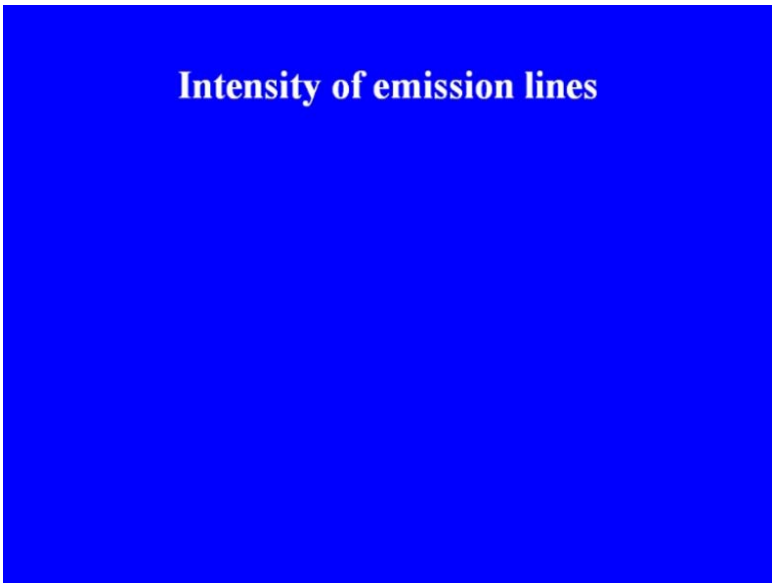

Now, intensity of emission lines will give quantitative measurement, and this will provide a particular like particular indication, about how much or number of atoms are present in there.

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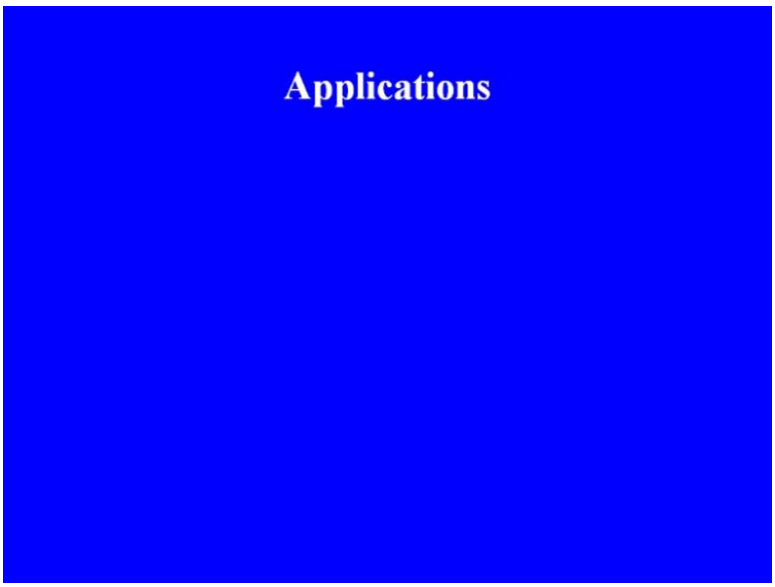

There are lots of applications for FES, it is a very simple sensitive, and easy technique considered little less sensitive as compared to absorption spectroscopy, but in absorption spectroscopy alkali metals cannot be done properly. So, FES here alkaline alkali trace earth metals lot of these things could be seen it is a sensitive method for detection of

metals, which is alkali alkaline earth as well as several transition metals could be analyzed in here.

Like I said it is could be attached with gas chromatography or other methods, it has found wide application in agricultural, environmental, industrial, clinical, applications and whether various others industries it is applied. So, both techniques atomic absorption, and as well as atomic emission spectroscopy are quite widely used for analysis of different types of metals and some non metals, both in qualitative and quantitative terms.

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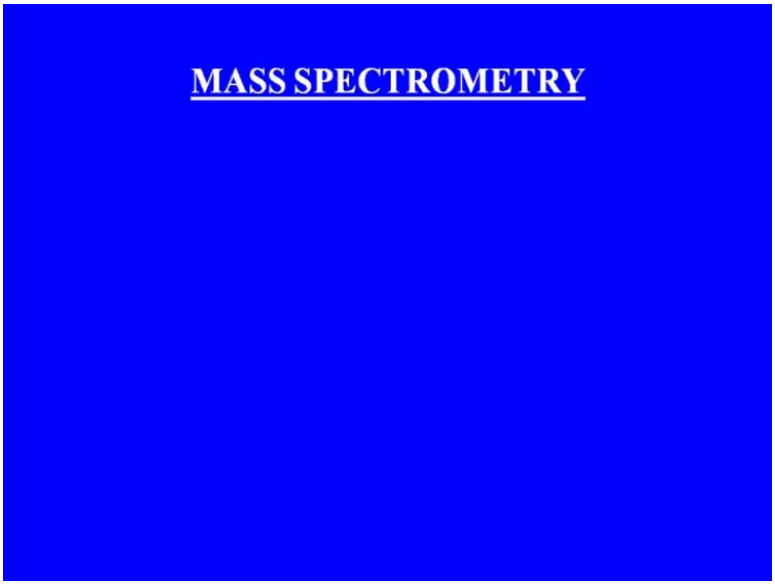

So, we will move on to the next technique now, that is mass spectrometry, now mass spectrometry is a technique used for analyzing molecules in on the basis of their mass to charge ratio. So, mass spectrometry is one very important technique, for analyzing and for different applications, a mass spectrometer determines the mass of a molecule by measuring the mass to charge ratio, that is m y z ratio of it is ion.

Ions are generated by inducing either the loss or gain of a charge from a neutral species, once formed ions are electrostatically directed into a mass analyzer, where they are separated according to m y z ratio, and finally detected. The result of molecular ionization ions separation and ion detection is a spectrum that can provide a molecular mass, and even structural information, so in a mass spectrometer the generated ions are separated into the mass analyzer and they are detected by an ion detectors.

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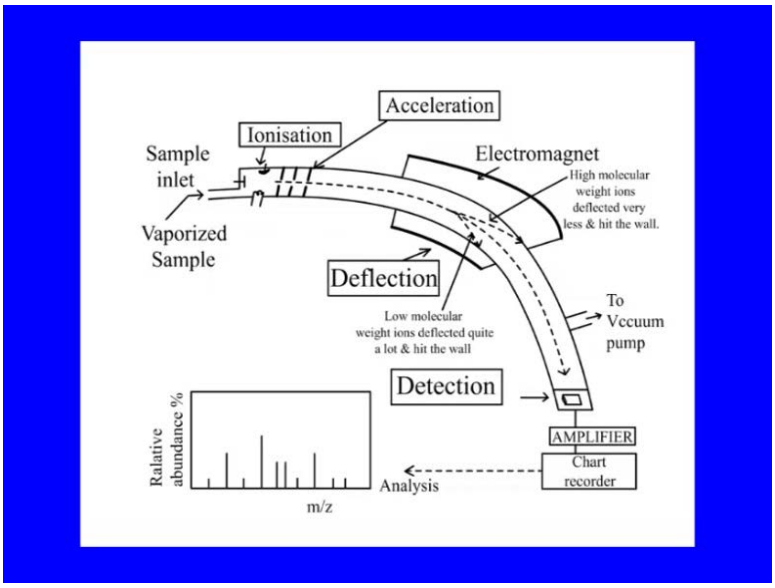

So, if you see here very simple this is a sample inlet here, and this there is a ionization source on ionization, this will be like passing through a particular mass analyzer.

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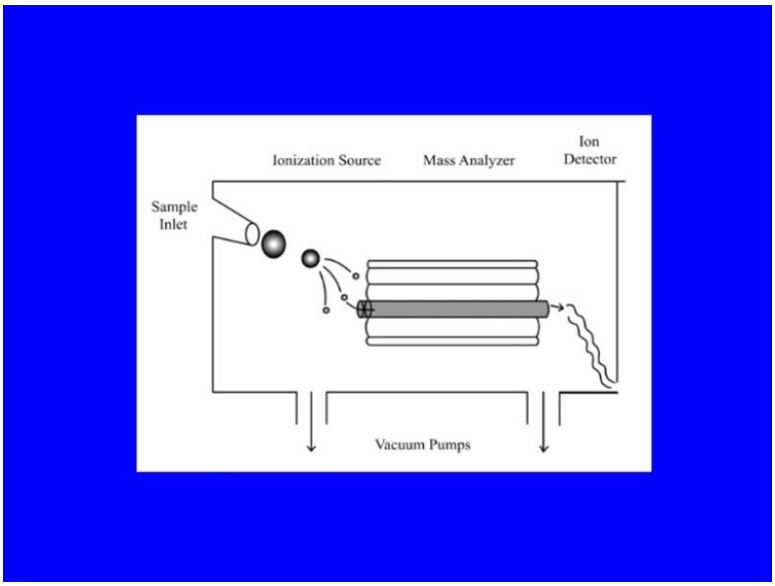

And it will be separated on the basis of their mass to charge ratio and finally, it will be gone to detector, and the spectra will be recorded or the it will be analyzed and detected in a ion detector. So, four basic components as we have seen in mass spectrometer is the sample inlet, an ionization source, a mass analyzer, and ion detector. Some instruments combine the sample inlet and the ionization source, while other combines the mass

analyzer and detector, so all samples molecules undergoing the same process regardless of instrument configuration.

Like instrument configuration could be any, but the process is same, sample molecules are introduced into the instrument through a sample inlet. Once inside the instrument the sample molecules are converted to ions in the ionization source, and before being electrostatically propelled into the mass analyzer ions are then separated according to their m to z ratio within the mass analyzer. So, that is regardless of instrument configuration these are the procedure which is followed, detector will convert the ion energy into the electrical signals which are then transmitted to a computer.

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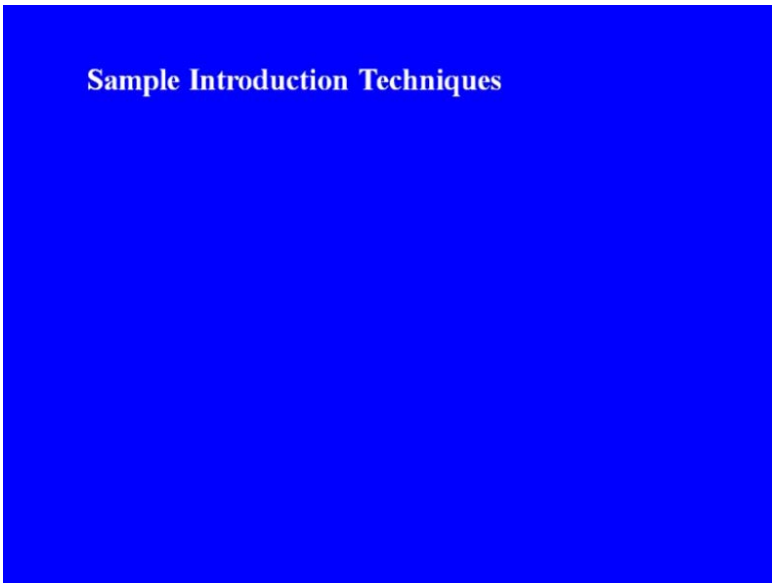

Sample introduction is an challenge in first challenge in mass spectrometry, and in order to perform mass analysis on a sample, which is initially at a atmospheric pressure, it must be introduced into the instrument, in such a way that the vacuum inside instrument remains unchanged. So, there are various methods like direct insertion using an insertion probe of plate, it is a very simple way to introduced sample through probe or plate. Sample is first plate on to placed on to a probe and then inserted into the ionization region of the mass spectrometer, and typically through a vacuum interlock.

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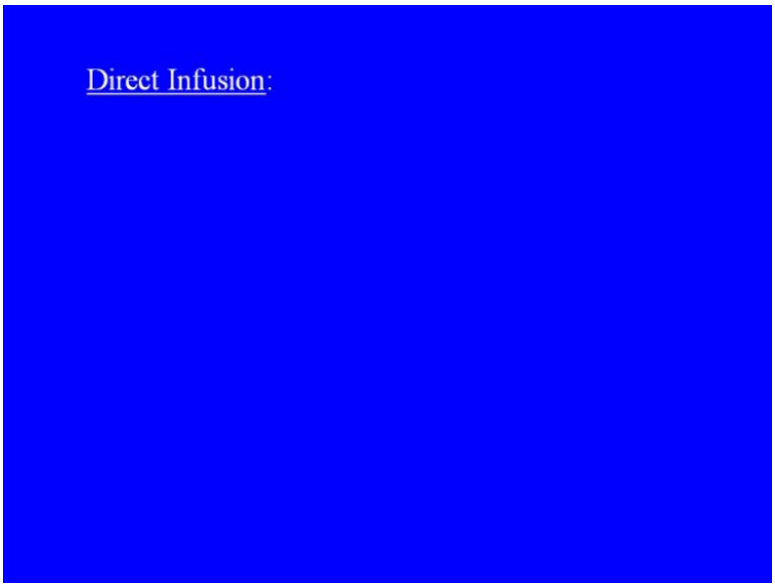

So, sample is then subjected to any number desorption process, such as laser desorption or direct heating to facilitate the ionization or vaporization, then there is direct infusion or simple capillary or capillary column is used to introduce sample as a gas or in solution. Direct infusion is also useful, because it can directly introduce a small quantities of sample into a mass spectrometry, without compromising the vacuum, capillary columns are routinely used to interface separation techniques with ionization source of a mass spectrometer. So, these techniques may also may include gas chromatography, and liquid chromatography which serves to separate a solutions different components prior to mass analysis, so these are coupled to mass spectrometer.

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Now, ionization method next part ionization method refers to the mechanism of ionization, and while the ionization source is the mechanical device that allows ionization to occur. Now, different ionization methods are there like which might work either ionizing neutral molecule through electron ejection, or through electron capture protonation or deprotonation, so lot of different methods are there.

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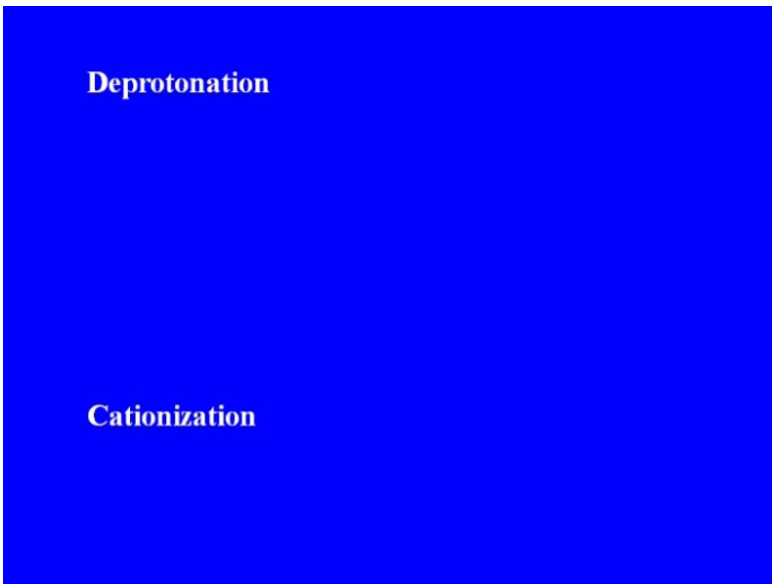

For this protonation, if we consider protonation is a method of ionization by which a proton is added to molecule producing a net positive charge for every proton added.

Deprotonation is the reverse of that is an ionization method by which the net negative charge will be achieved through the removal of a proton. Cationization is the method of ionization that produces a charge complex by non covalently adding positively charge ion to the neutral molecule.

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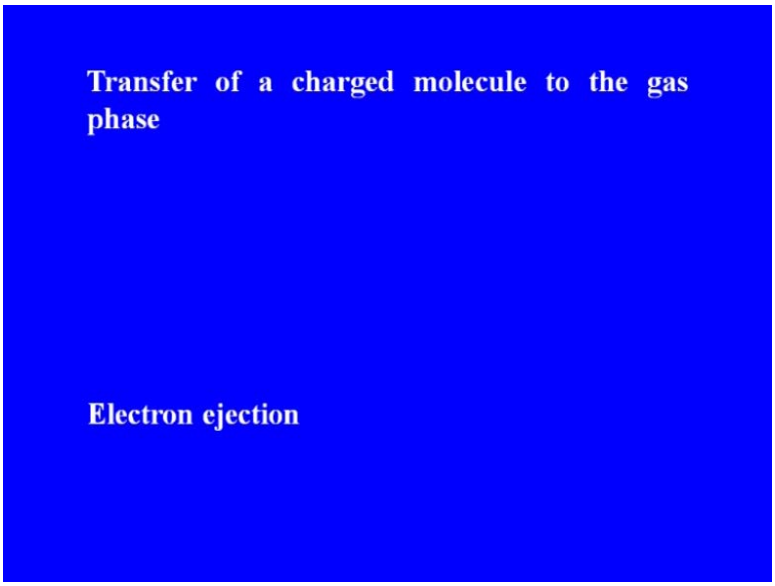

Then there is a transfer charged molecule to the gas phase, so transfer of compounds already charged in solution is normally achieved though desorption or injection, ejection of the charged species from condensed phase into the gas phase. Electron ejection achieves ionization through the ejection of an electron, to produce a net positive charge often forming radical cations.

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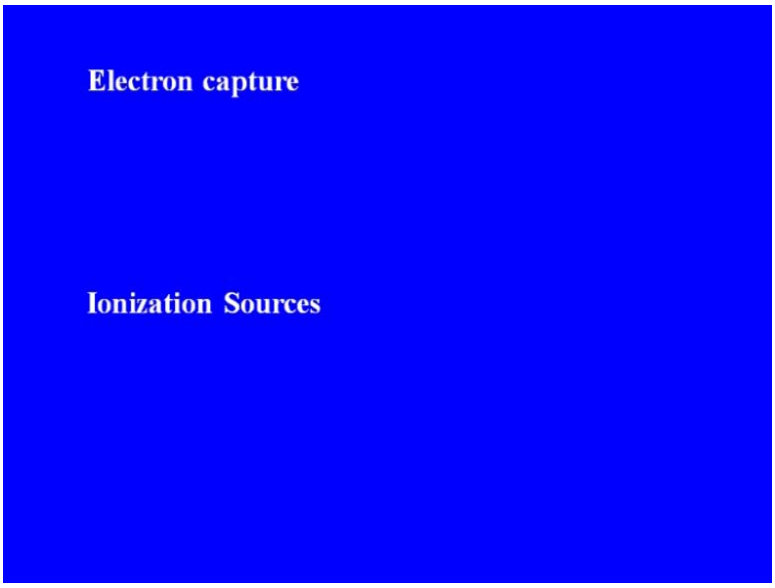

Electron capture here ionization method, we are net negative charge is achieved with absorption or capture of electron, there are lot of different ionization sources. Now, the ionization technique including like there are fast atom or ion bombardment technique, then there are matrix assisted laser desorption or ionization maldi is there, then electrospray ionization. So, there are whole lot of techniques in there, among these ESI and maldi have evolved to be the methods of choice, when it comes to the biomolecular analysis.

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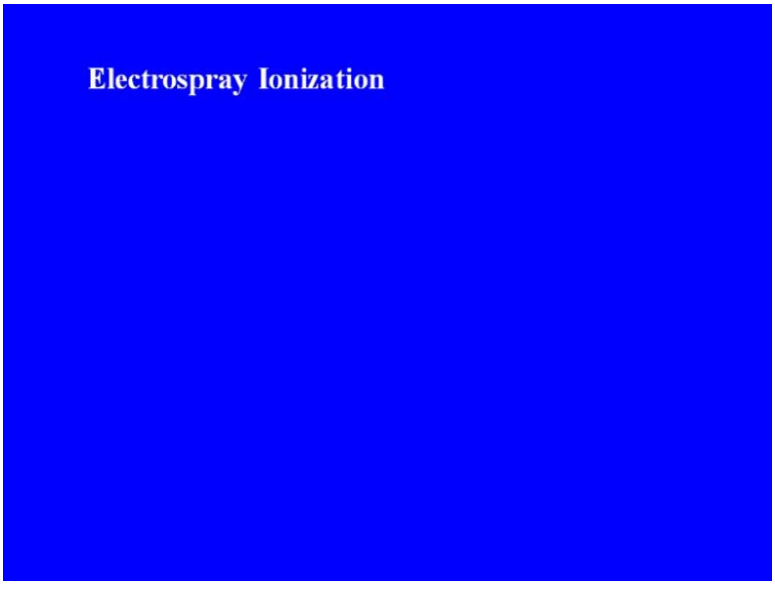

Now, electrospray ionization or ESI more physical explanation of ESI is that needle voltage produces an electrical gradient on the fluid, which separates the charge at the surface. This force since the liquid to emerge from the needle, as the cone the tip of the cone protrudes as the filament until the liquid reaches the rayleigh limit, where the surface tension and electrostatic repulsions are equal and the highly charged droplets leave the filament.

So, that is the one way that is kind of spray, the droplets that break away from the filament are attached to the entrance of the mass spectrometer, due to the high opposite voltage at the mass analyzers entrance. As, the droplet moves towards the analyzer the columbic repulsion on the surface exceeds the surface tension and the droplet explodes into small droplets ultimately releasing ions, so this is method of electrospray ionization as you can see here.

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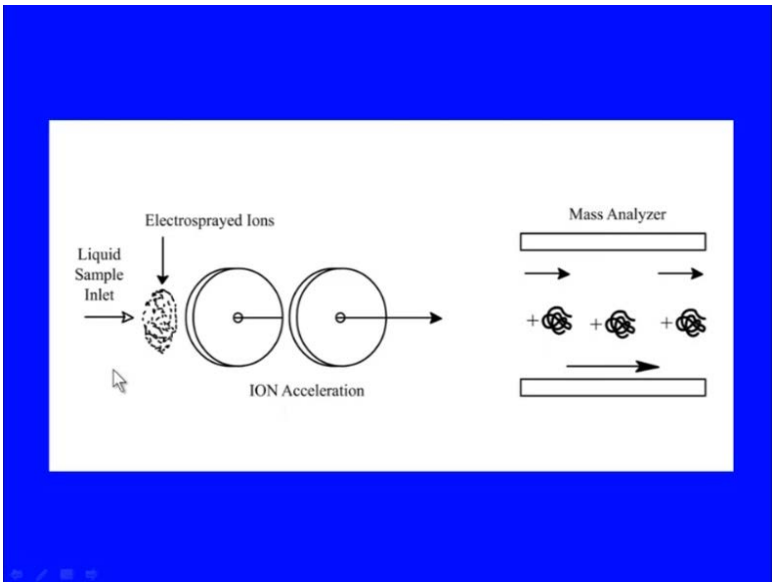

That this is liquid sample these are electrosprayed ions and these are ion acceleration occurs and then finally, the mass analyzer analyzes the sample.

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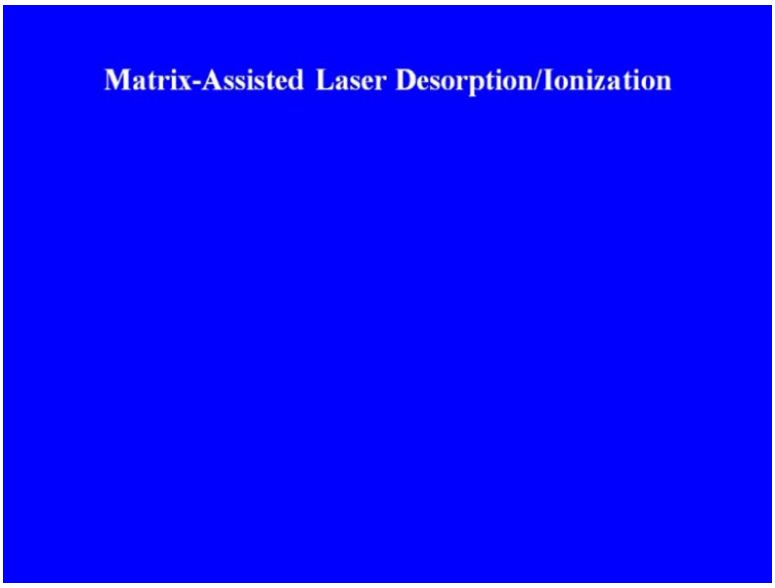

Then there is matrix assisted laser desorption ionization, where it is efficient and directed energy transfer during a matrix assisted laser induced desorption event, and just provides high ion yield of the intact analyte. And allows for the measurement of the compounds with sub peak mold sensitivity, in addition the utility of maldi for analysis for hydrogen samples makes it very attractive, for the mass analysis of complex biological samples, like such as proteins or proteolytic digests.

It is generally believed that maldi causes the ionization and transfer of sample from the condensed phase to the gas phase via laser excitation, and ablation of the sample matrix, that is maldi matrix nonvolatile solid material, which facilities the desorption and ionization process by absorbing the laser radiation. So, as a result both matrix and any sample embedded in the matrix are vaporized, the matrix also serves to minimize, sample damage from laser radiation by absorbing most of the incident energy.

So, this is like very important, so it is done by laser excitation, in maldi analysis the analyte is first cocrystallized with a large molar axis of matrix compound. Usually, UV absorbing week organic acid radiation of this analyte matrix, mixture by a laser results in the vaporization of the matrix, which carries the analyte with it. The matrix plays a key role in this technique, the cocrystallized sample molecule also vaporizes, but without having to directly absorb energy from the laser, molecule sensitive to the laser light are therefore, protected from directed from UV laser excitation.

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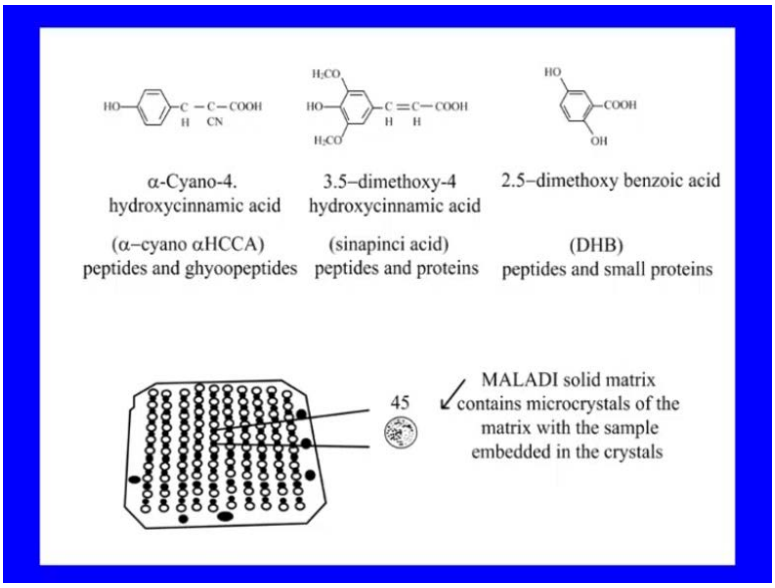

So, here there are some common maldi matrices, which are like hydroxycinnamic acid different hydroxycinnamic acid alpha-cyano-4-hydroxycinnamic acid, 3 4-dimethoxy-4-hydroxycinnamic acid, and 2 5 dimethoxybenzoic acid are utilized. These are different things like peptides, or proteins peptides, and the small proteins this is the matrix maldi solid matrix which contains microcrystals of the matrix with the sample embedded in the crystal.

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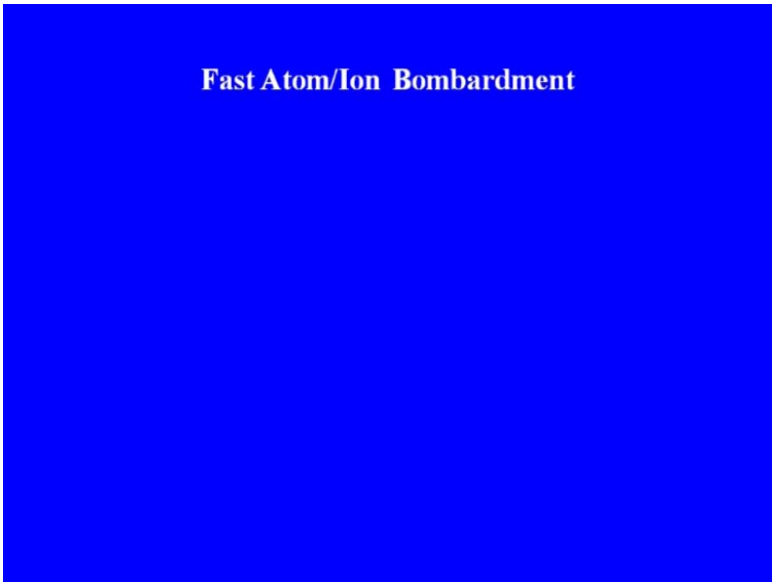

Then there are other methods like fast atom ion bombardment method, where ionization source similar to maldi in that, it uses a matrix and a highly energy of beam of particle to dissolve ions dissolve ion from surface. It is important; however, to point out that differences between maldi and feb are there, for maldi the energy beam is pulsed laser light while for f this fast atom bombardment, it uses the continuous ion beam. So, with maldi the matrix is typically a solid crystalline, where is for feb for this one it is a liquid matrix, so it is very important to note that fast atom bombardment is about 1000 time less sensitive then maldi.

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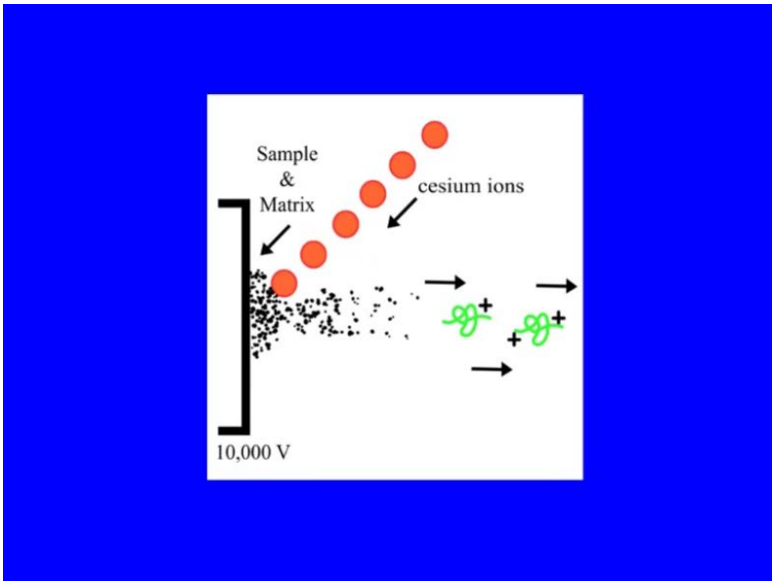

So, if you see here this is schematic of how this ionization occurs in fast atom bombardment, where you have a continuous ions and which bombards and then ions are sample is dissolved from here.

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Now, in mass analysis most in very simple terms, a mass analyzer measures gas phase ions with respect to their mass to ratio, where the charge is produced by the addition or loss of protons, cations, anions, or electrons. The addition of charge allows the molecule to be affected by electrical fields thus along it is mass measurements, this is an important aspect to remember about mass analyzer, that they measure m to z ratio and not the mass as such.

There are lot of detectors or mass analyzers you can say, which are quadrupole mass analyzers, which are connected in parallel to a radiofrequency generator and a DC potential. And at a specific RF field only ions of a specific mass to z ratio can pass through the quadrupole, and where only the ion of m to z ratio 100 or, so means it is like different depending on RF field particular kinds of ions are passed through quadrupoles.

It offers a few advantages or also they tolerate little bit higher pressure, quadrupoles have a significant mass range with capability of analyzing up to m z of 4000, which is useful, because of electrospray ionization of proteins. And other biomolecules commonly produced charge distribution which is in range of 1000 to 3500, final then quadrupole mass spectrometers are relatively low cost instruments.

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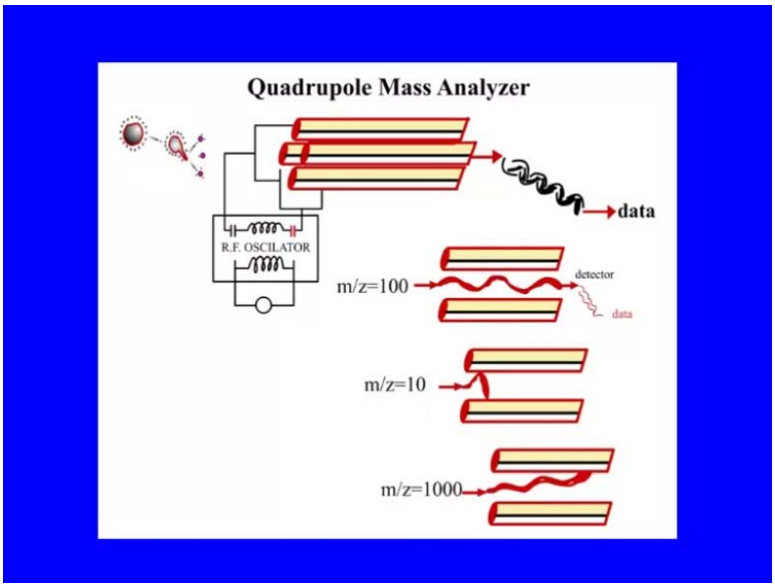

This is typical quadrupole mass analyzer, where you have a RF oscillator there is a mass analyzer, and you get data at different at different frequencies you will get different m to z ratio detected.

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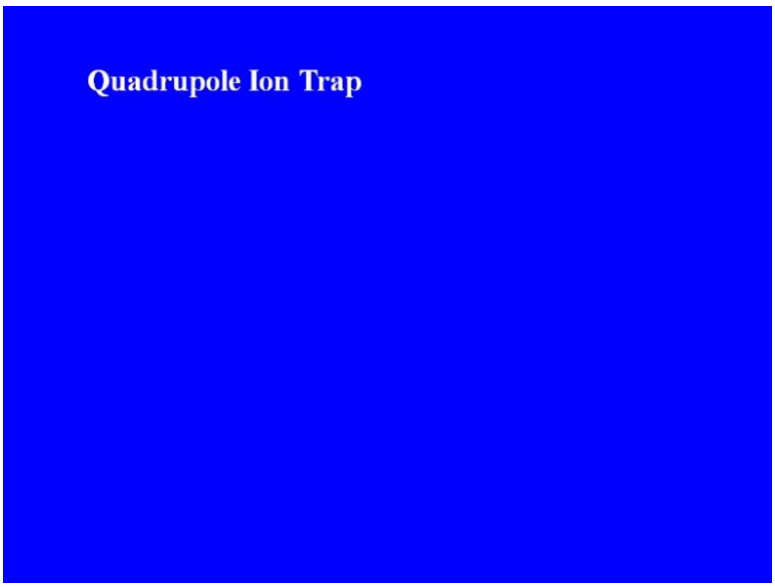

Then there are quadrupole ion trap, the quadrupole and the physics behind quadrupole or quadrupole ion trap are similar; however in an ion trap rather passing through a quadrupole analyzer with superimposed radio frequency field. The ions are trapped in a radio frequency quadrupole field, the quadrupole ion trap typically consists of a ring

electrode and two hyperbolic and kept electrodes. The motion of these ions induced by the electric field on these electrodes allows ions to be trapped or ejected from the trap.

So, in the normal mode the radio frequency is scanned to resonate excite, and therefore eject ions through small holes, in the end cap to a detector, as the RF is scanned to higher frequency higher m to z ions are excited ejected and detected. So, depending on what is the frequency at which they are scanned very useful feature of the ion trap is that it is possible to isolate one ion species by ejecting all other from the trap.

The isolated ions can subsequently be fragmented by collision activation and the fragment detection, this is very important term time of flight. Time of flight analysis which is done in maldi tof actually, based on accelerating a group of ions to detector, where all of these ions are given the same amount of energy through an accelerating potential. And because the ions have the same energy, but the different mass, the lighter ions will reach the detector first because of their greater velocity, while the heavier ions will take longer to and due to their heavier mass and lower velocity.

Hence, the analyzer is called time of flight because the mass is determined from the ions, time of arrival and mass charge in kinetic energy of the ion all play a part in the arrival time of the detector. So, time of flight is now widely used for ESI maldi and more recently for electron ionization like in GCMS applications, and it combines time of flight, which an electrostatic mirror.

The reflectron serves to increase the amount of time ions need to reach the detector, while reducing their kinetic energy distribution, they were reducing the temporal distribution that is delta t. Since, resolution is defined by the mass of a peak divided by the width of a peak, so increasing the t and decreasing the delta t and results in a higher resolution.

So, time of flight reflector on offers the higher resolution over simple tof instrument by increasing the path length, and kinetic energy focusing through the reflectron, it should be noted that increased resolution and sensitivity on a top reflectron does decrease significantly at higher masses, that is above 500 mass to charge ratio. So, this is time of flight analyzer like if you can use maldi or electrospray and it passes through and time of flight is calculated depending on that, they will reach the detector at different times and peak will be analyzed.

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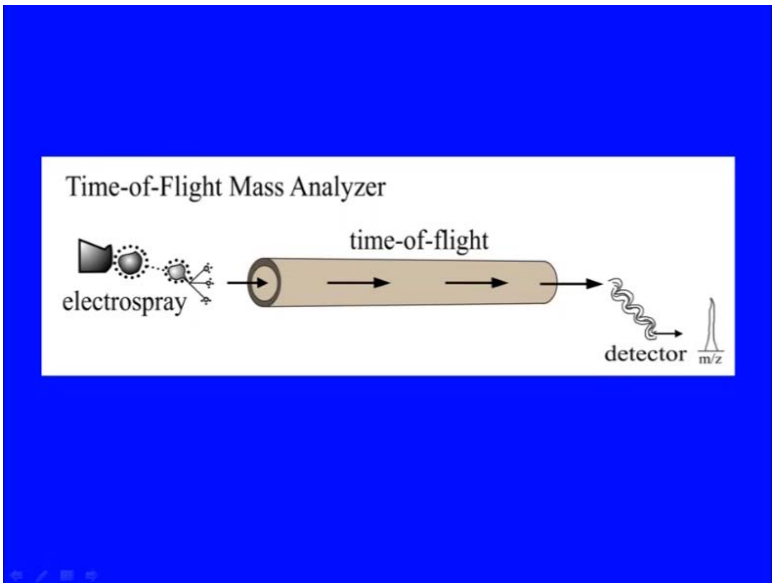

So, after mass analyzers there are different types of detectors ones the ions are separated by the mass analyzer, the reach the ion detector, which generates a current signal from the incident ions.

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The most commonly used detector is the electron multiplier which transfers the kinetic energy of the incident ions to a surface, that in turn generates secondary electrons; however, a variety of approaches are used to detect ions depending on the type of mass spectrometer.

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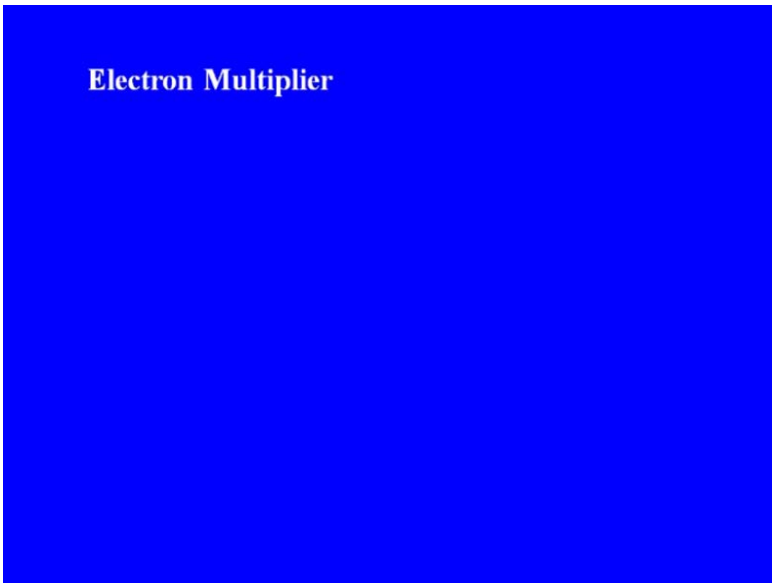

There could be electron multiplier which is most common means of detecting ions involves an electron multiplier, which is made up of a series of aluminum oxide dynodes maintained at ever increasing potential, ions strike the first anode surface, causing an emission of electrons. And these electrons are then attracted to the next anode held at a higher potential, and therefore more secondary electrons are generated. Ultimately, as numerous dynodes are involved a cascade of electrons is formed and that results in an overall current gain of the order of one million or higher.

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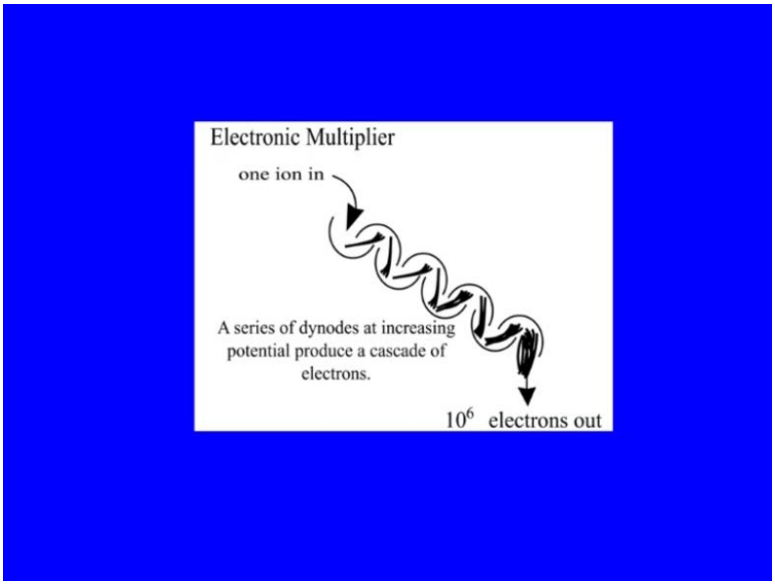

So, if you see here this is electron multiplier, so one ion in here and then through series of dynodes at increasing potential produces the cascade of electrodes and you get very high signal here. Then there is a faraday cup it involves an ion striking a dynode surface, which causes secondary electrons to be ejected, and this temporary electron emission induces a positive charge on the detector. And therefore, a current of electrons flowing towards the detector, and this detector is not particularly sensitive, and offering limited amplification of signal, yet it is a tolerant of relatively high pressure.

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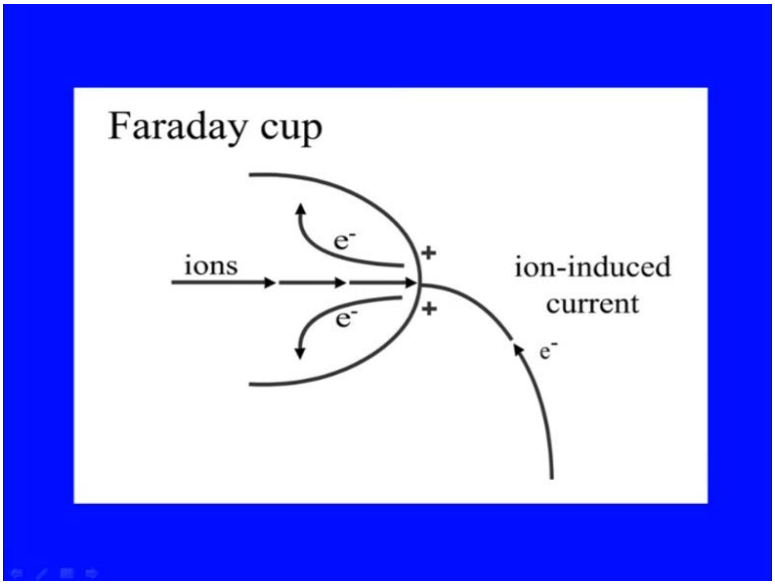

So, a faraday cup could be utilized here, and where secondary electrons are generated.

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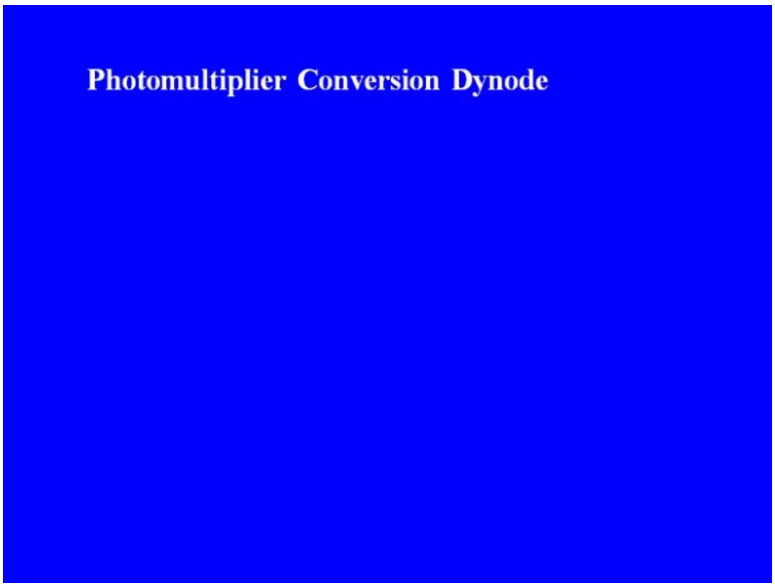

They could be photomultiplier conversion dynodes, the photomultiplier conversion dynode detector is not a commonly used as the electron multiplier, at similar in design. Whereas, secondary electron strikes a phosphorous screen instead of dynode and the phosphorous screen then releases photons, which are detected by the photomultiplier. And photomultiplier also operate like the electron multiplier, where the striking of the photon on a scintillation surface result, in the release of electrons that are then amplified using the cascading principle.

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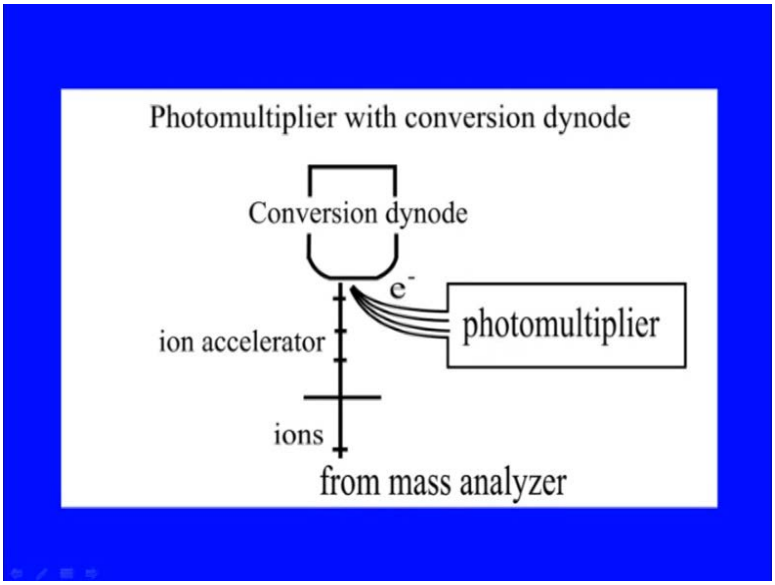

So, here photomultiplier with conversion dynodes is there and, so ions here as per rather than phosphorous screen is utilized, and the signal is collected. There are lot of applications as we have gone through the other arrangements could be like two mass spectrometers arranged in tandem, that is called MSMS or tandem mass spectrometry. In tandem mass spectrometry like one application here, that like say proteolytic fragments which are analyzed for their mass in first MS, they could be further degraded and protein the amino acids sequence of those peptides could be analyzed.

In second mass spectrometer, which can give you like maldi tof is available or other ESI MSMS are available, where one can know the amino acids sequence of peptides and by that the whole protein could also be sequenced. And it could be de novo sequence or otherwise, from databases it could be compared depending on the types of fragments, which are obtained. So, mass spectrometer has lot of applications, it is used to determine the isotropic composition of elements within a sample, like it is very high highly sensitive, and efficient technique, and it can differentiate between elements with very small differences in there mass.

So, isotropic components could be detected mass spectrometer is an important emerging method for the characterization and sequencing of proteins, the two primary ionization methods for ionization of whole proteins are electrospray ionization or maldi could be utilized, and where like as I said maldi tof tof or ESI MS could be utilized for sequencing.

Then there are lot of other approaches which could be used for characterizing, characterization of proteins in the first intact proteins are ionized by either of the two technique. And then introduce into the mass analyzer mass spectrometry, with it is low sample requirement and high sensitivity has been predominantly used in lot of different fields, like glycobiology for characterization and elucidation of structure like aglycone structure.

And even different forms like of proteins, where different alternations in proteins could be analyzed through mass spectrometry, it is used also for analysis of drug and it is in pharmacokinetics it is utilized. So, there are lot of applications of mass spectrometry in terms of determination of simple mass to sequencing that is peptide sequencing or to know elucidate a structure say in glycobiology, in or in protein bio chemistry.

Mass spectrometry has been key in proteomics research, where complete proteome of a cell or others could be identified by coupling it to mass spectrometry, like where in 2D gel electrophoresis, different proteins could be isolated. And then they could be picked up from the gel, and they could be proteolytically cleaved, and finally analyzed in mass spectrometry like in tandem mass spectrometry to derive their sequences.

So, like either they could be used for sequencing or protein all proteins in a proteome, or like if you are comparing 1 2 types of protein content of a shell in 2 conditions like say deceased, and normal condition. Then the reasons for like say over expression of a particular protein can be seen, and regions for the decease or at least basics about that could be found out. So, mass spectrometry is very useful technique, and have various applications, very powerful tool to for analysis, and it gives lot of information about different biomolecules, and it is being utilized for structural elucidation, mass analysis sequencing and in various areas of pharmacology and other analysis.

So, this completes our section on spectroscopy, we have discussed various techniques, and we are tried to discuss about basic concepts and many, times we have not gone in detail too much, but it the main aim is to make you aware about these techniques. And then from here you can go and understand, and go into details of these techniques for further making use of these techniques for your specific applications.

Thank you.