Mass Spectrometry Based Proteomics
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Mod 01 Lecture Number 05

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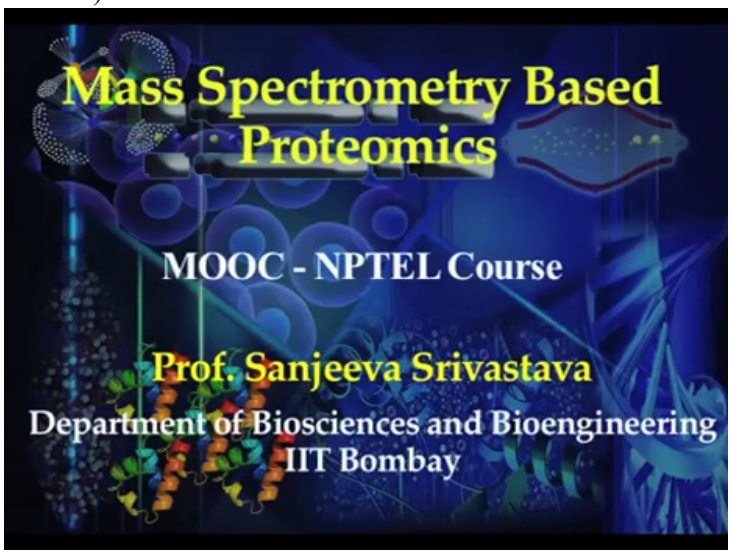

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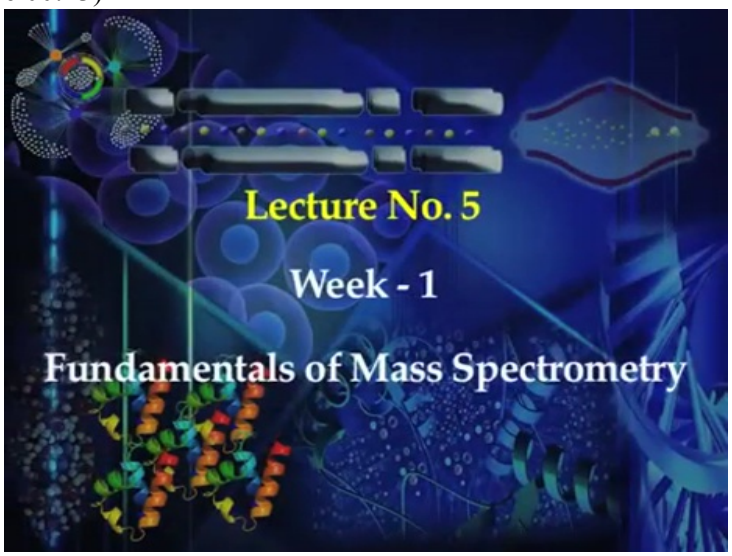

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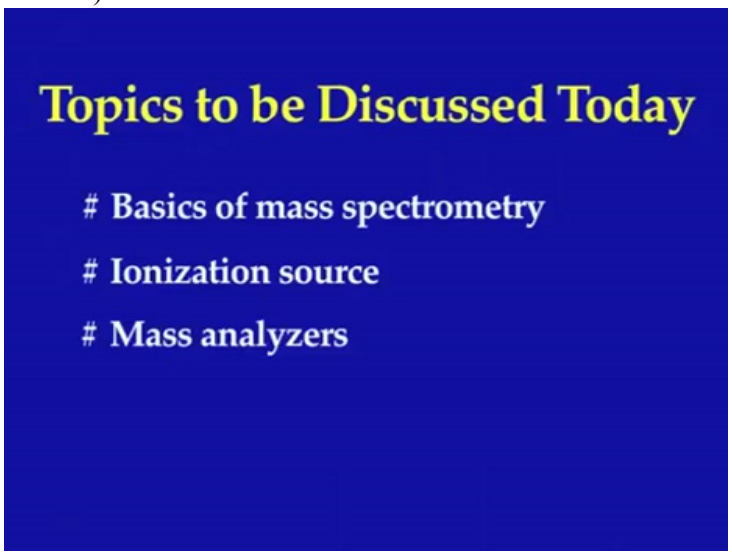

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So today first we will talk about fundamentals of mass spectrometry. I will describe you the role of MS and various basic concepts involved in understanding the mass spectrometry.

We will look at the individual components such as ionization source, mass analyzers as well other components. Then we will talk about Tandem Mass Spectrometry.

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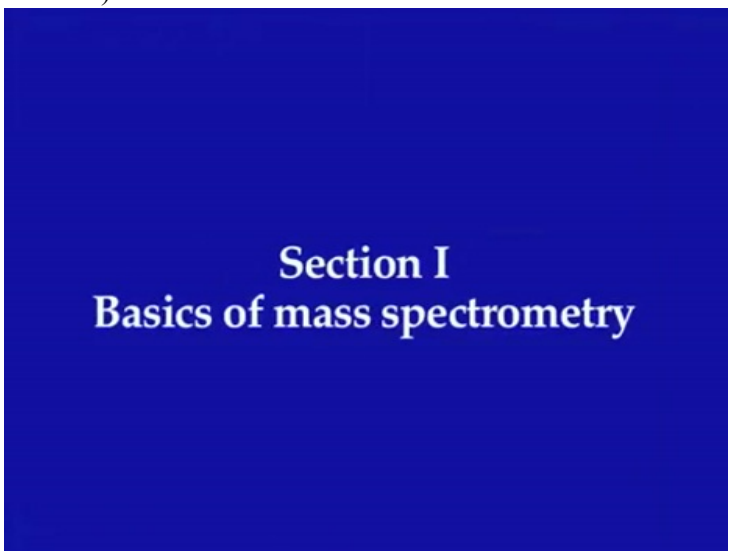

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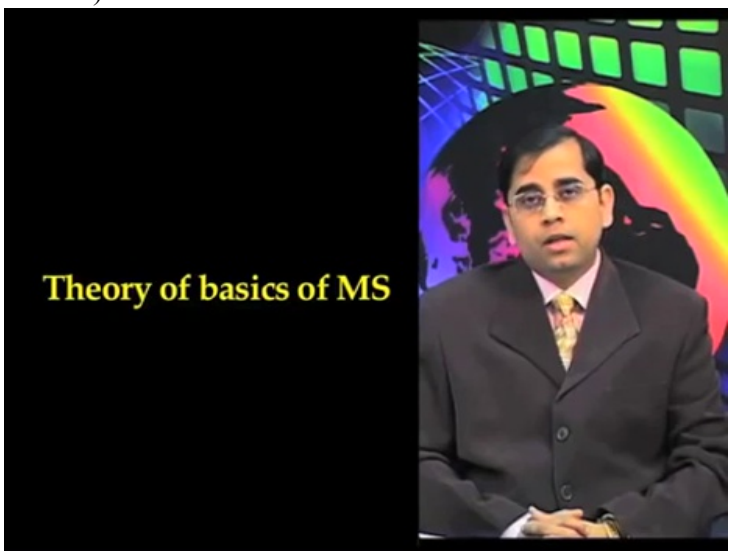

The scale, at which we want to study the proteome, requires much more analytical instrument capability. And mass spectrometry has ability to provide that platform for comprehensive coverage of proteome.

MS has become an important analytical tool in biology in general and in proteomics during the last decade. Now various applications have emerged using MS based platform. It offers high throughput, sensitive and specific analysis for many applications. Let's first look at some of the basic concepts of mass spectrometry.

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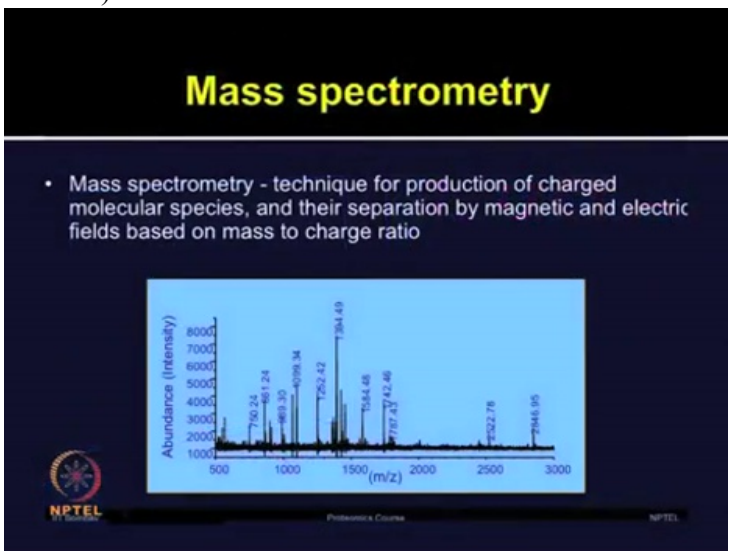

So first of all what is mass spectrometry? It is an analytical technique to measure the molecular mass of individual compounds and atoms accurately by converting them into the charged ions.

So by definition this is a technique for the production of charged molecular species in vacuum and their separation by magnetic and electrical fields based on mass to charge ratio.

You can see the MS spectrum shown in the slide m by z and the intensity, abundance on y-axis. So what are the unique features of mass spectrometry?

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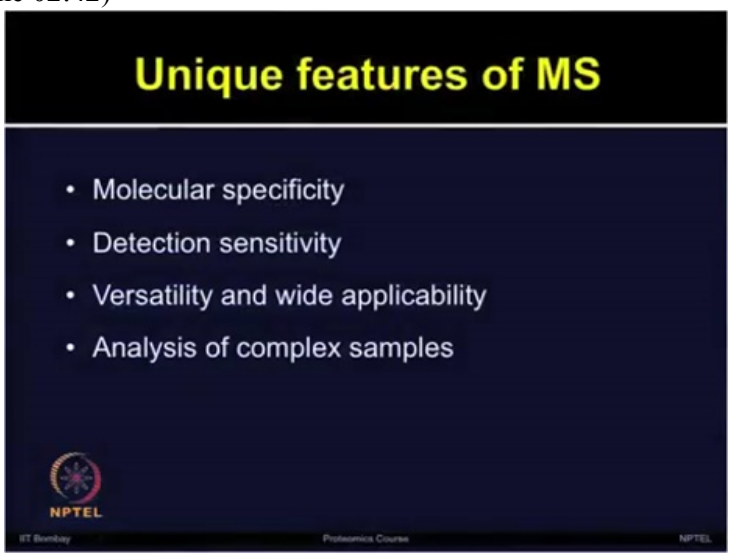

Molecular specificity, due to its unique ability to accurately measure molecular mass and provide fragment ions of analyte, mass spectrometry offers molecular specificity. It provides ultra high detection sensitivity. In theory MS can detect even a single molecule and its sensitivity at atomole and femtomole has also been demonstrated.

It provides versatile platform to determine the structure of compounds and it is applicable to all the elements, all type of samples whether it is volatile, non-volatile, polar, non-polar as well as solid, liquid or gas. So analysis of complex samples such as proteome is very much possible by using MS

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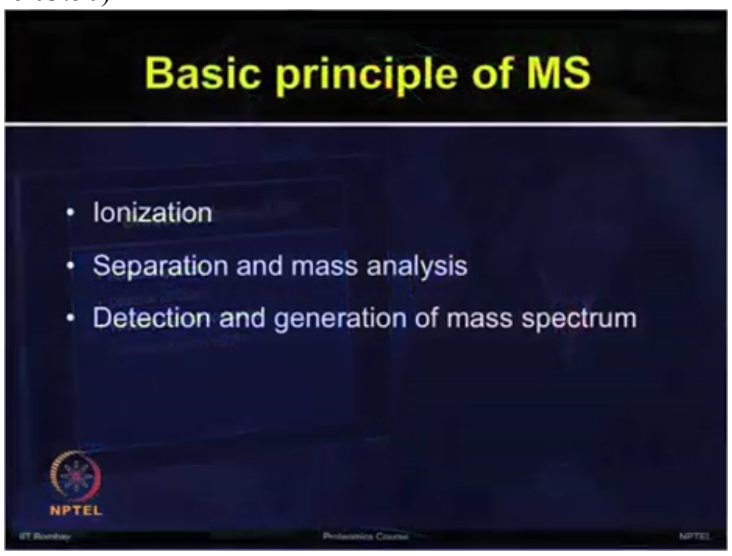

. What is the basic principle of mass spectrometry?

The first step is ionization. To convert analyte molecules or atoms into the gas phase ionic species. It removes or adds electrons or protons. The second step is separation and mass analysis of molecular ions and charge fragments on the basis of mass to charge ratio.

The final step is detection and generation of mass spectrum. These are the main steps involved in the mass spectrometry operation. Now let's discuss about general properties of MS.

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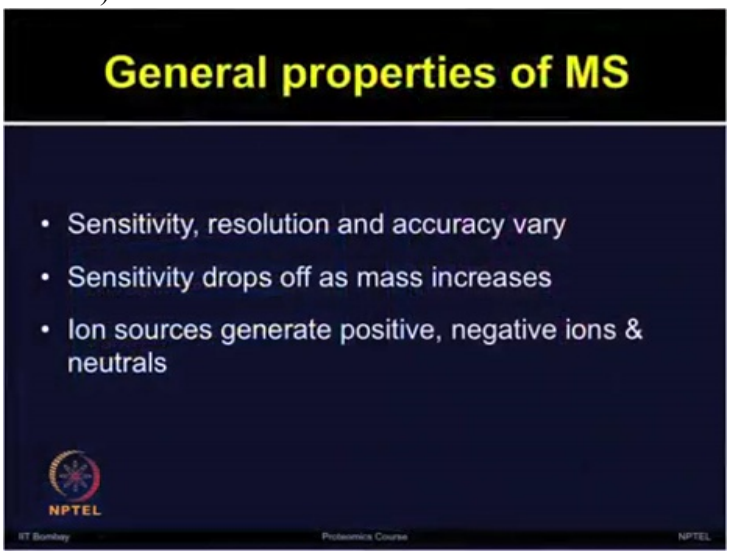

The sensitivity, resolution and accuracy vary among various mass spectrometers. The sensitivity drops off as the mass increases. And as I mentioned the sensitivity for protein detection can as low as atomolar or femtomolar range. Ion sources, they generate positive, negative and neutrals.

The neutrals obviously cannot be focused or accelerated by the ion optics. So, one can analyze either positive or negative ions. The positive ions have an adduct, which is typically a proton and the sensitivity for negative ions is generally lower.

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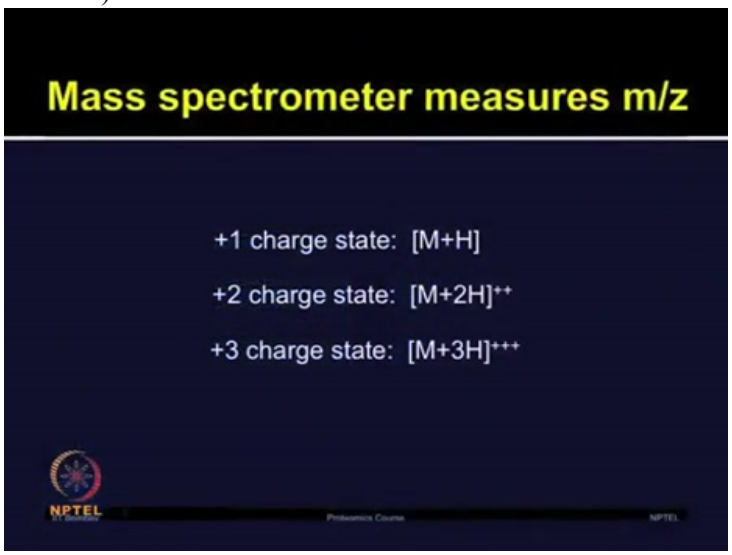

So the mass spectrometer measures m by z. The MS data is presented as mass to charge ratio, which is mass of an ion "m" divided by the number of charges "z" it carries. So, the total charge on ion is represented by q equals to "z""e", where e is the charge on an electron

How you can calculate the m by z of any peptide? As I mentioned here you can have multiple charge states; +1 charge state, +2, +3 or multiple charge states are possible. So if you need to calculate m by z you need to add m+h or m+2h or m+3h as shown and then divide by 2 or 3 depending on the how many charge state it carries.

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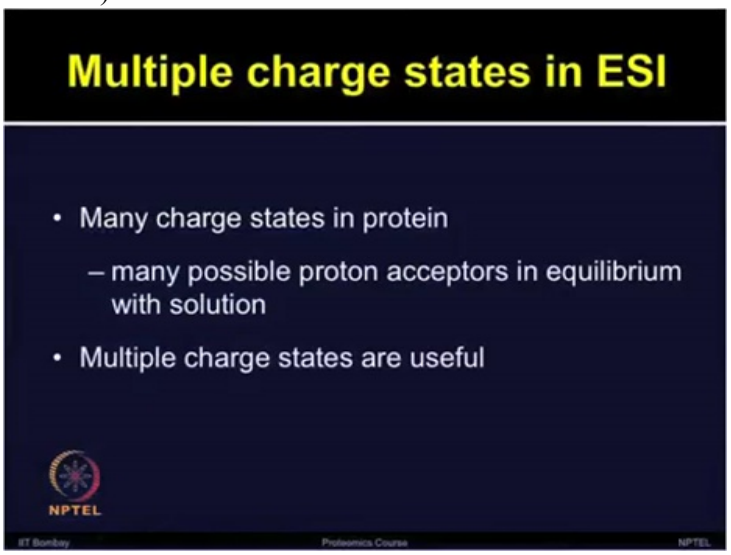

I will talk in more detail about how various types of ionization sources work that will be in the later part of the lecture. But just in this context I would like to mention that there are multiple charge states present in the ElectroSpray Ionization.

So in many charge states in proteins there many possible proton acceptors in equilibrium with the solution. So the multiple charge states are quite useful because they form ions which are in the mass range of mass analyzers such as TOF, Quadrupoles, Ion Traps etc.

Now during the initial part we are trying to cover some of the basic terminology and basic concepts involved in the mass spectrometry. So let's talk about what is average and monoisotopic masses of amino acids.

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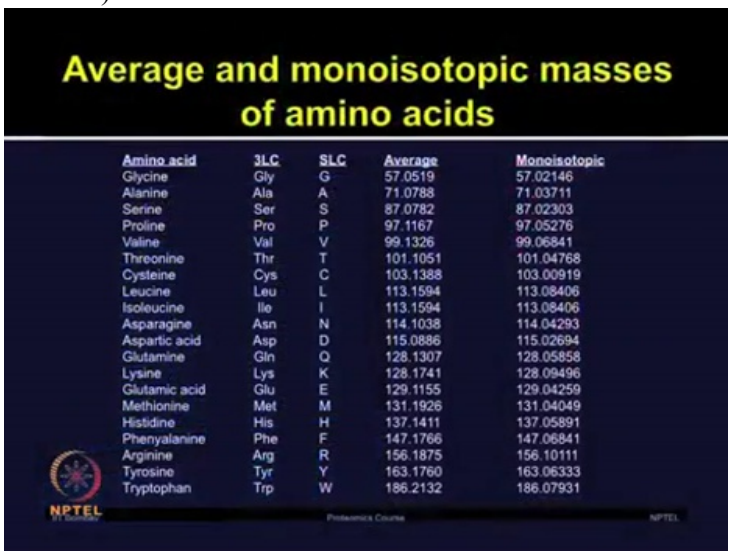

As shown in the table here; which shows amino acids, 3 letter codes, single letter code, average and the monoisotopic masses; you can use this for your reference later on which can be used in the data analysis and the calculations. So what is monoisotopic mass of a protein?

It is sum of masses for most abundant isotope of each element. The average mass of an element is the average of isotopic masses of each element weighted for the isotopic abundance. I hope you are able to distinguish the average and monoisotopic masses of amino acids.

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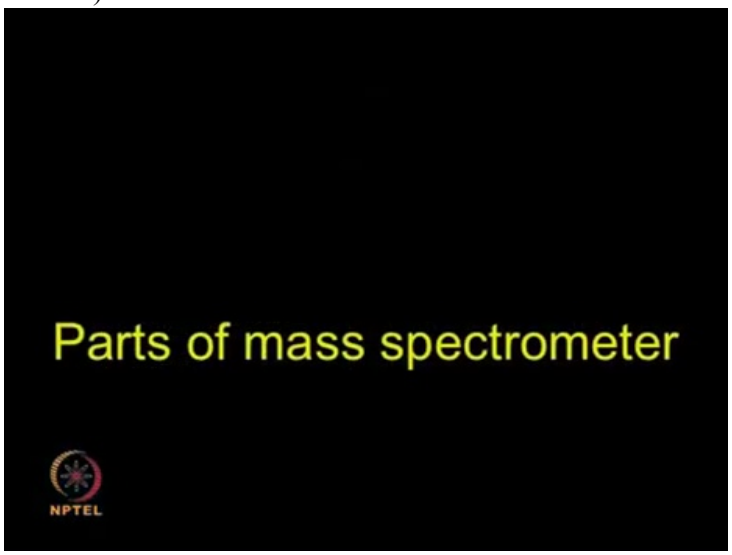

Now let's talk about different parts of mass spectrometer. So the major components include the sample inlet, ion source, mass analyzer, detector, signal processing components and data output. Let's look at each of these components in more detail.

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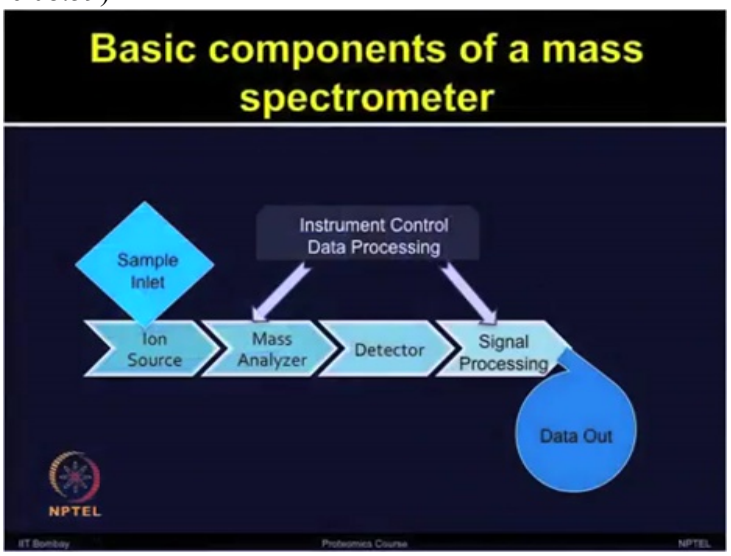

Sample inlet, it transfers the samples into the ionization source. The ion source or the ionization source, it converts neutral sample molecules into gas phase ions.

Mass analyzer, it separates and analyzes mass of the ionic species. There are various types of mass analyzers available which we will discuss in more detail during the subsequent part of the lecture. One need to maintain the vacuum condition, a very low pressure is maintained inside the mass spectrometer.

Detector measures and amplifies the ion current of mass resolved ions and then we need the electronics to control the operation of various units. The data system, it records, processes, stores and helps to display the data output

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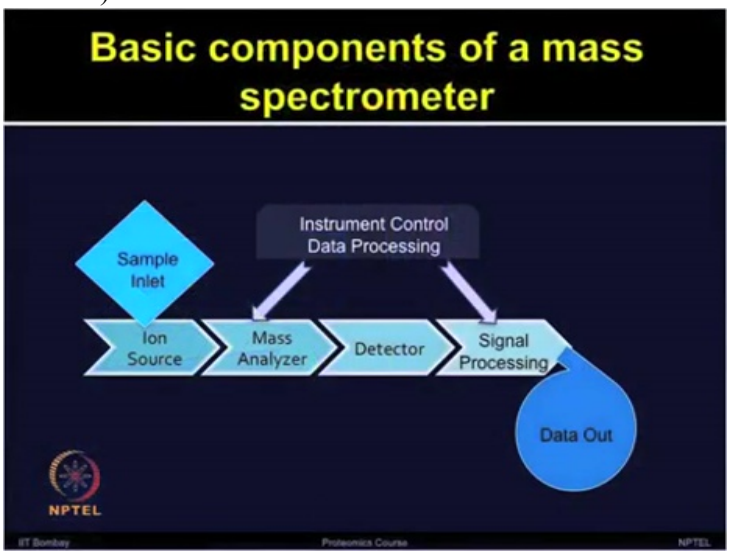

So although there are 3 major components involved; ionization source, mass analyzer and detector, but then there are some accessory components which are also equally important for doing the mass spectrometry based experiments.

So various components included sample introduction, one can be couple with HPLC or CHIP-based technologies for doing the liquid chromatography based separations, sample ionization

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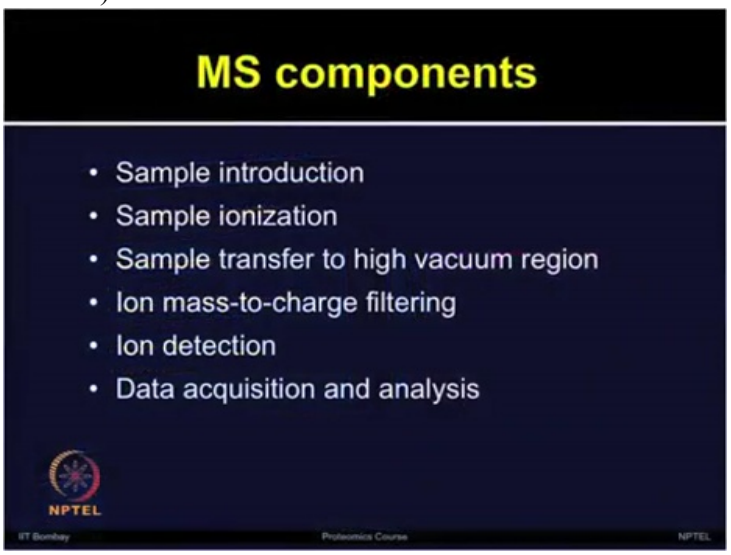

There are various types of ionization sources currently available, sample transfer to the high vacuum region, so the ion mass to charge filtering can be performed by mass analyzers, ion

detection by using detectors and then data acquisition and analysis by using data system. All these are integral part of MS components

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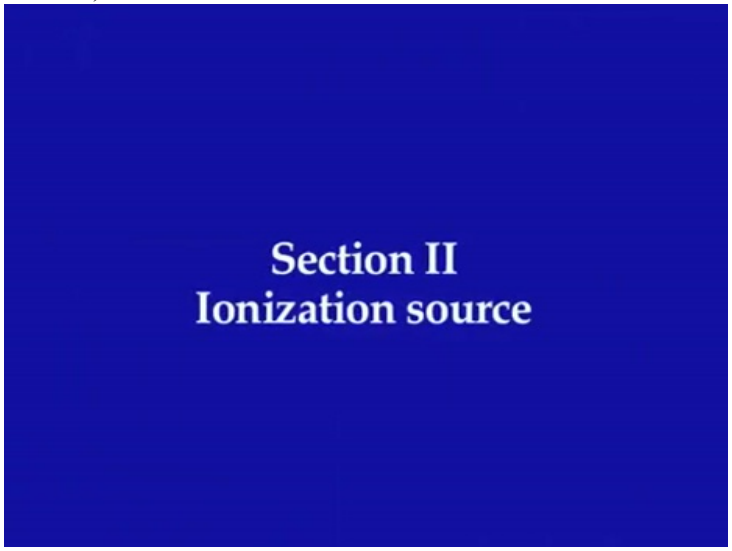

Some of the different types of ionization sources involved in the MS analysis. The success of MS experiment lies in efficiency of converting a neutral compound to a gas phase ionic species.

There are various types of options currently available. You can select what type of ionization source you want for your specific application. So the choice of particular ionization source is dictated largely by the nature of sample with which one wants to investigate.

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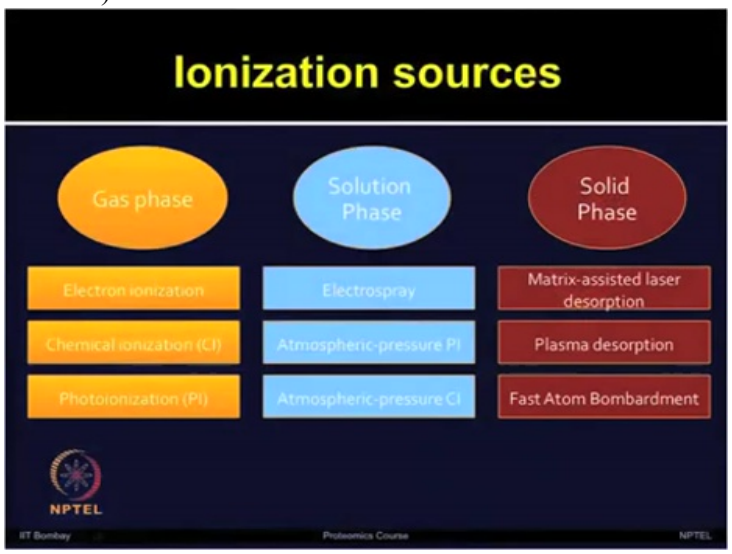

With the gas phase, electron ionization, chemical ionization and photo ionization, these are the commonly used ionization sources.

With solution phase, electrospray, atmospheric pressure and atmospheric pressure CI, these are the more commonly used ionization sources.

With solid phase, MALDI or Matrix-Assisted Laser Desorption Ionization, plasma desorption are the more commonly used solid phase ionization sources.

The traditional ionization sources used for the small molecule chemical application relied on the chemical or electrical ionization.

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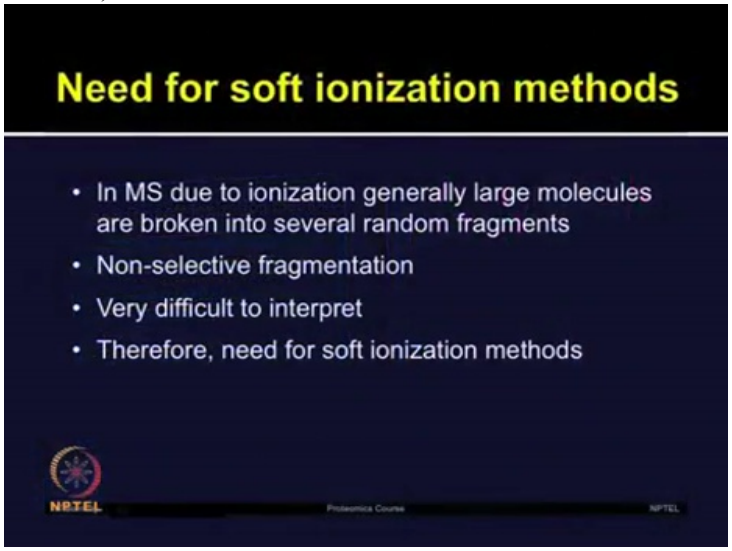

But these processes are too energy-rich to ionize intact large biomolecules and they lead to the unpredictable analyte decomposition. So for proteomic application, there was need for the soft ionization methods in mass spectrometry.

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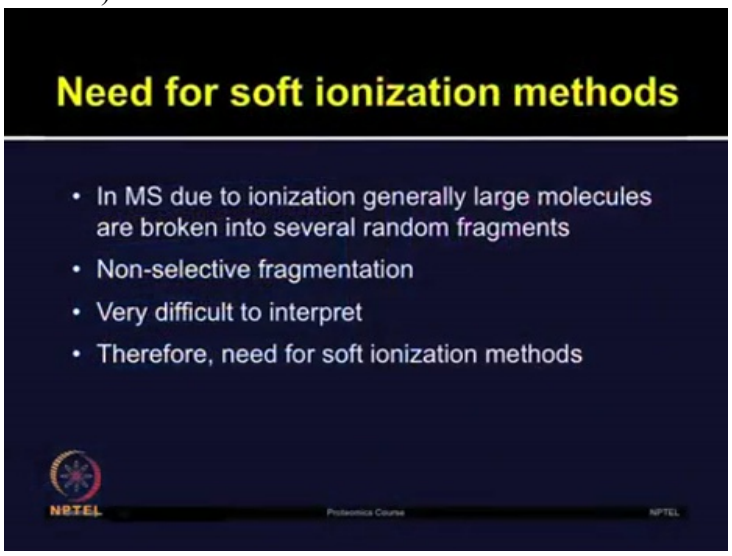

These are non-selective fragmentation. The hard ionization is very difficult to predict. So, therefore it led to the need for soft ionization methods in proteomics. What are different properties of ionization sources?

The main function of an ion source is to convert sample molecules or atoms into the gas phase ionic species.

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Now in the animation I will show you two most commonly used soft ionization methods; MALDI and ESI.

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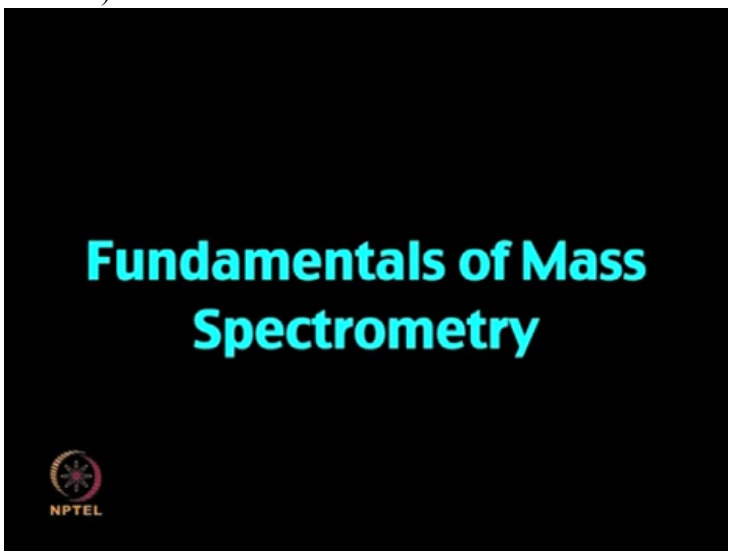

Fundamentals of mass spectrometry

Mass spectrometry is a technique for protein identification and analysis by production of charged molecular species in vacuum and their separation by magnetic and electric fields based on mass to charge ratio.

MS has increasingly become the method of choice for the analysis of complex protein samples in proteomics studies due to its ability to identify thousands of proteins.

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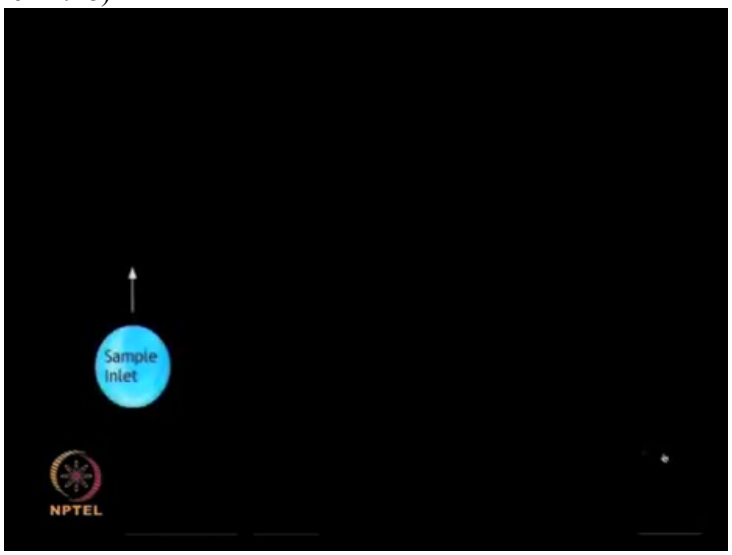

Mass spectrometer …

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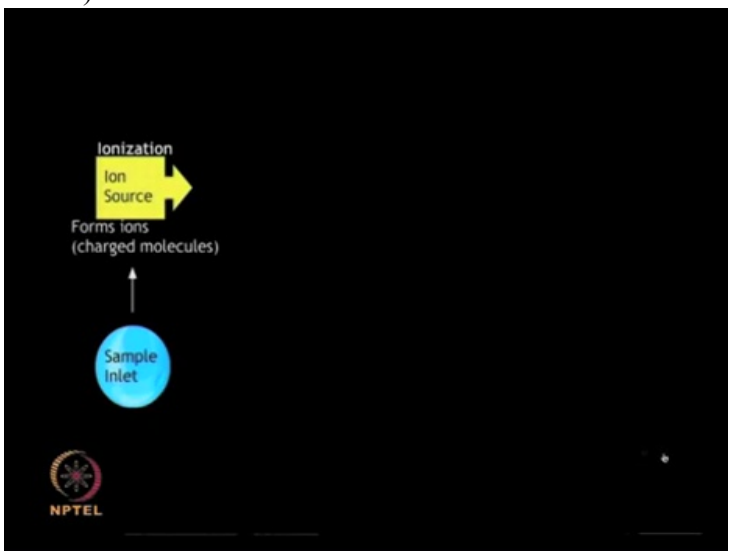

… is an instrument that produces ...

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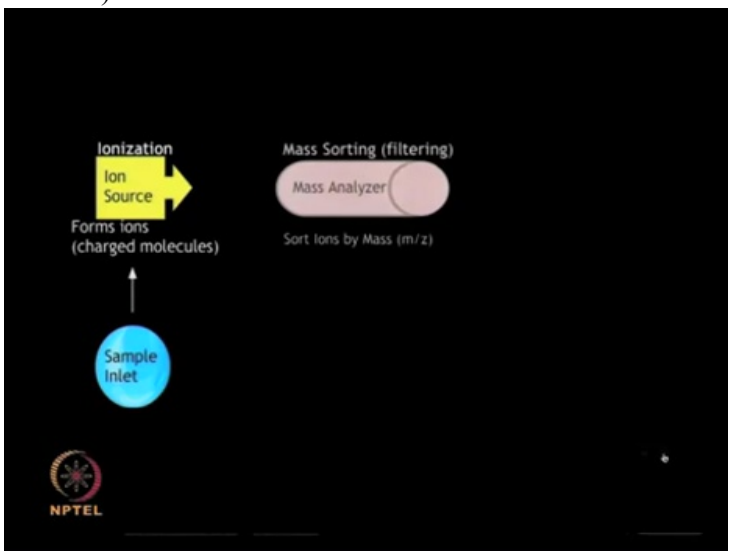

…charged molecular species in vacuum, separates them

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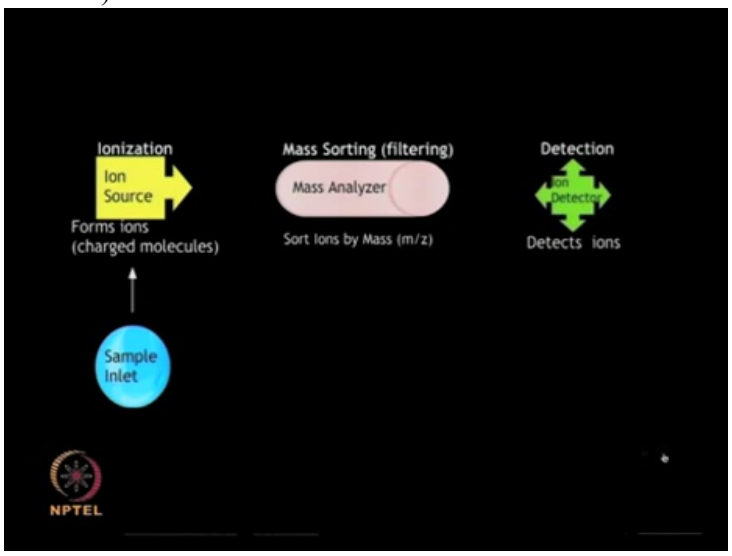

… by means of electric and magnetic fields …

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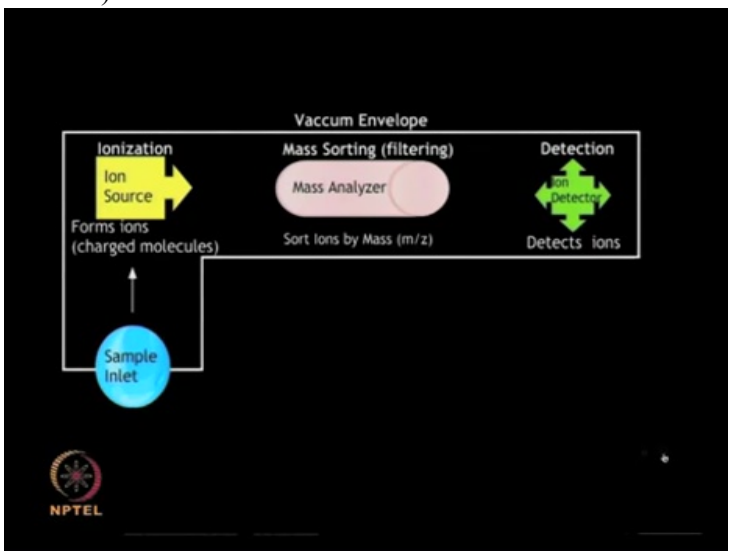

…and measures the mass to charge ratio…

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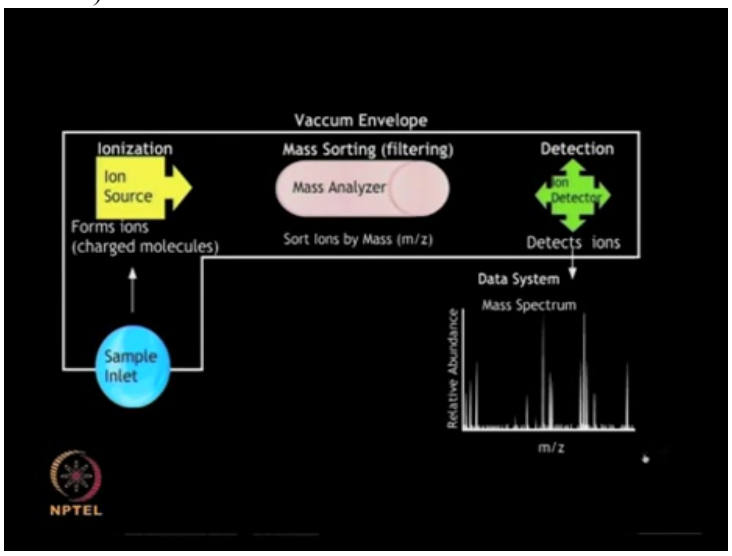

….and relative abundance of the ions thus produced.

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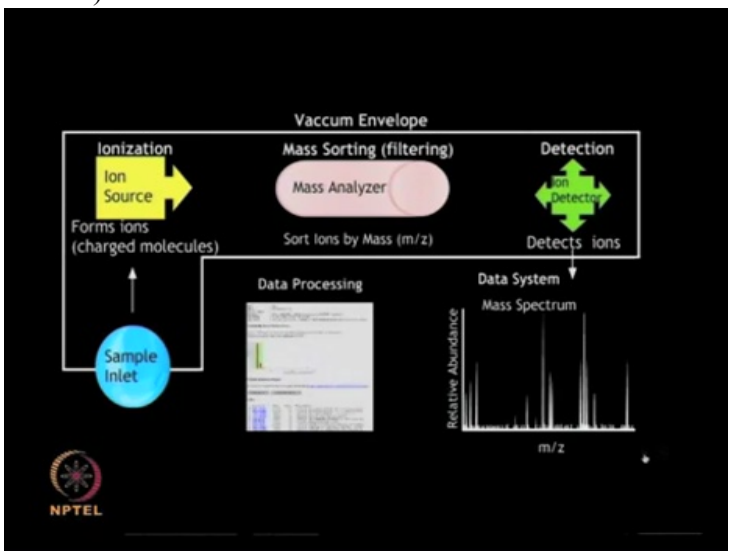

It is being increasingly used for detection and analysis of proteins from the complex samples. The various components which are involved in the mass spectrometry experiments are shown here

Starting from the sample inlet, the ionization source, mass analyzer, detector and then data analysis and data processing

Let us first define these terms so that our understanding for each component becomes more clear when we come to the advanced concepts.

Sample inlet: This is the first point of contact where the sample is introduced within the mass spectrometer either as liquid nano-droplets or the mixture of matrix.

Ionization source: The ionization source is responsible for converting the analyte molecules into gas phase ions in vacuum. Ionization source enables the ionization which can be further integrated with the mass analyzers.

The technology that enables is known as the soft ionization for its ability to ionize non-volatile biomolecules while ensuring minimum fragmentation and thus easier interpretation.

The commonly used ionization source include MALDI, Matrix-Assisted Laser Desorption Ionization and ESI or ElectroSpray Ionization.

Mass analyzers: The mass analyzers resolves the ions produced by the ionization source on the basis of their mass to charge ratio. There are various types of mass analyzers available including Time Of Flight, Quadrupole, Ion Trap etc.

Detector: The ion detector determines the mass of ions that are resolved by the mass analyzer and generates data, which can be further analyzed. The electron multiplier is a most commonly used detection technique.

Now let us look at the function of each of these components in more detail.

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Let us first start with the ionization source. The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum.

This has been made possible by the development of soft ionization techniques, which ensures that the non-volatile protein sample is ionized without completely fragmenting it. The most commonly used ionization sources are

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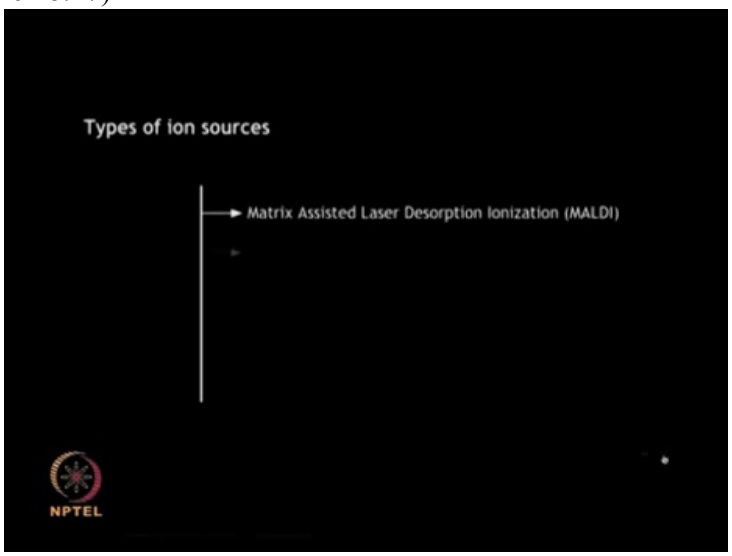

….MALDI…

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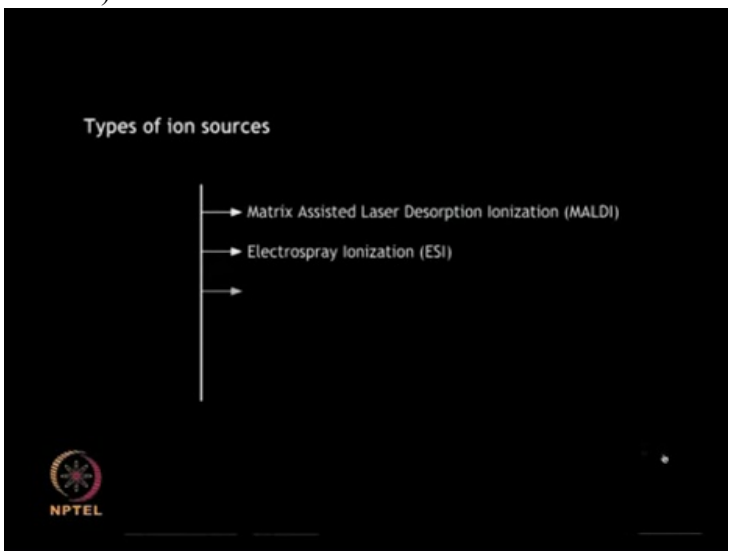

…and ESI.

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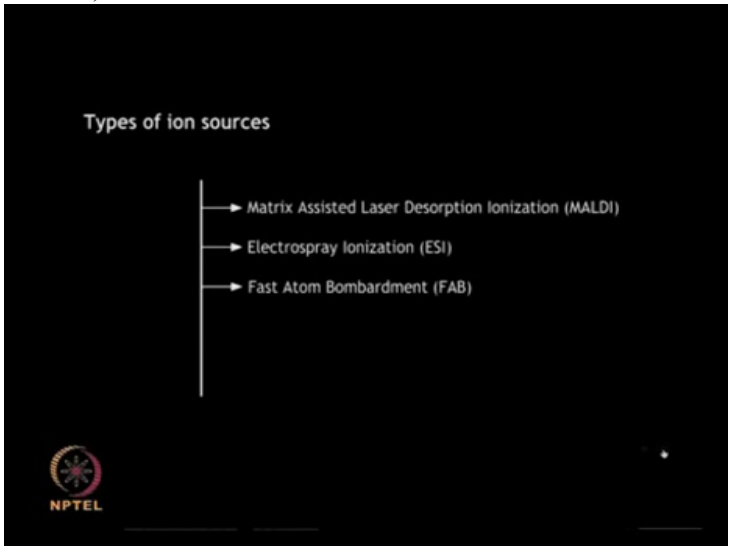

Additionally there are other ionization sources such as Fast Atom Bombardment FAB…
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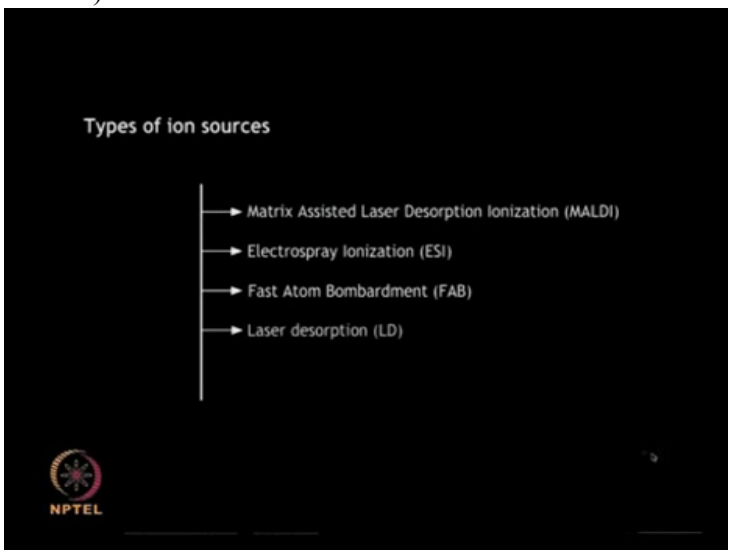

…. Laser Desorption LD….
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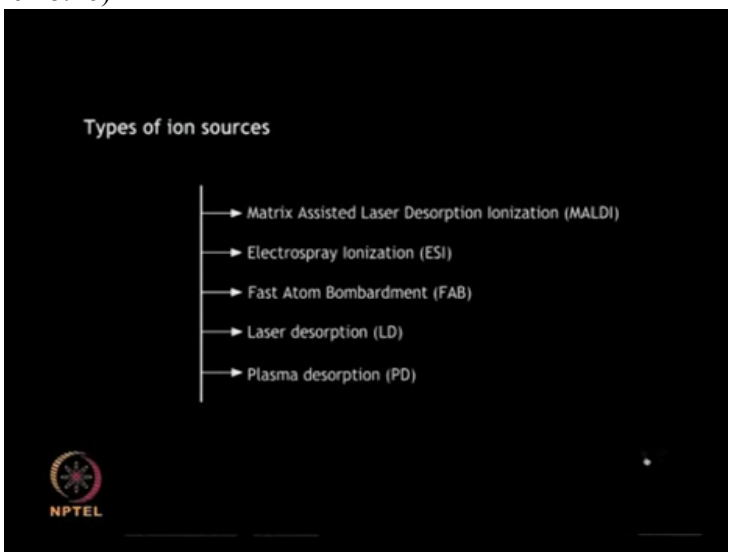

… Plasma Desorption PD.

We will discuss the two most commonly used soft ionization techniques MALDI and ESI in more detail.

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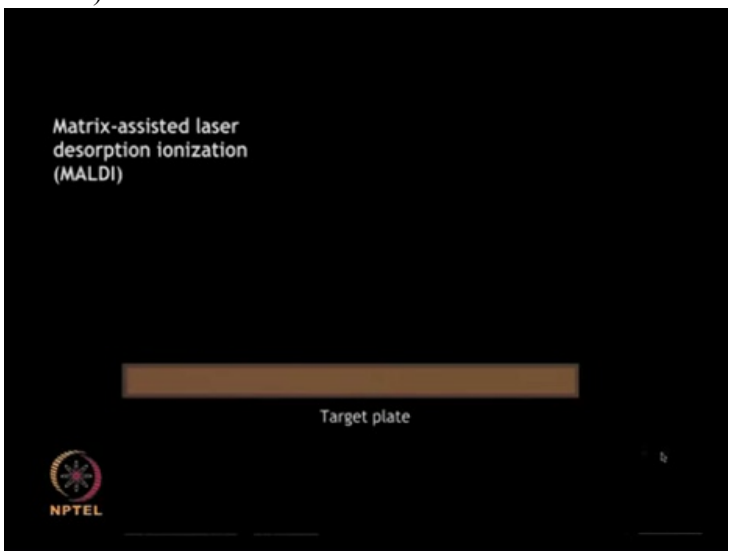

In MALDI …

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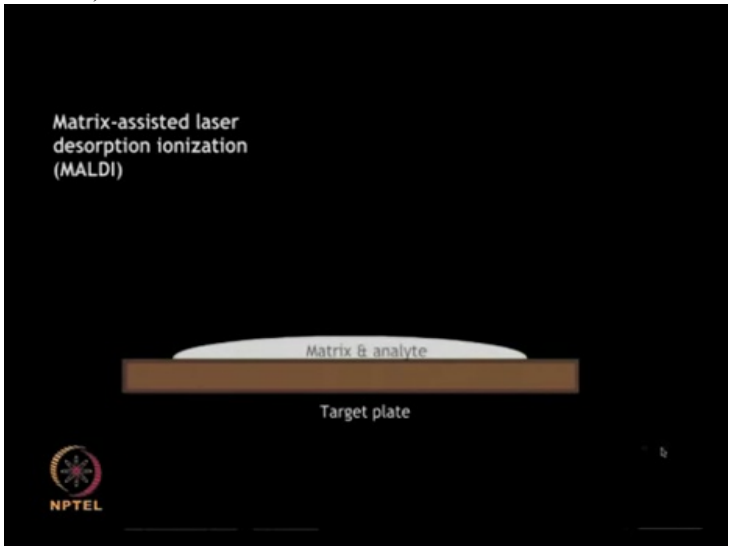

the analyte of interest is mixed with an aromatic matrix compound such as
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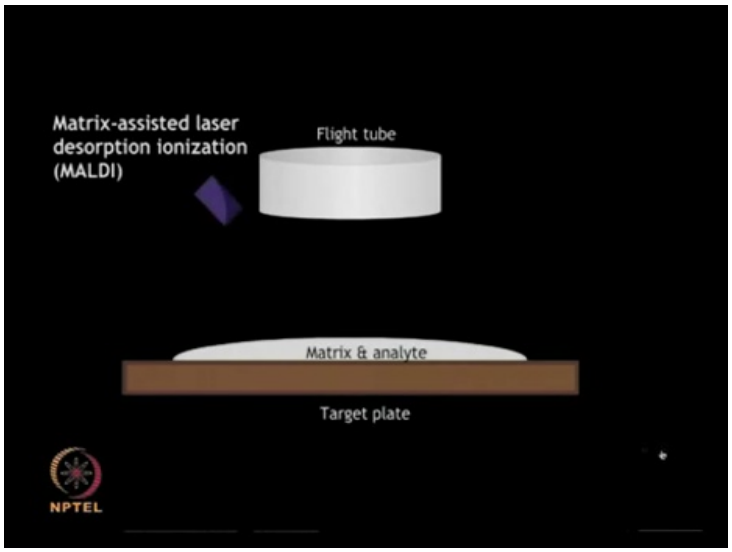

…alpha-Cyano-4-hydroxycinnamic acid or Sinapinic acid

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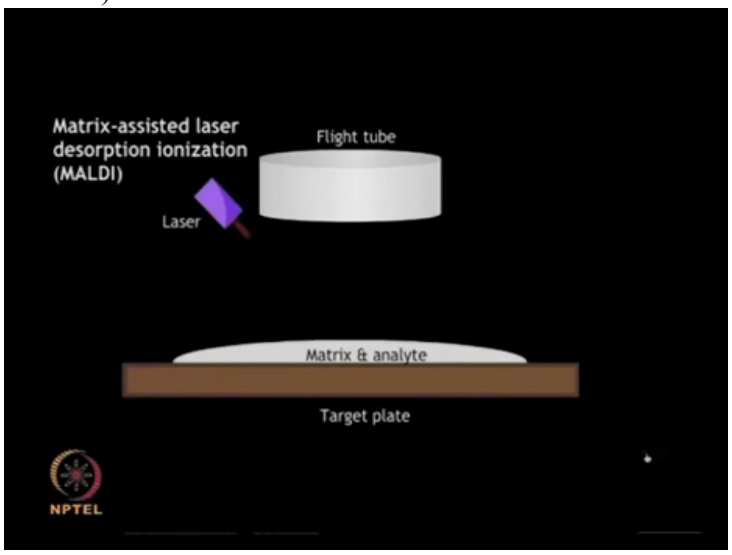

This is dissolved in an organic solvent and placed on a metallic sample plate.

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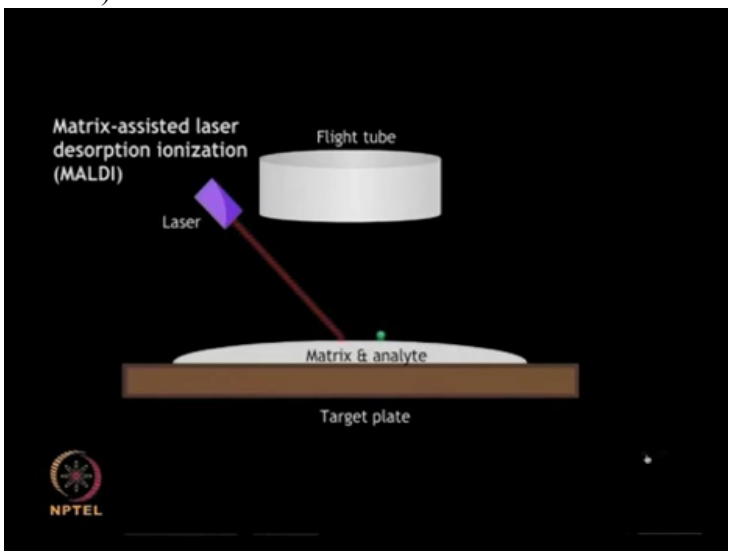

The evaporation of solvent leaves the analyte embedded in the matrix. The target plate is placed in a vacuum chamber with high voltage…

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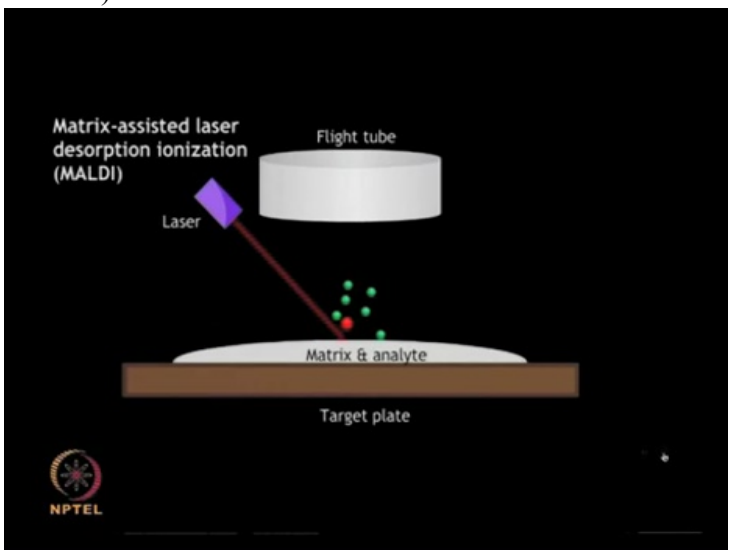

…and short laser pulses …

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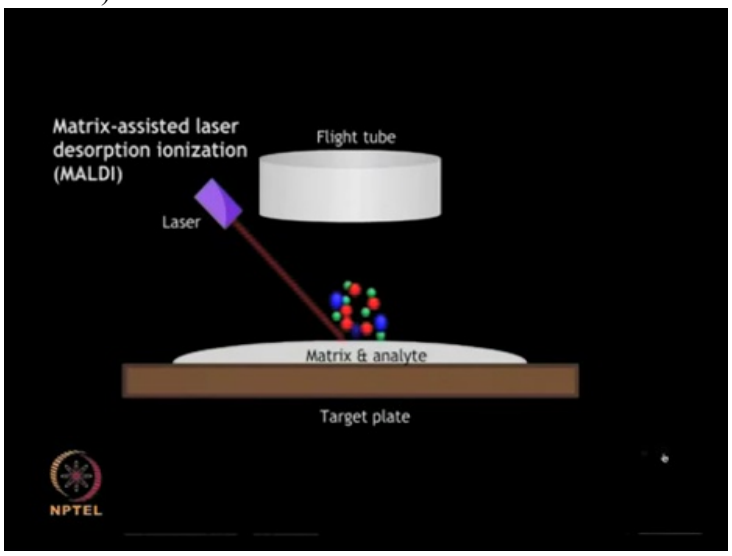

…are applied.

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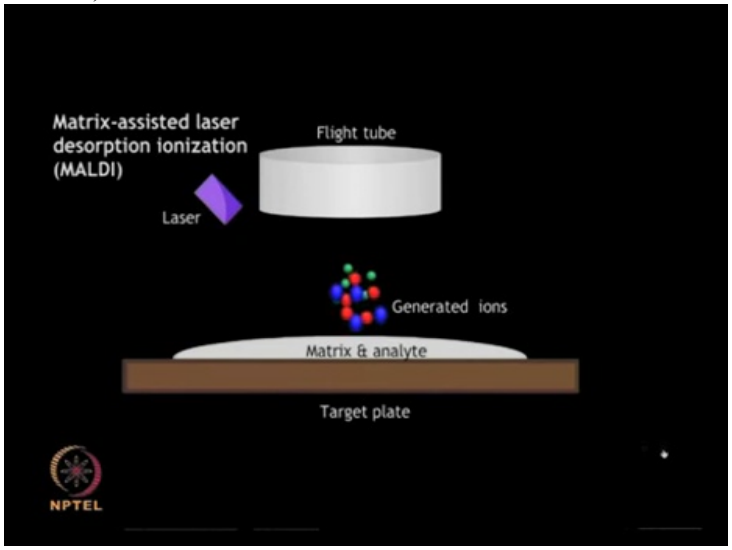

The laser energy…

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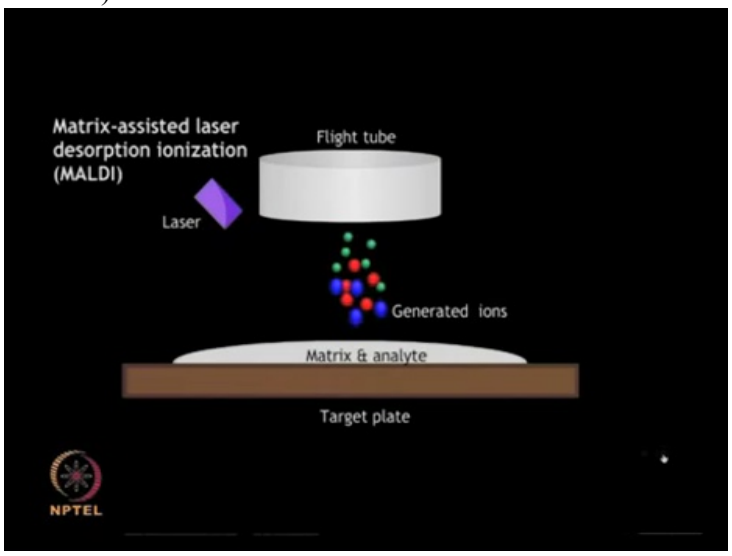

…gets absorbed by the matrix and is transferred to the analyte molecules…

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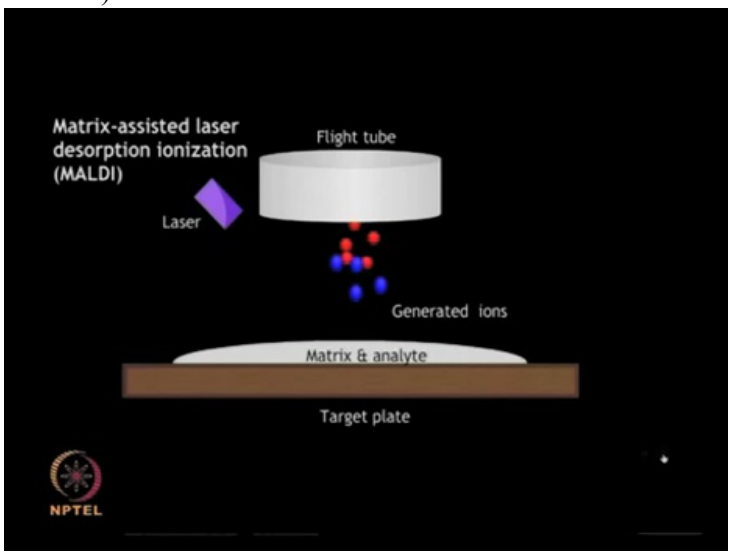

… which undergo rapid sublimation resulting in gas phase ions.

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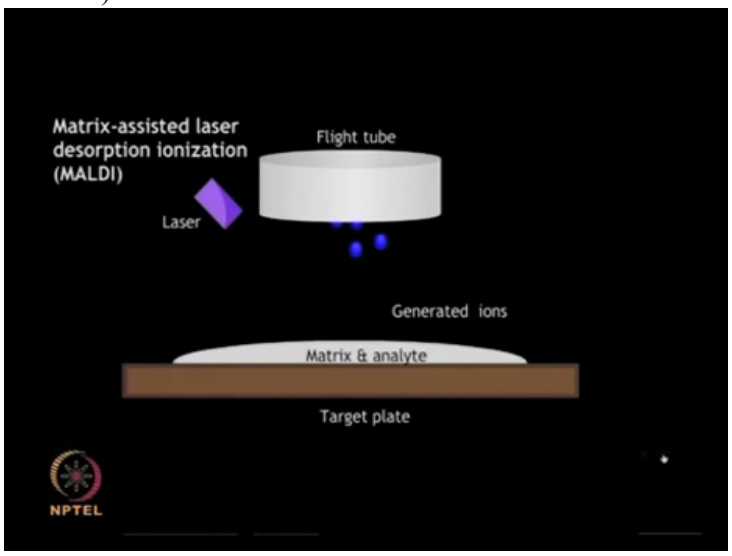

These ions then accelerate towards the mass analyzer based on their mass-to-charge ratio.

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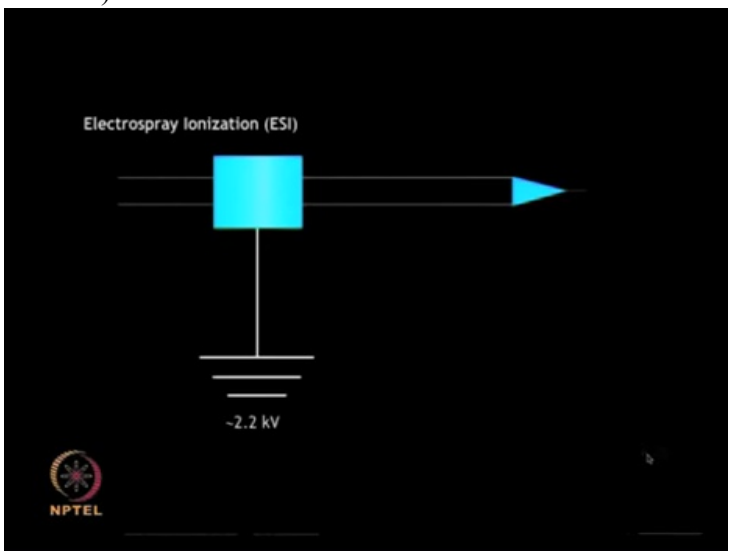

In ElectroSpray Ionization, the sample is present in the liquid form and ions are created by

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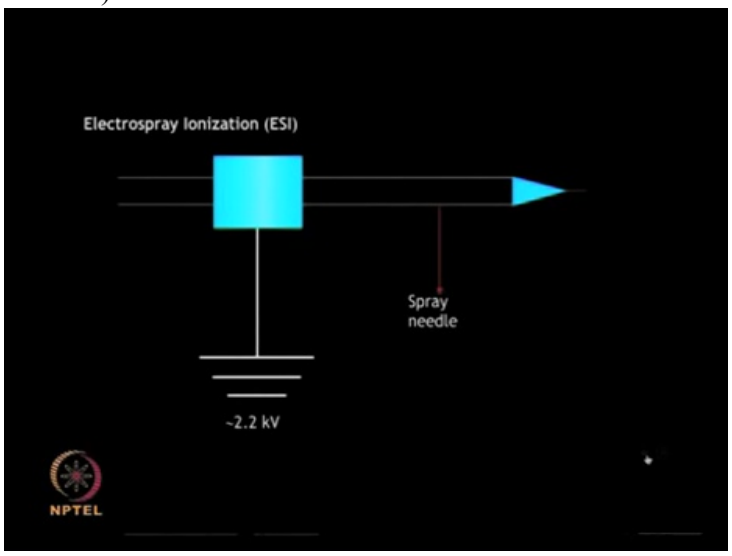

… spraying a dilute solution of the analyte at atmospheric pressure …

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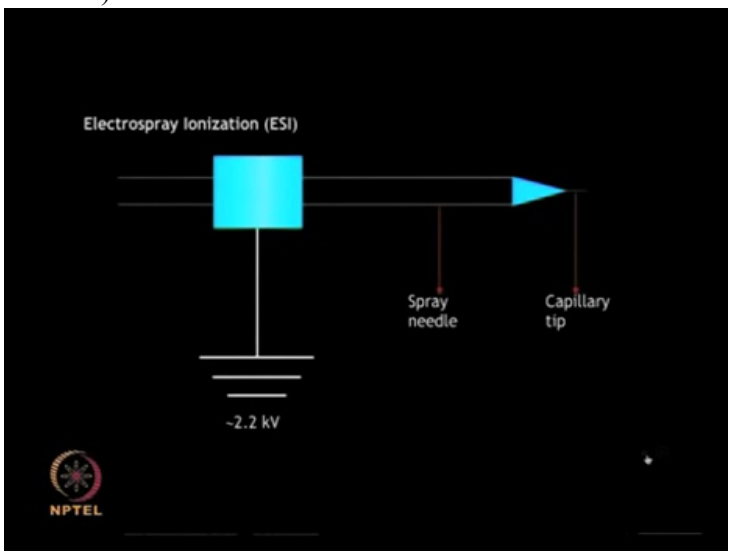

….from the tip of a fine metal capillary….

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…creating…

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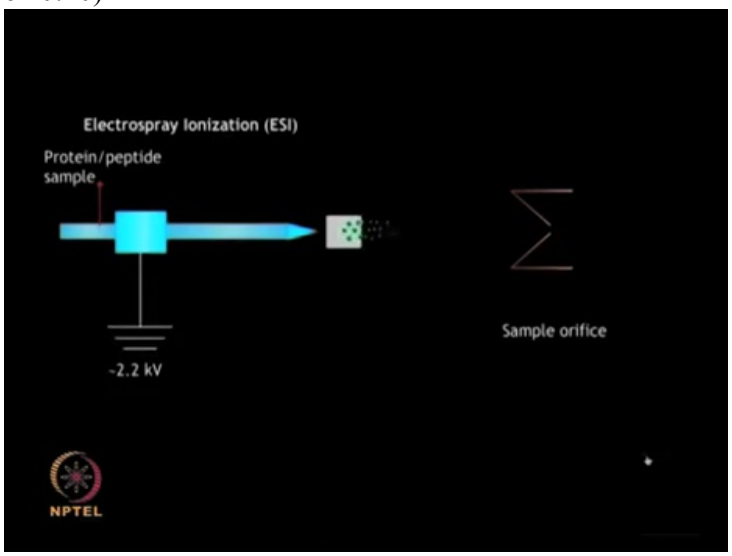

…a mist of droplets…

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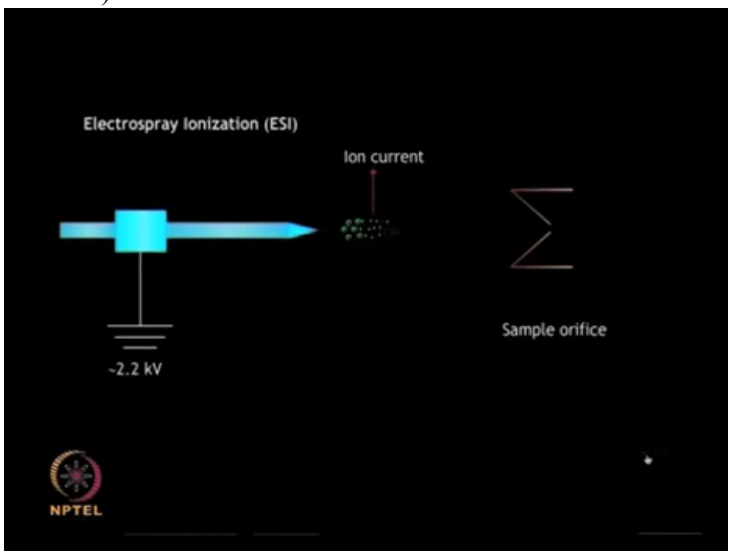

The droplets are formed in a very high electric field and become highly charged.

As the solvent evaporates, the peptide and protein molecules in the droplet….

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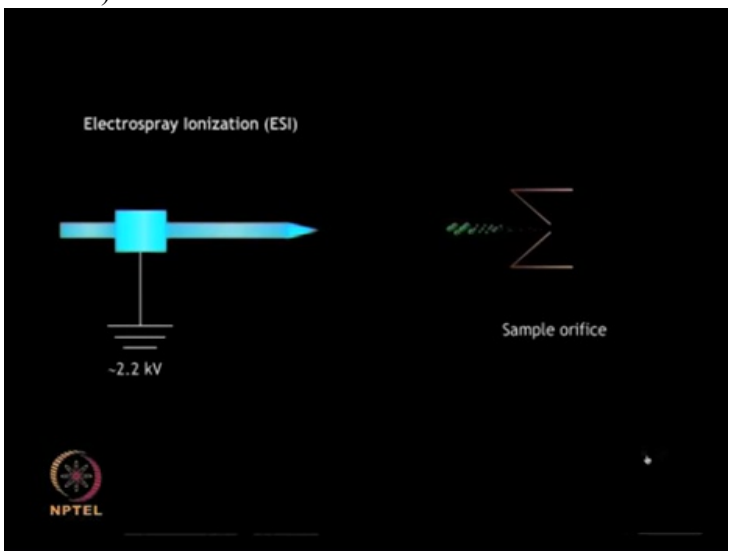

… pick up one or more protons from the solvent to form charged ions

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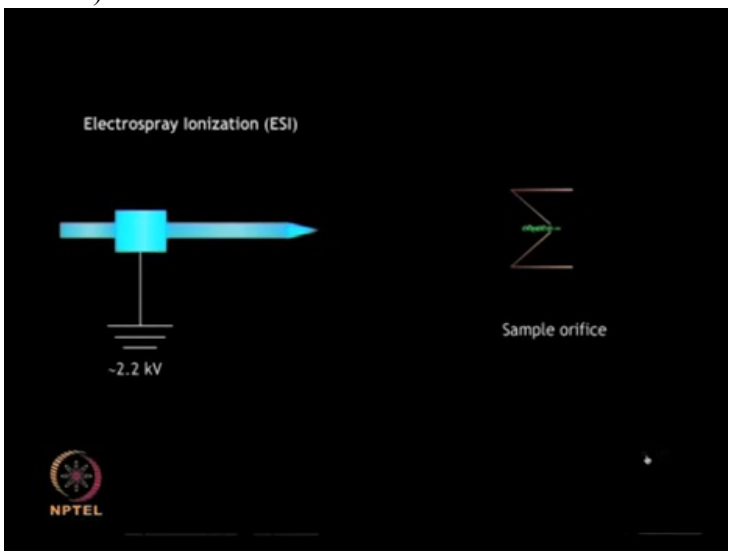

These ions are then accelerated towards the mass analyzer depending upon their mass and charge.

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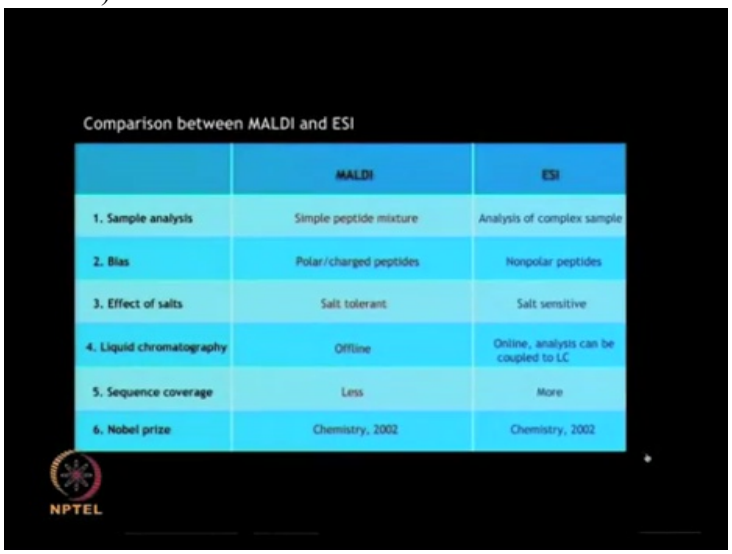

Let's have a comparison between MALDI and ElectroSpray Ionization and discuss their pros and cons which can be used for the analysis of different types of protein samples.

In MALDI, the sample analysis is for the simple peptide mixture whereas ESI can be used for the analysis of complex samples.

There is the bias towards the polar or charged peptides in MALDI where as it is for the non-polar peptide in ESI.

MALDI is more salt tolerant where as ESI is more salt sensitive.

Liquid chromatography can be performed offline where as in ESI it is online and analysis can be coupled to the liquid chromatography.

For the proteomic applications, the sequence coverage is less in MALDI as compared to the ElectroSpray Ionization.

Both MALDI and ESI development were awarded the Nobel Prize.

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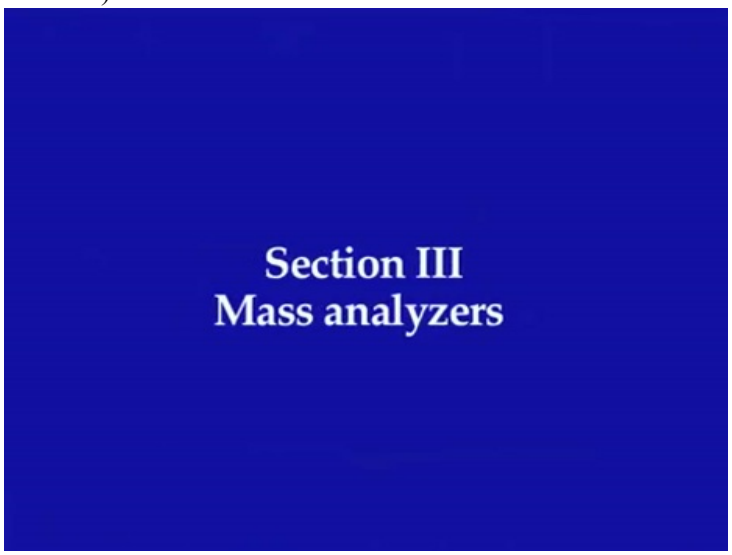

So we are talking about an MS experiment and I am trying to give you an overview of various steps involved. First of all we looked at liquid chromatography-based prefractionation, in-gel digestion, different types of ionization sources and now let's move on to the mass analyzers.

A mass analyzer plays two most important functions.

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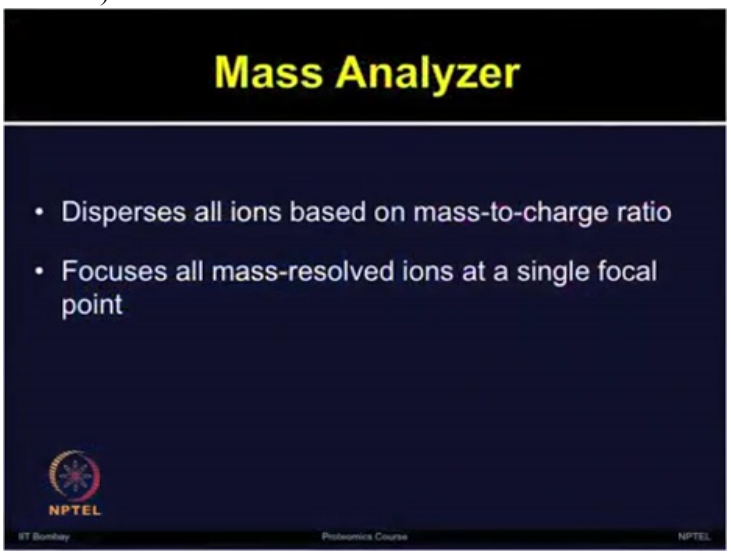

First of all it disperses all the ions based on their mass to charge or m by z ratio. Secondly, it focuses all the mass-resolved ions at the major, single focal point. So therefore all the ions enter in mass spectrometer, it can maximize their transmission.

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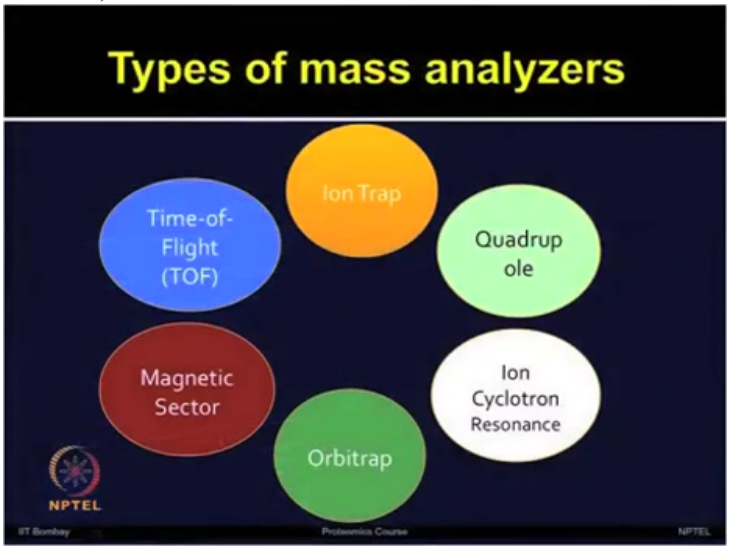

There are several different types of mass analyzers are currently available that use the same basic properties which we discussed. Some of the popular mass analyzer configurations are shown in this slide; which include Time Of Flight (TOF), Ion Trap, Quardupole, Magnetic Sector, Orbitrap and Ion Cyclotron Resonance.

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Let me show you few available mass analyzers in following animation.

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It resolves the ions produced by the ionization source on the basis of their mass to charge ratio. Various characteristics such as resolving power, accuracy, mass range and speed determine the efficiency of these mass analyzers. Let us discuss few most commonly used mass analyzers for the proteomics applications.

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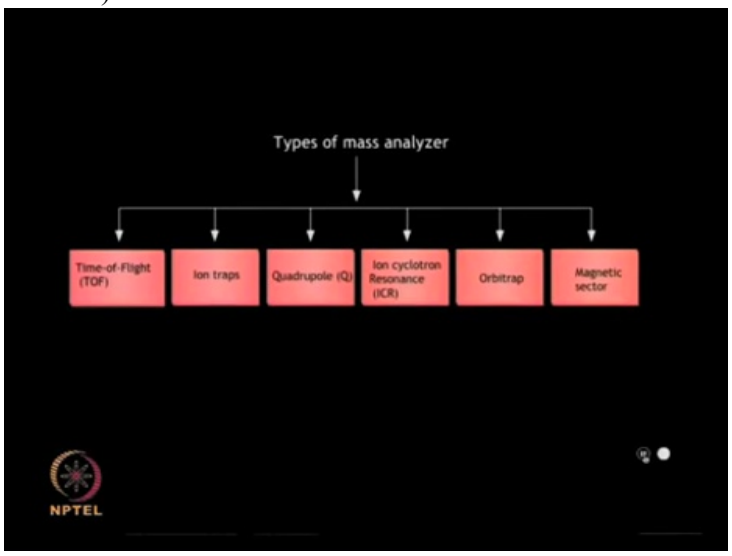

Currently various types of mass analyzers are available including Time Of Flight, Ion Traps, Quadrupole, Ion Cyclotron Resonance, Orbitrap and Magnetic Sector.

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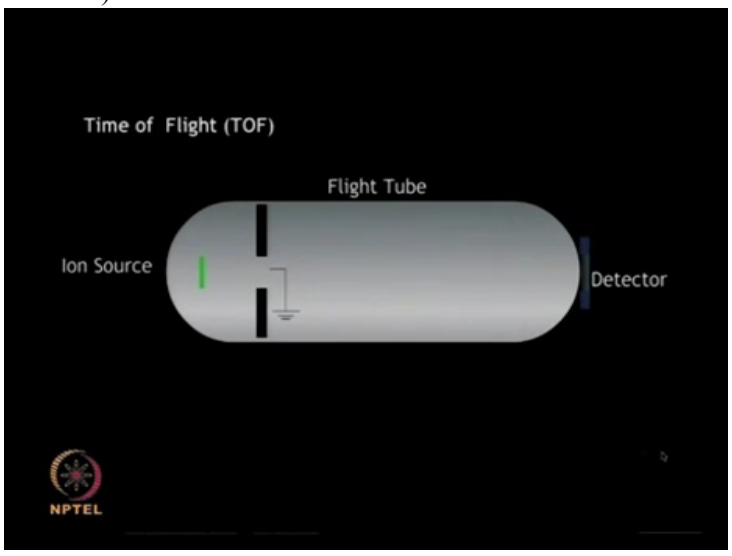

The Time Of Flight analyzer accelerates the charged ions generated by the ionization source…

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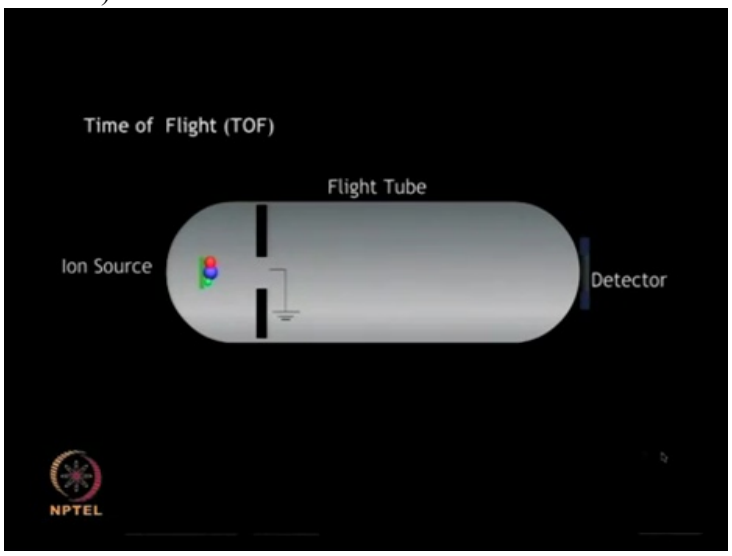

… MALDI along the long tube known as the Flight tube or TOF

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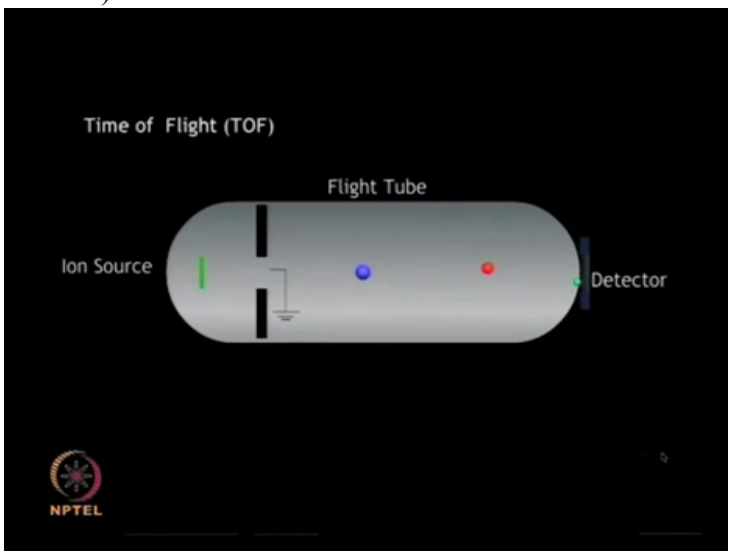

Ions are accelerated at different velocities depending on their mass to charge ratios.

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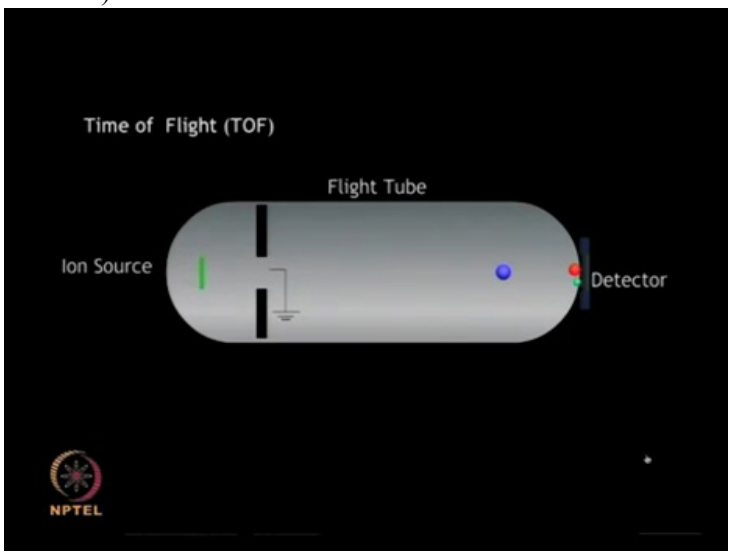

Ions of lower masses are accelerated to higher velocities and reach the detector first. The time of flight under such circumstances is inversely proportional to square root of molecular mass of the ion. The TOF analyzer …

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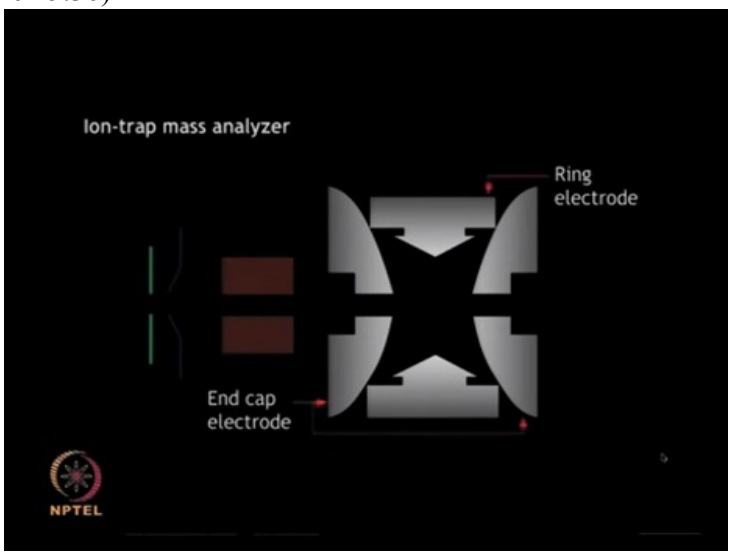

… has several applications in proteomics. Now let's discuss the next mass analyzer, Ion Trap.

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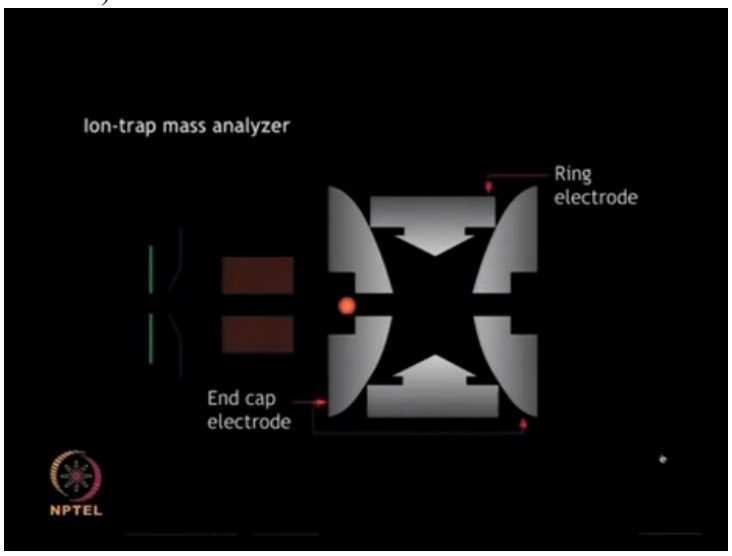

An ion trap makes use of a combination of …

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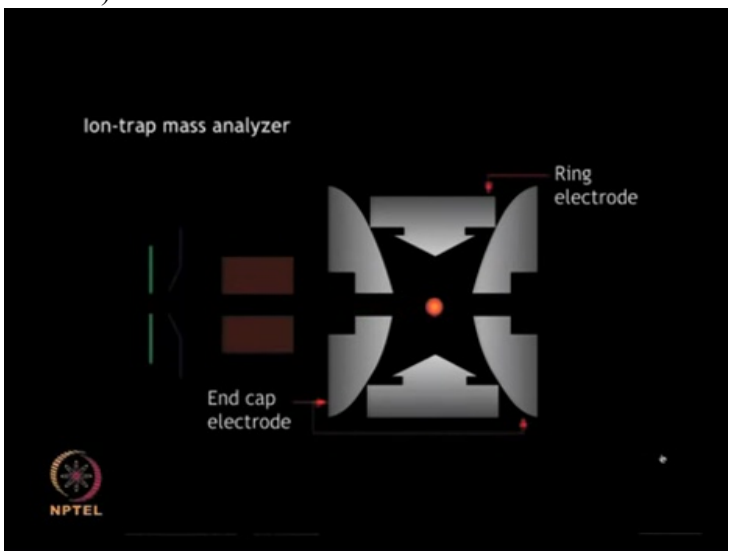

…electric and magnetic fields and captures ion in a region of vacuum system or tube Ion trap traps the ion …

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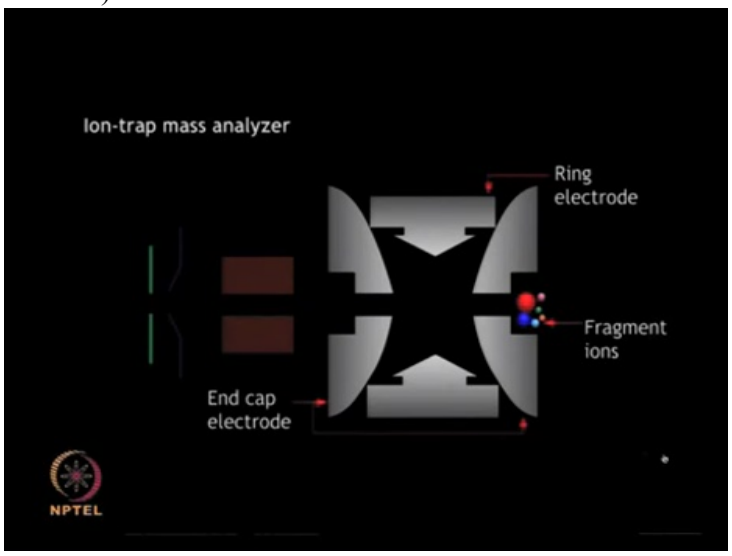

….using the electric field and measures the mass by selectively ejecting them to a detector.

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Quadrupole, Quadrupole mass analyzers use oscillating electric fields …

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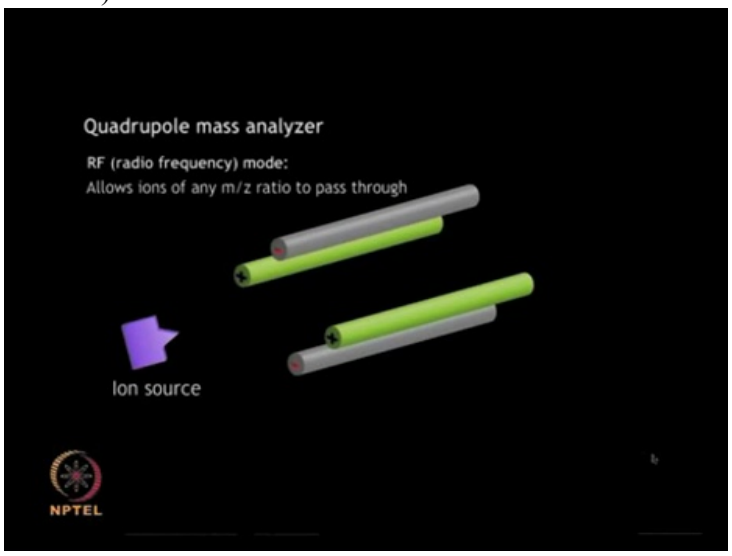

… to selectively stabilize or destabilize the paths of ion passing through a radio frequency RF quadrupole field. The Quadrupole mass analyzer can be operated …

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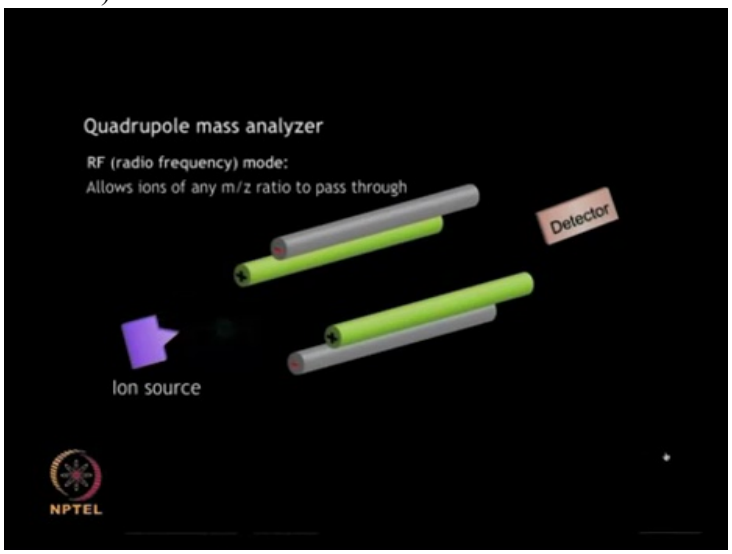

…in either radio frequency or scanning mode

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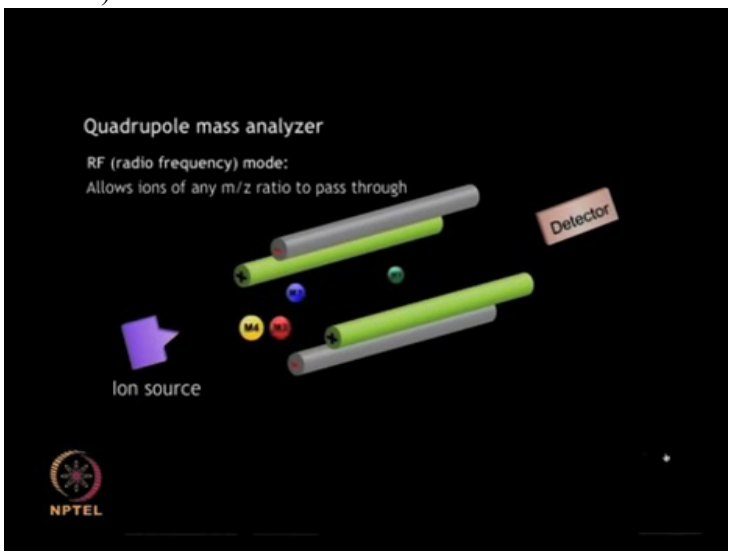

In RF mode or radio frequency ions of all m by z are allowed to pass through …

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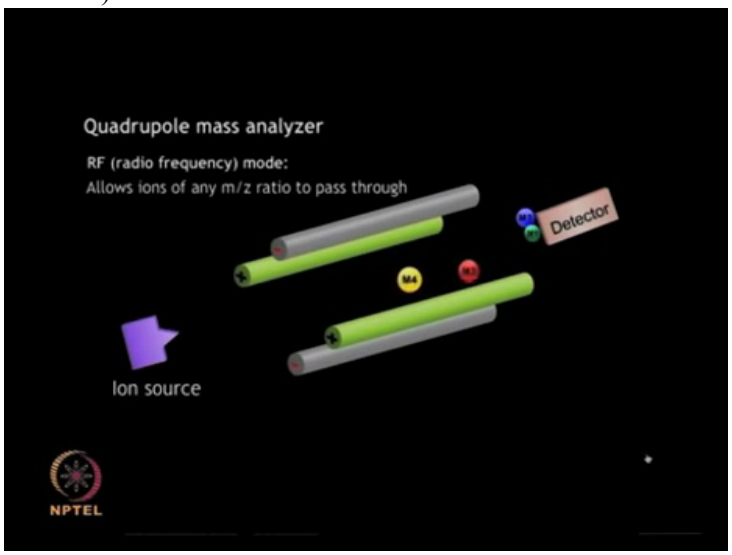

…which are then detected by a detector

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In the scanning mode the Quadrupole Analyzer selects ions of specific m by z value …

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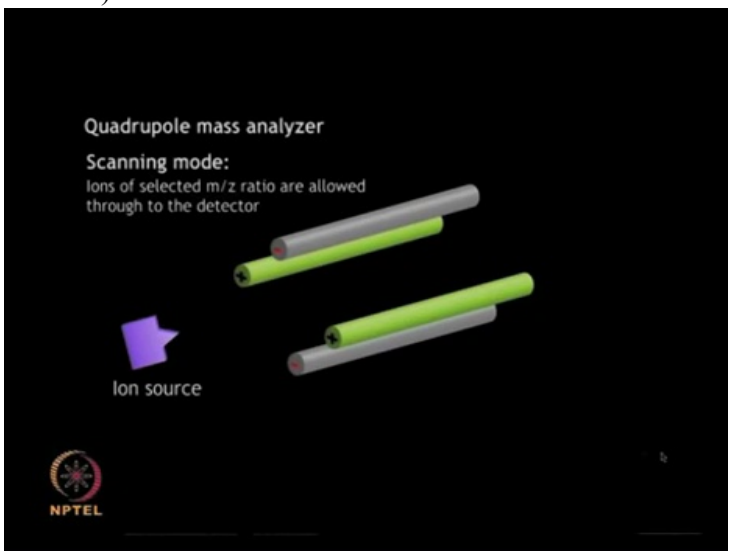

…as set by the user

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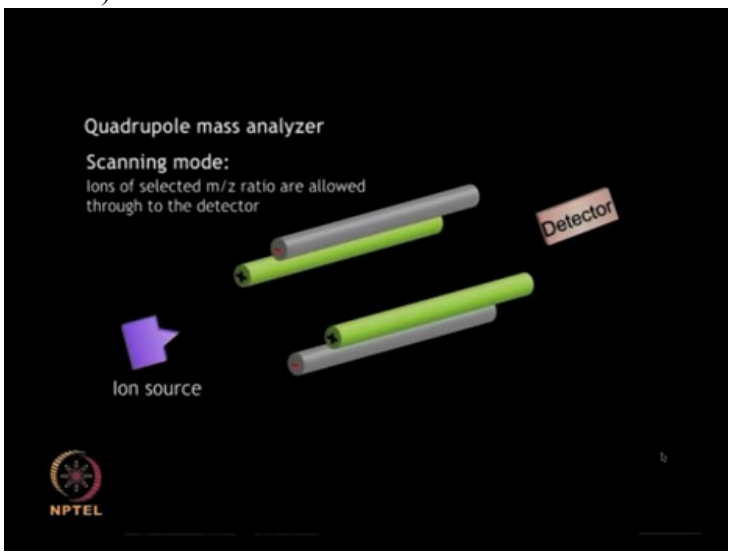

A range can also entered in which case only those specific ions …

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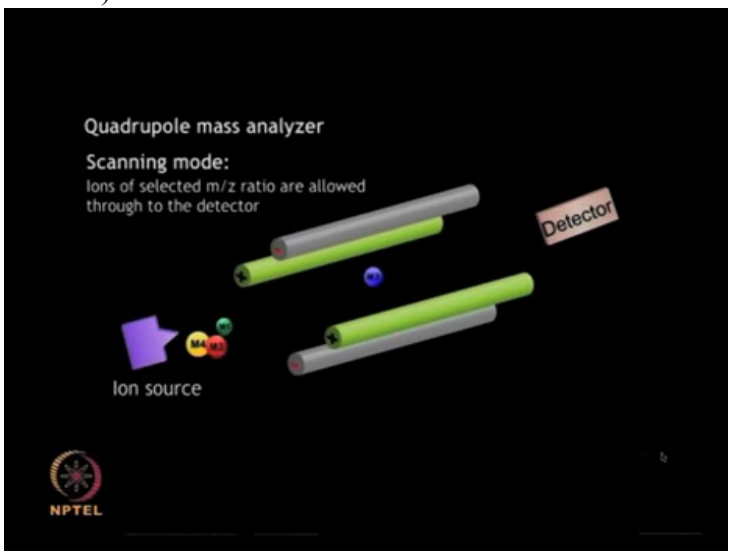

…which satisfy the criteria …

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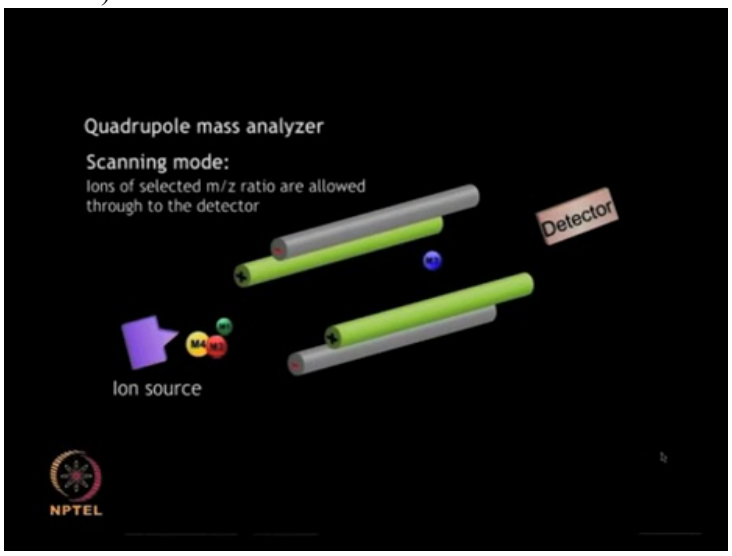

…will move towards the detector and the rest can be filtered out.

So in summary, today we talked about...

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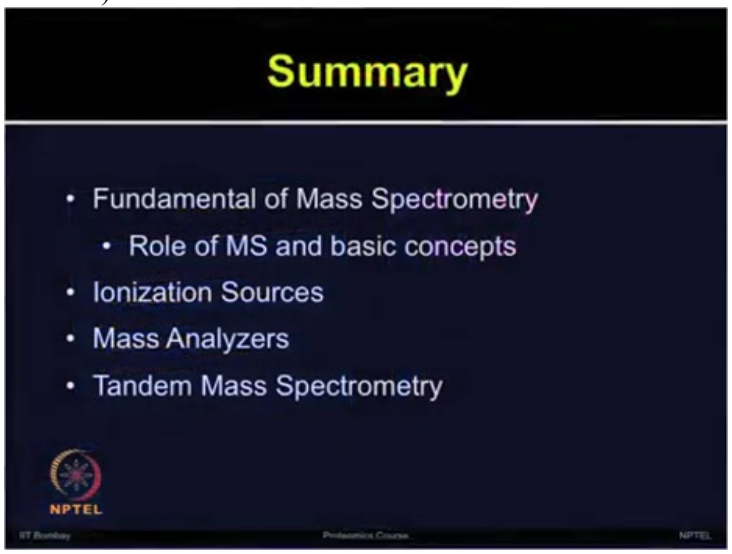

… some of the fundamental concepts involved in the mass spectrometry We talked about different types of ionization sources, mass analyzers, detectors, different type of terminology involved in evaluating the performance of these instruments.

So what an ideal MS should be?

It should possess wide mass range, high sensitivity, high resolution, high mass accuracy, true MS/MS and MRM capabilities, wide linear dynamic range, multiple charge separation capability, polarity switching capability with rapid or low, complementary ionizations, one can also use the modular where different types of sources can be combined such as ESI and APCI. Targeted analysis can be performed for PTM, label-free quantifications or MRM type of assays.

So from your ideal mass spectrometer you would like to have wide range of applications and that is only possible if it has very, very high specifications.

So from today's lecture I hope you are able to understand some of the fundamental concepts involved in mass spectrometry, very briefly we touched upon ionization sources and mass analyzers and then we talked about Tandem MS configurations.

In the subsequent lectures we will talk in more detail about some of these ionization sources, mass analyzers and different types of mass spectrometry configurations and its applications. Thank you

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