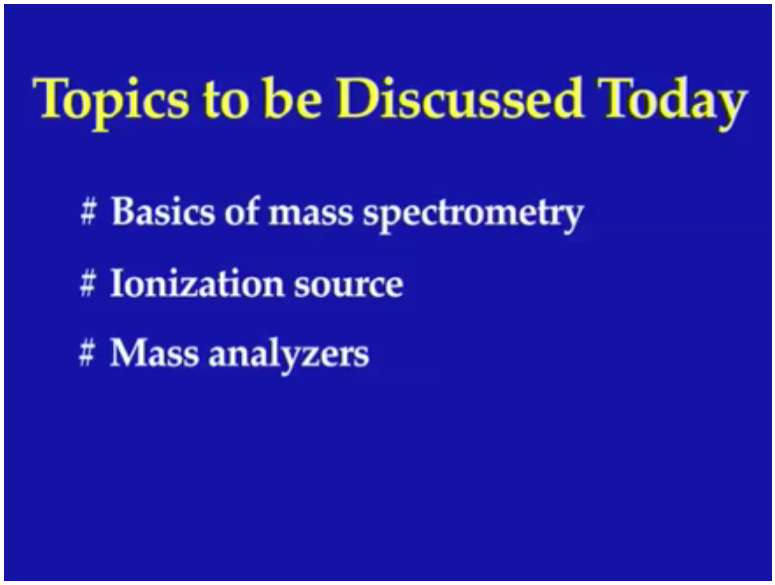
**Introduction to Proteomics**
**Dr. Sanjeeva Srivastava**
**Department of Biosciences and Bioengineering**
**Indian Institute of Technology – Bombay**

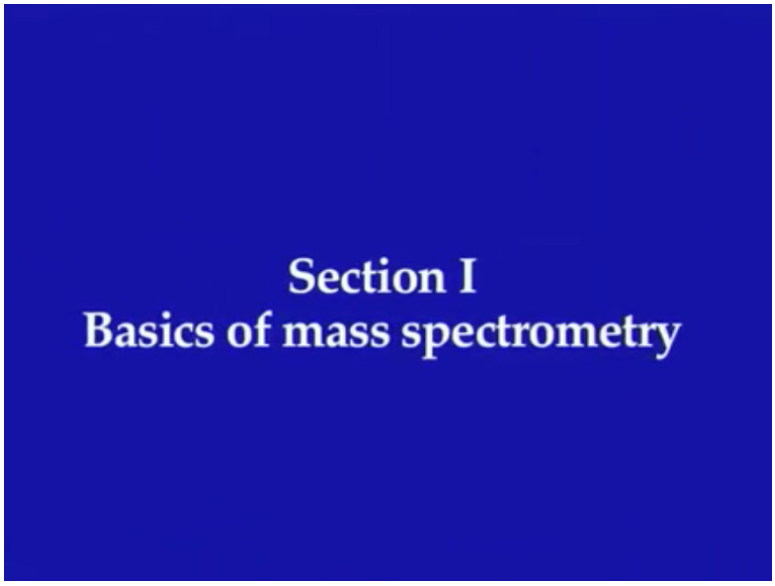
**Lecture – 21**
**Fundamentals of Mass Spectrometry**

**(Refer Slide Time: 00:18)**



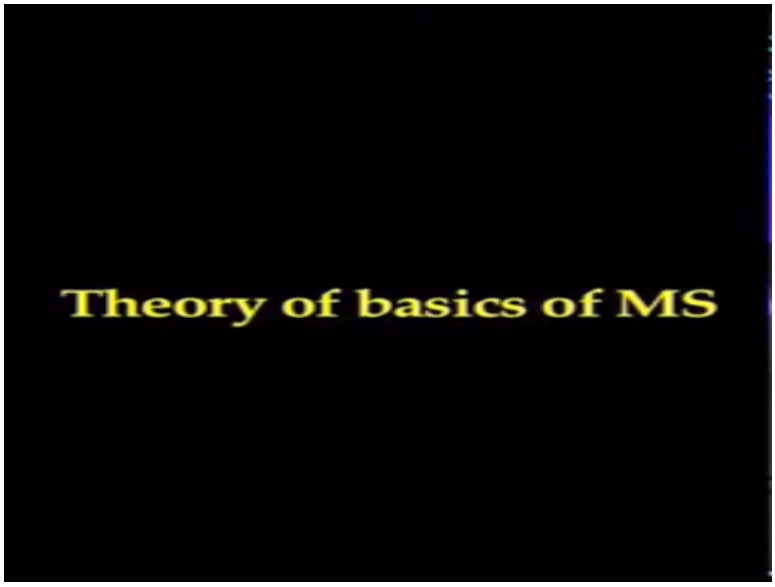

So today first we will talk about fundamentals of Mass Spectrometry and describe the role of MS and various basic concepts involved in understanding the Mass Spectrometry. We will look at the individual components such as ionization source,mass analyzers, as well as other components. Then we will talk about Tandem Mass Spectrometry.

**(Refer Slide Time: 00:54)**



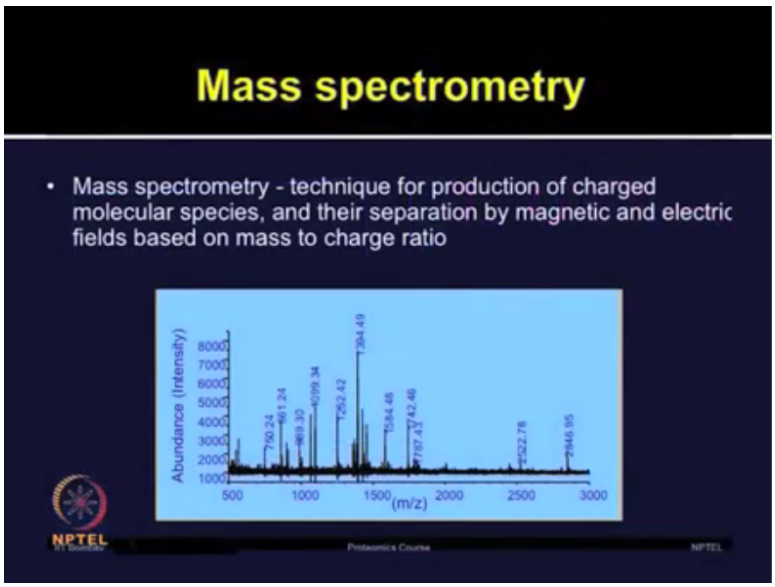
The skill at which we want to study the proteom requires much more analytical instrument capability and Mass Spectrometry has ability to provide that platform for comprehensive coverage of proteom.

**(Refer Slide Time: 01:17)**




MS has become an important analytical tool in Biology in general and Proteomics during the last decade. You know various applications have emerged by using MS based platform. It offeres high throughput, sensitive and specific analysis for many applications. Let us first look at some of the basic concepts of Mass Spectrometry.

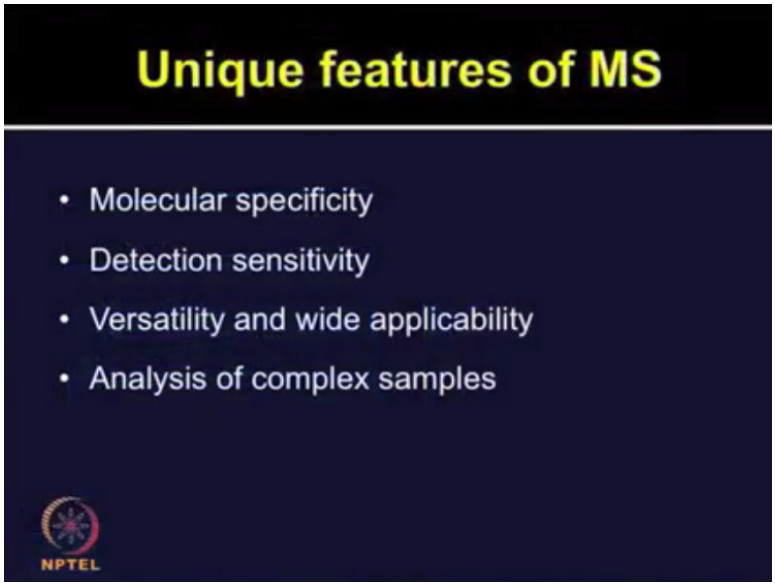
**(Refer Slide Time: 01:54)**




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 the technique for production of charged molecular

species in vacuum and their separation by magnetic and electrical field based on mass to charge ratio. You can see the MS spectrum shown in the slide m/z and intensity abundance plotted on the y - axis. So what are the unique features of Mass Spectrometry?

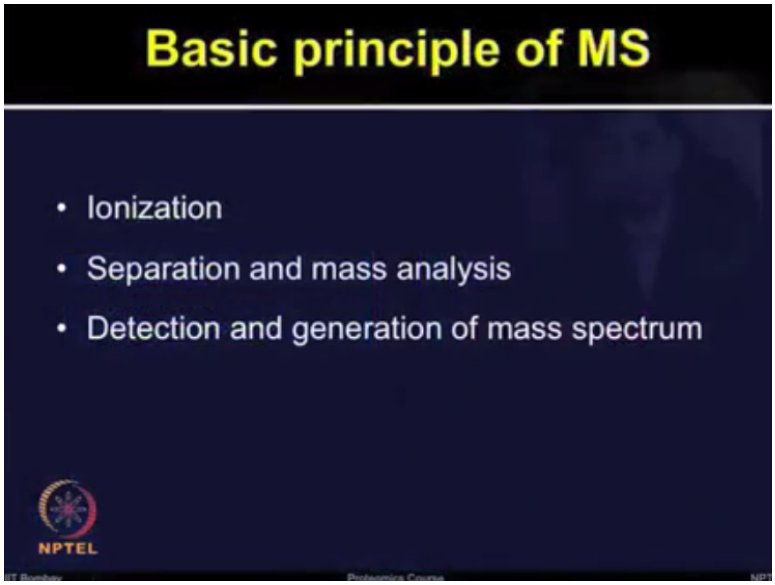
**(Refer Slide Time: 02:42)**




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 even sensitivity at the atomole and femtomole has been also demonstrated.

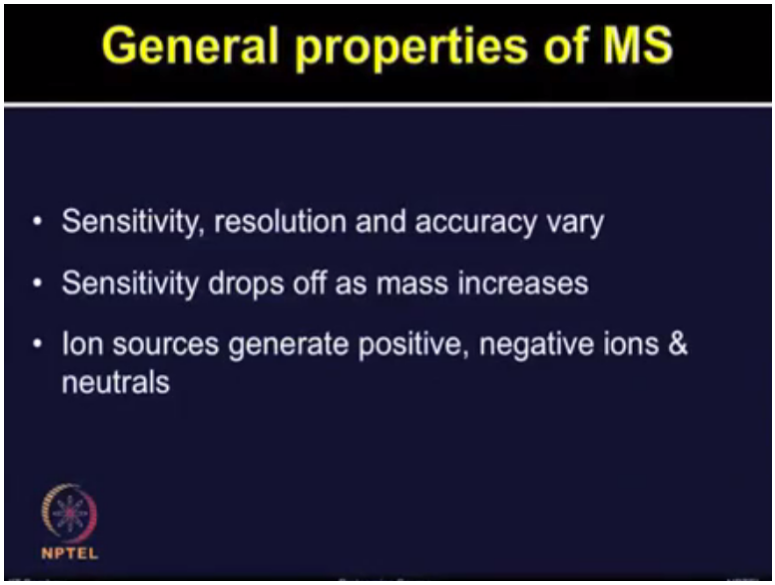
It provides a versatile platform to determine the structure of compounds and it is applicable to all the elements, all type of samples whether it is volatile, non – volatile, polar, non – polar, as well as solid, liquid or gases. So analysis of complex samples such as proteom is very much possible by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

**(Refer Slide Time: 03:51)**




Now what are the basic priciple of Mass Spectrometry? So 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.

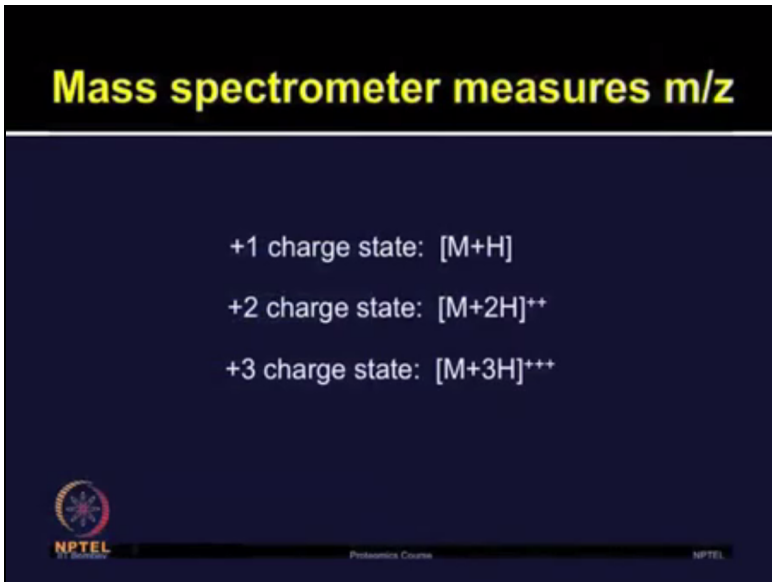
**(Refer Slide Time: 04:45)**




Now let us discuss about general properties of MS. 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 be as low as in the atomolar or femtomolar range. Ion sources generate positive, negative and neutral. 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 sensitivity for negative ions is generally lower.

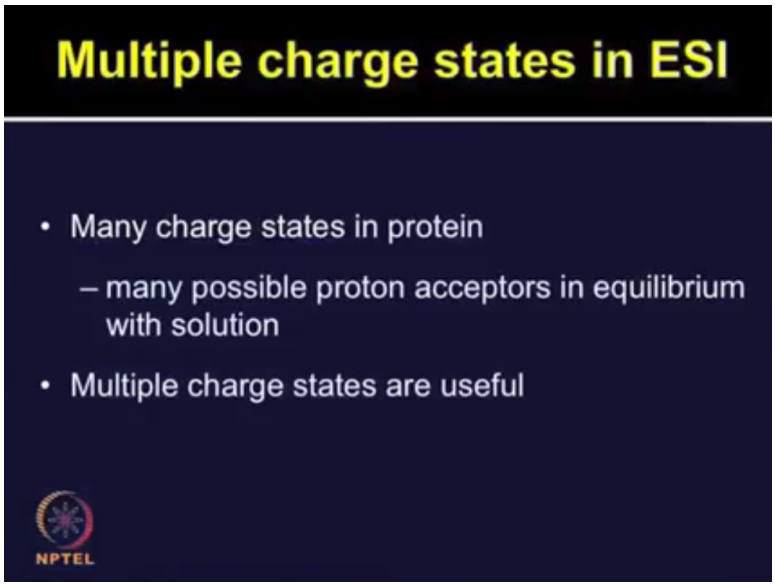
**(Refer Slide Time: 05:39)**




So Mass spectrometer measures the m/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 total charge on ion is represented by q = ze, where e is the charge on an electron. Now, how you can calculate the m/z of any peptide? As I mentioned here, you can have multiple charged states +1 charged state, +2, +3 or multiple charged states are possible.

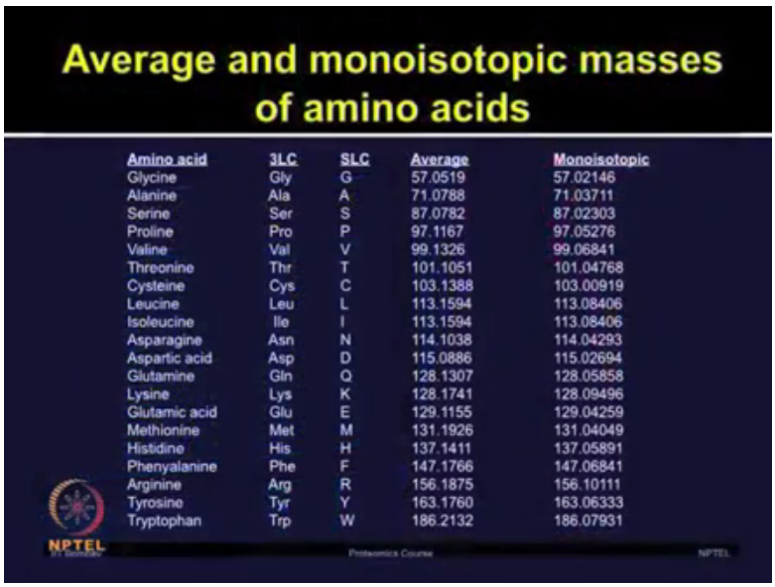
So if you need to calculate the m/z, you need to add M+H or M+2H or M+3H as shown here and then divide by 2 or 3 depending on the how many charged states it carries.

**(Refer Slide Time: 06:44)**



I will talk more in detail about how various types of ionization source works, that will be much later part of the lecture. But just in this context I would like to mention that there are multiple charged states present in the electrospray ionization. So many charged states in proteins there are many possible proton acceptors in the equilibrium with the solution. So the multiple charged states are quite useful because they form ions which are in the mass range of mass analyzers such as TOF, quadrupoles, ion traps, etc.

**(Refer Slide Time: 07:43)**



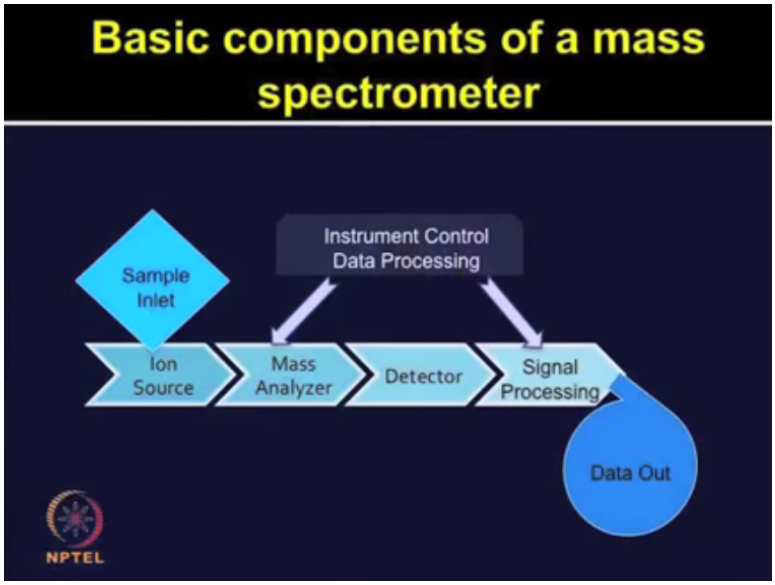

Now during the initial part we are trying to cover some of the basic terminology and basic concepts involved in the Mass Spectrometry. So let us talk about what is average and monoisotopic masses of aminoacids. I have shown a table here which shows aminoacids, 3 letter codes, single letter code, average and the monoisotopic masses.

You can use this for your reference later on which can be used for the data analysis and calculations. So what is monoisotopic mass of protein? It is sum of masses for most abundant isotope of each element. The average mass of an element is the average of the isotopic masses of each element weighted for the isotopic abundance. I hope you are able to distinguish average and monoisotopic masses of aminoacid.

**(Refer Slide Time: 08:35)**


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

**(Refer Slide Time: 09:00)**

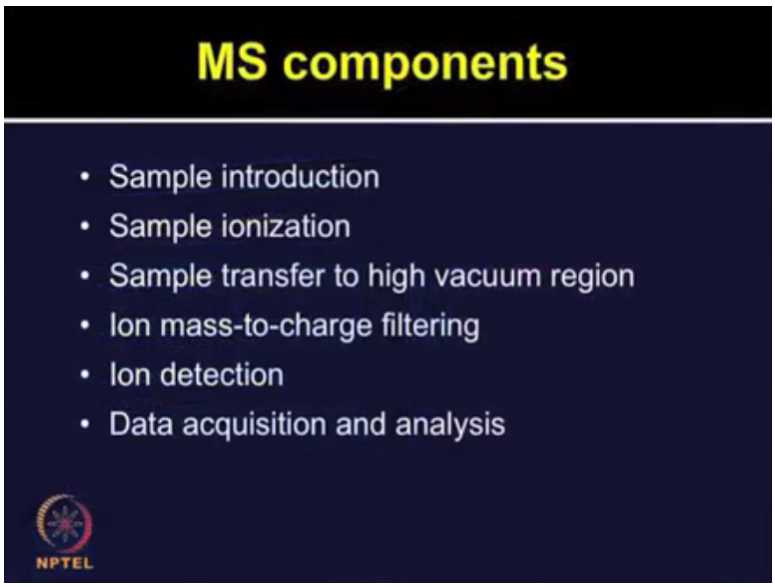



Sample inlet: It transfers a sample into the ionization source. The ion source or ionization source: It converts neutral sample molecules into the gas phase ions. Mass analyzers: It seperates and analyses mass of the ionic species. There are various type of mass analyzers available which we will discuss in more detail during the subsequent part of the lecture. Now I 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 electronics to control the

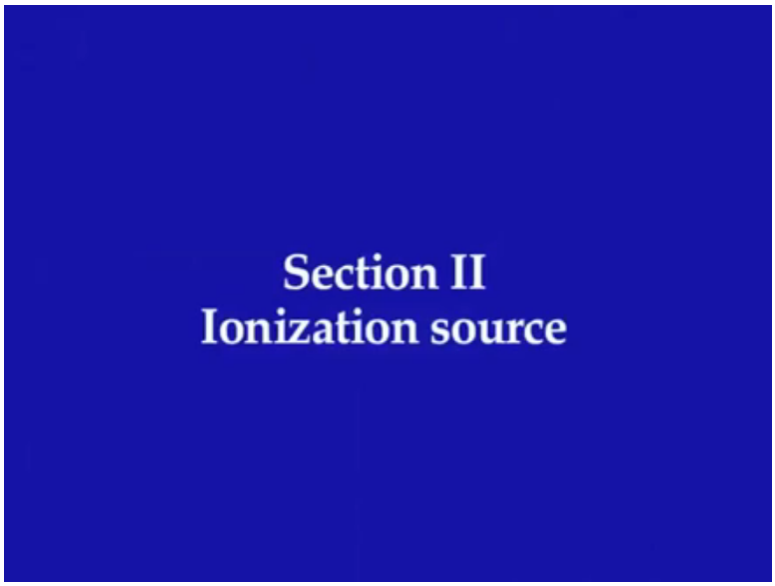
operation of various units. The data system it records, processes, stores and help to display the data output. Although there are 3 major components involved ionization source, mass analyzer and detector but then there are some accessory component which are also equally important for doing the Mass Spectrometry based experiments.

**(Refer Slide Time: 10:36)**



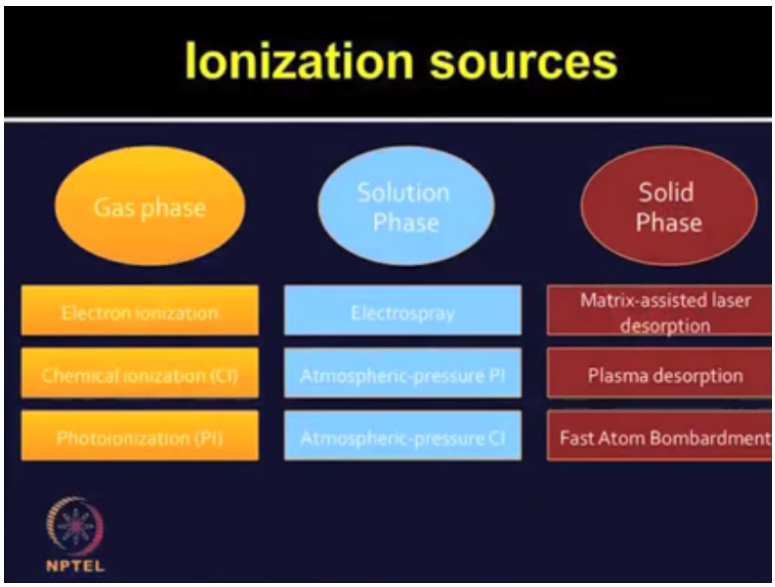

So various components includes sample introduction, one can couple that with HPLC or CHIP based technologies for doing the liquid chromatography based seperations. Sample ionization, there are various types of ionization sources currently available. Sample transfer to the high vacuum region. So there are ion mass – to – charge filtering can it performed by the mass analyzers, ion detection by using detectors and then data acquisition and analysis by using data system. All these are integral part of the MS components.

**(Refer Slide Time: 11:04)**



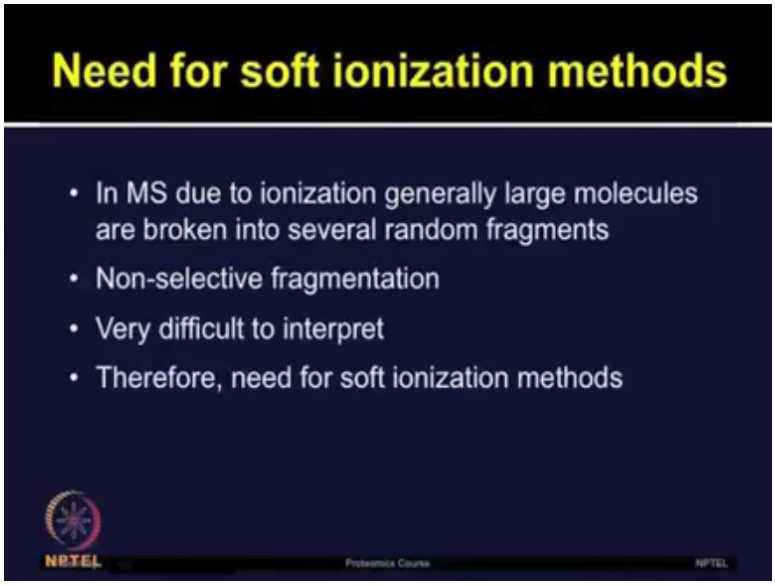
So what are different type of ionization sources involved in the MS analysis. So success of Mass Spectrometry experiment lies in efficiency of converting a neutral compound to a gas phase ionic species. So you have various type of options currently available. You can select what type of ionization source you want for your specific application. So the choice of particular ionization source is dictated largely by the nature of sample which one wants to investigate.

**(Refer Slide Time: 11:52)**




With the gas phase electron ionization, chemical ionization and photoionization; these are the more 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.

**(Refer Slide Time: 12:44)**

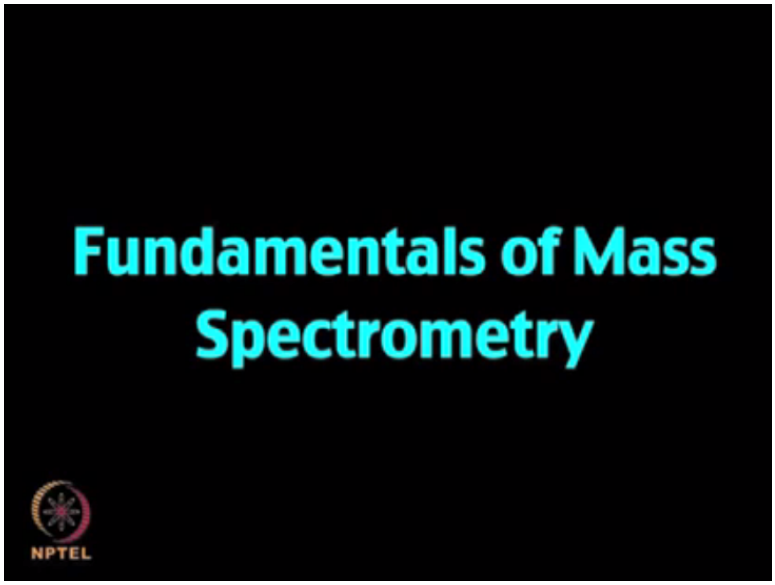



The traditional ionization sources used for the small molecule chemical application relied on the chemical or electrical ionization. But these processes are too energy rich to ionize intact large biomolecules and they lead to the unpredictable analyte decomposition. So far protemic application, there was a need for the soft ionization methods in Mass Spectrometry. These are non – selective fragmentation. The hard ionization is very difficult to predict. So therefore, it led to the need of soft ionization methods in proteomics.

**(Refer Slide Time: 13:37)**

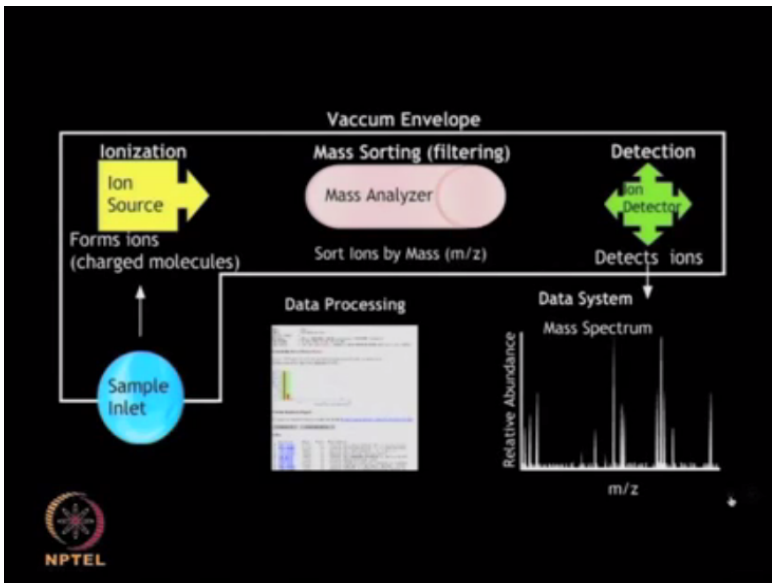

What are the different properties of ionization source? The main function of an ion source is to convert sample molecules or atoms into the gas phase ionic species. Now in the animation I will show you 2 most commonly used soft ionization methods; MALDI and ESI.

**(Refer Slide Time:13:48)**




Fundamentals of Mass Spectrometry: Mass Spectrometry is the 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 analysis of complex protein samples in proteomic studies due to its ability to identify thousands of proteins.

**(Refer Slide Time: 14:31)**




Mass spectrometer is an instrument that produces charged molecular species in vacuum, separates them by means of electric and magnetic fields and measures the mass to charge ratio and relative abundance of the ions that produced. 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 experiment 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 and then we come to the advance concepts.Sample inlet: This is first point of contact where the sample is introduced within the mass spectrometer either as liquid nano droplets or as a mixture with the 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.

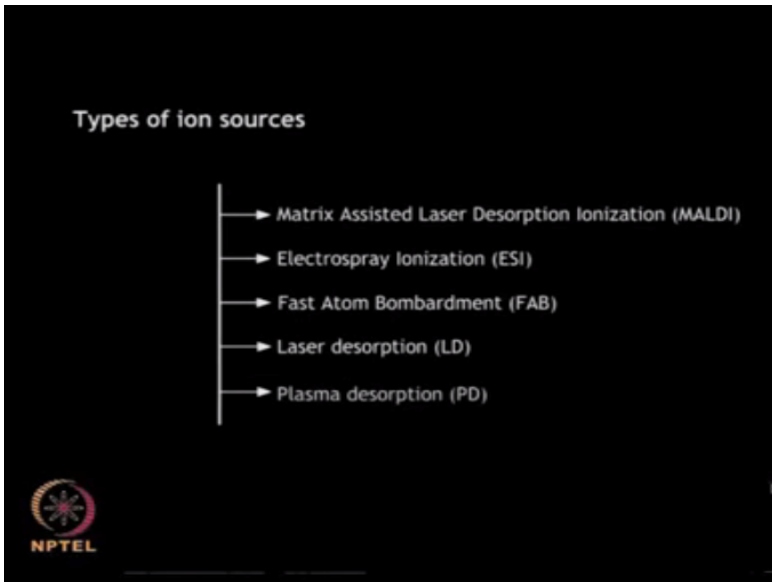
The commonly used ionization source include MALDI – Matrix Assisted Laser Desorption Ionization and ESI or Electrospray Ionization. Mass analyzers: The mass analyzers resolves the ions produced by the ionization source on the basis of their mass to charge ratios. There are various type of mass analyzers availble including Time of Flight, quadraple, ion trap, etc.

Detector: The ion detector determine the mass of ions that are resolved by the mass analyzer and generate data which can be further analyzed. The electron multiplier is the most commonly used detection technique. Now let us look at the function of each of these components in more detail.

**(Refer Slide Time: 17:44)**

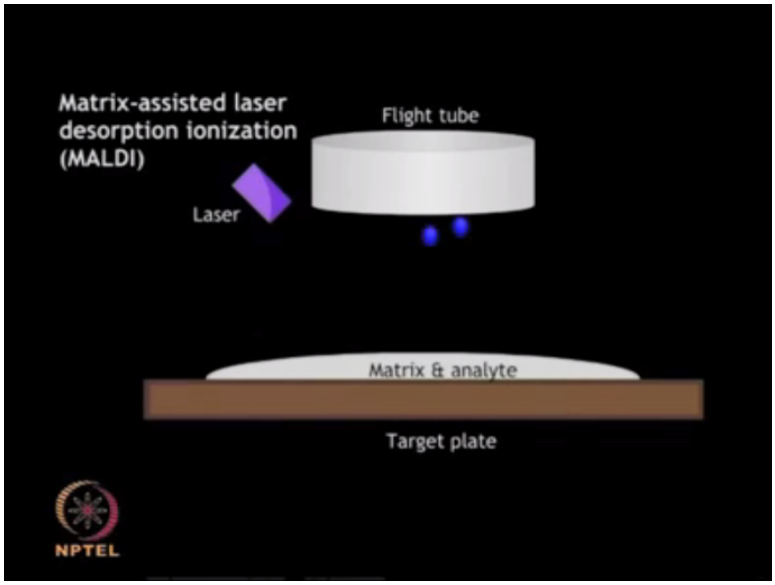
Let us first start with the ionization source. The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum. This has been made possible by the development of soft ionization techniques which ensures that the non – volatile protein sample is ionized without completely fragmenting it. Most commonly used ionization sources are MALDI and ESI.

**(Refer Slide Time: 18:16)**




Additionally, there are other ionization sources such as Fast Atom Bombardment (FAB), Laser Desorption (LD), Plasma Desorption (PD).

**(Refer Slide Time: 18:39)**

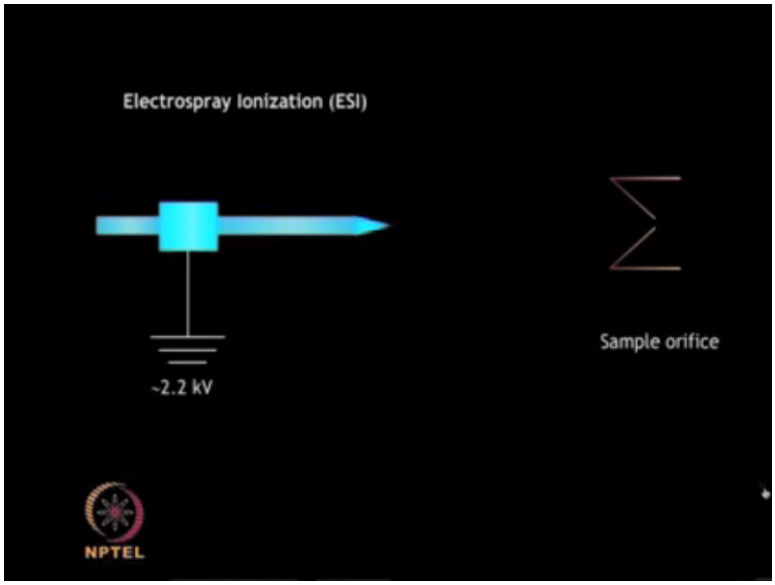



Let us discuss the 2 most commonly used soft ionization techniques MALDI and ESI in more detail. In MALDI, the analyte of interest is mixed with an aromatic matrix compound such as

alpha – cyano – 4 – hydroxycinnamic acid or sinapinic acid. This is dissolved in an organic solvent and placed on a metallic sample plate.

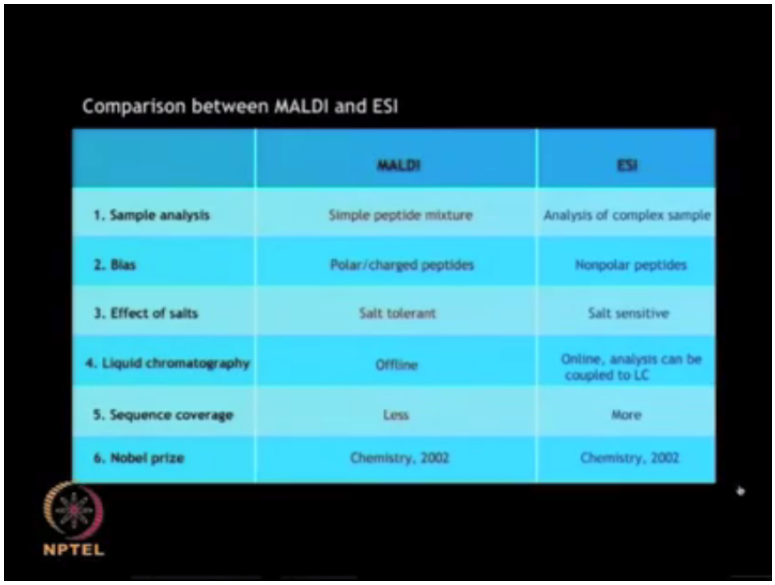
The evaporation of solvent leaves the analyte embedded in the matrix. Target plate is placed in a vacuum chamber with high voltage and short laser pulses are applied. The laser energy gets absorbed by the matrix and is transfered to the analyte molecules which undergo rapid sublimation resulting in gas phase ions. These ions then accelerate towards the mass analyzer based on their mass to charge ratios.

**(Refer Slide Time: 19:56)**




In Electrospray ionization, the sample is present in the liquid form and ions are created by spraying a dilute solution of the analyte at atmospheric pressure from the tip of a fine metal capillary creating a mist of droplets. The droplets are formed in a very high electric field and becomes highly charged. As the solvent evaporates, the peptide and protein molecules in the droplet pick up one or more protons from the solvent to form charged ions. These ions are then accelerated towards the mass analyzer depending upon their mass and charge.

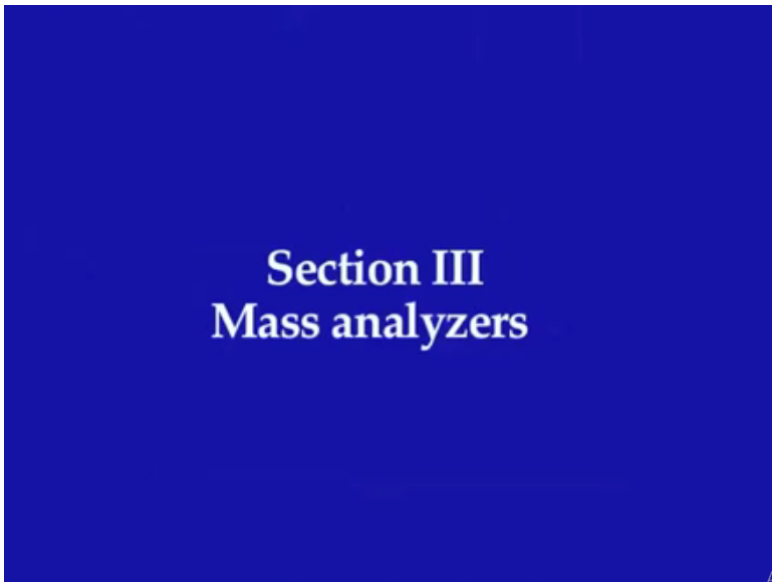
**(Refer Slide Time: 20:49)**




Let us 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 sampless. In MALDI, the sample analysis is for the simple peptide mixture where as in ESI it can be used for the analysis of complex samples. There is a bias towards the polar or charged peptides in MALDI where as it is for the nonpolar peptides in ESI.

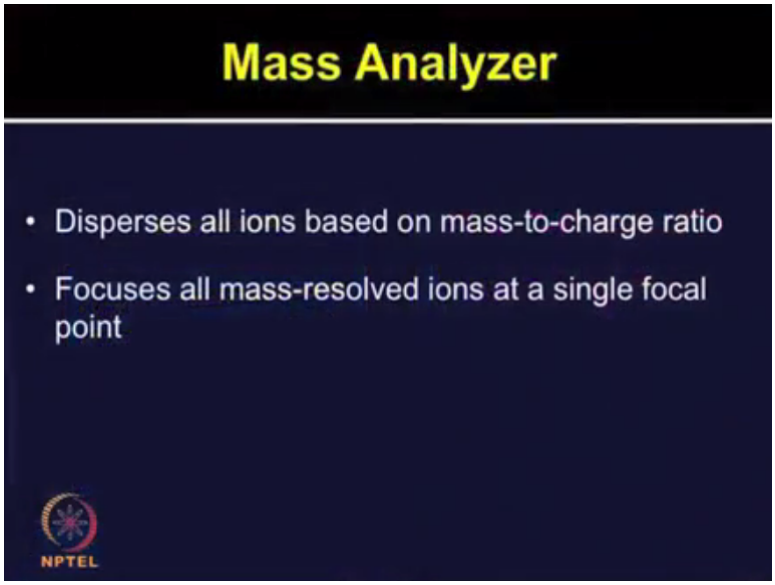
MALDI is more salt tolerant where as ESI is more salt sensitive. The liquid chromatography can be performed offline where as in ESI it is online and analysis can be coupled to the liquid chromatography. For the proteomic applications the sequence coverage is less in MALDI as compared to the Electrospray ionization. Both MALDI and ESI development were awarded with the Nobel Prize.

**(Refer Slide Time: 22:21)**



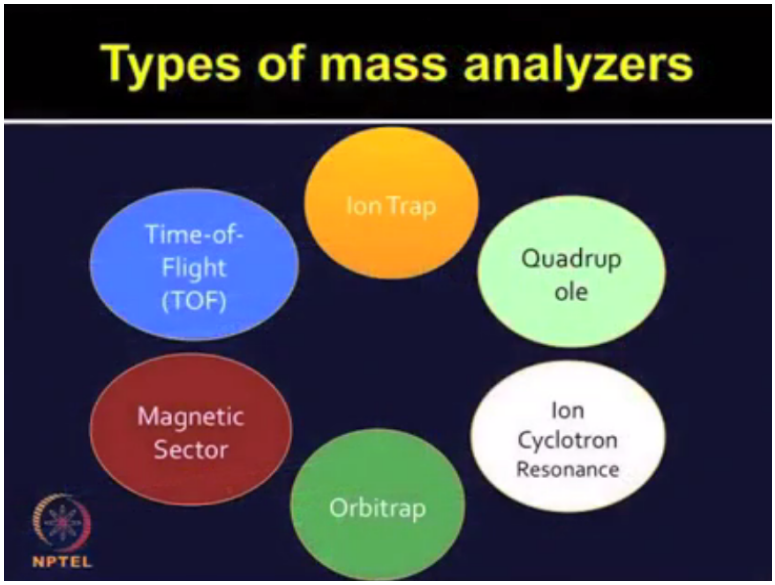
So we are talking about an MS experiment and I am trying to give you an overview of various steps involved. First of all we looked at liquid chromatography based prefractionation, in – gel digestion, different type of ionization sources and now let us move on to the mass analyzers. A mass analyzer plays 2 most important functions.

**(Refer Slide Time: 22:56)**




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

**(Refer Slide Time: 23:24)**



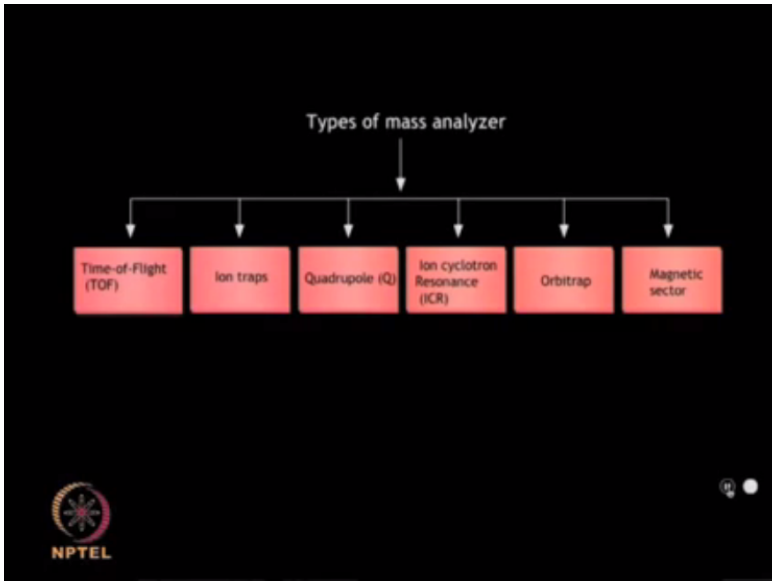

Several different types of mass analyzers are currently available. They choose the same basic properties which we discussed. Some of the popular mass analyzer configurations are shown

in the slide which include Time of Flight or TOF, ion traps, quadrupole, magnetic sector, orbitrap and ion cyclotron resonance.

**(Refer Slide Time: 23:51)**

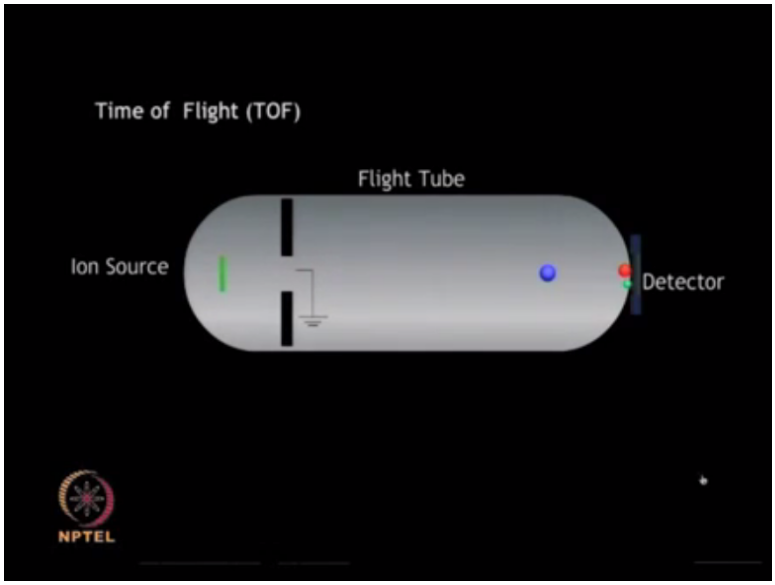

(()) (23:54) show you few available mass analyzers in the following animation. It resolve the ions produced by the ionization source on the basis of their mass to charge ratio. Various characteristics such as resolving power, accuracy, mass range and speed determine the efficiency of these mass analyzers. Let us discuss few most commonly used mass analyzers for the proteomic applications.

**(Refer Slide Time: 24:27)**



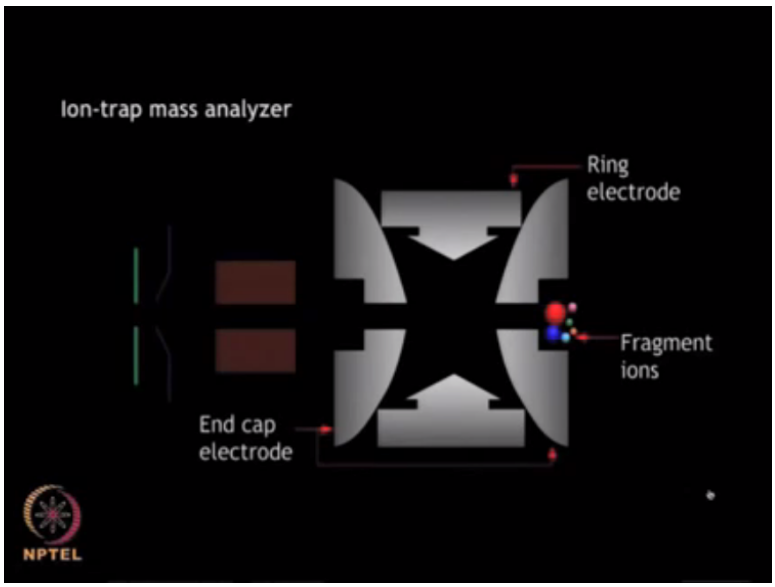

Currently various type of mass analyzers are available including Time of Flight, ion traps, quadrupole, ion cyclotron resonance, orbitrap, and magnetic sector.

**(Refer Slide Time: 24:29)**



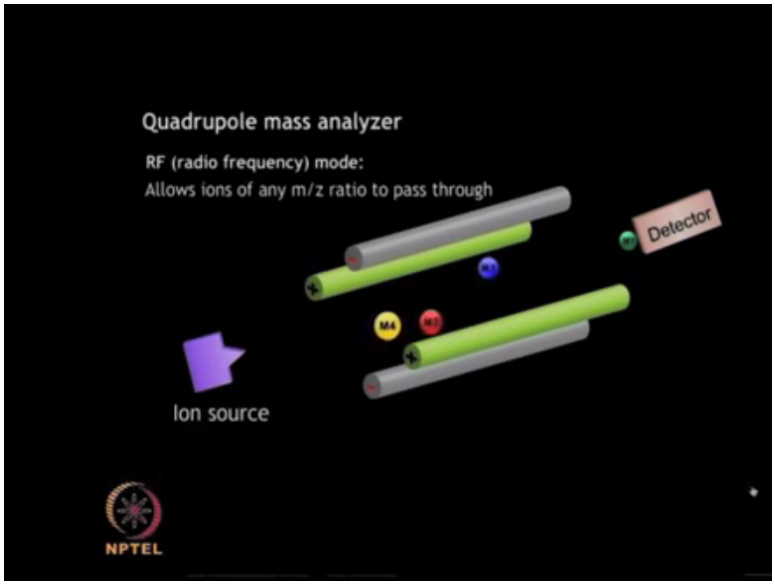

The Time of Flight accelerates charged ions generated by the ionization source MALDI along a long tube known as the flight tube or TOF. Ions are accelerated at different velocities depending on their mass to charge ratios. Ions of lower masses are accelerated to higher velocities and reach to the detector first. The Time of Flight under such circumstances is inversely proportional to the square root of molecular mass of the ion. The TOF analyzer has several applications in the proteomics.

**(Refer Slide Time: 25:41)**



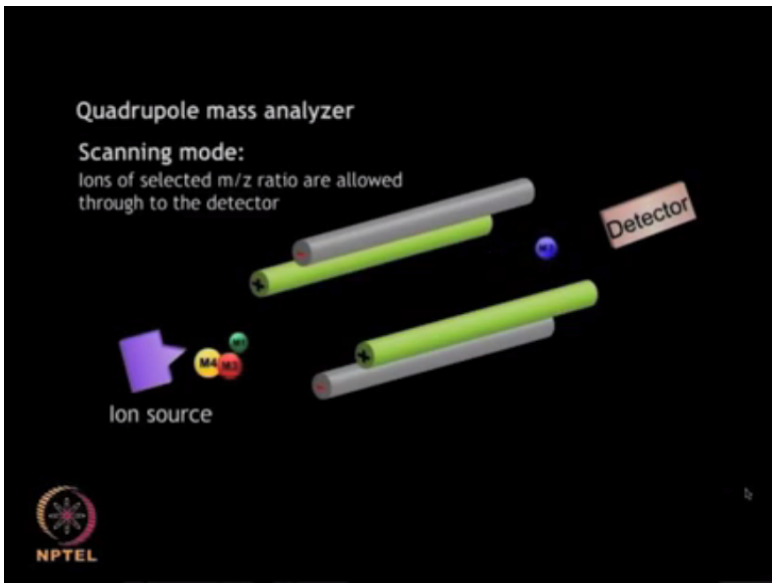

Now let us discuss the next mass analyzer, ion - trap. An ion - trap make use of a combination of electric and magnetic fields and captures ion in a region of a vacuum system or tube. Ion - trap traps the ion using electric field and measures the mass by selectively ejecting them to a detector.

**(Refer Slide Time: 26:09)**



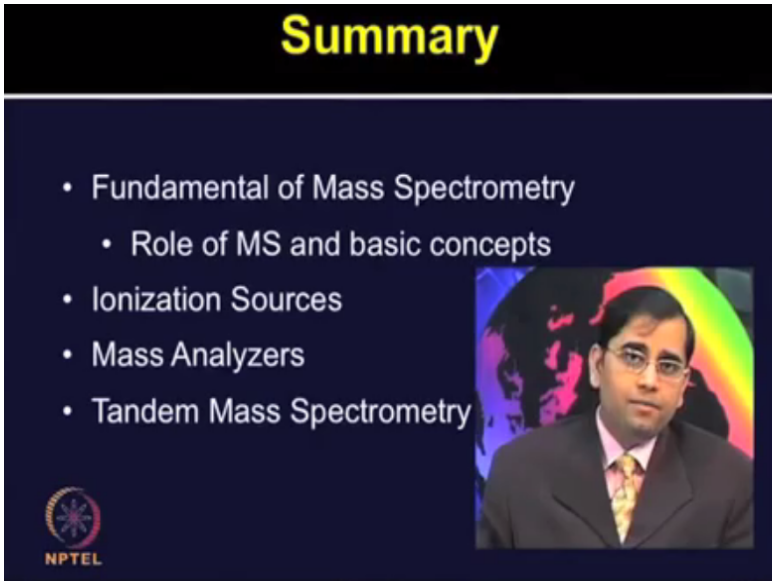

Quadrupole: The quadrupole mass analyzer use oscillating electric fields to selectively stabilize or destabilize the parts of ion passing through a radio frequency quadrupole field. The quadrupole mass analyzer can be operated in either radio frequency or scanning mode. In RF mode or radio frequency, ions of all m/z are allowed to pass through which are then detected by a detector.

**(Refer Slide Time: 26:53)**




In the scanning mode, the quadrupole analyzer selects ions of specific m/z value as said by the user. A range can also be (()) (27:10) in which case only those specific ions which satisfy the criteria will move towards the detector and rest can be filtered out.

**(Refer Slide Time: 27:20)**




So in summary, today we talked about some of the fundamental concepts involved in the mass spectrometry. We talked about different type 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 capablities, wide linear dynamic range, multiple charge separation capability, polarity switching capablity with rapid or low complementary ionizations, one can also use the modular or different type of ionization sources can be combined such as ESI and CI.

Targetted analysis can be performed for PTM, label free quantification 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 high specifications. So from todays lecture, I hope you are able to understand some of the fundamental concepts involved in the 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 source, mass analyzers and different type of Mass Spectrometry configurations and its applications. Thank you.