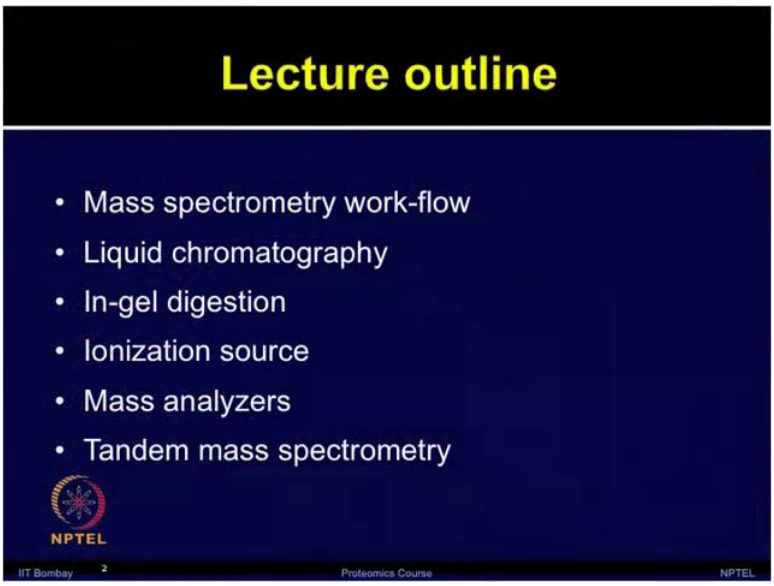
Proteomics: Principles and Techniques
Prof. Sanjeeva Srivasatava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Lecture No. # 22
Liquid Chromatography-Mass spectrometry (LC-MS/MS)

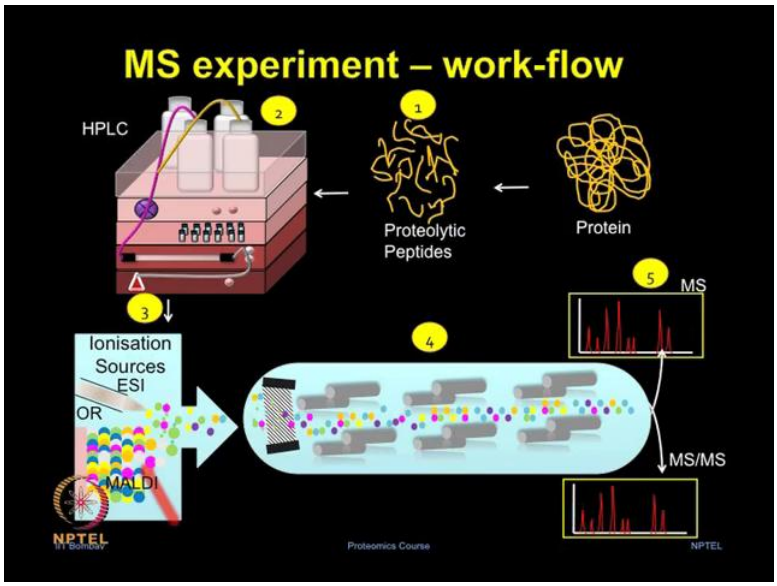
Welcome to the proteomics course; in this module, we are discussing about mass spectrometry techniques. In the previous lectures, we talked about basic concepts of mass spectrometry, then discussed about Maldi TOF MS. And now, today we will talk about liquid chromatography, mass spectrometry or LC MS MS; MS is based on production of ions, which are subsequently separated according to their m by z ratio. The resulting mass spectrum provides a plot of relative abundance of generated ions as a function of m by z, MS provides most versatile platform and comprehensive analytical technique for the proteomic scientist for wide variety of applications.

(Refer Slide Time: 01:38)



So, today's lecture, I will first give you an overview of the mass spectrometry workflow, we will then talk about individual components in some more detail, such as liquid chromatography, ionization sources, mass analyzer, and then tandem mass spectrometry.

(Refer Slide time: 01:53)

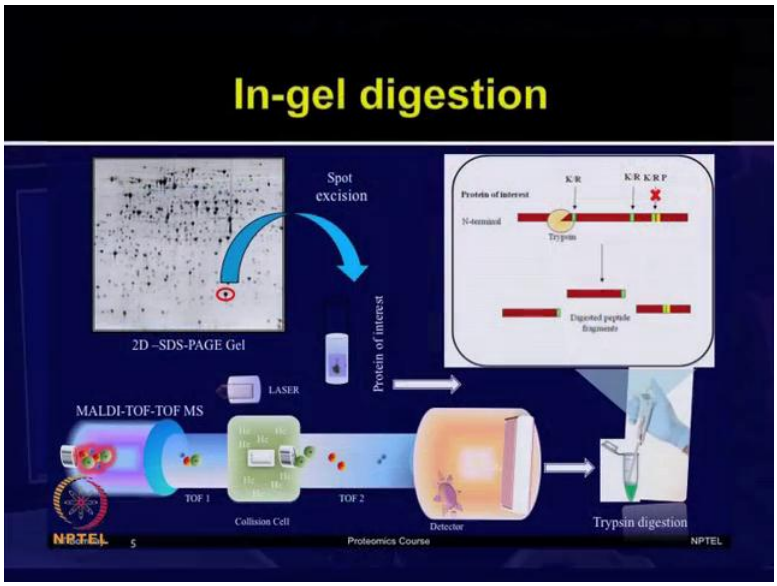


So, we can see in the slide, in the MS experiment work flow the multiple steps are involved, I have numbered those from one to five. So, protein samples are digested using Trypsin and Peptide mixtures are fractionated by using liquid chromatography or LC. These fractions are subjected to an electric potential which results into a spray formation, in ESI or electro spray ionization it leads to desolvation and ionization of peptides. The mass to charge ratio are measured in mass analyzer which is shown a step four, specific ions are randomly selected in the collision cell, and then based on the collision induced dissociation the resulting fragments ion are further measured in the second mass analyzer.

The MS makers or ion intensity can be used for peptide quantification, and MS MS ion fragmentation can be used for the sequencing formation and protein identification. So, this work flow all the steps are equally important starting from first step; the trepid digestion, second prefractionation strategies using liquid chromatography, then ionization sources, mass analyzer and then spectrum generation MS or MS MS.

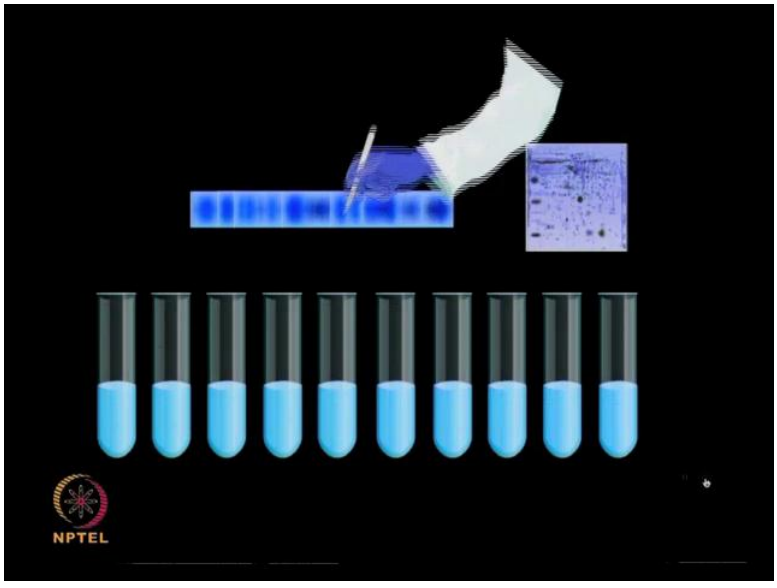
So, first step In-gel digestion, it is a multistep procedure which includes spot selection, if you have taken the gels from the two d gel or if you have used the gel fill techniques you can still run the samples on the gel excise a band, and then excise those bands. After that you have to remove the stinks, if you had used with the coomassie staining, then reduce alkyl ate and then perform the photolytic cleavage, and finally peptides can be extracted.

(Refer Slide time: 04:05)



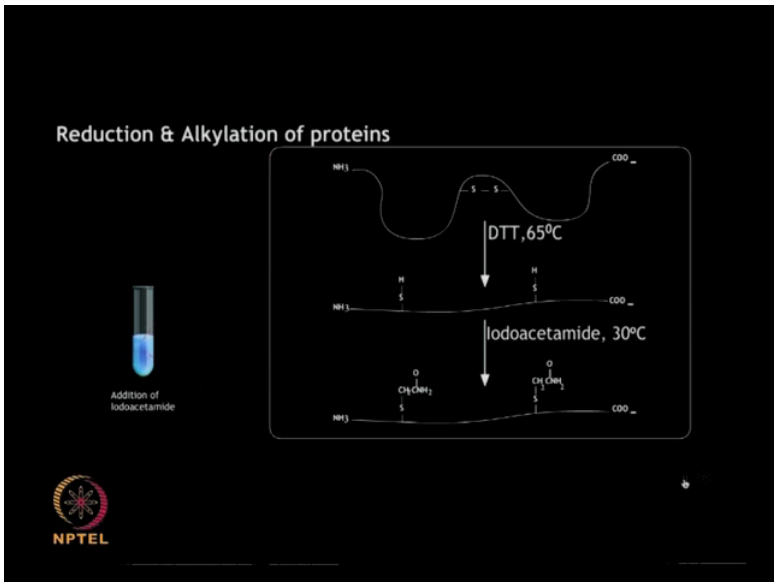
This slide gives you an overview of various steps involved in the In-gel digestion. I have discussed this in more detail, in the previous lecture while discussing about the Maldi TOF MS, so you can refer to more detail over there, but just to refresh with the refresh with the concept I will talk this concept again in the following animation of In-gel digestion.

(Refer Slide time: 04:35)



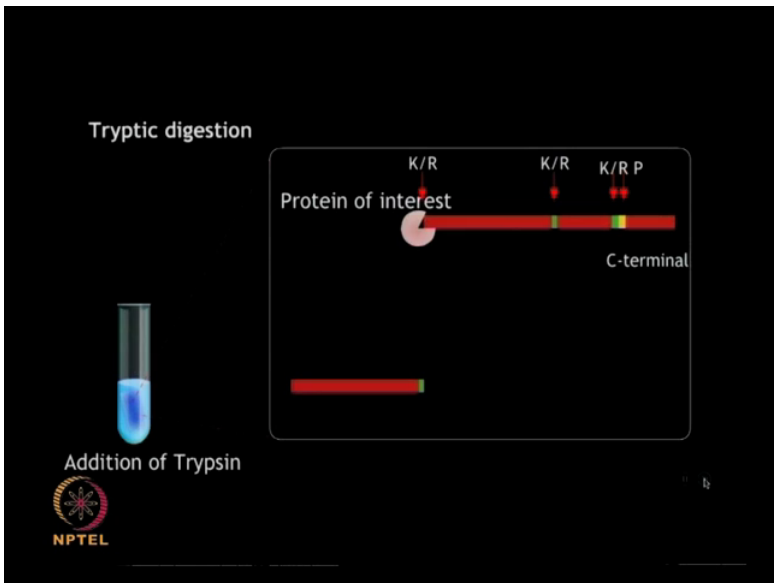
Electrophoresis separation of a protein mixture results in distinct protein bands, these proteins can be used for analytical purposes by carrying out In-gel digestion. Entire gel is fragmented into small pieces, and each piece is dissolved in a suitable buffer.

(Refer Slide time: 05:13)



To simplify the complex proteome, it is good idea to chop down the overall separate proteins into the smaller pieces; the protein solution is treated with a reducing agent, like dithiothreitol DTT which cleaves the disulphide bond in the protein. This is followed by treatment with iodoacetamide or IAA, which alkylates the sulphide group, and thereby prevents the reformation of disulphide bonds.

(Refer Slide time: 05:46)

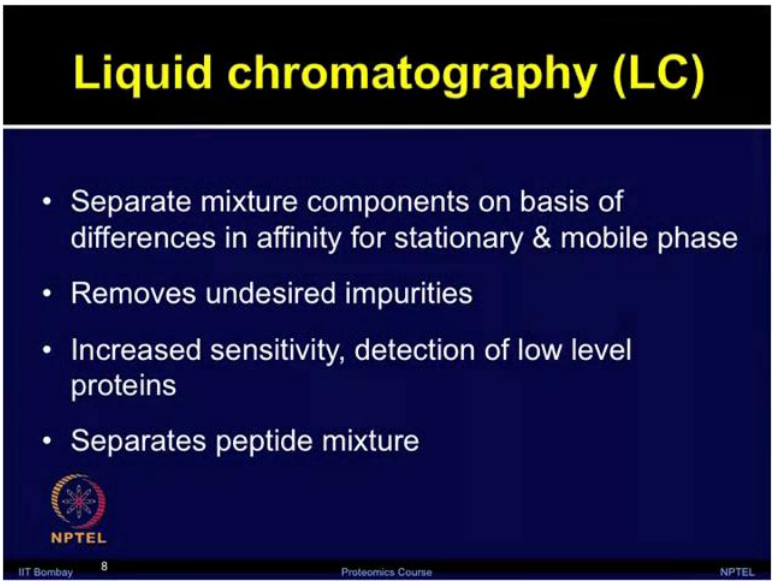


After cleavage of the disulphide bonds, the protein is treated with a photolytic enzyme; the most commonly used enzymes include Trypsin. Trypsin cleaves the protein at specific

residues argentine and lysine, and generates smaller peptide fragments, this Tryptic digestion is used for further purification and analysis.

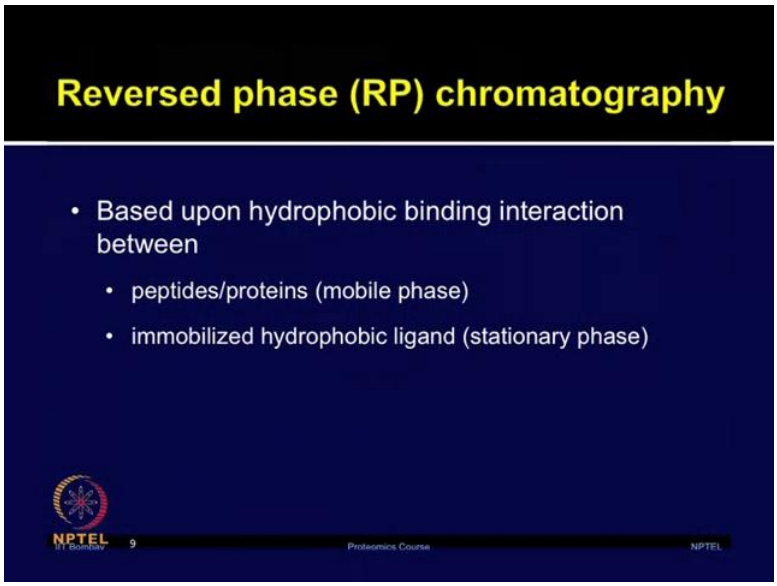
So, after doing the In-gel digestion now let us move on to second part; the separation technology, liquid chromatography or L C. So, chromatography in general is a physical separation method, in which components for separation are selectively distributed in two immiscible phases, a mobile phase flowing through the station rephrase. Now, depending upon the mobile phase the technique is termed as, either liquid chromatography or gas chromatography etcetera.

(Refer Slide time: 07:04)



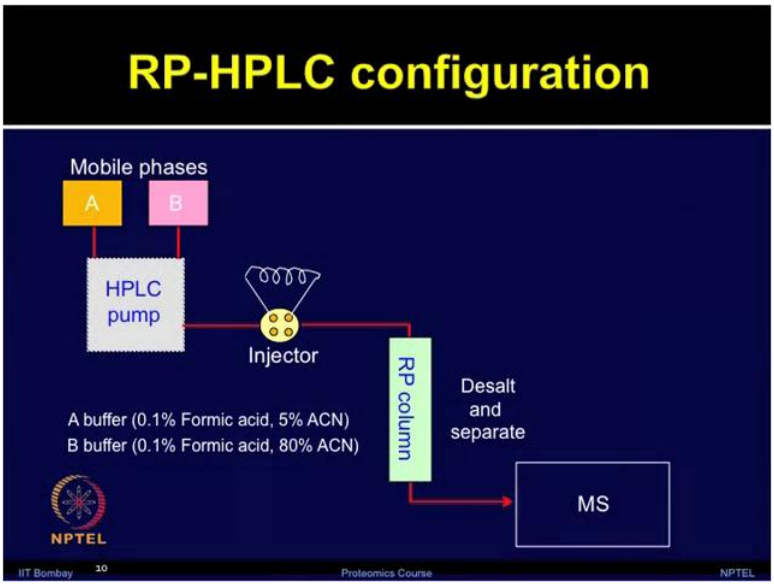
So, what is liquid chromatography? The Peptide mixtures can be fractionated in line with the instrument prior to the introduction into mass spectrometer, that is I think one of the advantage for doing the proteomic applications. So, in LC, it can separate mixtures or components on the basis of differences in the affinity for stationary and mobile phase. Liquid chromatography is also useful for removing the undesirable impurities; it also increases reproducibility in the samples, as well as robustness of the MS measurement. L C along with further enrichment can help into the concentration of the diluted samples, it also helps in increased sensitivity, detection of low level proteins, and further it can separate peptide mixtures.

(Refer Slide time: 08:11)



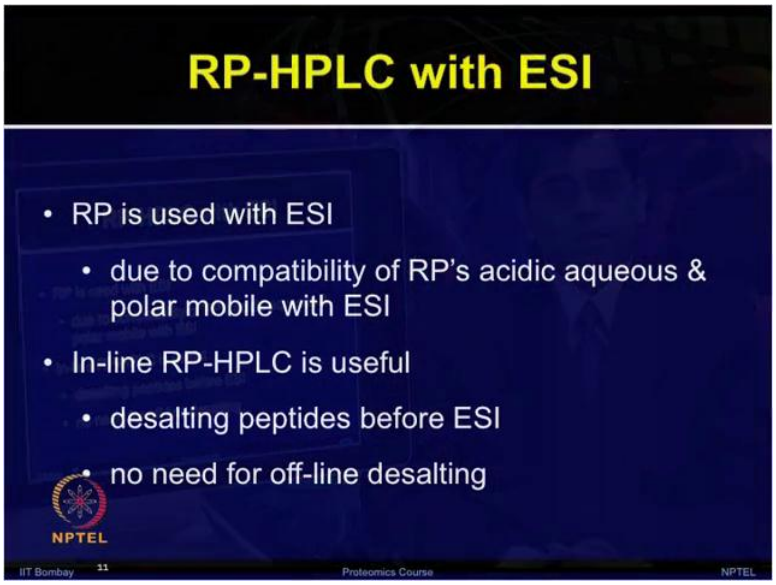
So, there is various type of chromatography one can use for different applications, in proteomics the most commonly used method for the peptide fractionation is reverse phased liquid chromatography or RP L C, which separates peptides based upon the hydrophobic bonding of interaction between the peptides or proteins in the mobile phase, and immobilized hydrophobic ligands in the stationary phase. By utilising this hydrophobicity, one can separate the peptides; however, if your proteome mixture or the peptides are very complex, then one can further use another type of chromatography method such as strong cationic exchange, as well as different type of multi dimension separation can be employed.

(Refer Slide time: 09:13)



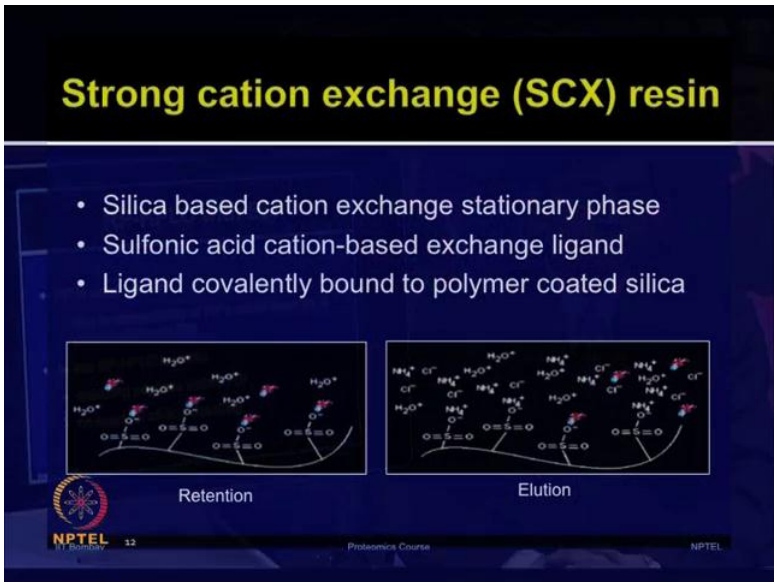
In this slide I have shown you the configuration for reverse phase HPLC, as we can see the two components in the mobile phase A and B lane with the HPLC pump. A buffer can provide between the range of point 0.1 percent of formic acid to 5 percent of acetonitrile, and in B 0.1 percent to 80 percent of acetonitrile. So, first of all equilibrate the system in buffer A, then load the peptides and wash those, then run the gradient of increasing mobile phase B, now wash the reverse phase with buffer B and then requilibrate in A. In this way the peptides can be separated, and prior to MS analysis it can be desalted so that there is no interference of salt.

(Refer Slide time: 10:39)



Now, reverse phase is most commonly employed with the electro spray ionization, because of its compatibility of reverse phased acidic aqueous, and polar mobile with electro spray ionization. As I mentioned in the proteomics, one can use ESI and liquid chromatography in line. So, directly samples can be prefractionated and further analysed using mass (( )). The inline reverse phase HPLC is very useful, because it can do the desalting of peptides prior to ionization in ESI. There is no need for doing separate offline desalting and prefractionation, it can focus peptides from the dilute samples into the narrow chromatographic bands, and it also enhances the sensitivity.

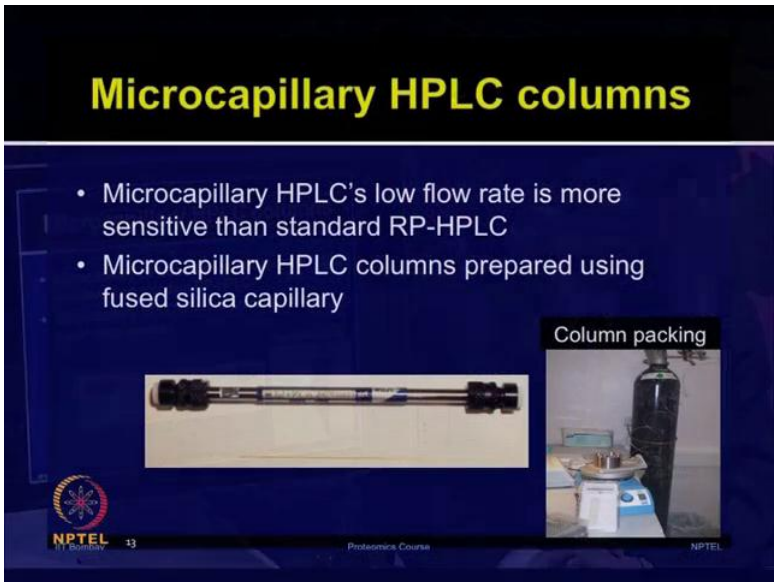
(Refer Slide time: 11:28)



Let us now talk about another separation technique, which is also commonly employed in proteomics which is strong cationic exchange or SCX. So, in SCX resin the silica based cationic exchange using the stationary phase, the sulfonic acid cation based exchange ligands are used, these ligands are covalently bound to the polymer coated silica.

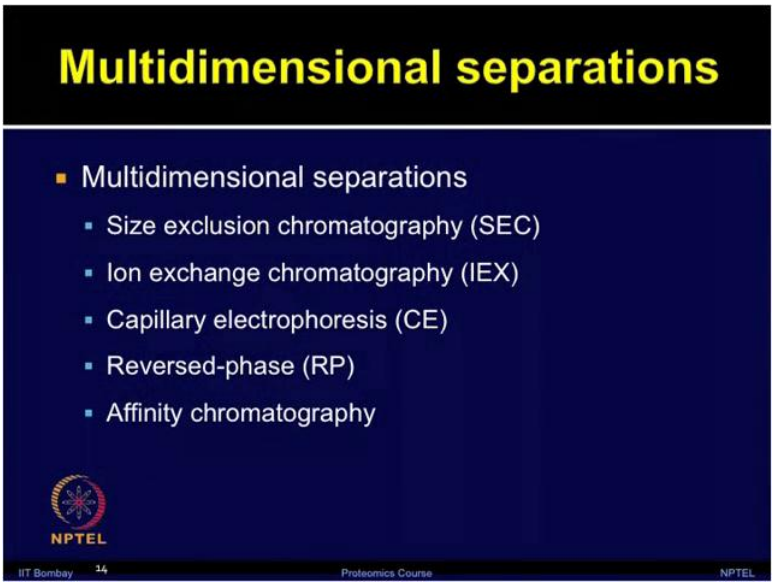
Now, as I have shown in the slide the two important phenomenons here; one is retention, other is elution. In retention, that is based on the electrostatic attraction between the negatively charged sulfonic acid and positively charged peptides. The elution can be performed by an exchange of peptides for cation of mobile phase additive, the ammonium ions, and this is a reaction to the high concentration of cations.

(Refer Slide time: 12:56)



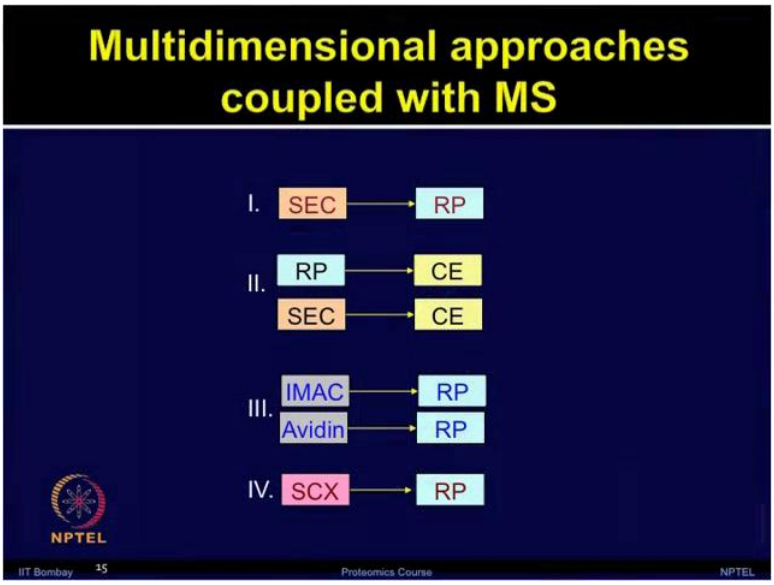
Now, HPLC can be micro capillary, it can be nano L C or it can also be different type of chip based chromatography separation. Now, for proteomics various type of microcapillary, nano L C and different type of chip technologies are currently used. In the microcapillary HPLC, the low flow which is less than one micro litre per minute is more sensitive as compared to the standard reverse phased HPLC, which is around 50 micro litres per minute. The microcapillary HPLC is required for the analysis of low femtomole amount of the peptides, one can prepare the microcapillary HPLC's by using few silica capillaries, and then pack with the reverse phased packing material.

(Refer Slide time: 13:53)



For prefractination multi dimension separations are used, the different type of principles are involved for separating these peptides, one can use size exclusion chromatography which separates based on the molecular weight or the size. Ion exchange chromatography based on the charge, capillary electrophoresis based on charge, reversed phase based on the hydrophobicity, affinity chromatography which is based on the biological interactions.

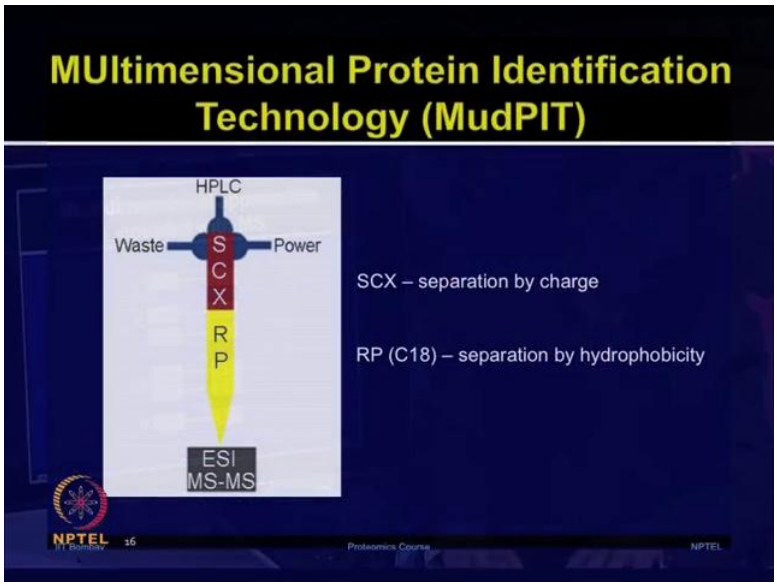
(Refer Slide time: 14:36)



Now, multi dimensional approaches can be coupled with the mass spectrometry. So, one can use various type of liquid chromatography methods in tandem, and then do the multi dimension separation. Different type of approaches have been tried for example, size exclusion chromatography followed by reverse phase chromatography, but it has resulted into the poor resolution of peptides in size exclusion chromatography, so that is not very widely used. Other approaches include reverse phase chromatography followed by the capillary electrophoresis, or size exclusion chromatography followed by the capillary electrophoresis.

Due to the limited capacity, and low loading volume of the capillary electrophoresis, again it is not very popular. The affinity chromatography based separations, such as IMAC or evidence followed by the reversed phase chromatography, those are commonly used. And a strong cation exchange followed by the reversed phase is most commonly used.

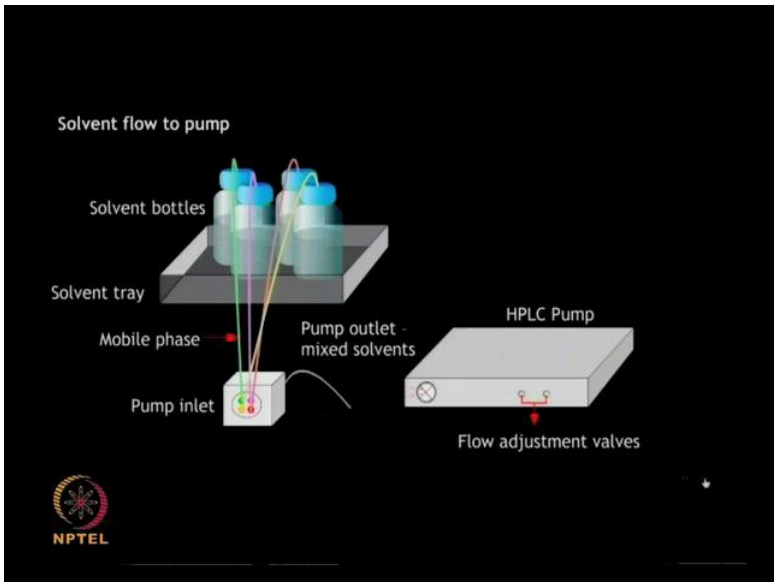
(Refer Slide time: 15:47)



So, this slide gives you an overview of various type of methods; liquid chromatography separation, which can be employed prior to injection into the ionization source. As I mentioned, the strong cation exchange as well as reverse phase chromatography, together can be employed for various proteomic application, which have been demonstrated in the multidimensional protein identification technology or mud pit. In this technique a strong cation exchange separates by the charge, which provides low resolution fractionation in the beginning, and then reverse phase C18 column which separates peptides based on the hydrophobicity, and it provides high resolution gradient.

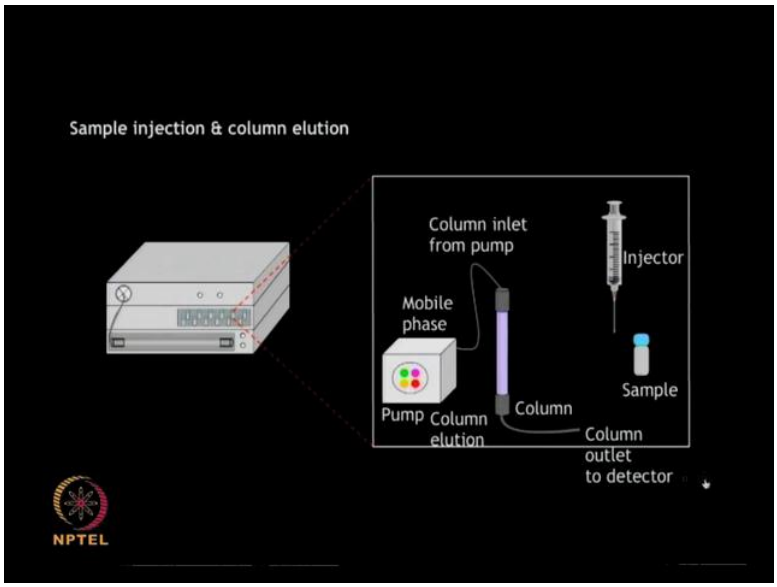
Let me describe some of the concepts involved in the liquid chromatography in following animation. I will also discuss mud pit and some of the chip based approaches, which are integrated for proteomic application with the liquid chromatography, so let us discuss these concepts in following animation. A typical liquid chromatography set up consists of solvent bottles, degasifier dual or quaternary perms, sample injector, column and detectors.

(Refer Slide time: 17:08)



Here you can see the various components which are involved in performing the liquid chromatography; different solvents can be placed in the solvent bottles depending upon the purification requirements.

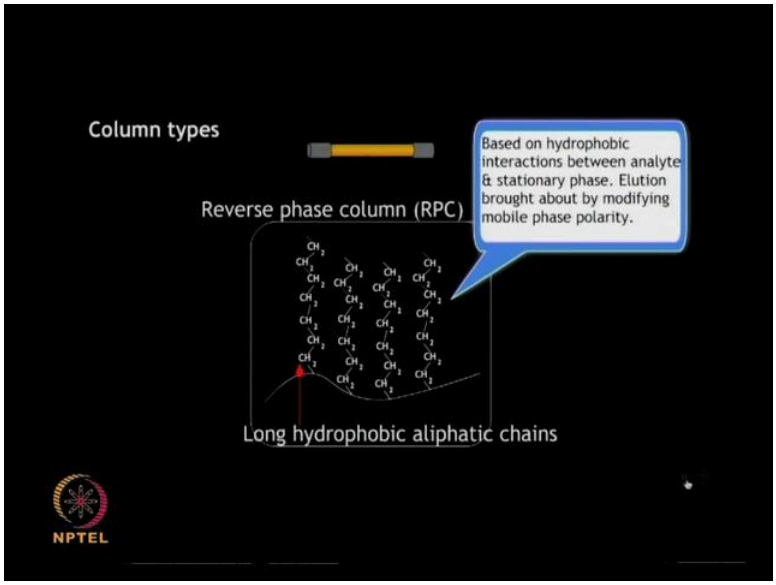
(Refer Slide time: 17:39)



These solvents are mixed in the desired ratio, and pumped into the column during elution, after removal of any trapped air inside by means of the degasified. The sample injector system may be automatic or manual, the automatic sample uses a syringe to inject the sample, which is placed in a while directly into the column. When the sample is injected,

mobile phase flows into the column through the pump, the column consist of a stationary matrix that preferentially binds certain analysis.

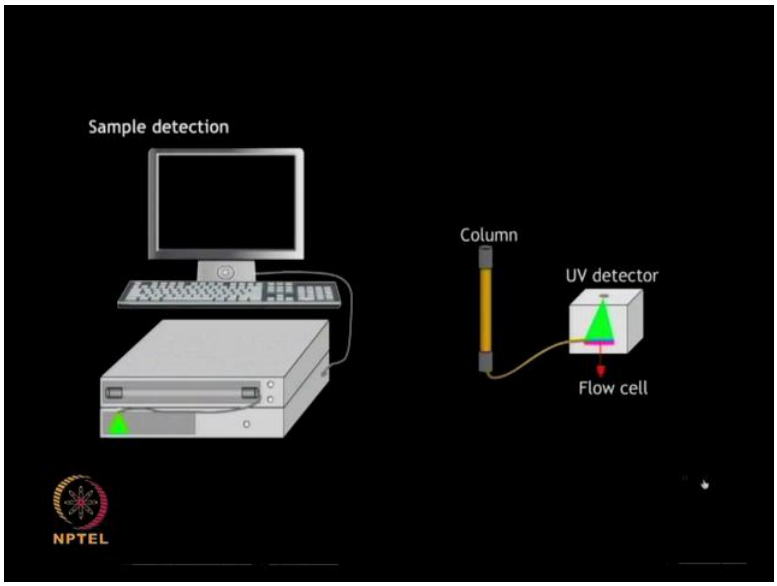
(Refer Slide time: 18:38)



The outlet from the column enters the flow cell where it can be detected. There are various station rephrase matrices are available, that separate the components of the mixture based on different principles. One of the most commonly used matrices include a strong cationic exchanger or SCX, which separates charged peptides based on their electrostatic interaction, with negatively charged sulphonic acid groups on the regime surface.

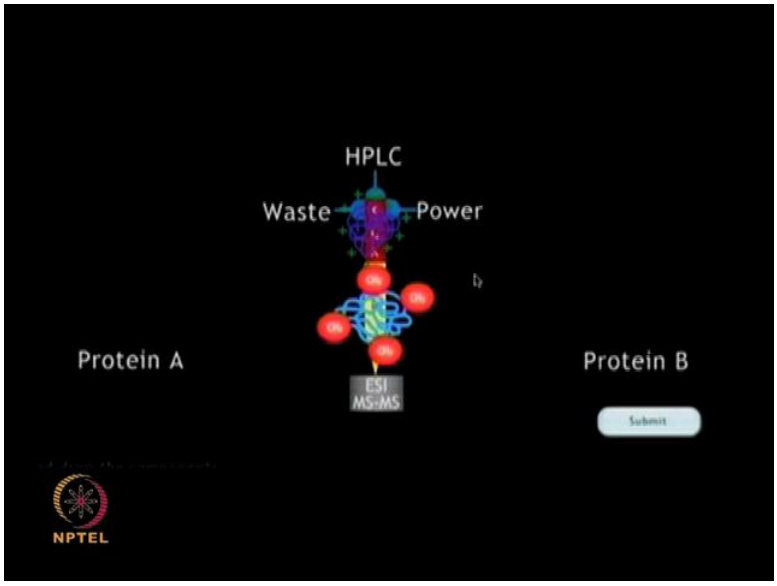
Now, elution can be caused by the addition of positively charged mobile phase. The reverse phase chromatography is another commonly used tool, which uses a hydrophobic matrix consisting of long aliphatic carbon chains; these regime analytes separate on the bases of hydrophobic interactions, and can be eluted by changing the polarity of the solvent.

(Refer Slide time: 19:55)



The nano liquid chromatography which makes use of C18 capillary columns has gain popularity for the proteomic studies due to their ability to achieve final separations. For these separated components passed from the column outlet into the flow cell, which is present in the detector. The most commonly used detector for protein analysis is; u v detector, which analyzes the protein absorbance at 280 nanometres, and plots a graph of retention time against intensity.

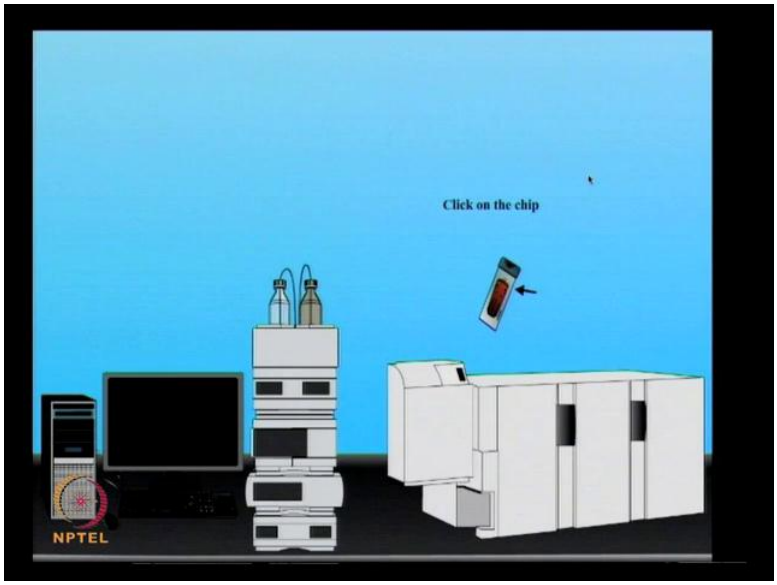
(Refer Slide time: 20:48)



Multi dimensional protein identification technology or mud pit is a widely adopted strategy that carries out two consecutive protein separations, based on different principles as shown in the animation here, shown on either side is a protein with different properties. Earlier, we have talked about how one can make use of different properties of strong cation exchange, and reverse phase chromatography to separate out peptides. Now, use those basic concepts, and drag and drop the proteins that will interact with the SCX and RP regions of the columns respectively.

So, your answer is correct, you can use the protein properties and separate those in the multidimensional protein identification technology. Mud pit is a non gel technique to separate and identify individual components of complex proteins, and peptide mixtures of a proteome. It has been shown that mud pit has potential to be used as a substitute of traditional two-dimensional gel electrophoresis; since, it can separate peptides in the two d liquid chromatography. Mud pit technique allows greater separation of peptides, which can directly be interfaced with mass spectrometry ionization source, it also avoids band broadening which is one of the drawbacks of many chromatographic methods.

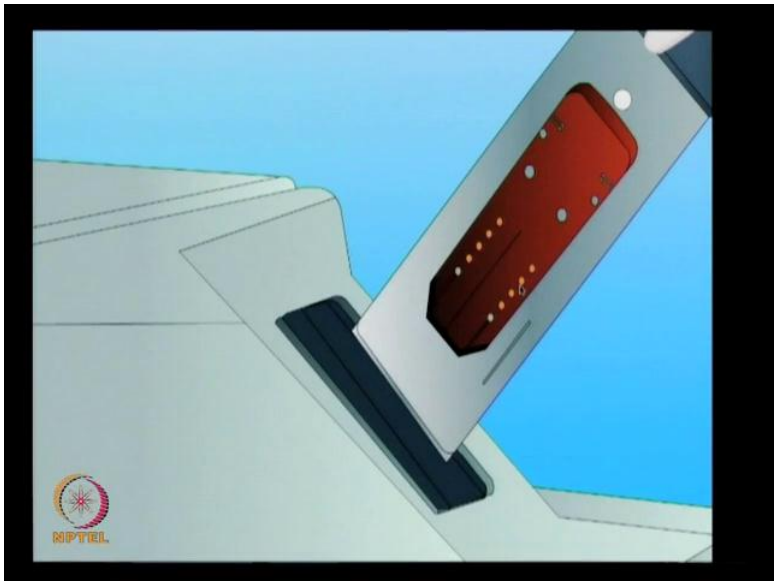
(Refer Slide time: 22:50)



We have discussed different type of liquid chromatography systems, in a traditional nano flow L C MS system, there are several fittings and connections are required, which is one of the major limitation of using those traditional systems. They are certain commercial advancements available to improve these type of limitations, agilent HPLC chip

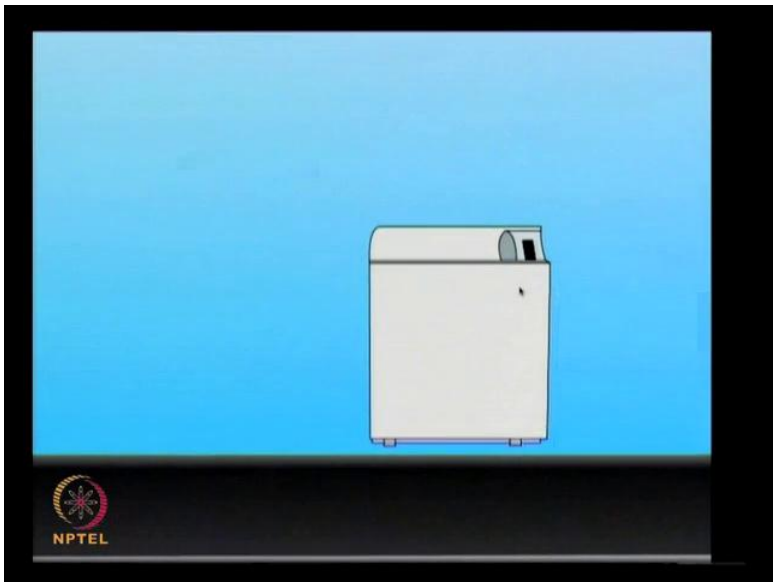
technology is a micro fluidic device which carries out nano flow high performance liquid chromatography, and reduces limitations of several fittings and connections.

(Refer Slide time: 23:39)



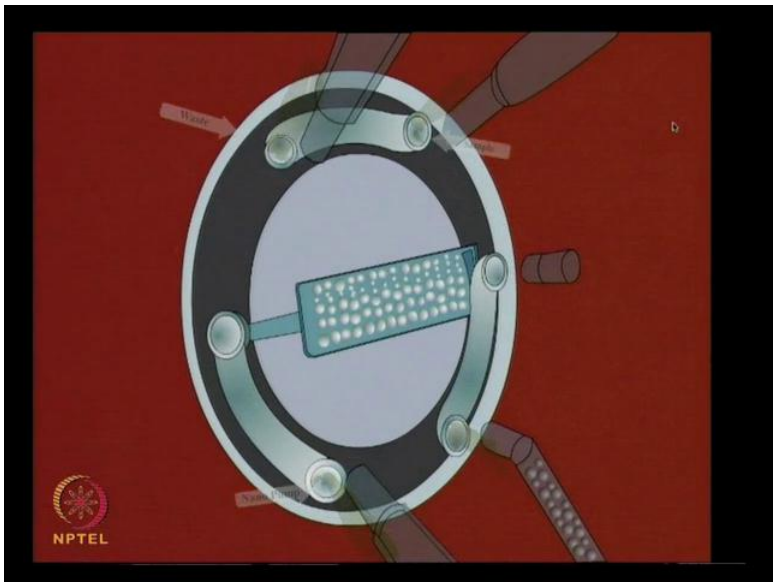
The micro fluidic device contains circuits of tiny closed channels, and wells which are edged onto a glass or plastic microchip.

(Refer Slide time: 23:57)



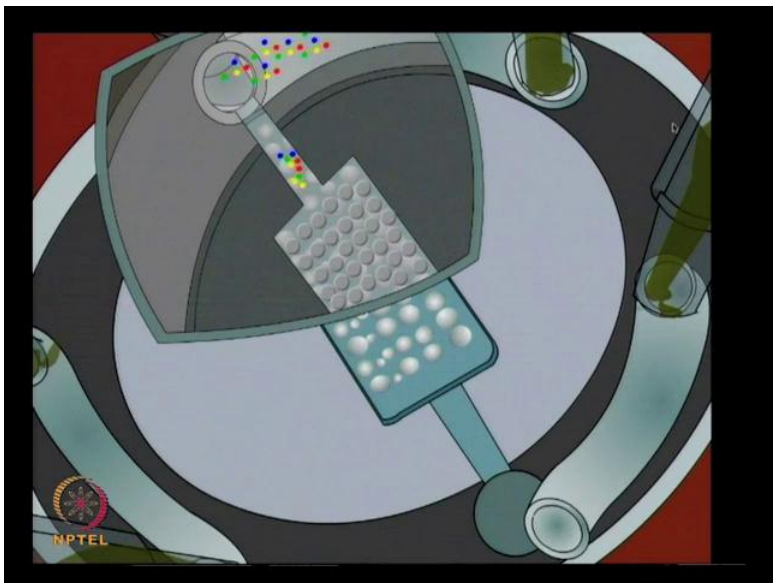
Different forces, such as pressure or or electro kinetic can push a small volume of fluids in a defined manner.

(Refer Slide time: 24:07)



This technology integrates functional components onto a reusable biocompatible chip, which integrates sample enrichment and analytical nano columns.

(Refer Slide time: 24:22)

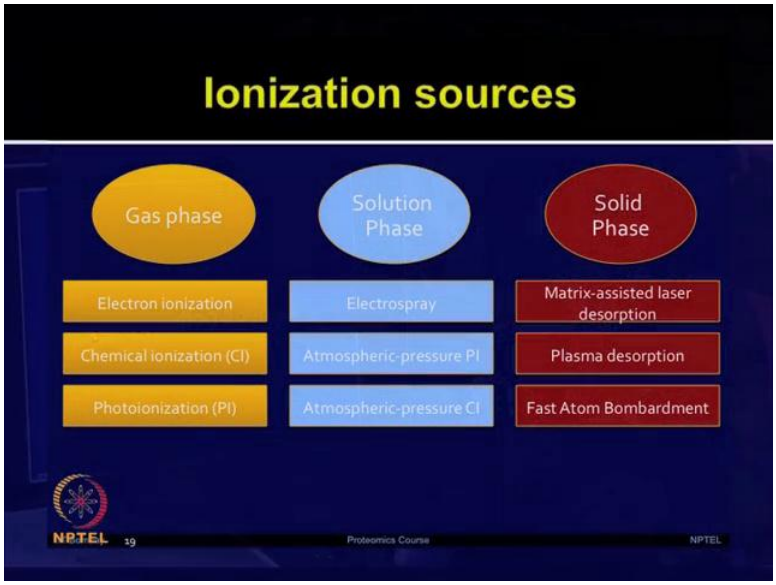


Nano spray emitter, fittings and connection capillaries on a reusable biocompatible polymer chip. The chip based technology reduces the possibility of leads and dead volumes; it improves sensitivity and reliability during the liquid chromatography steps. By making the integrated system, it avoids several pit falls which are associated with separate tubing's and fittings. Another important component of this technology is the

HPLC chip MS interface, a chip is inserted into the interface which mounts on a mass spectrometer. So, this design configuration ensures that the electrospray trip is in the optimal position for mass analysis, when the chip is inserted in the mass spectrometer.

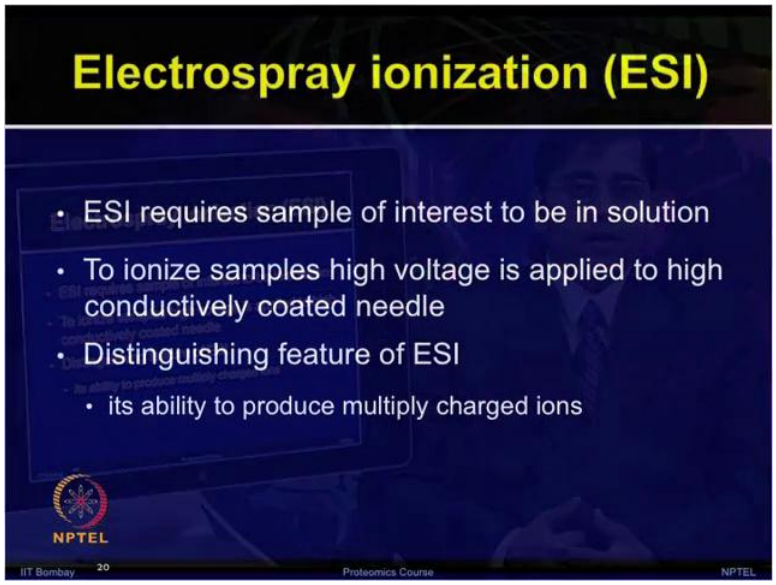
So, compare to the conventional nano spray techniques, this technology can achieve maximum sensitivity with minimum sample size by integrating sample preparation, separation and electrospray trip on the single chip technology. So, far we have talked about trips ionization or doing the peptide cleavage, then we talked about prefractionation strategies liquid chromatography.

(Refer Slide time: 26:25)



Now, these samples are ready to be injected into the ionization sources. In the previous lecture when we talked about basic concepts, and on overview of the different type of procedures involved, I gave you a list of various type of ionization techniques available. We also discussed that soft techniques, soft ionization techniques are required for the proteomic application, so soft ionization techniques such as electrospray ionization ESI, and matrix assisted laser desorption ionization or Maldi, these were introduced in the late80s, and now they have overcome the problem of hard ionization, and now these are widely adopted for the proteomic applications.

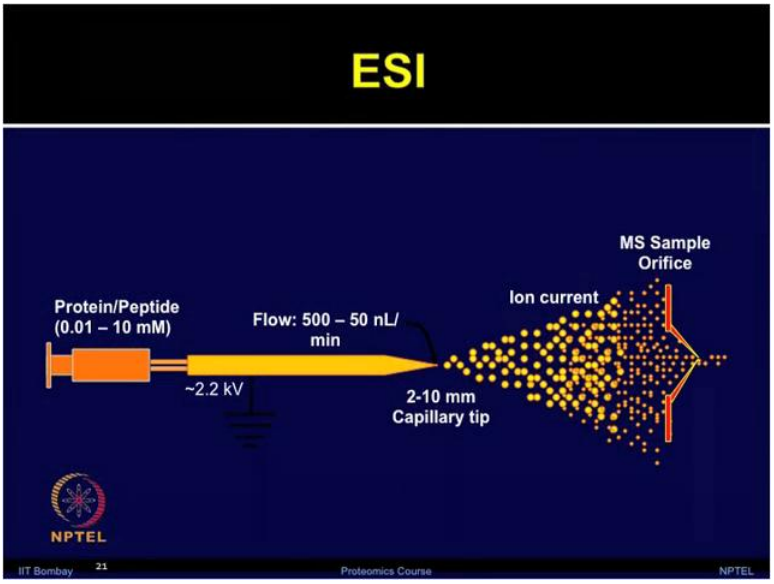
(Refer Slide time: 27:41)



So, we will focus more onto the ESI and Maldi. Since, we discussed Maldi in more detail in the previous lecture; I will focus more on the ESI or electrospray ionization in today's lecture. So ESI, it requires sample of interest to be in solution, and that is why we mentioned that we can use the inline separation along with the liquid chromatography.

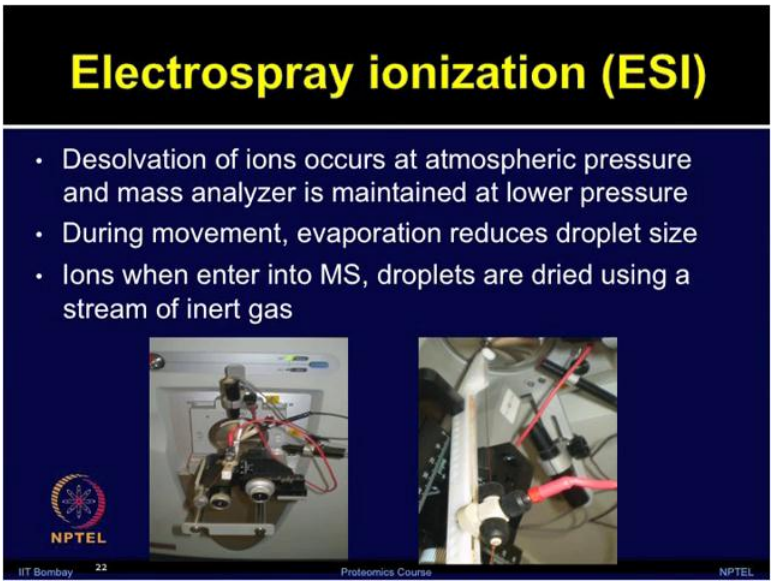
To ionize the sample high voltage is applied to high conductively coated needle, so this voltage results into the sample becoming charged, either positive or negative. The positive ions are primarily used for the analysis of proteins, the distinguishing features of electrospray ionization includes its ability to produce multiple charged ions, the number of charges that can be accepted by a particular molecule depends on its basicity and its size.

(Refer Slide time: 28:21)



Now, here you can get an overview of the process involved in the electro spray ionization, this small droplet of solutions are generated by the Taylor cone which contain the peptide analyse, protons from the acidic solution provides droplets; the positive charge, so that it can move from the needle to the negatively charged instrument.

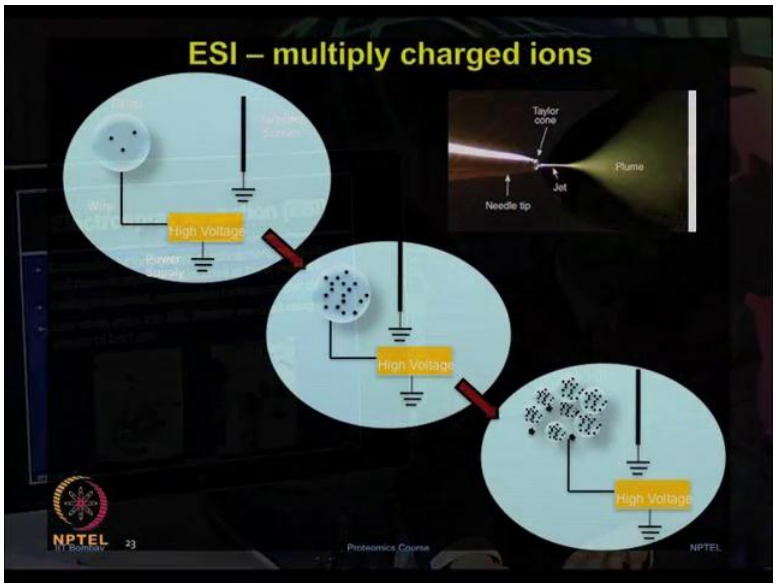
(Refer Slide time: 28:48)



In ESI, the desolvation of ions occurs at the atmospheric pressure and mass analyzer is maintained at a lower pressure so that ions can be drawn into the MS based on the pressure differential. During movement, the evaporation reduces a size of droplets, and

then it splits into the small charge droplets. Ions when enter into the mass spectrometer, the droplets are dried using a vacuum of the inert gas, which results into a gas phase ion acceleration through analyzer towards the detector.

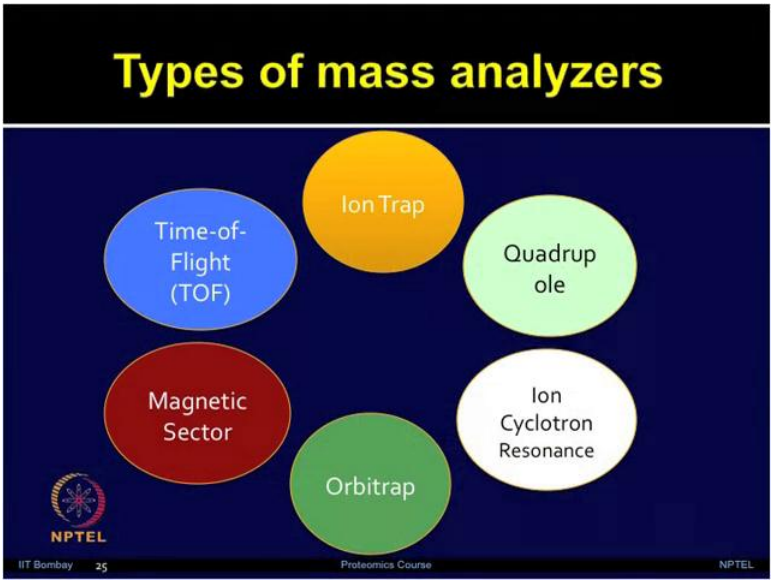
(Refer Slide time: 29:35)



You can see this process with much clarity here in this slide. The top panel showing the Taylor cone generation, and then centre it is shown that production of multiple charge ions, usually it is coupled to the MS via real time liquid separation. After discussing three important components, now let us move onto the fourth part; the mass analyzers. There are different types of mass spectrometers currently available, but for proteomics they are two configurations, which are most commonly or most oftenly used.

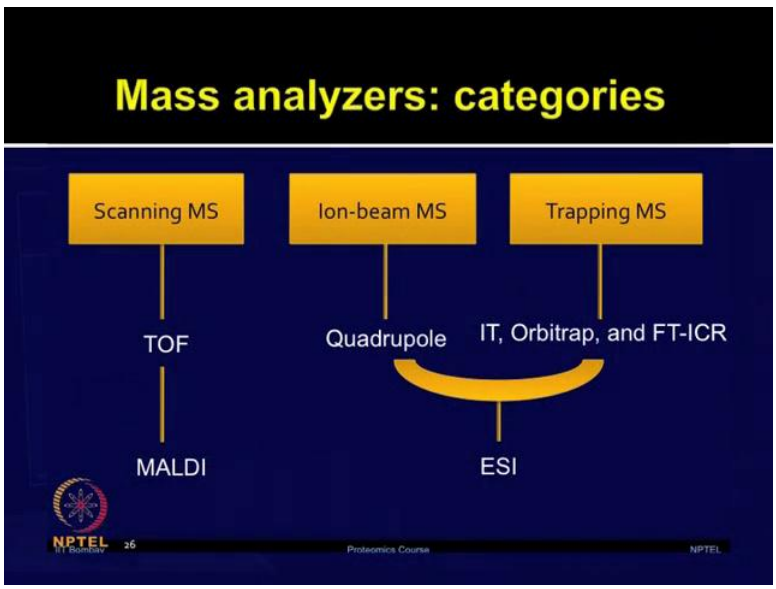
The quadruple time of flight or acute of base configuration, and hybrid linear ion orbit rap instruments. The TOF configurations separate peptides in time as they reach on the detector, so the time of flight is measured. Whereas the orbitrap mass analyzer, they measure frequency of peptide ions which are oscillating in the ion trap. Now, different type of resolution and sensitivity can be obtained from each of these configurations.

(Refer Slide time: 31:02)



In the previous lecture, I gave you an overview of different type of mass analyzers currently available, each of those have its own unique properties in mass range, analysis speed, resolution, sensitivity, the ion transmission and dynamic range. The time of flight analyzers used time flight, ion trap, orbitrap and ion cyclotron resonance, this separate ions based on their massed charge resonance frequency, where as quadrupoles or q they use oscillating electrical field for selective stabilization of ions. This just gives you an overview of various type of mass analyzers, and briefly we discussed about their principle.

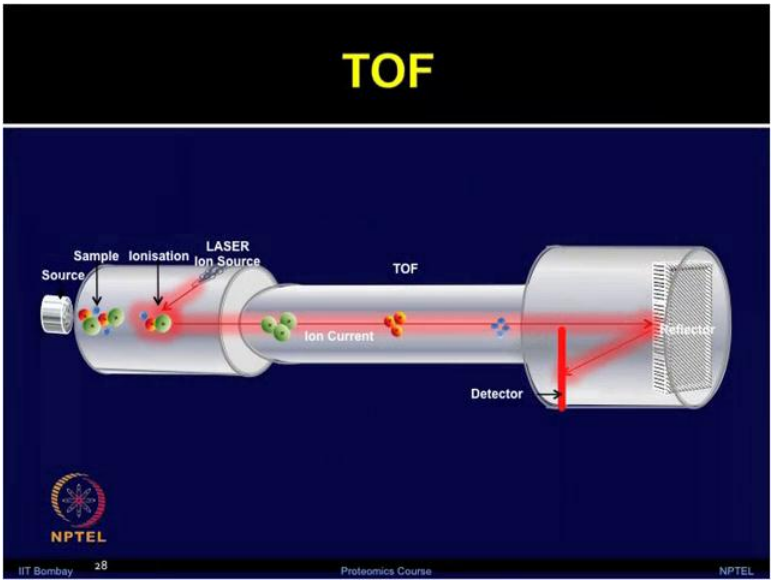
(Refer Slide time: 31:58)



Now, mass analyzers can be categorized broadly into the scanning MS, ion beam MS and trapping MS. Scanning MS is more commonly used with the TOF, which is further coupled with the Maldi ionization sources. The ion beam MS is commonly used for the quadrupoles, where as trapping MS for the ion traps, orbitraps and FT-ICR. All these can be coupled with the electrospray ionization ESI.

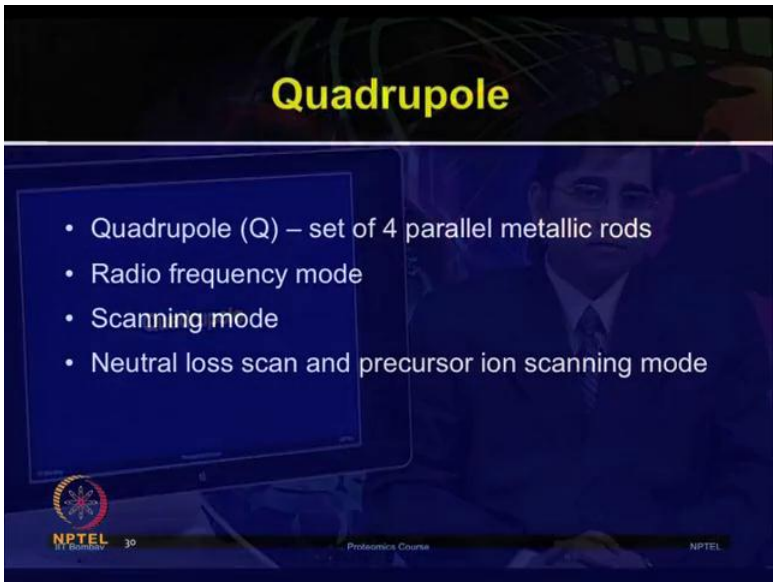
Now, some of the important mass analyzers, let us discuss a little more detail. First, talk about time of flight; which is one of the simplest mass analyzer currently used in combination with the Maldi. The TOF has immerged as one of the main stream technique for the analysis of biomolecules, and it is widely used for various applications.

(Refer Slide time: 33:04)



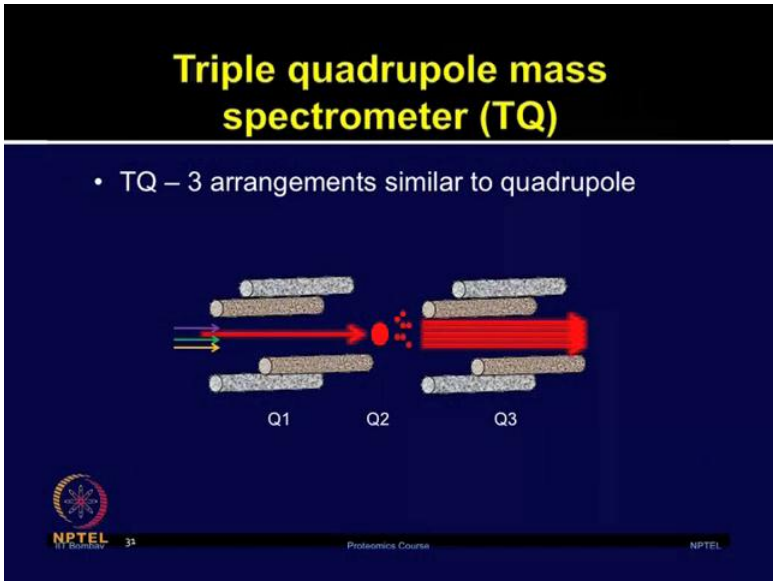
In TOF, the ions are accelerated to high kinetic energy, and due their different velocities they are separated in a flight tube. One can also use the reflector mirror, so that ions can turn around into a reflector, and it can compensate for minor differences in the kinetic energy and provide long separation. Another commonly used mass analyzer is quadrupole, the q instruments are one of the most widely used type of mass analyzers currently used in proteomics, it consist of four massed parallel metal rods, and mass separation is accomplished by the stable vibratory motion of ions in a high frequency oscillating electric field, that is created by applying direct current and radio frequency potentials to these electrodes.

(Refer Slide time: 34:11)



So as I talked, quadrupole is set of set four parallel metallic rods with opposite periods are electrically connected, the different modes one can use for this analysis RF or radio frequency mode, which allows ions of any m by z ratio to pass through scanning mode. Ions of selected mass by charge can be allowed by the detector, the potential difference applied and instrument can be used as a mass filter.

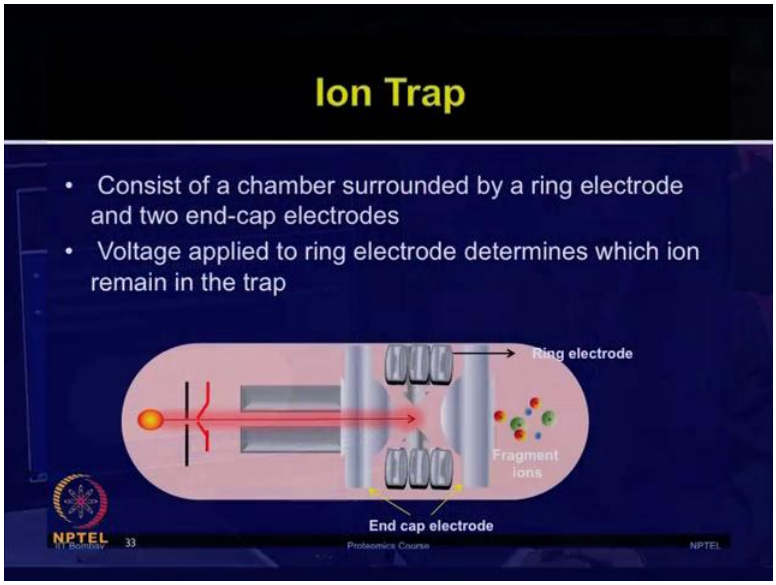
(Refer Slide time: 35:02)



The neutral loss scan and precursor ions scanning method, they are used for the phosphorylation to distinguish the phosphorylated and nonphosphorylated peptides. Now

triple quads, which is arrangement of quadrupoles is widely used for the proteomics. In triple quad, the Q1 is scans ion streams; it directs ions of selected m by z ratio into the second's quadrupole, Q2 which is collision cell. As you can see in this slide, the collision cell operates in the radio frequency mode, the fragmentation of intact peptide ions can be induced by colliding with inert gases, and then selected ions are further moved into the Q3. Q3 scans the stream of ion fragments, which are emerging from the collision cell to generate a collision induced dissociation spectrum; the mass spectrum of fragments derived from one peptide after one analysis is complete, then q one directs a different intact peptide into the collision cell.

(Refer Slide time: 36:36)

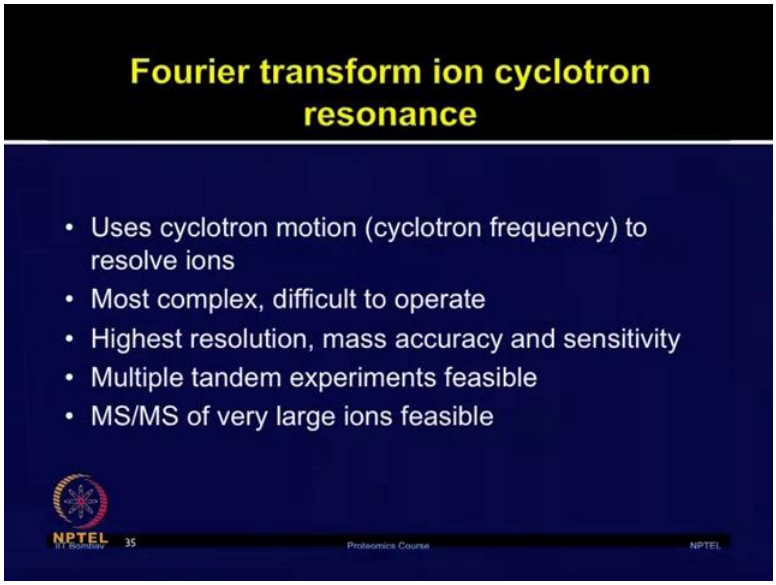


So, in this equation manner, it can process various peptides. Now, let us talk about another important mass analyzer; ion trap. The ion trap, traps ion using electrical fields and it measures by selectively ejecting them to a detector. It consist of a chamber, which is surrounded by a ring electrode and two end cap electrodes, as you can see in this figure here.

The voltage applied to the ring electrode determines which ions remain inside the trap. So, ions above a threshold of m by z ratio, they remain inside the trap and other can be ejected through a small hole. Theoretically, ion trap can provide MS n analysis, and it can also provide a mass filter. One important mass analyzer is; Fourier transform ion cyclotron resonance or FT-ICR. Due to its high resolution and MS MS capabilities,

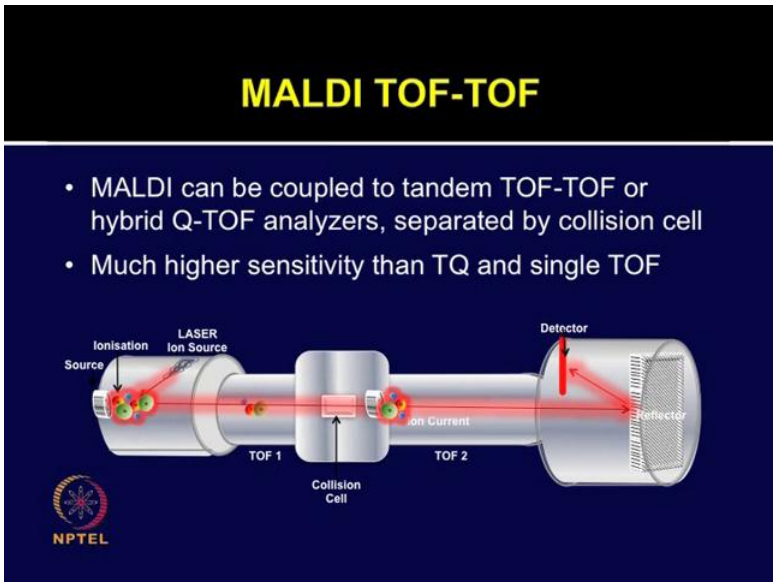
application of FT-ICR MS in combination with electrospray ionization has been employed for the large biomolecules, and now it is also used in the proteomics.

(Refer Slide time: 37:45)



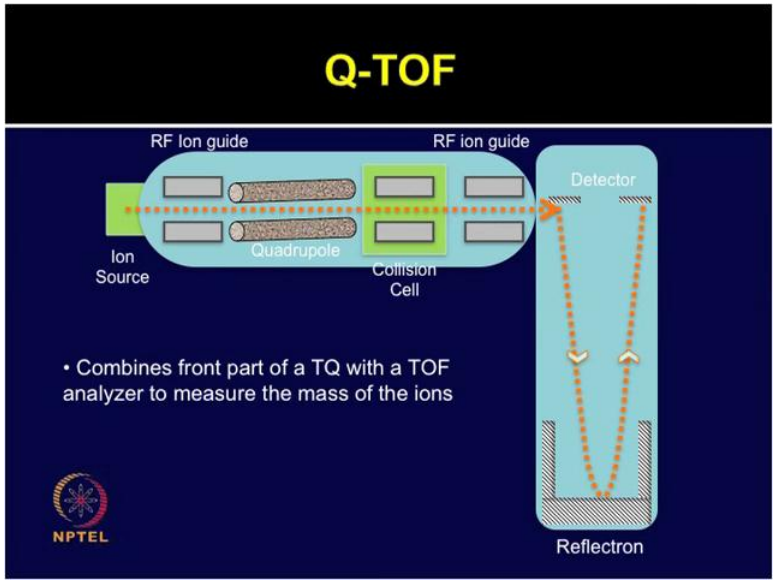
An FT-ICR MS can be considered as an ion trap system, were ions are trapped in the magnetic field. It uses cyclotron motion or cyclotron frequency to resolve the ions, although operationally it is very complex and not very easy to operate, but it provides highest resolution mass accuracy and sensitivity. It also provides a capability of multiple tandem experiments, and MS MS of very large ions are possible by suing FT-ICR MS. So, we have discussed all the important components of liquid chromatography mass spectrometry, now one can apply these configurations in tandem, one can select different type of mass analyzer and use it based on their applications.

(Refer Slide time: 38:55)



So, now we will look at some of the popular hybrid MS and MS MS configurations. Maldi TOF TOF, that is one of the widely used tandem MS configuration, in this one the TOF Tof or true time of flight tubes, as well as hybrid quadrupole time of flight analyzers can be used. We have discussed the Maldi TOF TOF system in some more detail in the previous lecture,

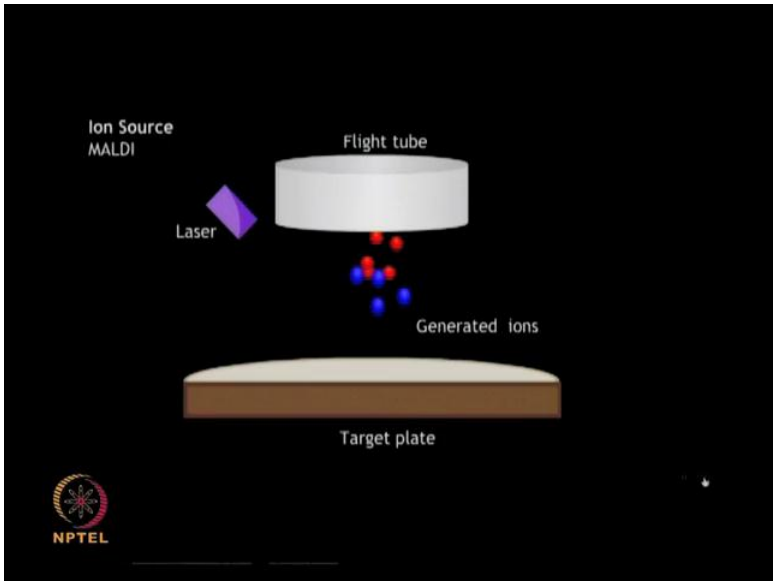
(Refer Slide time: 39:27)



Shall move on to some other configurations which is Q-TOF. The Q-TOF, it combines front part with quadrupole or it can be triple quad TQ along with the TOF analyzers to

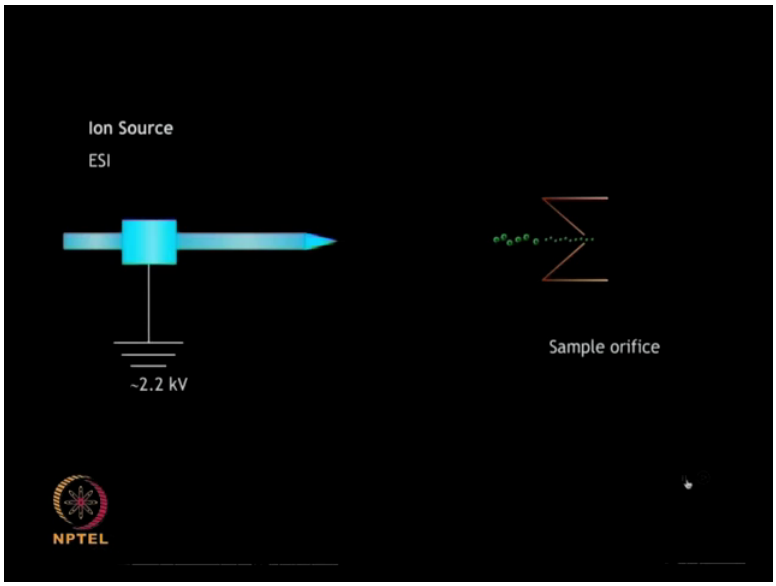
measure the mass of ion. Some of the important concepts involved in ionization, mass analyzers and tandem MS, I will describe those in following animation.

(Refer Slide time: 40:21)



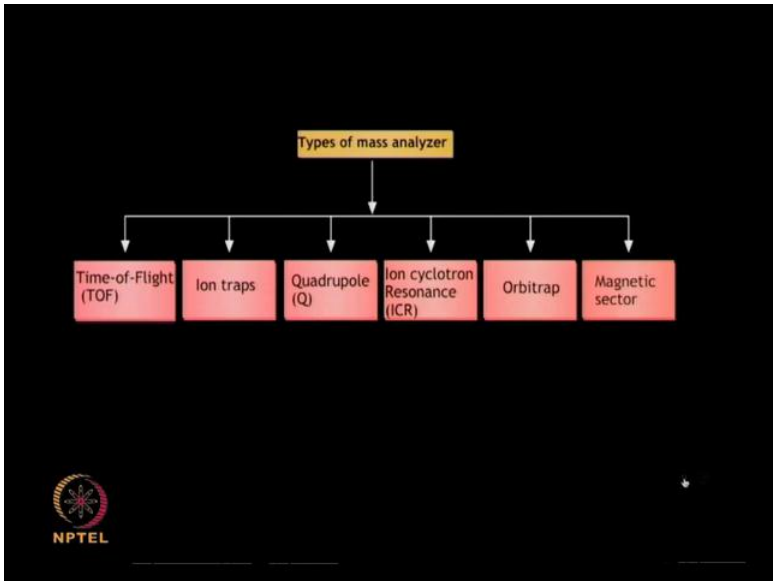
Tandem mass spectrometry, let us first discuss the individual components; the ionization source, mass analyzers, and then how one can make use of various type of ionization sources, and mass analyzers in tandem for the mass spectrometry analysis. The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum, this has been made possible by the development of soft ionization techniques, like matrix assisted laser desorption ionization Maldi and electrospray ionization ESI. In Maldi, the analyte of interest is mixed with an aromatic matrix and bombarded with short pluses of laser. The laser energy is transferred to the analyte molecules, which undergo rapid sublimation into gas phase ions.

(Refer Slide Time: 41:26)



Another electrospray ionization technique ESI; in ESI 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, which creates a mist of droplets. These ions are then accelerated towards the mass analyzer depending upon their mass to charge ratio.

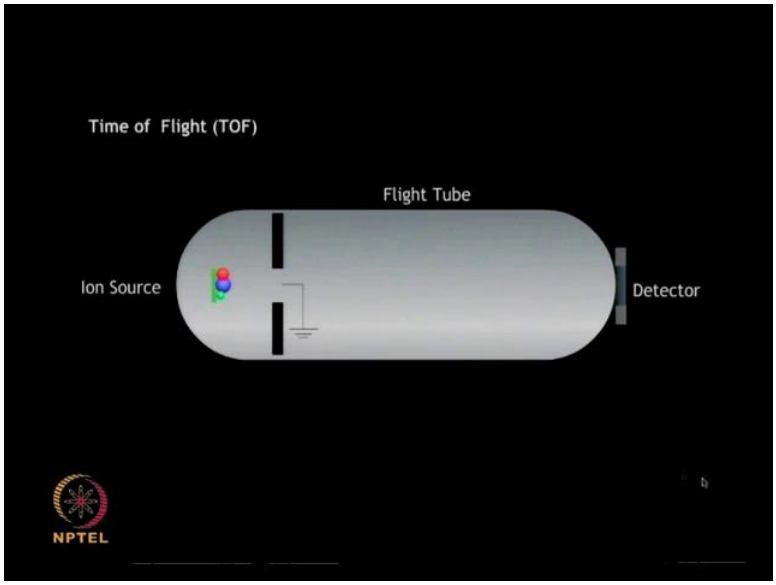
(Refer Slide Time: 42:06)



Now, let us discuss about mass analyzers; the mass analyzer resolves ions produced by the ionization source on the basis of their mass to charge ratios, there are various

characteristics; such as resolving power, accuracy, mass range and speed, which determine the efficiency of these mass analyzers.

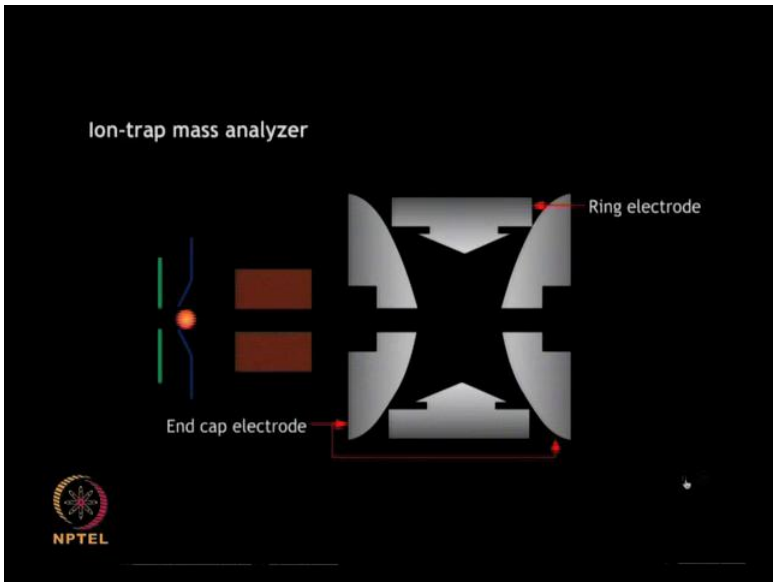
(Refer Slide Time: 42:58)



The commonly used mass analyzers include; time of flight TOF, ions traps, quadrupole, ion cyclotron resonance, orbitrap, magnetic sector etcetera. The time of flight analyzer accelerates charged ions generated by the ionization source along a long tube, known as the flight tube. Ions are accelerated at different velocities depending on their mass to charge ratios.

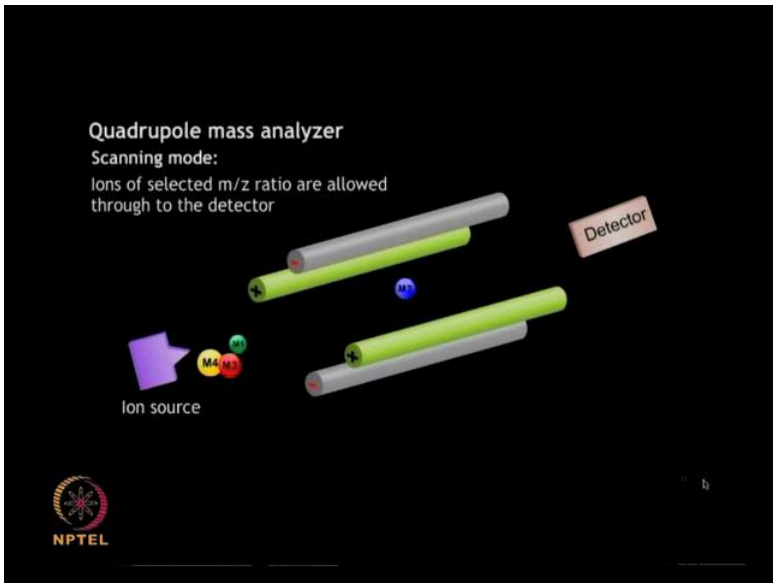
Ions of lower masses are accelerated to higher velocities and reach to the detector first. The TOF analyzer is most commonly used with the Maldi ionization source, since Maldi tends to produce singly charged peptide ions. The time of flight, under such circumstances is inversely proportional to square root of molecular mass of the ion.

(Refer Slide Time: 43:59)



An ion trap makes use of a combination of electric and magnetic fields, and captures ion in a region of a vacuum system or tube. It traps ions using electric fields, and measure the mass by selectively ejecting them to a detector.

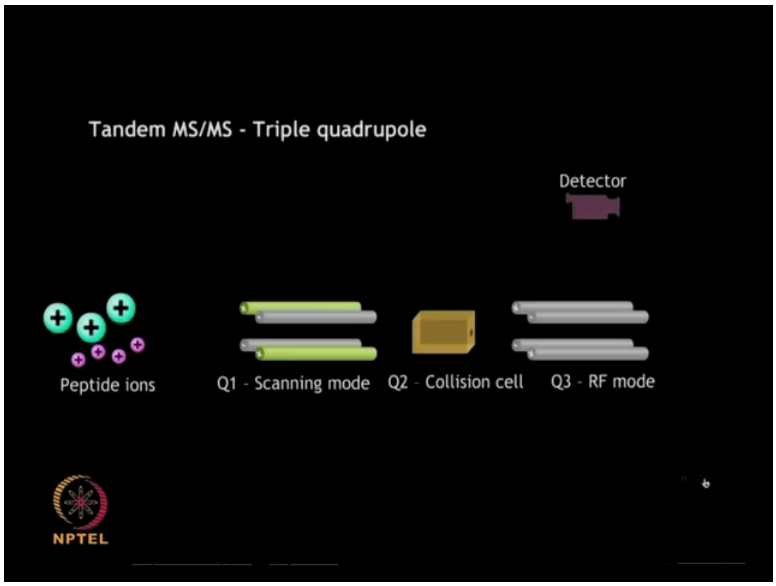
(Refer Slide Time: 44:29)



Quadrupole mass analyzers, they use oscillating electrical fields to selectively stabilize, or destabilize the paths of ions through a radio frequency or RF quadrupole field. The RF mode allows ions of any m by z ratio to pass through, so there are two modes in which the quadrupoles can be operated; the radio frequency or the scanning mode. The RF

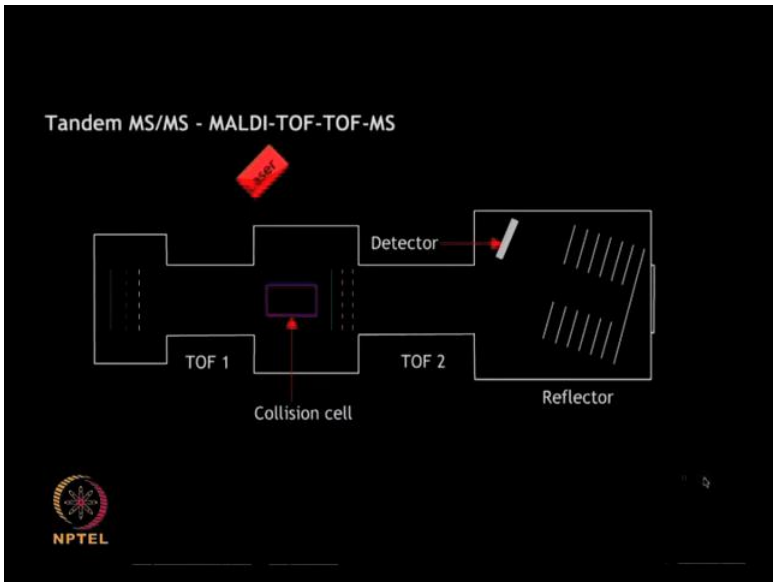
mode can allow all the ions to pass through whereas, the scanning mode, the quadrupole analyzer selects ions of a specific m by z value as defined by the user. A range can be entered, in which case only specific ion satisfying the criteria moves towards the detector and rest can be filtered out.

(Refer Slide Time: 45:58)



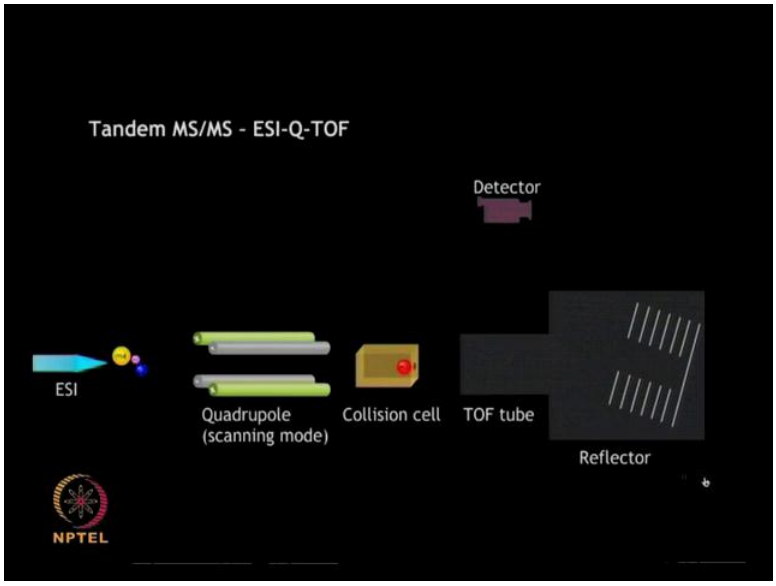
The triple quadrupole consist of two sets of parallel metallic rods interspersed by a collision cell, the first quadrupole scans the ions coming from the ionization source, and allows only ions of a particular m by z ratio to pass trough. Once the ions are selected, these ions enter the collision cell where they are fragmented by collision against an inert gas, like argon. The smaller fragments then enter the third quadrupole, which scans all the ions in a radio frequency or RF mode to generate a spectrum based on the varying behaviour of ions in an oscillating electrical field.

(Refer Slide Time: 47:11)



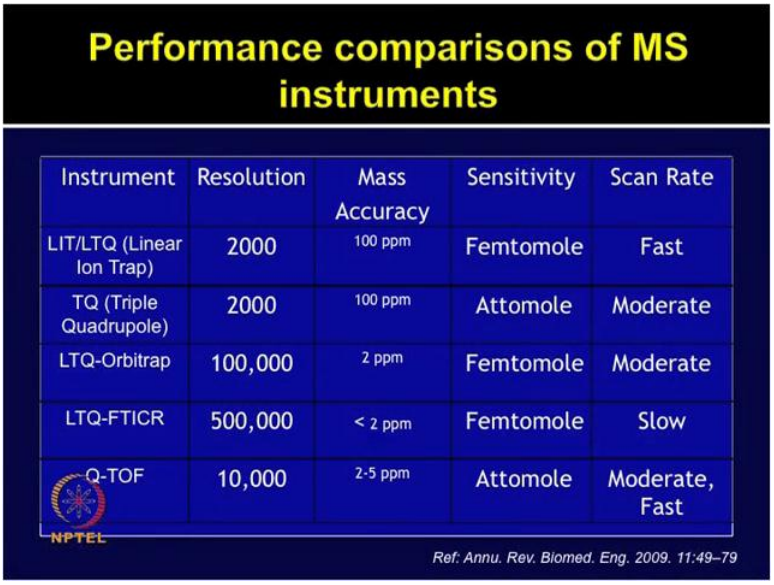
The different type of tandem MS MS configurations; such as Maldi TOF Tof. The Maldi TOF TOF MS is a common tandem MS configuration, in which the ions are first resolved on the basis of their time of flight in the first TOF analyzer; the selected ions enter the collision cells where they are further fragmented. The fragmented ions are accelerated, and further resolved on the basis of their m by z values in the second time of flight tube after which they can be detected.

(Refer Slide Time: 48:12)



ESI-Q-TOF is another common tandem MS configuration that first selects ions in radio frequency mode. Selected peptides are fragmented in collision cells, and resulting ions are accelerated and resolved on the basis of their time of flight.

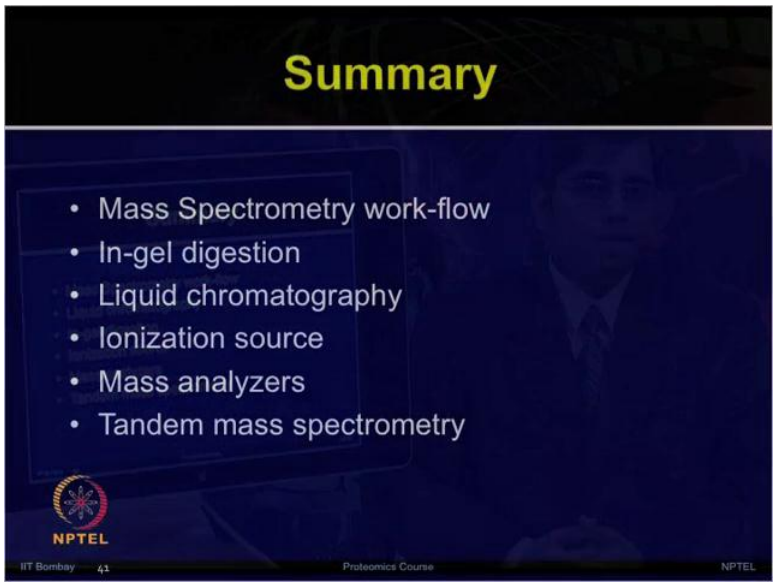
(Refer Slide Time: 48:43)



So finally, there are so many mass spectrometers currently available commercially. So, now, depending on individual's application one can select different type of configuration. Based on an excellent review from (( )) and colleagues, I provided this performance comparison of MS instruments in following slide.

Here you can see the linear ion traps are LIT or l TQ's, they have resolution of 2000, mass accuracy 1000 ppm, mass accuracy 100 ppm, sensitivity femtomole and scan rate is very fast. The triple quadrupoles or TQ's with resolution of 2000, mass accuracy 100ppm, sensitivity is attomole and scan rate is moderate. The LTQ-Orbitraps, they can provide high resolution 1000000, mass accuracy 2 ppm, sensitivity in femtomole and scan rate is moderate to low. LTQ-FTICR; they can provide very high resolution of 5000000, mass accuracy less than 2 ppm, sensitivity in femtomole range, slow scan rate. The quadrupole time of flight, they provide resolution more than 10000, mass accuracy 2 to 5 ppm, sensitivity in atom mole range and scan rate is moderate to fast.

(Refer Slide Time: 50:35)



I hope, in today's lecture you got various concepts together how one can use in use in mass spectrometry experiment, we have discussed individual components of In-gel digestion, liquid chromatography, ionization source, mass analyzers, and how they can be used in tandem. And then finally, we compared various commercial configurations currently available, so that one can get an idea that what type of resolution sensitivity (( )) speed etcetera, they can provide. We will continue our discussion on mass spectrometry and data analysis in following lecture. Thank you.