Mass Spectrometry Based Proteomics
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Mod 02 Lecture Number 08

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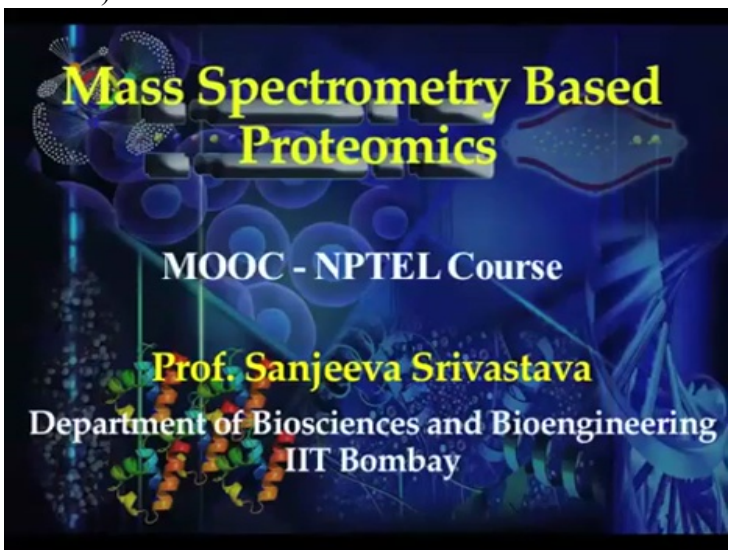

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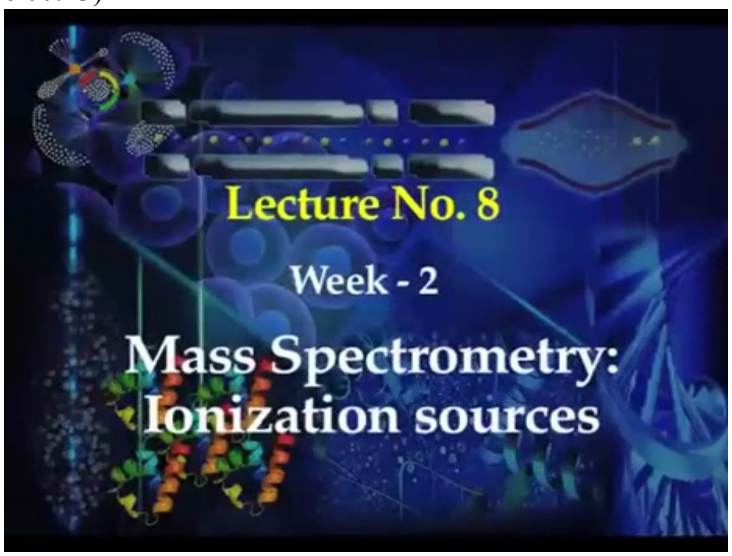

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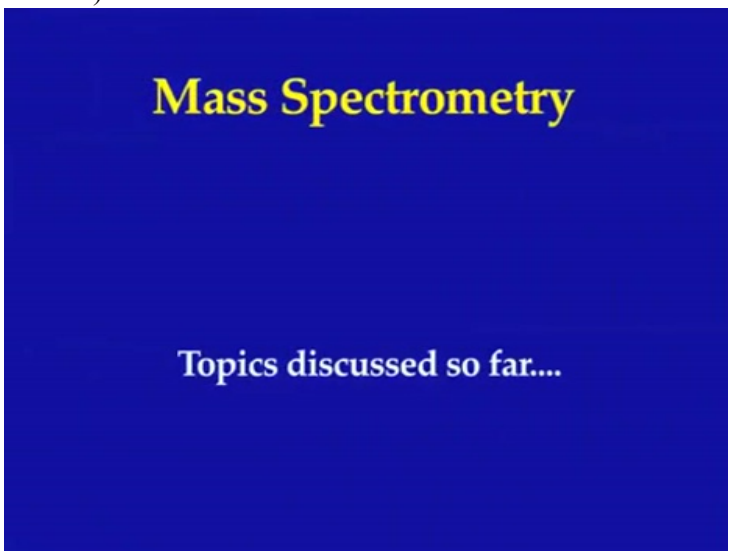

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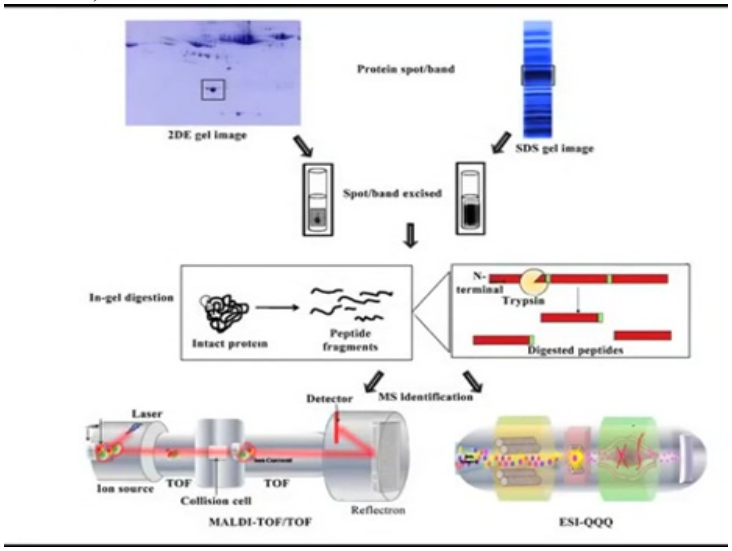

This slide gives you an overview of in-gel digestion process. We will talk in more detail about each of these steps as we go along in the next lectures talking more specifically about more specific types of mass spectrometry techniques.

Today I am giving you sort of an overview various types of concepts and various steps involved in performing the mass spectrometry experiments.

So in-gel digestion, regardless of you have done the gel based proteomics or you want to perform the gel-free proteomics, you can resolve the protein on the gels, simplify the proteome, excise either the spots or the bands of your interest and then subject those to the in-

gel digestion or the in-solution digestion, so that proteins can be cleaved into small peptides and these can be then further analyzed by using ionization source and mass analyzers.

The slide shows you an overview of workflow involved in performing MS based experiment.

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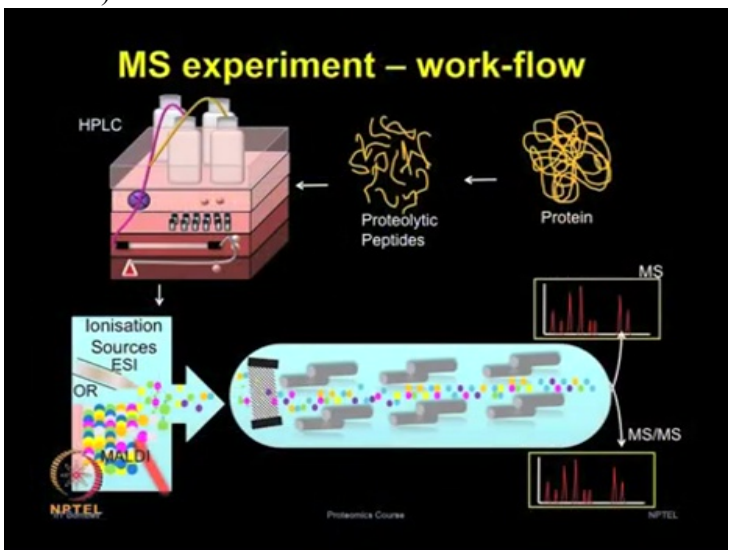

First of all, you can pre-fractionate your sample by using liquid chromatography. Or one can also try different methods of doing the pre-fractionation and then applying that for doing the further MS analysis.

After pre-fractionation, then in-gel digestion or proteolytic cleavage can be performed by using various enzymes such as Trypsin and then the sample can be injected into the ionization source, whether it can be ESI or MALDI most commonly used for proteomics as well as other type of ionization sources.

And then these ions can be further resolved into the mass analyzers. Again there are various types of mass analyzers. And then data can be analyzed in MS or MS/MS mode.

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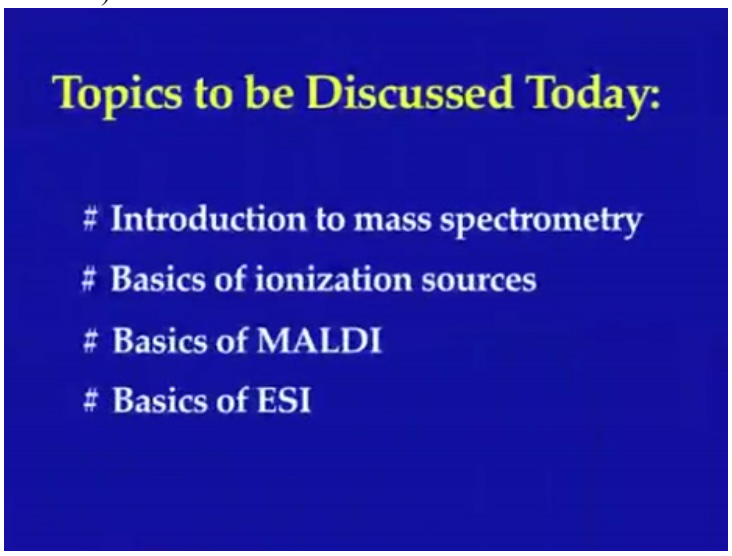

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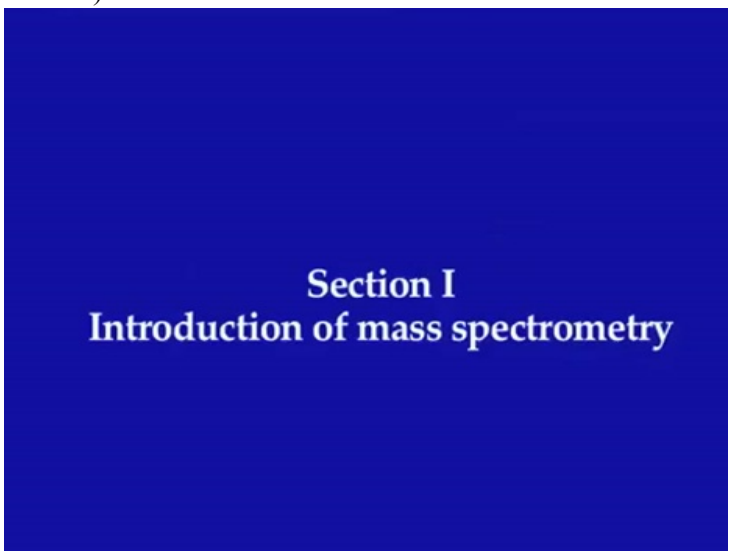

Now let's talk about different parts of the mass spectrometer.

So the major components include the sample inlet, the ion source, the mass analyzer, the detector, signal processing components and data output. Let's look at each of these components a little more detail.

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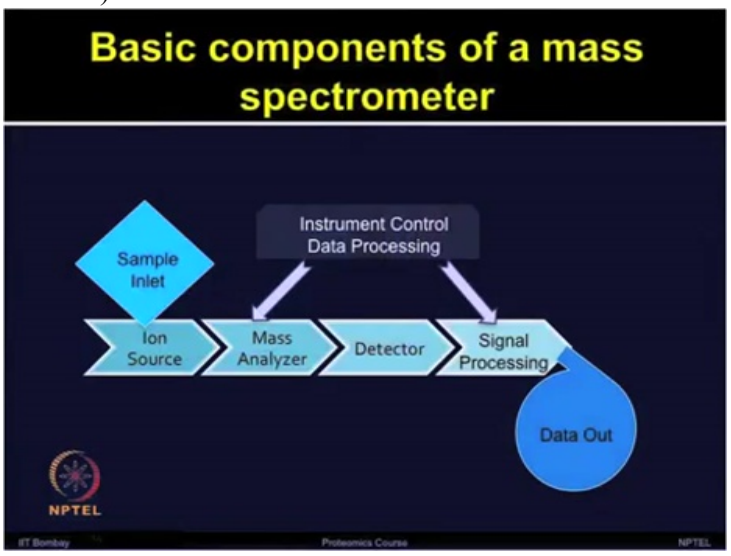

Sample inlet, it transfers the samples into the ionization source.

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

Mass analyzers, it separates and analyses 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.

Now 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 the mass-resolved ions.

And then we need 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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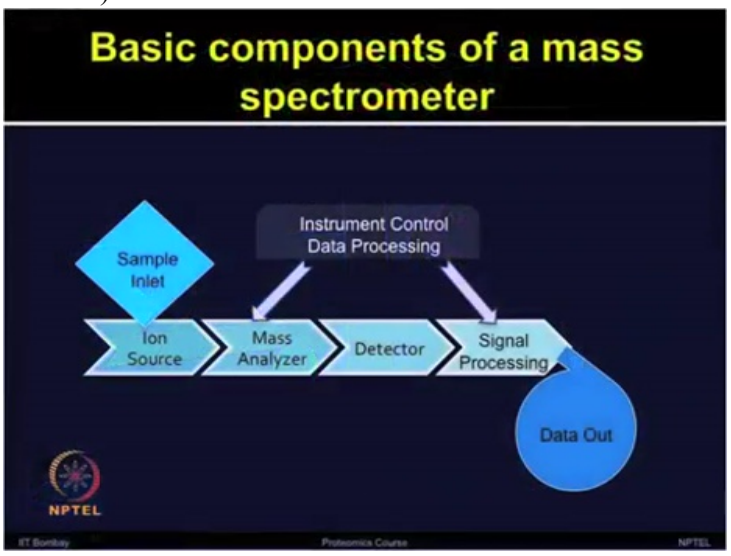

So although there are 3 major components involved, the ionization source, mass analyzer and detector, but then there are some accessories components which are also equally important for doing the mass spectrometry based experiments.

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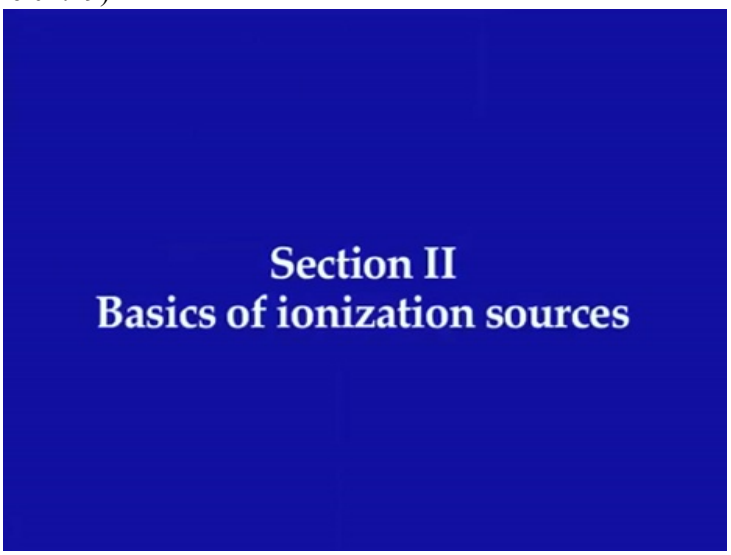

So, what are different ionization sources involved in the MS analysis? The success of mass spectrometry experiment lies in efficiency of converting a neutral compound to gas phase ionic species. So we have various types of options currently available.

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 which one wants to investigate.

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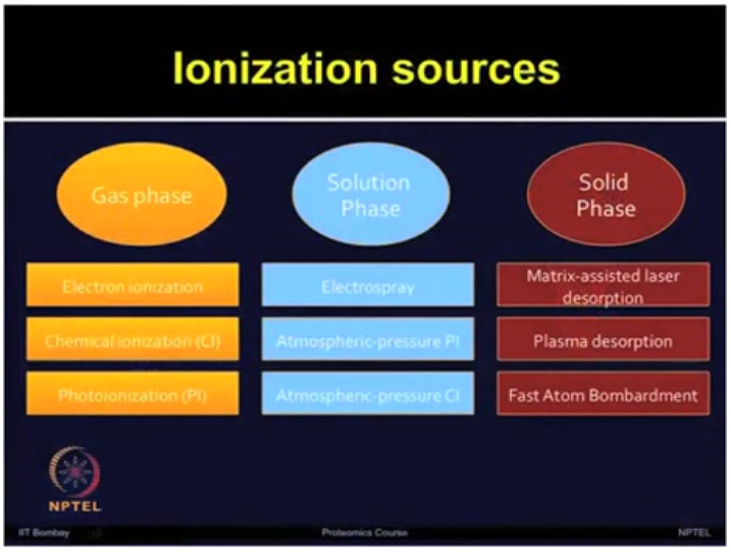

In the gas phase electron ionization, chemical ionization and photo ionization, these are the more commonly used ionization sources.

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.

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

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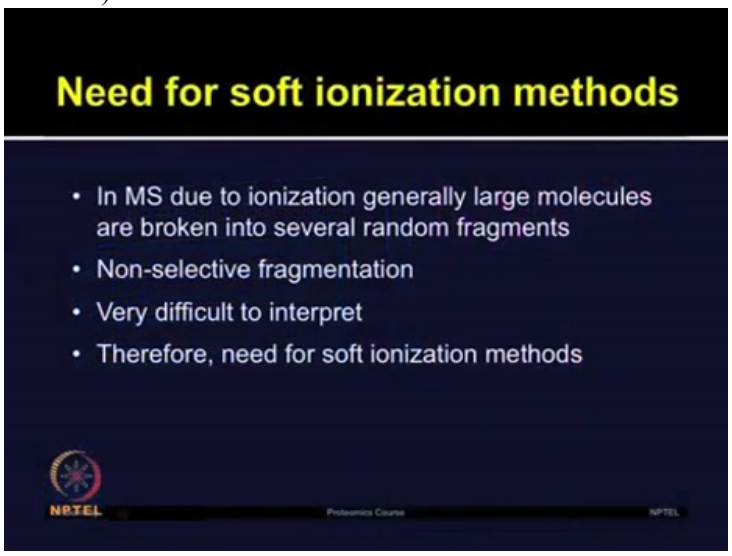

But these processes are too energy-rich to ionize intact large biomolecules and they lead to the unpredictable analyte decomposition. So for proteomic application, there was need for the soft ionization methods in mass spectrometry.

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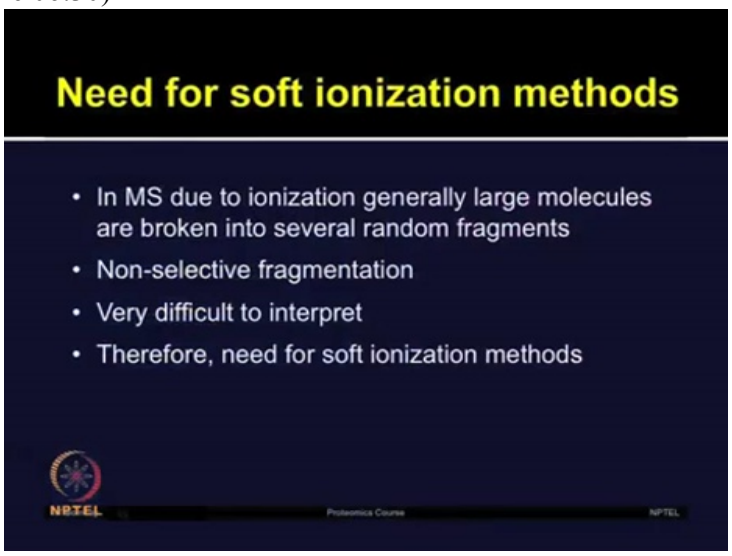

These are non-selective fragmentation. The hard ionization is very difficult to predict. So, therefore it led to the need of soft ionization methods in proteomics. What are different properties of ionization sources?

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

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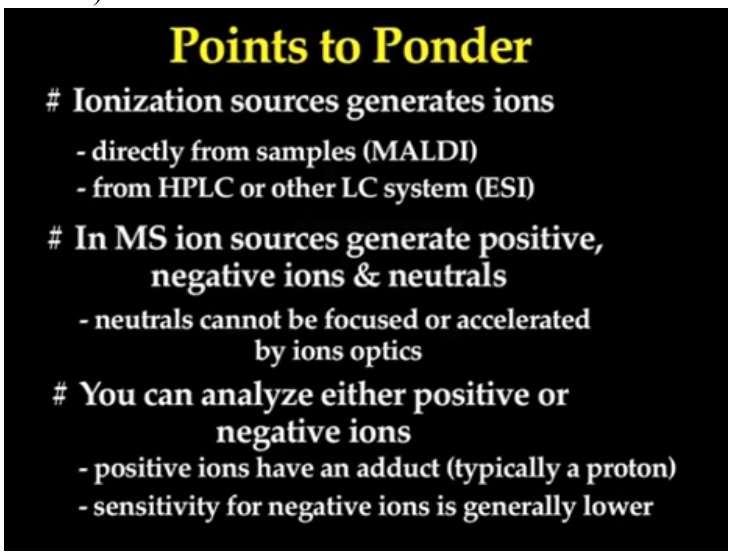

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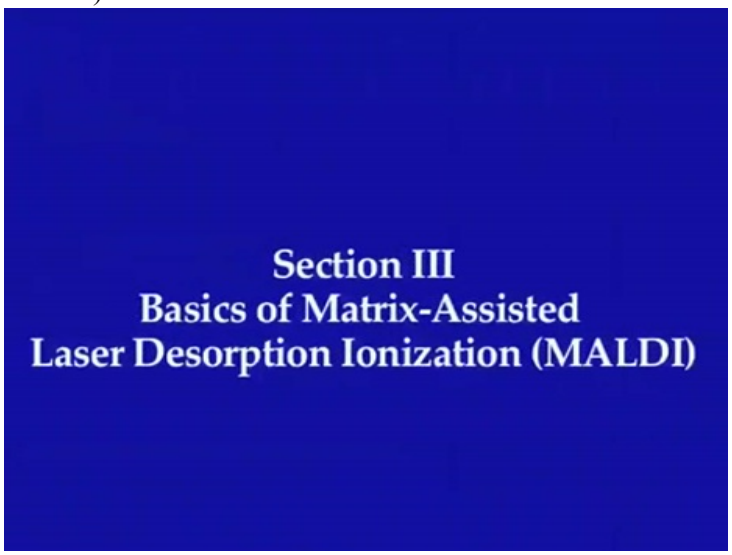

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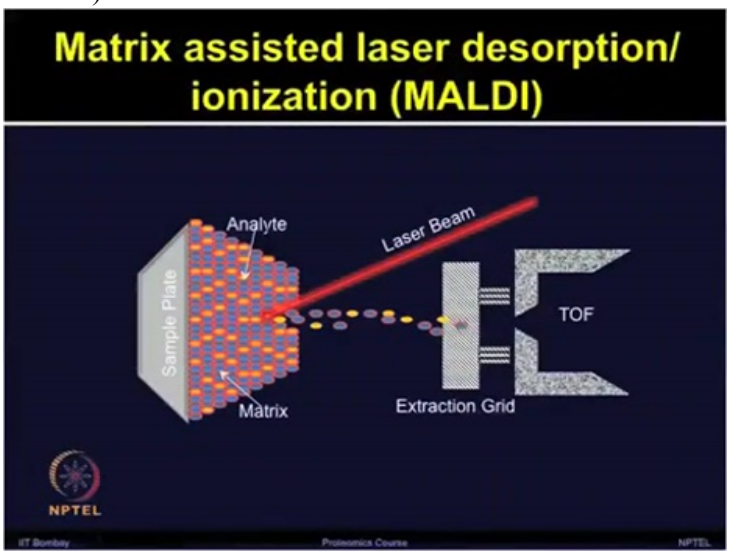

The ionization source is responsible for converting analyte molecules into gas phase ions in vacuum. This has been made possible by the development of soft ionization techniques, which ensure that the non-volatile protein sample is ionized without completely fragmenting it. Most commonly used ionization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

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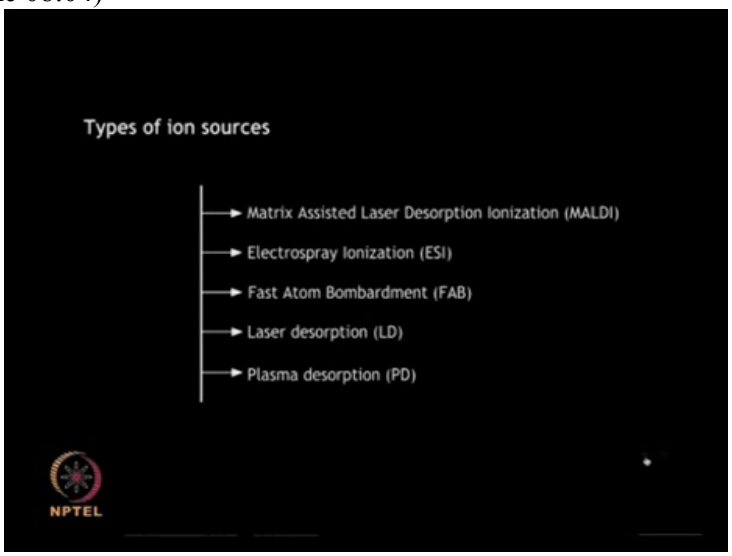

Additionally there are other ionization sources such as Fast Atom Bombardment FAB, Laser Desorption LD, Plasma Desorption PD.

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

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So MALDI is an efficient process for generating gas phase ions for peptides and proteins for mass spectrometric detection. MALDI is one of the most widely used ionization techniques currently applicable in the proteomics area.

This ionization method was independently developed by two scientists; Koichi Tanaka and Hillenkamp. Tanaka also received a Nobel Prize for his novel contribution into soft ionization technique such as MALDI.

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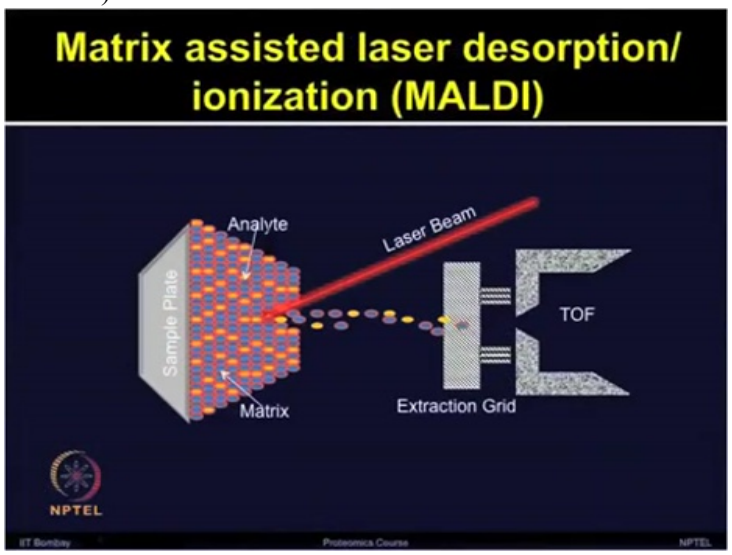

Let's first talk about Matrix-Assisted Laser Desorption Ionization or MALDI. So analyte or proteins of interest are mixed with the matrix which is usually an aromatic compound. There

are various types of matrices available, which we will talk about in detail when we come to the sample preparation and matrix selection.

But just for your reference you can use 2-5-dihydroxy benzoic acid, we can use sinipinic acid and there are several other choice.

Once you have selected a matrix for the experiment then analyte and matrix can be dissolved in an organic solvent after which then it can be placed on a metallic target.

As you can see in the slide, the first left section shows you how to place the analyte and matrix together on the sample plate.

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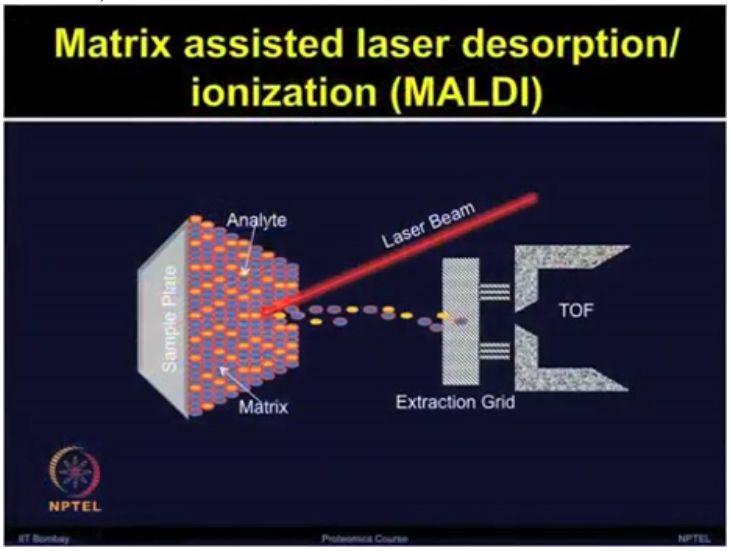

Now, once you have placed the matrix and the analyte on the target plate you can put that in the vacuum chamber and apply high voltage.

Now these crystals are targeted with the short laser beams, as you can see in the slide, then rapid sublimation can convert analyte into the gas phase ions.

Now these ions once generated, they can accelerate away from the target plate through the mass analyzer which is Time of Flight TOF tube and they can reach the detector.

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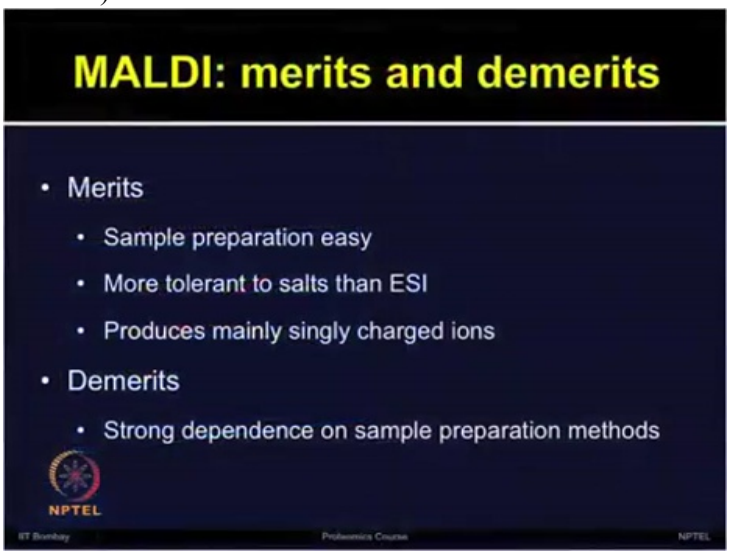

There are various advantages and disadvantages of using MALDI as an ionization source. The sample preparation is very easy. The MALDI provides high tolerance to salt as compared to electrospray ionizer methods.

MALDI produces single charged species. Most analytes can accept the single photon. The single charged characters can result in some molecules having large mass-to-charge values. So, therefore MALDI is typically integrated with the TOF mass analyzers, which can provide the m by z range for the large ions as well.

Now these are various merits of using MALDI. Obviously, it has to be connected with the TOF. Now there are various demerits of using this system. There is strong dependence on how to prepare good sample for this analysis. So sample preparation methods heavily influence the uh, spectrum generated from these experiments.

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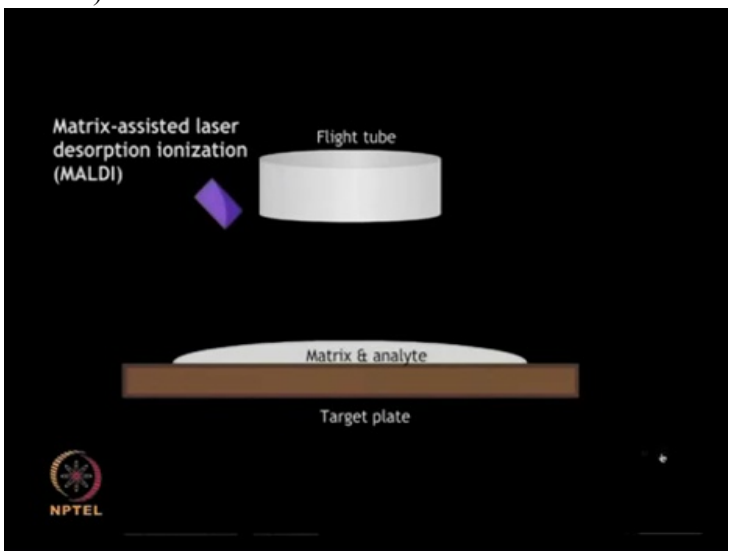

In MALDI, analyte of interest are mixed

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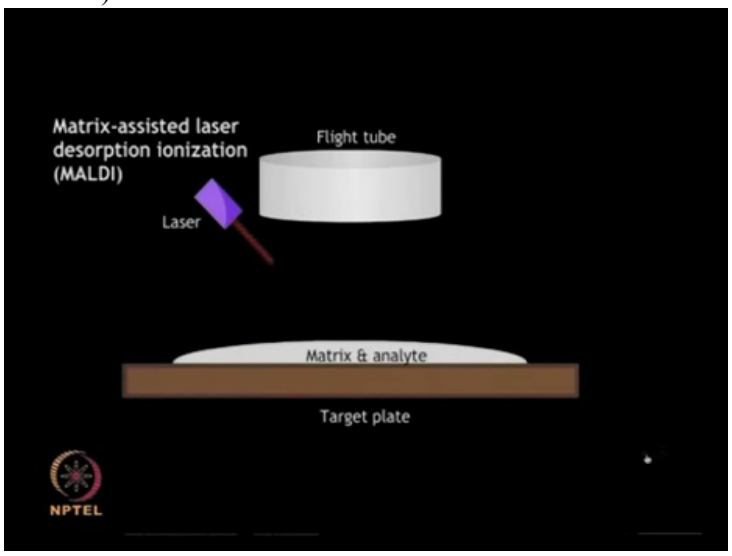

…with an aromatic compound such as alpha-Cyano-4-hydroxycinnamic acid or Sinapinic acid This is dissolved in an organic solvent and placed on a metallic sample plate. The evaporation of solvent leaves the analyte embedded in the matrix.

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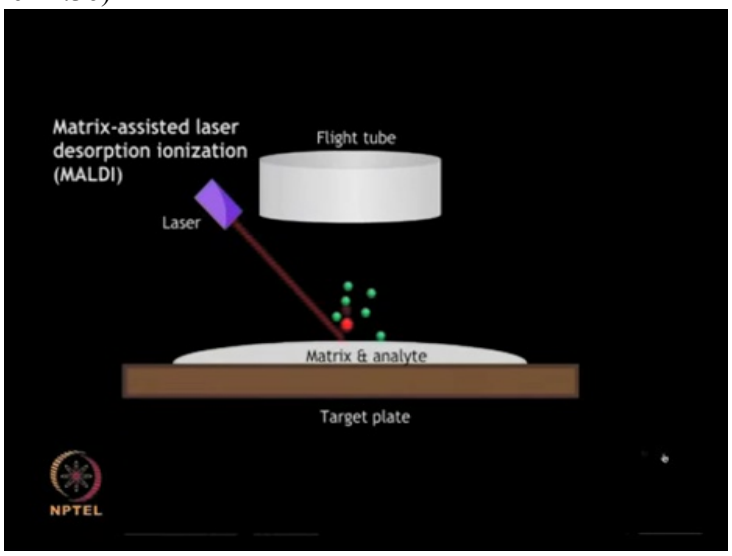

The target plate is placed in a vacuum chamber with high voltage and short laser pulses are applied.

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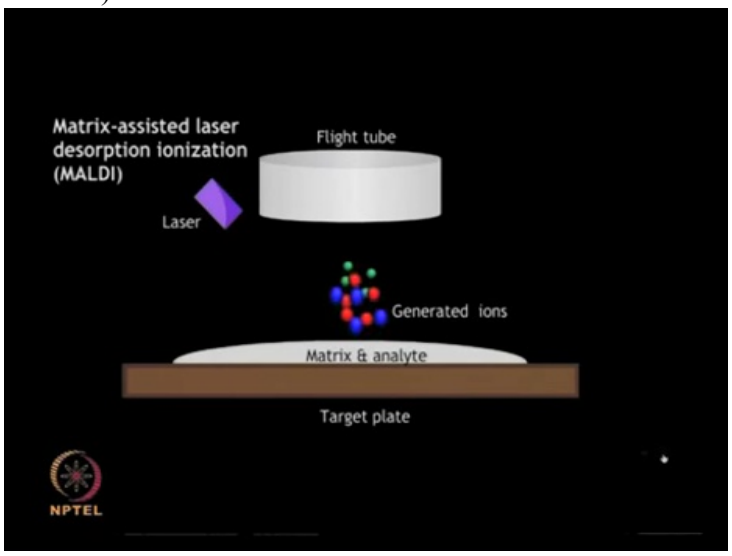

The laser energy gets absorbed

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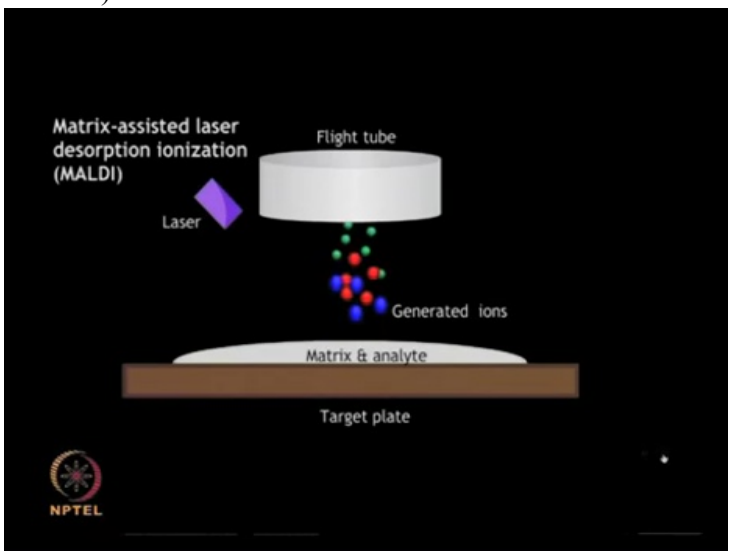

… by the matrix and is transferred to the analyte molecules which undergo rapid sublimation resulting in gas phase ions. These ions then accelerate towards the mass analyzer based on their mass-to-charge ratio.

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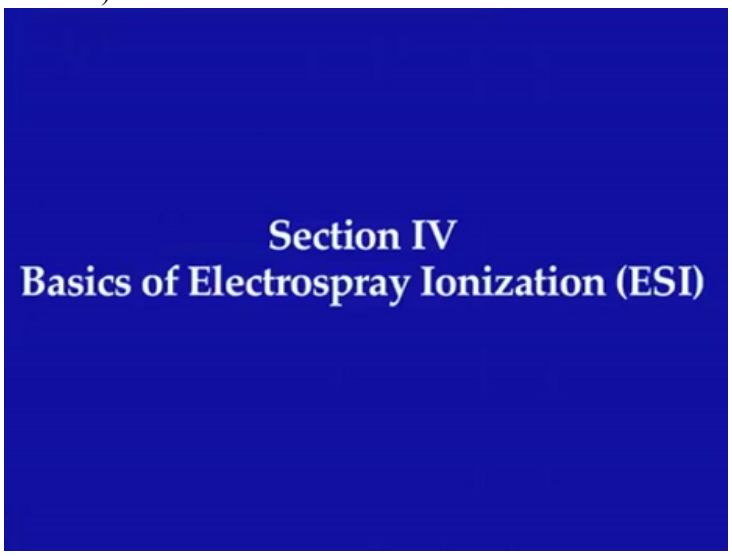

So ESI, it requires sample of interest to be in solution.

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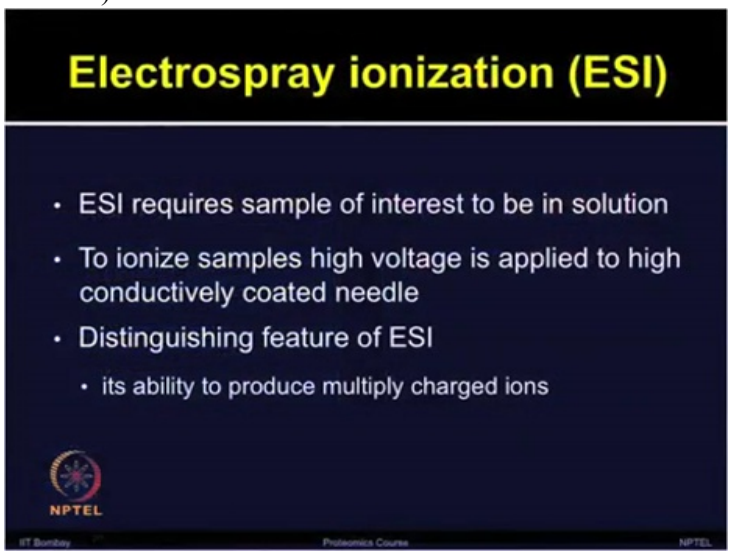

To ionize the samples, high voltage is applied to high conductively coated needle. So this voltage results into the sample becoming charged, either positive or negative. The positive ions are primarily used for the analysis of proteins.

The distinguishing features of Electrospray Ionization, includes its ability to produce multiple charge ions. The number of charges that can be accepted by a particular molecule depends on its basicity and its size.

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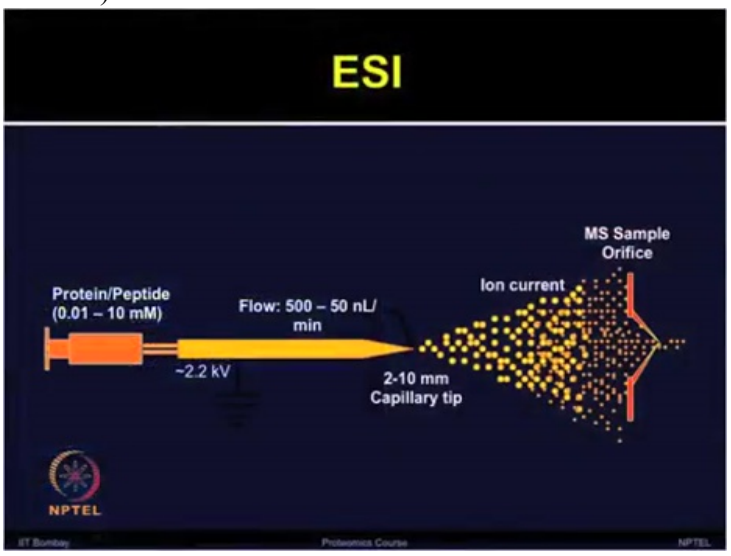

Now here you can get an overview of the process involved in Electrospray Ionization. The small droplet of solutions are generated by the Taylor cone which contains the peptide analyte. Protons from the acidic solution provides droplets the positive charge so that it can move from the needle to the negatively charged instrument.

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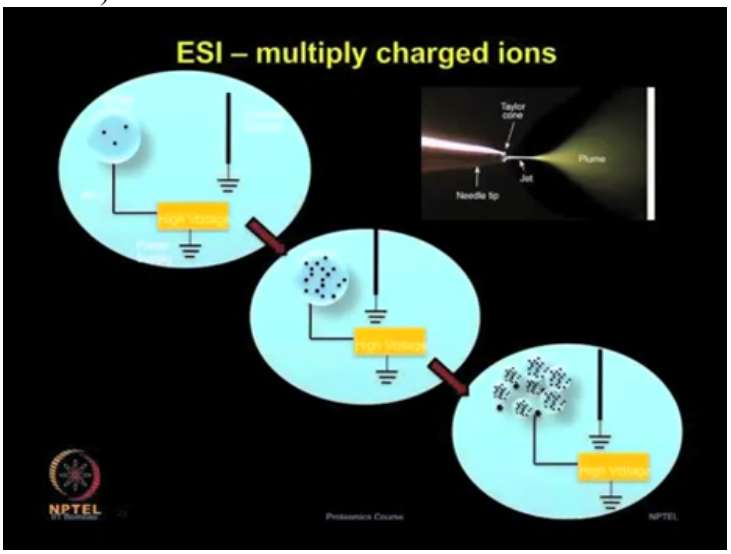

You can see this process with much clarity here in this slide.

The top panel showing the Taylor cone generation, and the center, it is shown that production of multiple charged ions. Usually it is coupled to the MS via real-time liquid separation.

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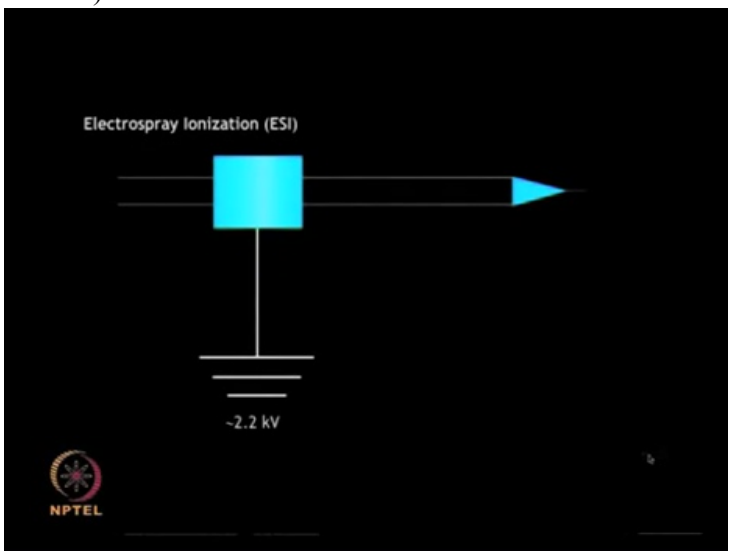

In Electro Spray Ionization the sample is present in the liquid form and ions are created by spraying a dilute solution of the analyte

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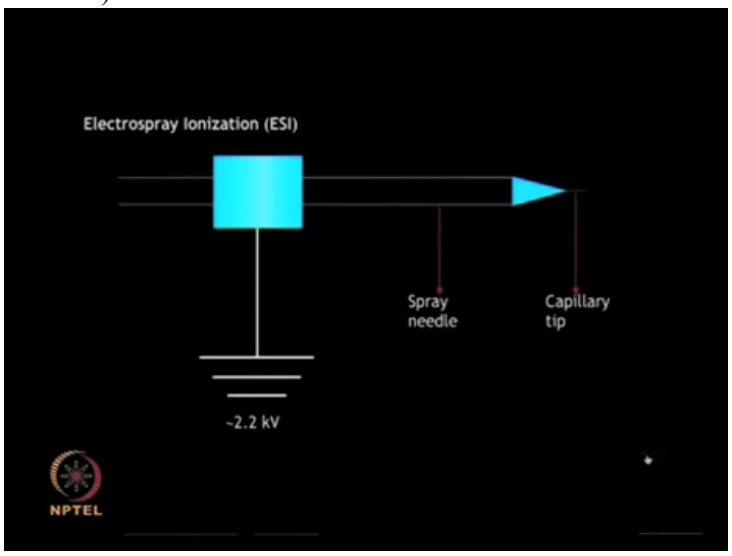

at atmospheric pressure from the tip of a fine metal capillary creating a mist of droplets

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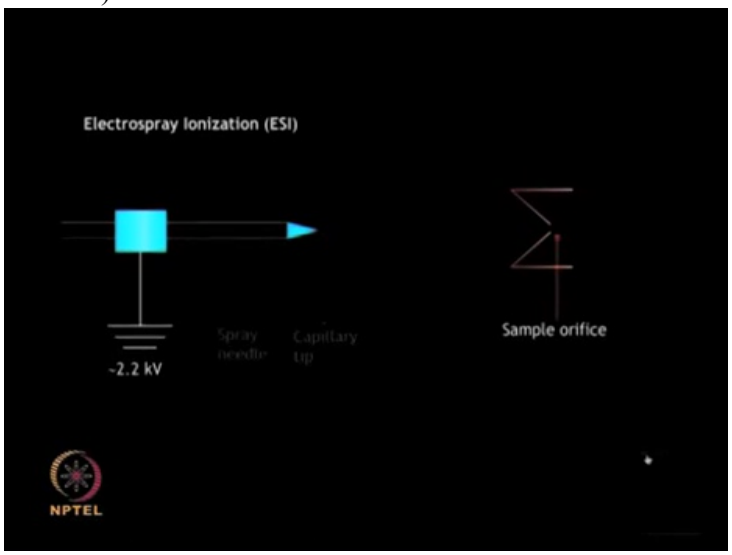

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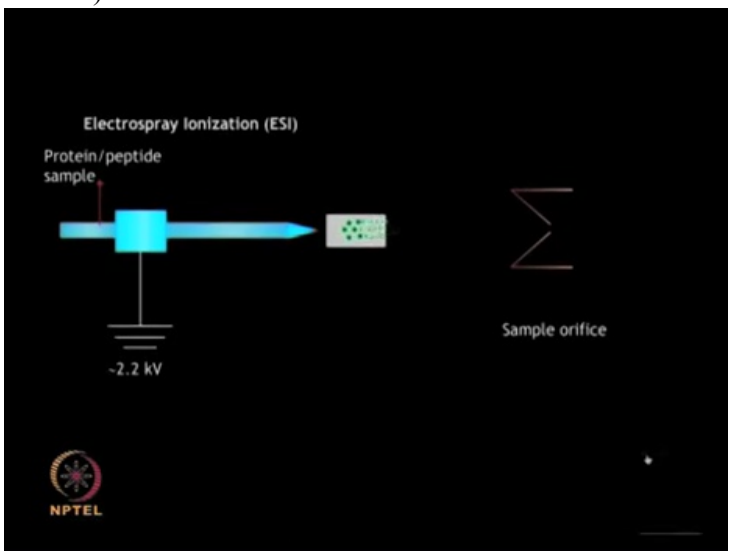

The droplets are formed

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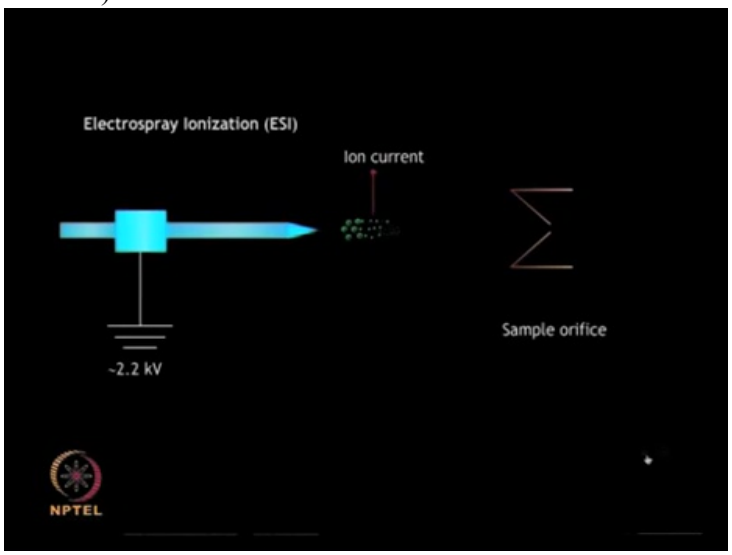

in a very high electric field and become highly charged

As the solvent evaporates the peptide and protein molecules in the droplet pick up one or more protons

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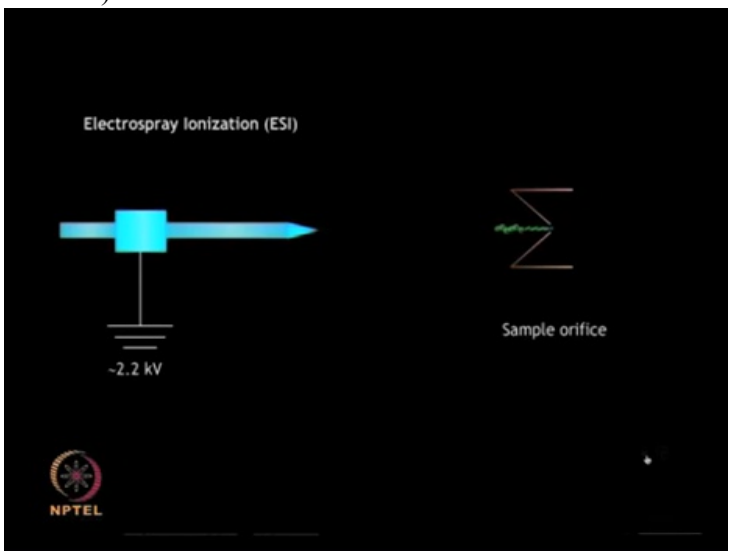

…. from the solvent to form charged ions These ions are then accelerated towards the mass analyzer depending upon their mass and charge

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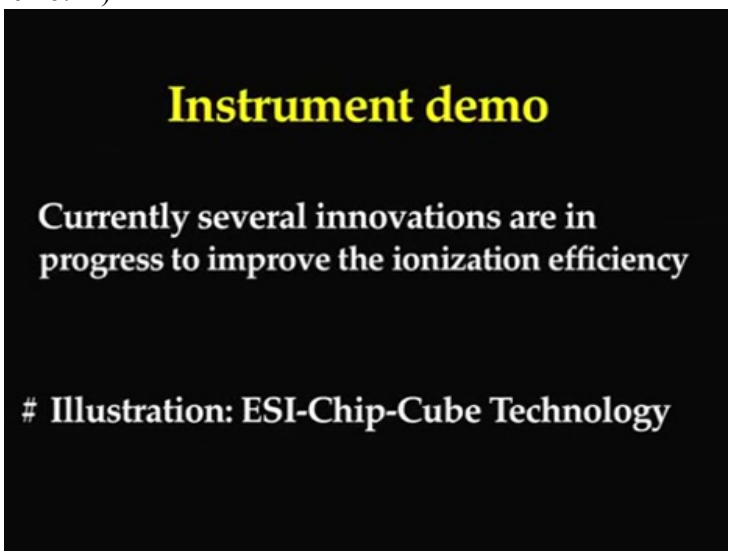

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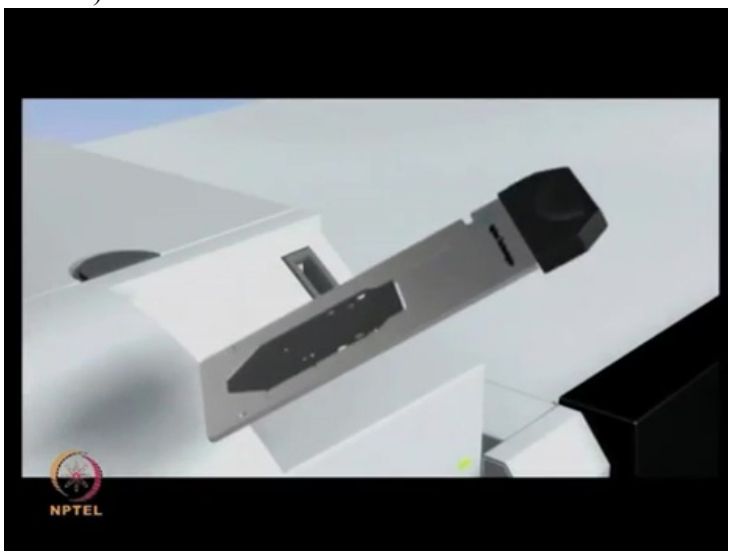

The Agilent HPLC-Chip integrates enrichment and analytical columns, micro-valve connections and

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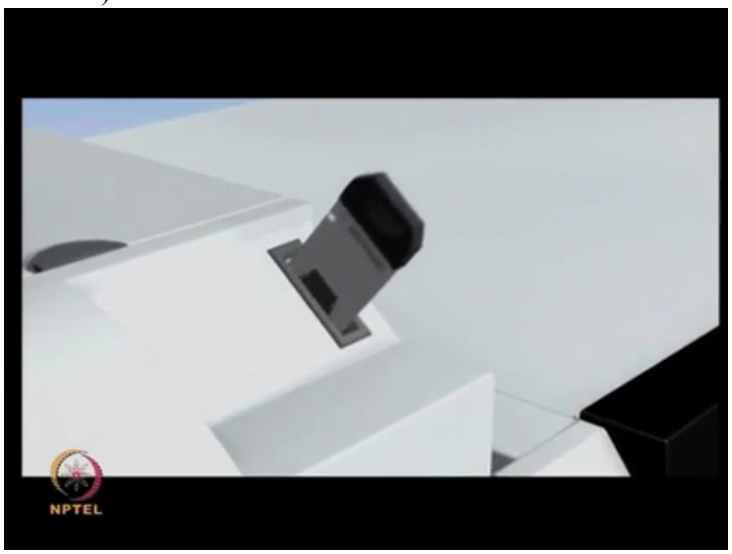

… metal-coated, nano-electro spray tip on an inert multilayer polyamide film and is smaller than a credit card.

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The compact architecture of the Agilent HPLC-Chip reduces peak dispersion and combines all steps from sample loading to compound ionization for a seamless operation. A closer look at HPLC-Chip

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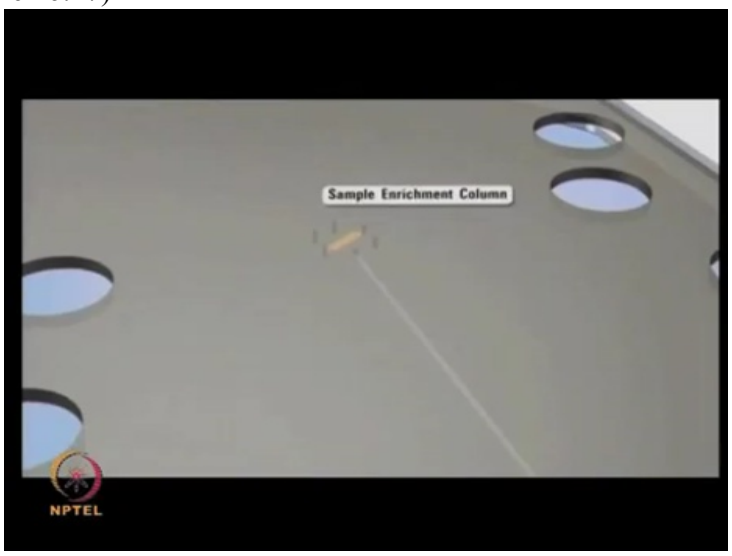

reveals that sample enrichment

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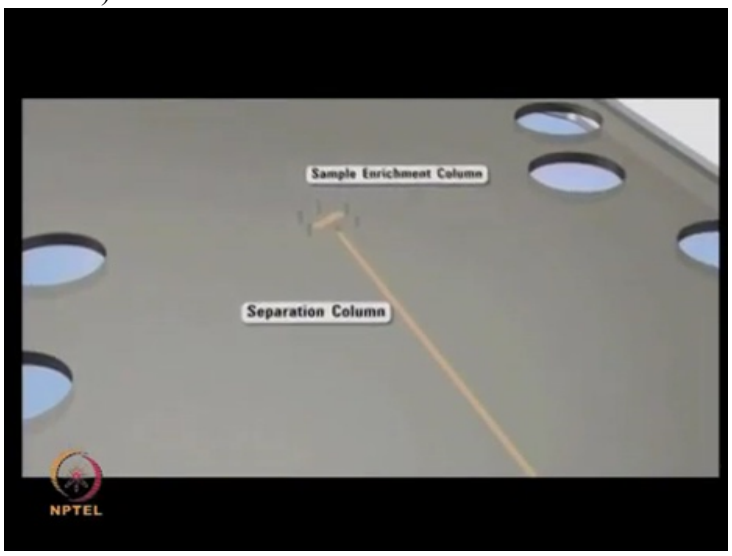

and separation columns of a nano-flow LC system are integrated with intricate connections and

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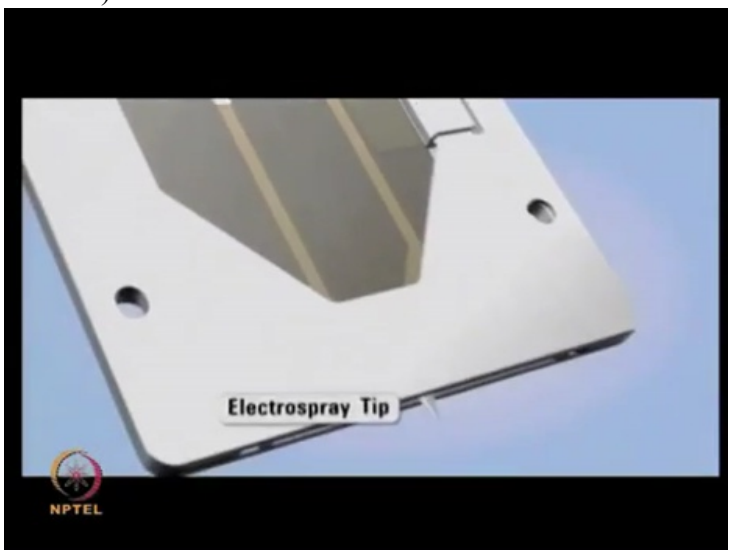

… nano-electro spray tip for compound ionization in mass spectrometry. This eliminates 50% of the traditional fittings and connections typically required in a nano-flow LC/MS system which dramatically reduces the possibility

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… of leaks and dead volumes and significantly improves ease of use, sensitivity, productivity and reliability. The HPLC chip also incorporates all electrical contacts

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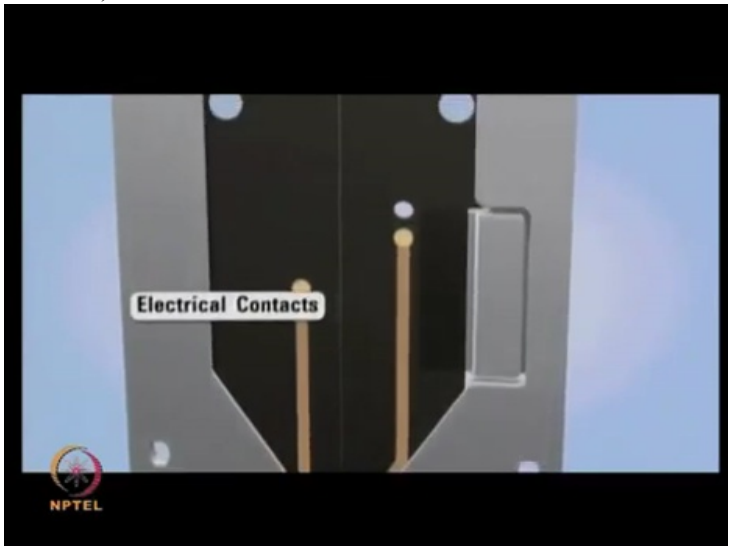

with a nano-electro spray tip

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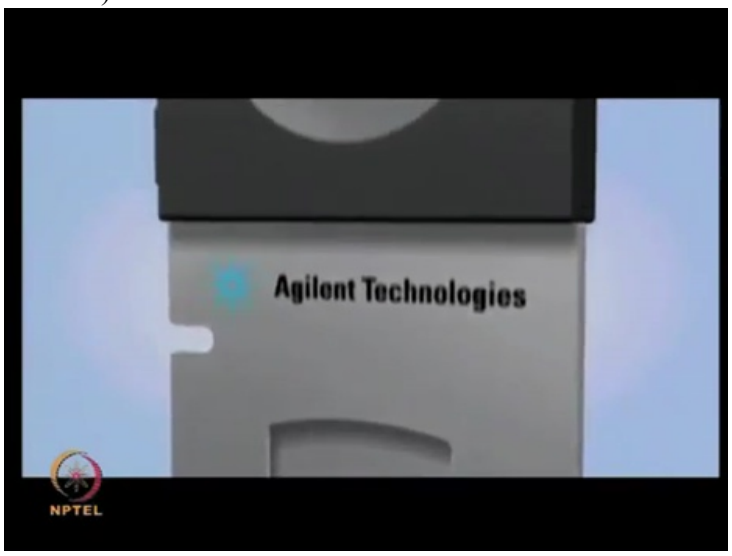

and features an embedded Radio Frequency ID tag

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that tracks the usage and operating parameters of the chip.

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The Chip cue includes an electro-spray ion source with optics for spray visualization, HPLC-Chip loading and ejection mechanism.

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Nano LC connections and micro-valve switching,

The HPLC-Chip loading mechanism precisely and optimally positions

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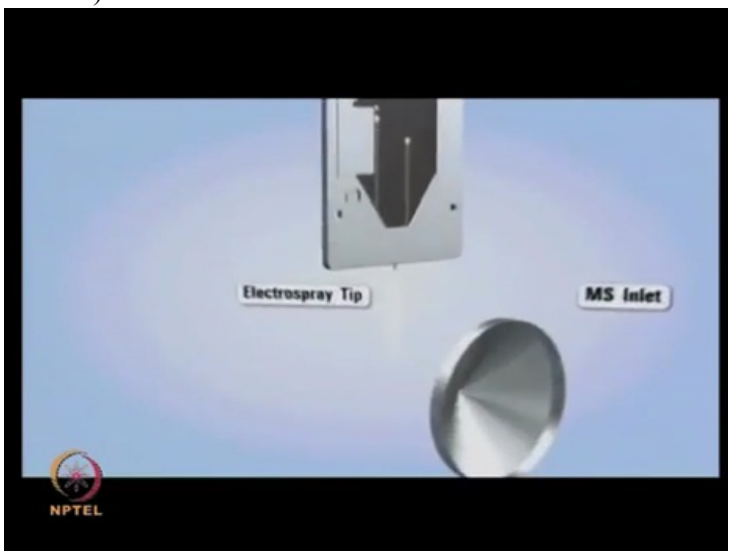

the electro spray tip orthogonal to MS inlet for maximum sensitivity and robustness

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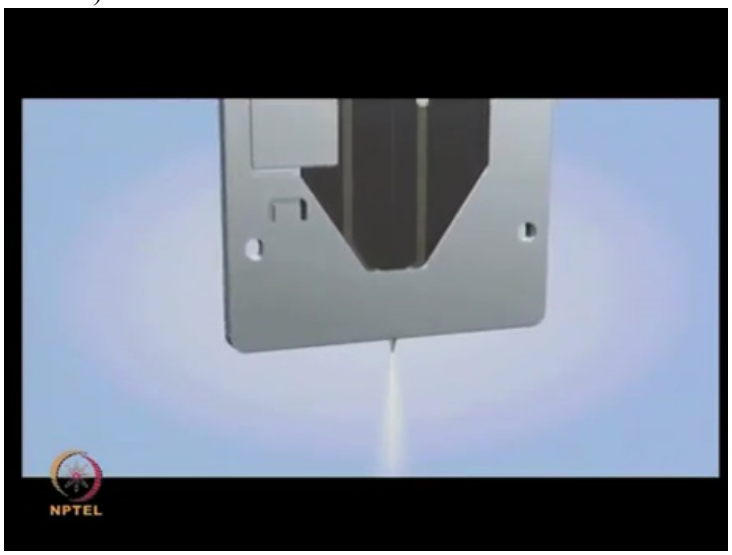

…day- in day-out With the Agilent 1200 series nanoLC system including micro-valve plate, auto sampler and loading pump connected directly to Chip cue and HPLC Chip is loaded and leak-tight fluid connections are

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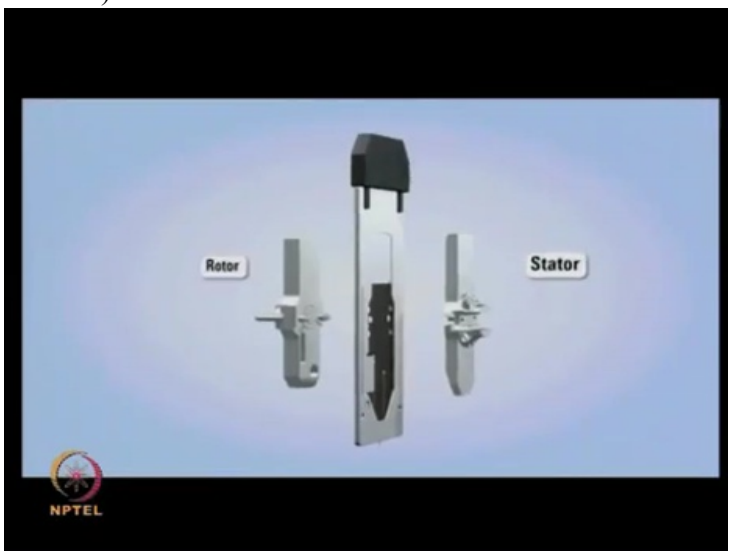

… established automatically by sandwiching the chip between the rotor and the stator of the built-in multi-port micro-valve The rotor and stator …

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… dock on to the chip and establish the flow path from nano-LC to the ports on the chip surface.

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Fast movement of the rotor ensures reliable switching between

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… sample loading and sample analysis positions on the HPLC-Chip

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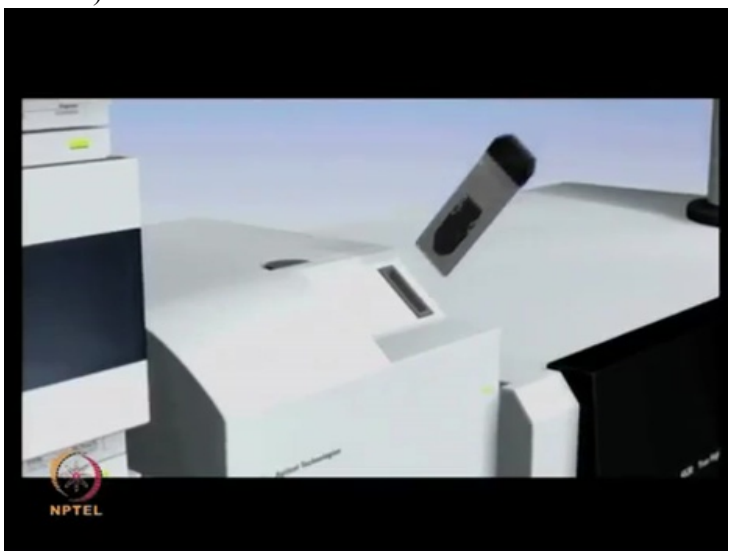

Replacement of the HPLC-Chip is simple and can be completed in a few seconds.

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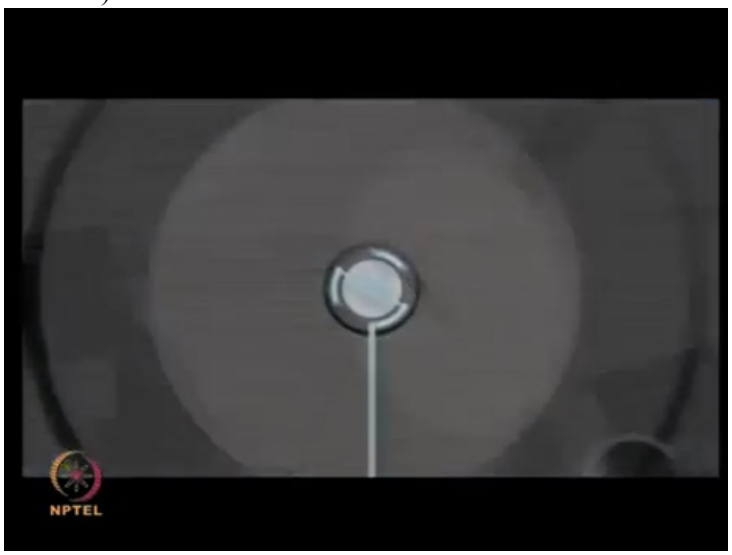

Let’s look at how Agilent 1200 series HPLC-Chip MS system can be applied

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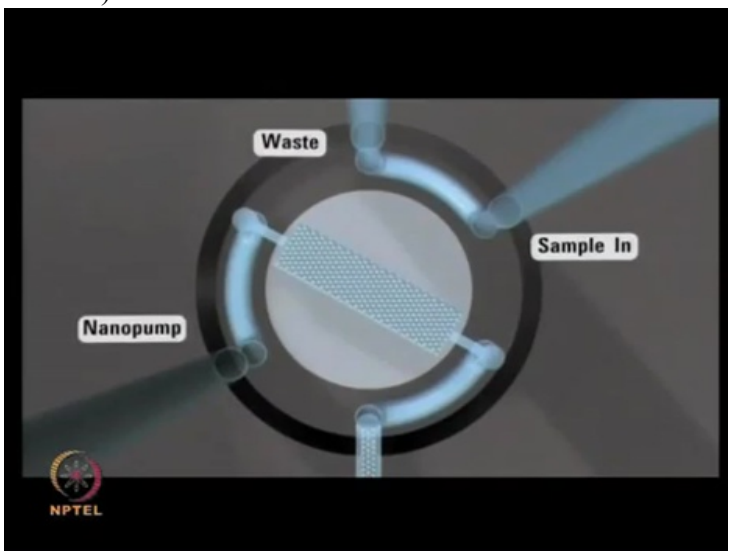

…to a typical protein identification analysis The Agilent microvalve plate….

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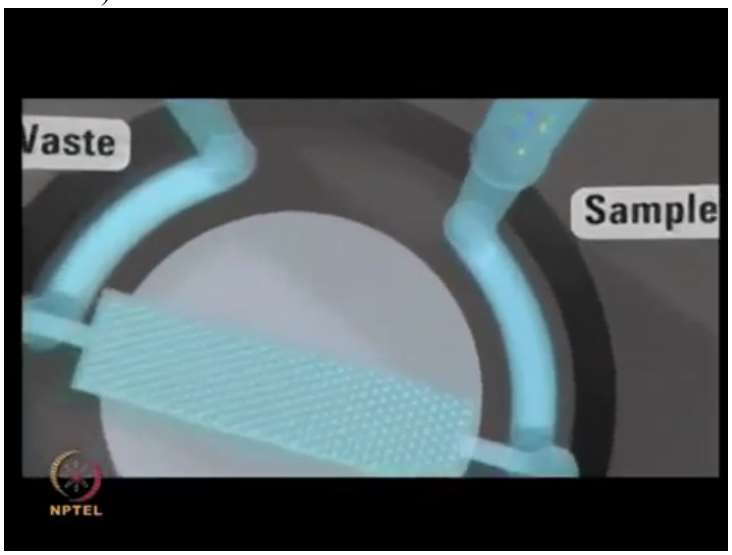

auto sampler loads the digested proteins.

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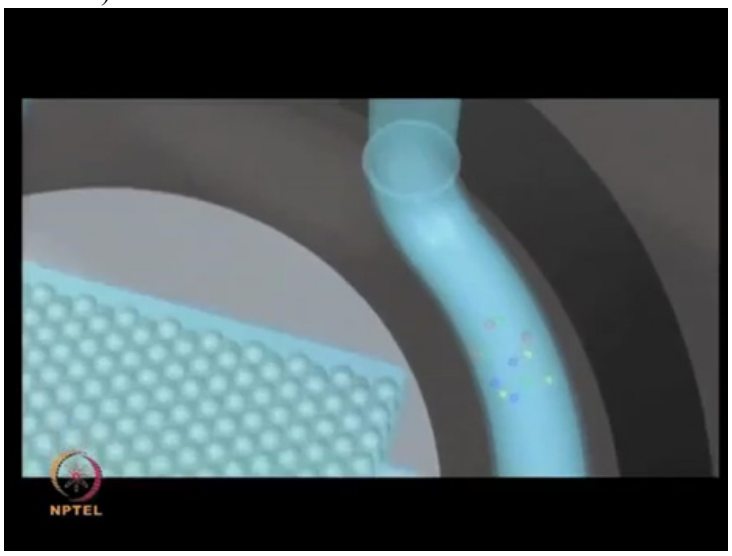

A solvent flow moves the peptides

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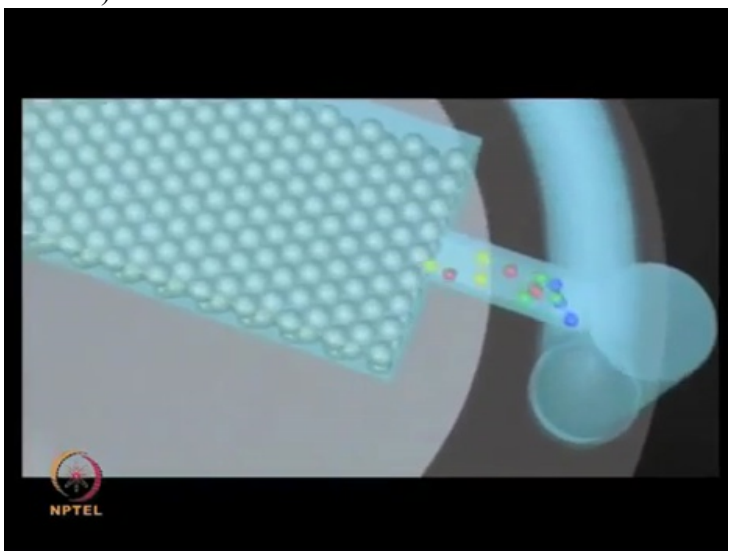

into the trapping column.

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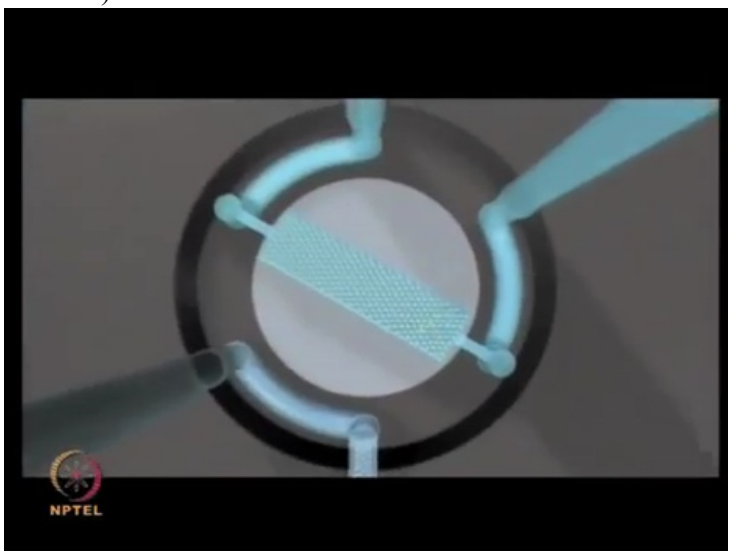

The microvalve changes the flow path.

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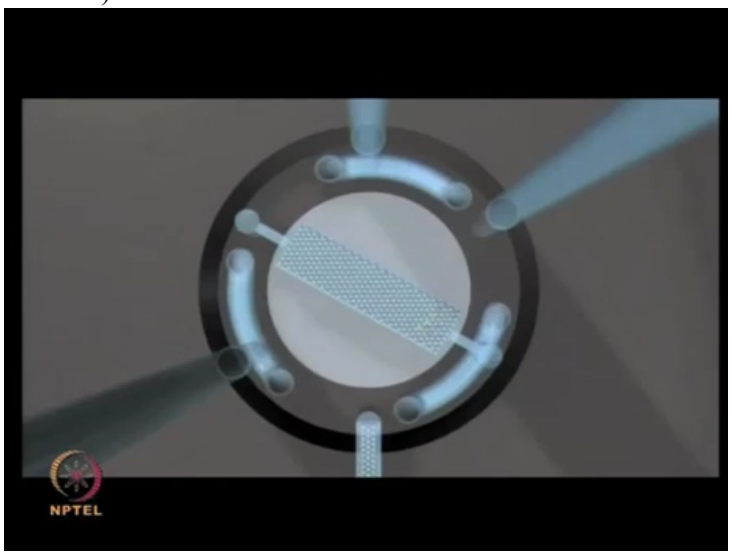

The gradient flow from the nano-flow pump

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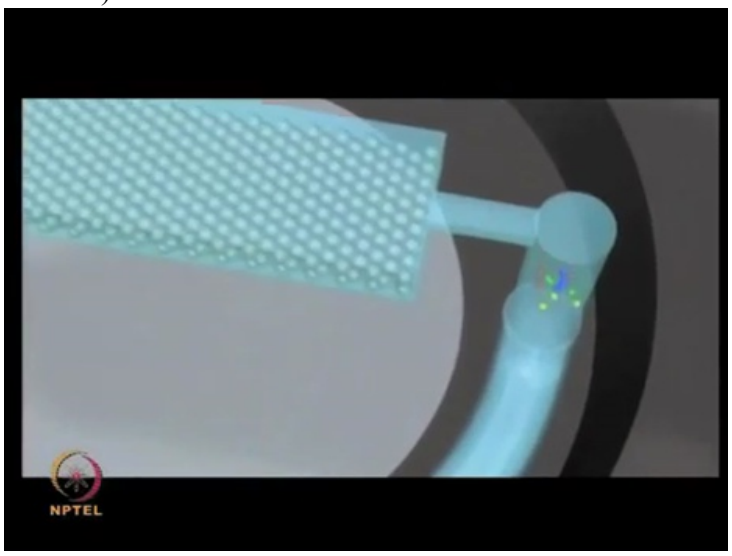

takes the enriched sample from the trapping column to

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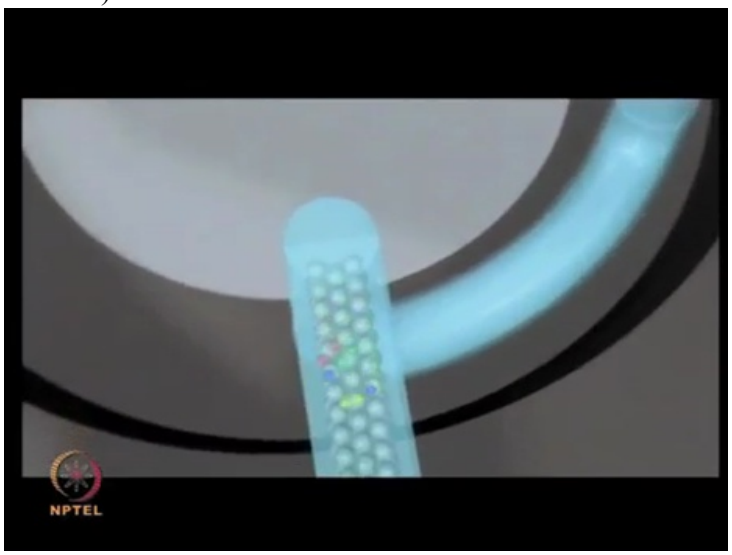

the separation column. The peptides are separated just like on a

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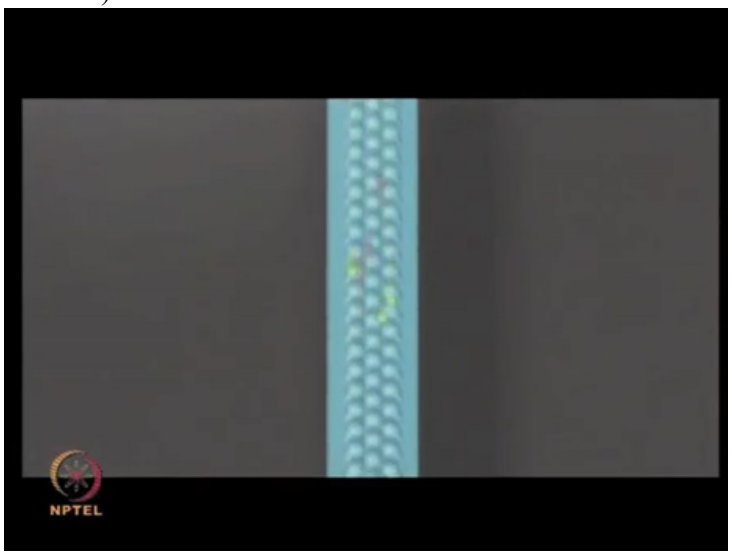

conventional nano-flow column. Reduced peak dispersion yields better separation efficiency and sensitivity.

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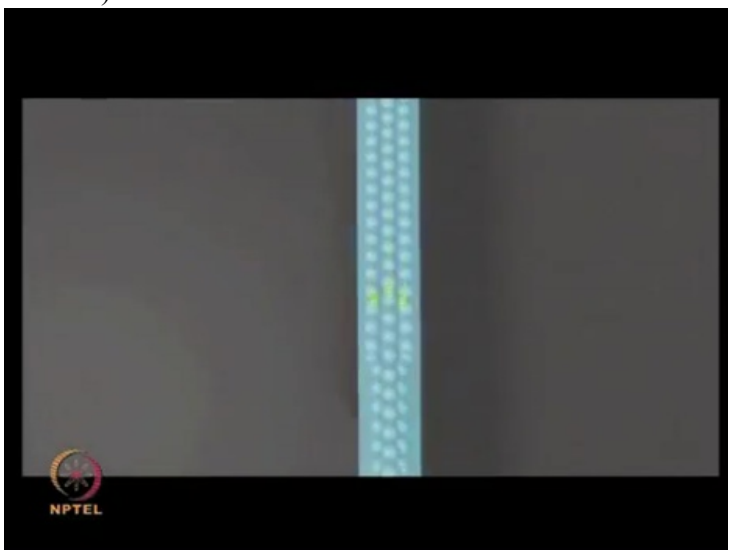

The integrated nano spray tip

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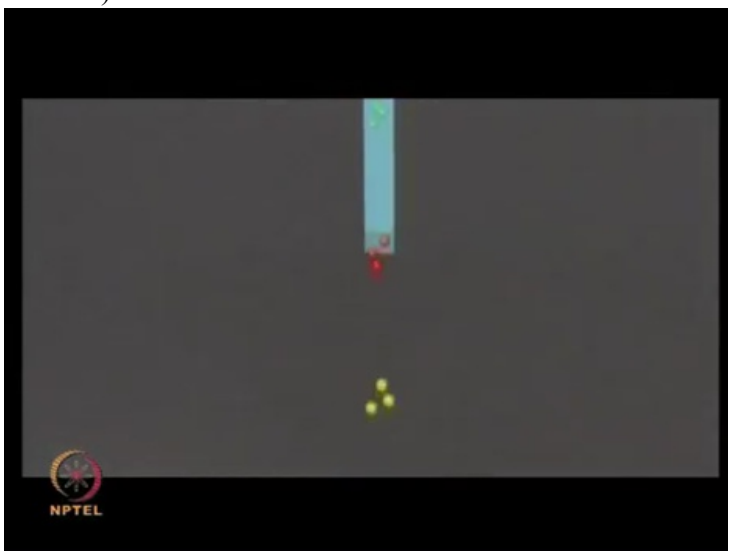

ensures reproducible nebulization of the effluent vital for

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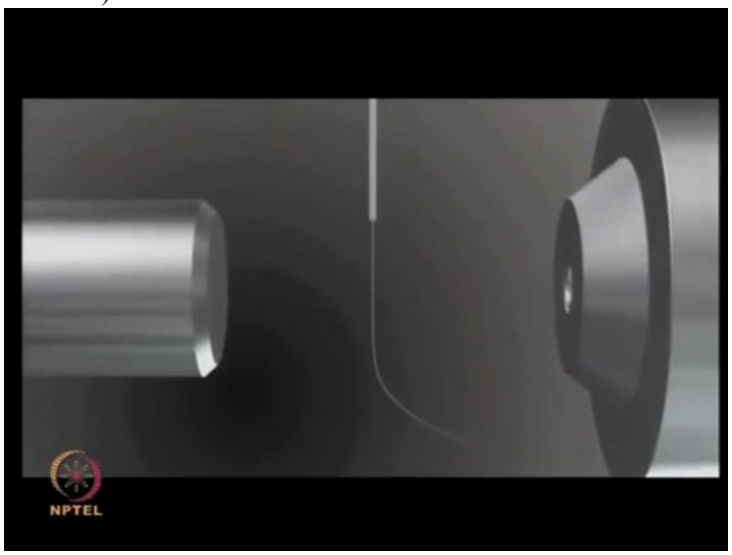

optimum ionization of compounds and best results. Proven nano-flow LC-MS technology and

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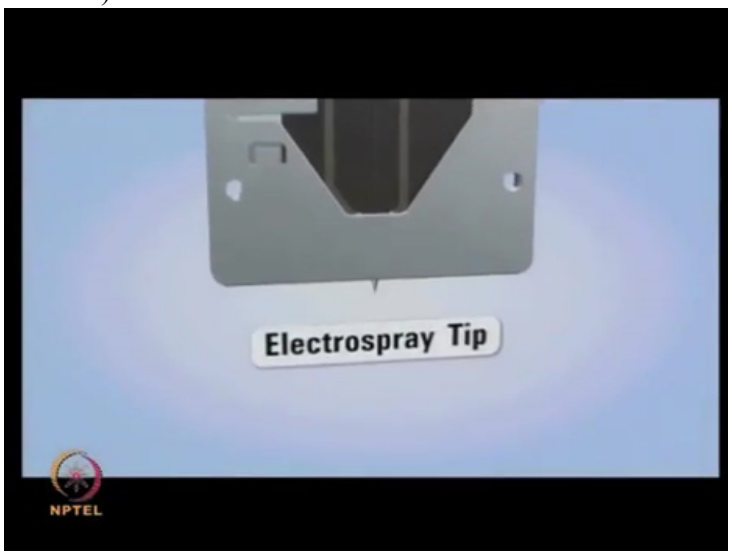

the new and exciting capabilities of micro-fluidics combined to form a system that is easy to set up.

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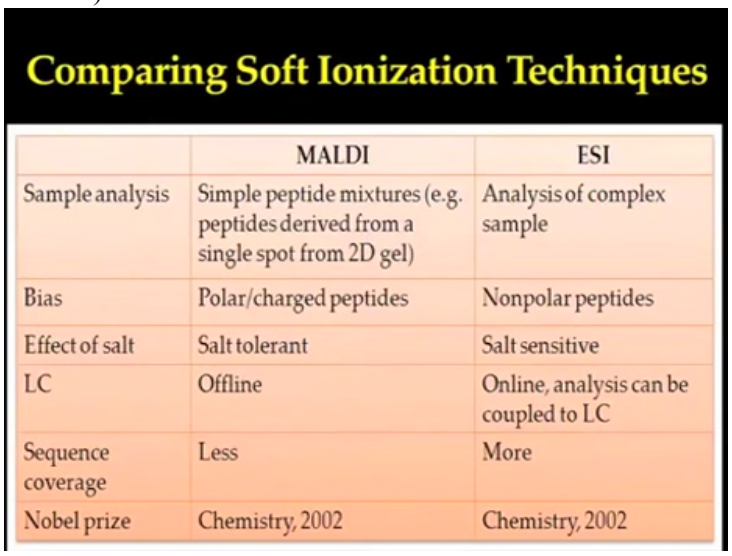

Let's have a comparison between MALDI and ElectroSpray and discuss their pros and cons which can be used for the analysis of different types of protein samples

In MALDI the sample analysis is for the simple peptide mixture whereas in ESI, it 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 peptides 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 Electro Spray Ionization

Both MALDI and ESI development were awarded with the Nobel Prize

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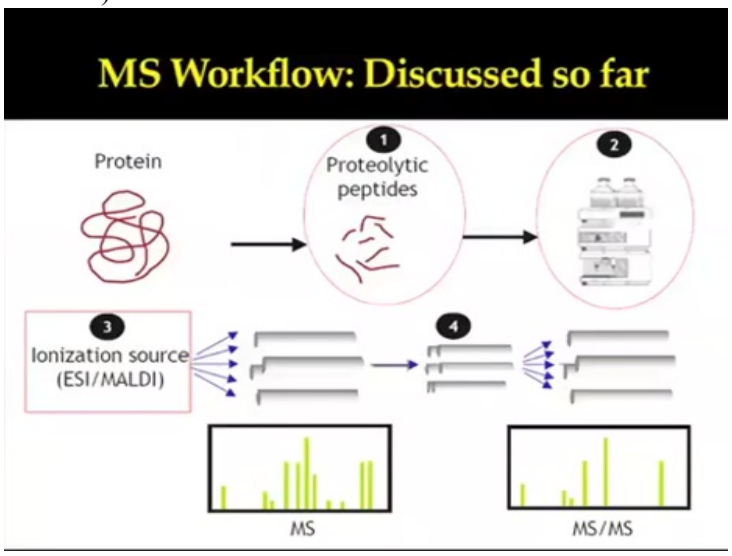

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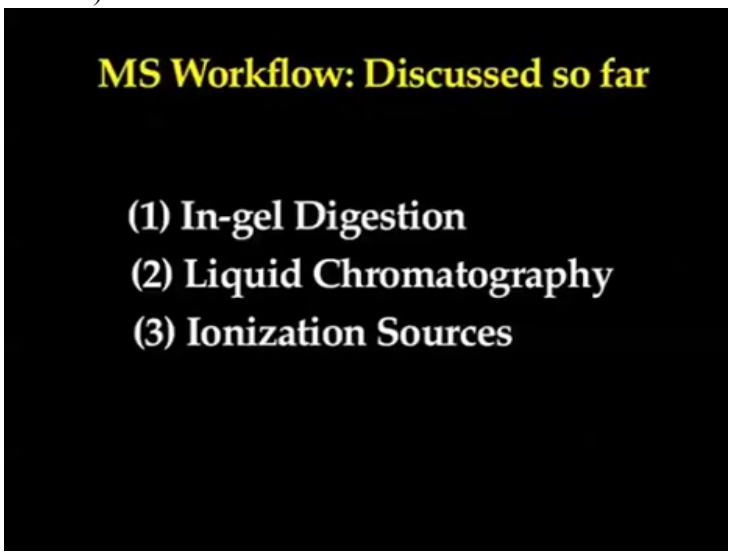

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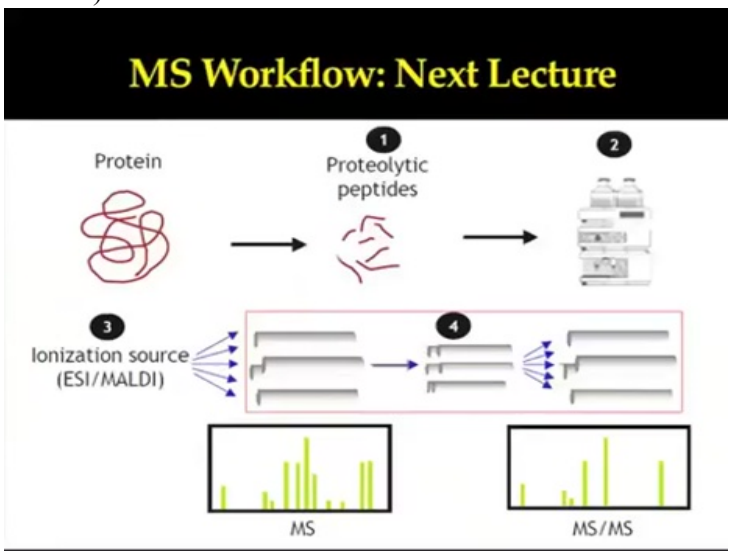

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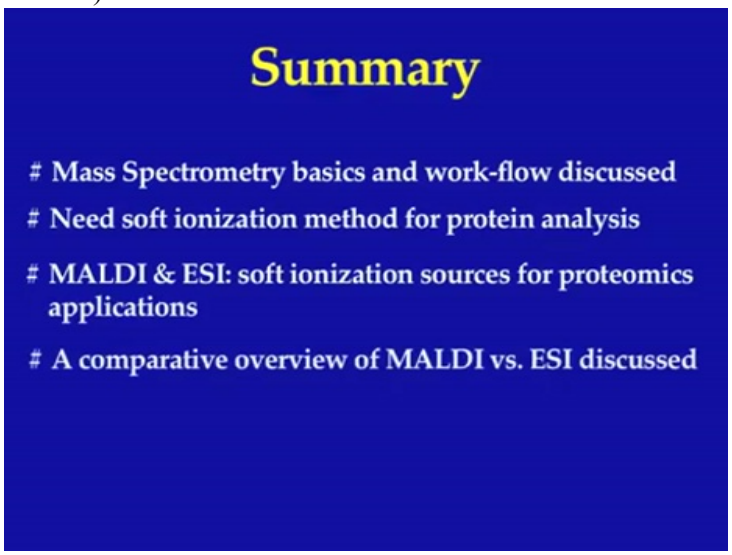

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