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A REVIEW ON SIZE EXCLUSION CHROMATOGRAPHY HYPHENATED WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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ABSTRACT

size exclusion chromatography (SEC) hyphenated to ICP-MS for detection of metal cofactors according to the molecular weight of the eluting biomolecules. Coupling SEC to elemental detection itself is not a new analytical technique; the first featured examples, reported in the 1980s, used atomic absorption and atomic emission spectrometry to detect trace metals in various biological fluids. Since then, a number of examples of SEC hyphenated to mass-specific detection have been used to study trace metals in a variety of physiological and disease states, including rat serum, cancerous human thyroid tissue, murine neurological tissue and platinum-treated human blood products to name just a few. Common themes of these foundation papers are lengthy runtimes and difficulties in acquiring truly quantitative data. Using standard metalloproteins, we were able to absolutely quantify the metal content of each eluting species, and have suggested the possible biological molecules associated with specific metal ions.

INTRODUCTION:

What is Size exclusion chromatography **Size exclusion chromatography** (**SEC**) is a chromatographic separation procedure that separated analyte molecules according to their size or geometry and some cases by their molecular weight. SEC principle is used mainly for the separation of macromolecules like Proteins, Enzymes, antibodies, nucleic acids (DNA and RNA), and industrial Polymers. Size exclusion Chromatography principle is based on the exclusion of analyte molecules through a column containing porous beads. When we used an aqueous solution for the transportation of samples through the column, the procedure is called gel-filtration or Permeation chromatography.

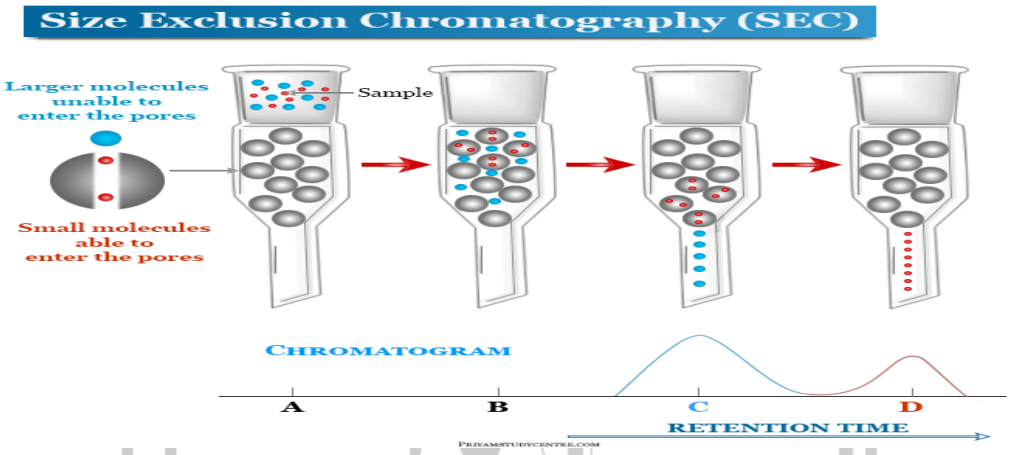

Fig1-principle involved in size exclusion chromatography

The selection of molecules on the basis of their molecular size and shape utilizes the molecular sieve properties of a variety of porous materials. Probably the most commonly used such materials are a group of polymeric organic compounds which possess a three-dimensional network of pores which confer gel properties upon them[1].
The general principle of exclusion chromatography is quite simple. A column of gel particles or porous glass granules is in equilibrium with a suitable solvent for the molecules to be separated. Large molecules which are completely excluded from the pores will pass through the interstitial spaces, while smaller molecules will be distributed between the solvent inside and outside the molecular sieve and will then pass through the column at a lower rate. Three stages in such a column are represented diagrammatically in figure 2.

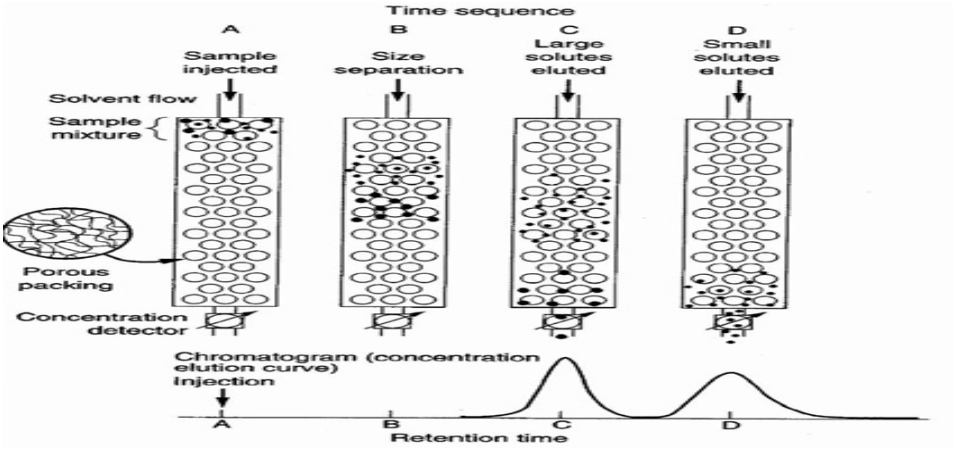

Fig2-mechanism of size exclusion chromatography

What is inductively coupled plasma?

Inductively coupled plasma is plasma that contains a sufficient concentration of ions and electrons to make the gas electrically conductive. The plasmas used in the spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free electron. In these plasmas, the positive ions are almost all singly charged and there are few negative ions, so there are nearly equal amounts of ions and electrons in each unit volume of plasma. Inductively coupled plasma (ICP) for spectrometry is sustained in a torch that consists of three concentric tubes, usually made of quartz. The end of this torch is placed inside an induction coil supplied with a radiofrequency electric current. A flow of argon gas (usually 14 to 18 litres per minute) is introduced between the two outermost tubes of the torch and an electric spark is applied for a short time to introduce free electrons into the gas stream. These electrons interact with the radiofrequency magnetic field of the induction coil and are accelerated first in one direction, then the other, as the field changes at high frequency. The accelerated electrons collide with argon atoms, and sometimes a collision causes an argon atom to part with one of its electrons. The released electron is in turn accelerated by the rapidly changing magnetic field. The process continues until the rate of release of new electrons in collisions is balanced by the rate of recombination of electrons with argon ions (atoms that have lost an electron). This produces a 'fireball' that consists mostly of argon atoms with a rather small fraction of free electrons and argon ions. The temperature of the plasma is very high, of the order of 10,000 K5. The ICP can be retained in the quartz torch because the flow of gas between the two outermost tubes keeps the plasma away from the walls of the torch. The second flow of argon (around 1 litre per minute) is usually introduced between the central tube and the intermediate tube to keep the plasma away from the end of the central tube. A third flow (again usually around 1 litre per minute) of gas is introduced into the central tube of the torch. This gas flow passes through the centre of the plasma, where it forms a channel that is cooler than the surrounding plasma but still much hotter than a chemical flame. Samples to be analysed are introduced into this central channel, usually as a mist of liquid formed by passing the liquid sample into a nebulizer6$_2$. As a droplet of nebulised sample enters the central channel of the ICP, it evaporates and any solids that were dissolved in the liquid vaporize and

then break down into atoms. At the temperatures prevailing in the plasma a significant proportion of the atoms of many chemical elements are ionized, each atom losing its most loosely bound electron to form a singly charged ion.

Fig3-inductively coupled plasma mass spectrometry

What is Mass Spectrometry?

It is the most accurate method for the determination of the molecular mass of a compound and its elemental composition. It can provide concurring that molecular of organic and inorganic compounds. The mass spectrometer is an instrument in which a substance in a gaseous or vapour state is bombarded with a beam of electrons(70 EV) to form positively charged ions(cations) which are sorted to mass to charge ratio to record their masses and relative abundance[3]

All mass spectrometers consist of three parts.

- Ion Source
- Mass Analyser
- Detector System

Stages within the mass Spectrometry:-

1. Producing ions from the sample.

2. Separating ions of differing masses.

3. Detecting the number of ions of each mass produced.

4. Collecting the data and generating the mass spectrum.

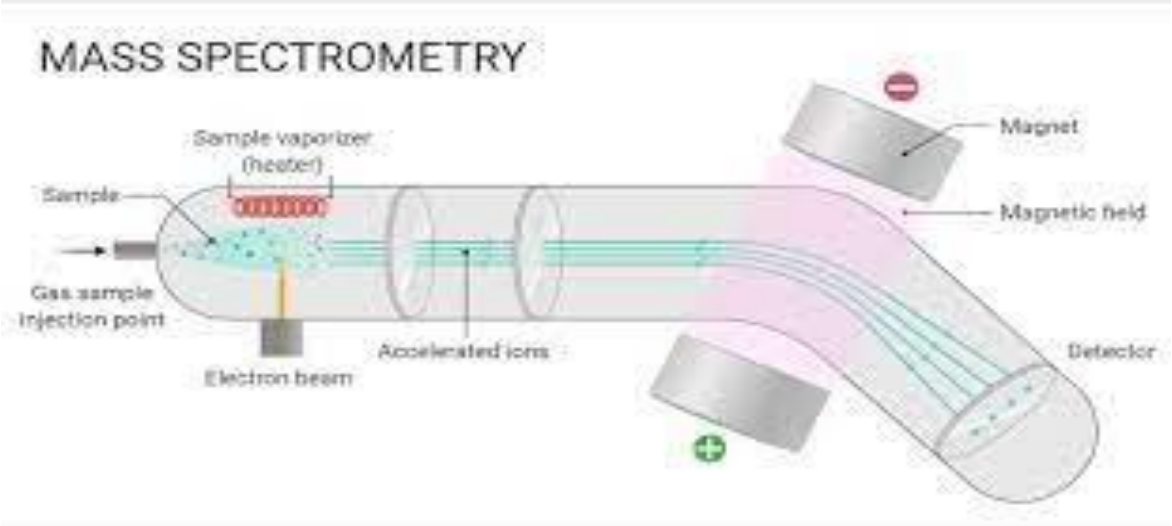

Fig4-mass spectrometry

INSTRUMENTATION

Components in SEC-ICP-MS are

- Stationary phase
- Mobile phase
- Sample delivery system
- Column
- Nebulizer
- Spray chamber
- Peristaltic pump
- Torch
- Mass spectrometry(quadrupole)
- Detector

Stationary phase:

Gels that are commonly used include cross-linked dextran, agarose, polyacrylamide, poly acryloyl morphine, and Polystyrenes. These are semi-permeable, porous beads with a well-defined range of pore sizes. Small pore sizes are used for the rapid desalting of proteins or protein purification. Intermediate pore size is used to separate relatively small proteins. Very large pore size is used for the purification of biological complexes. Before column packing, these gels or stationary phases are soaked overnight to prevent breaking or bursting of the column due to swelling of the gel.

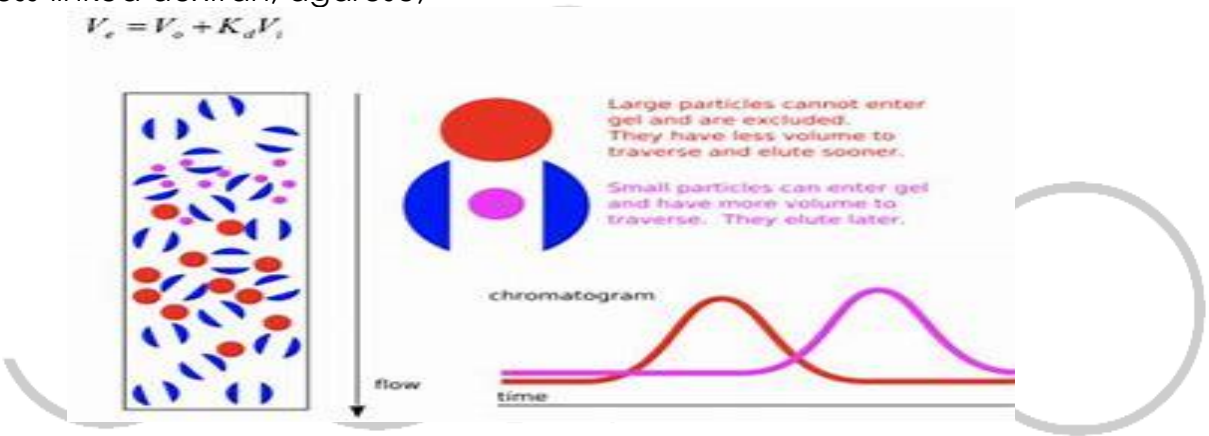

Fig5-particle size in the stationary phase

Mobile Phase

The liquid used to dissolve the biomolecules to make the mobile phase is usually known as a buffer. Then the mixture of biomolecules dissolved in the buffer is called the sample. The choice of mobile phase to be used in any separation will depend on the type of separation to be achieved and the components to be separated. The solvent must dissolve the sample completely.

E.g. Tetrahydrofuran, chloroform, dimethylformamide

Sample Preparation

The sample solutions are supposed to be prepared in dilute concentrations (less than 2 mg/ml). A good solvent can dissolve a sample in any proportion in a range of temperatures. Samples with broad molecular weight distribution may require higher concentrations. It is recommended to filter the sample solution before injecting it into the

columns to get rid of clogging and excessively high-pressure problems. Generally, filtration is required to remove insoluble impurities do not agitate and filter sample that contains very high molecular weight(> 1 million).

Column Packing

The packing of a column is based on either porous silica or semi-rigid(highly cross-linked) organic gels, in most cases copolymers of styrene and divinylbenzene.125A° pour size for analysis of small proteins and peptide molecules.250A° pour size for most protein samples.450A° pour size for very large proteins and nucleic acids. A column consists of a hollow tube tightly packed with extremely small porous polymer beads designed to have a force of different sizes. The larger the particle size the faster the exclusion. Increasing the column length enhances the resolution and increasing the column diameter increases the capacity of the column. proper column packing is important to maximize resolution. An overpacked column can collapse the process in beads resulting in a loss of resolution. An under-packed column can reduce the relative surface area of gel accessible to smaller molecules, resulting in the molecules spending less time trapped in pores.

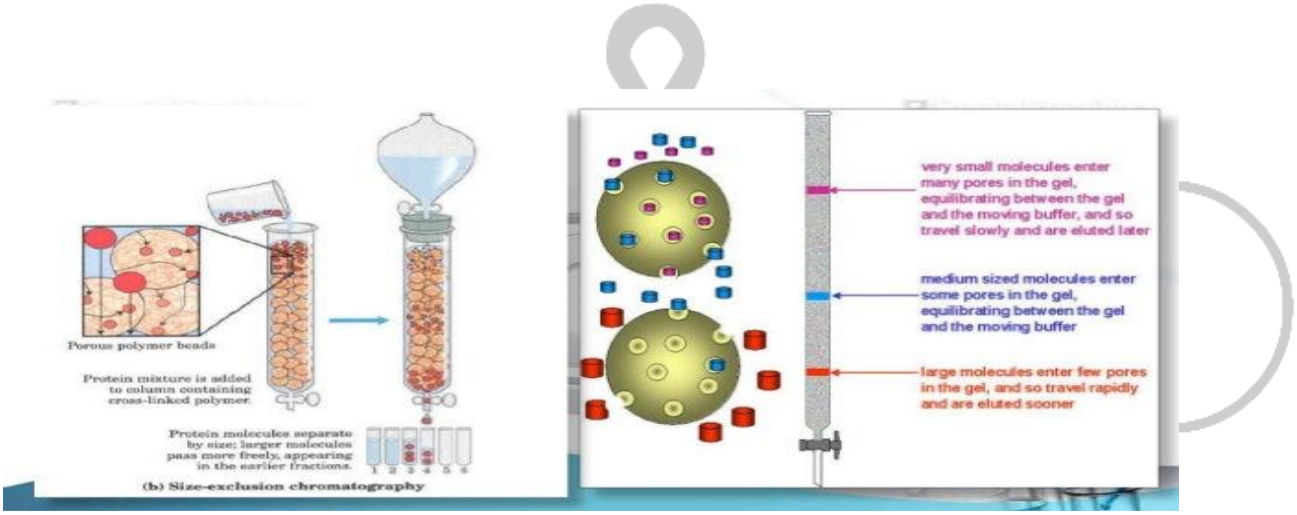

Fig6-column packing in size exclusion chromatography

INTERFACE-1

The Size-exclusion chromatography coupled with inductively coupled plasma mass spectrometry is the simplest form it is just the connection of the column outlet with the nebulizer of the sample introduction system through the transfer tubing.

SEC was implemented using an HPLC pump with an injection valve as a sample delivery system for sample volume.ICP mass spectrometer is used for the quantification of the mental content in extracts and for online monitoring is the HPLC eluate. The sample or mobile phase from the SEC column was introduced into the ICP-MS system through a nebulizer fitted in a Scott spray chamber.

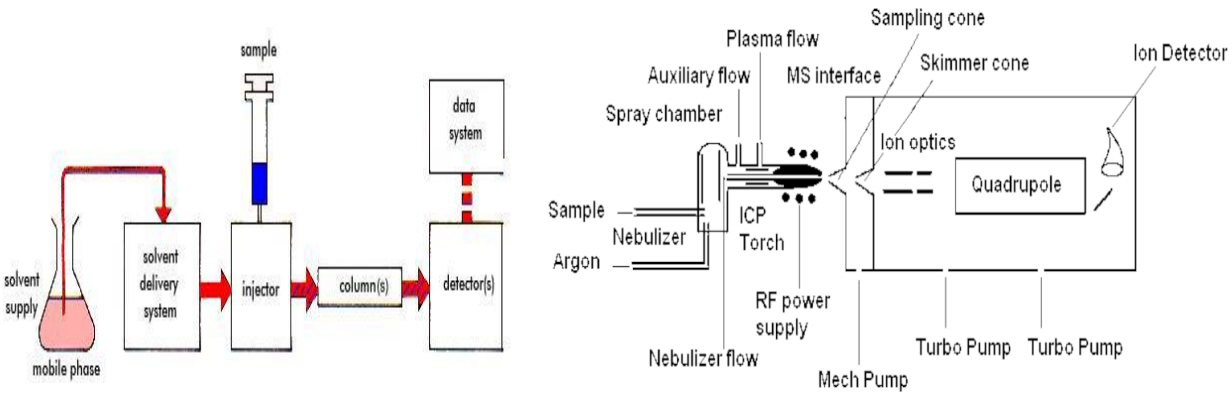

Fig7-interface 1 from column to nebulizer

PERISTALTIC PUMP

These pumps utilize a series of rollers that push the sample solution through the tubing using a process known as peristalsis. The pump itself does not come in contact with the solution, only with the tubing that carries the solution from the sample vessel to the nebulizer. The special tubing used with a peristaltic pump must be compatible with the sample that is passing through it. Most types of peristaltic pump tubing are compatible with weakly acidified aqueous media. Pumping strongly acidic solutions or organic solvents, however, usually requires the use of tubing made of specific materials.

Fig8-peristalic pump

Nebulizers

Nebulizers are devices that convert a liquid into an aerosol that can be transported to the plasma. The nebulization process is one of the critical steps in SEC- ICP-MS.

The ideal sample introduction system would be one that delivers all of the samples to the plasma in a form that the plasma could reproducibly desolate, vaporize, atomize and ionize, and excite. Because only small droplets are useful in the ICP, the ability to produce small droplets for a wide variety of samples largely determines the utility of a nebulizer for ICP-MS. Many forces can be used to break up a liquid into an aerosol; however, only two have been used Pneumatic, and ultrasonic nebulizers. The function of a nebulizer is to convert a liquid sample into an aerosol for introduction into the plasma. The aerosol facilitates uniform sample introduction for

both a reproducible signal output and stable operation of the plasma.

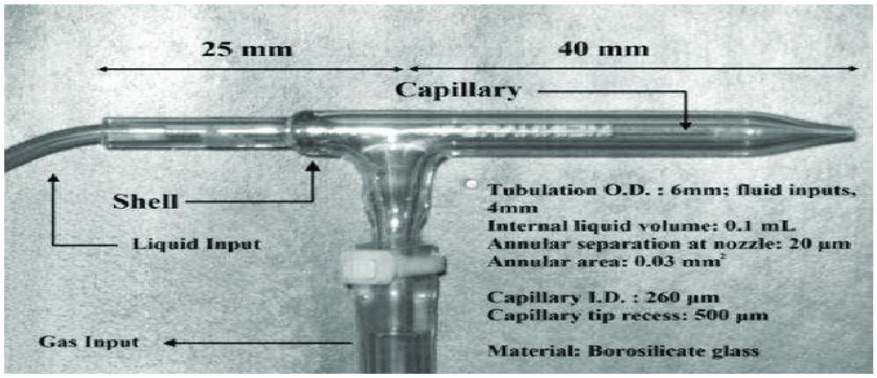

Fig9-pneumatic nebulizer

Scott double-pass spray chamber:-

The spray chamber is placed between the nebulizer and the torch. The primary function of the spray chamber is to remove large droplets from the aerosol. A secondary purpose of the spray chamber is to smooth out pulses that occur during nebulization general, spray chambers for the ICP are designed to allow droplets with diameters of about 10 m or smaller to pass to the plasma With typical nebulizers, this droplet range constitutes about 1 - 5% of the sample that is introduced to the nebulizer. The remaining 95 - 99% of the sample is drained into a waste container. The material from which a spray chamber is constructed can be an important characteristic of a spray chamber.

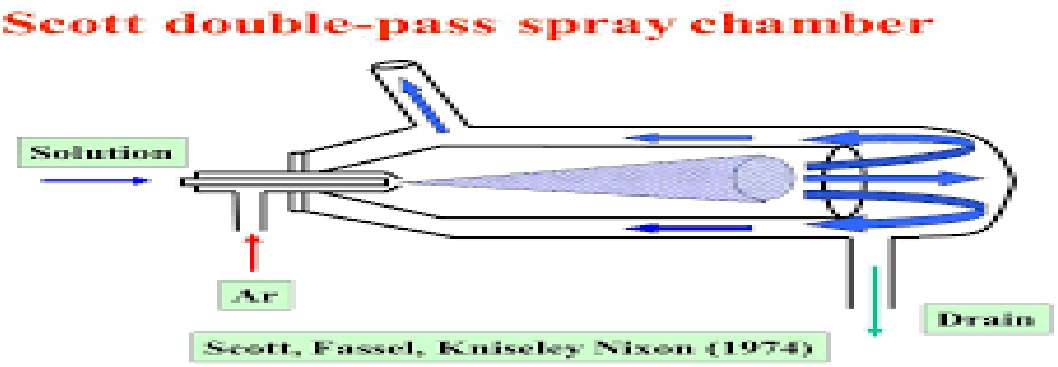

Fig10-Scott double pass spray chamber

TORCH

The ICP torch consists of a copper induction coil wrapped around concentric quartz. Argon gas is continuously flowing throughout the quartz torch, and a radio-frequency (RF) generator provides power to the RF coil at oscillating frequencies. Plasma (an electrical conducting gaseous mixture) generation occurs when the argon gas is

seeded with a spark from a Tesla unit. The spark ionizes some of the argon, and the cations and electrons produced from that accelerate towards the RF coil. The cations and electrons collide with other argon molecules during this acceleration, creating high temperatures. With ample argon supplied, the plasma will reach equilibrium and remain at a constant temperature of about 6,000°C for the duration of the analysis. The aerosol produced via nebulization enters this high-temperature plasma, where it is first dried to a solid, and then heated to a gas, referred to as atomization. These atoms will continue to travel through the plasma, absorbing energy until they release an electron, becoming ionized, referred to as ionization. These newly formed ions then travel out of the torch and come to the interface.

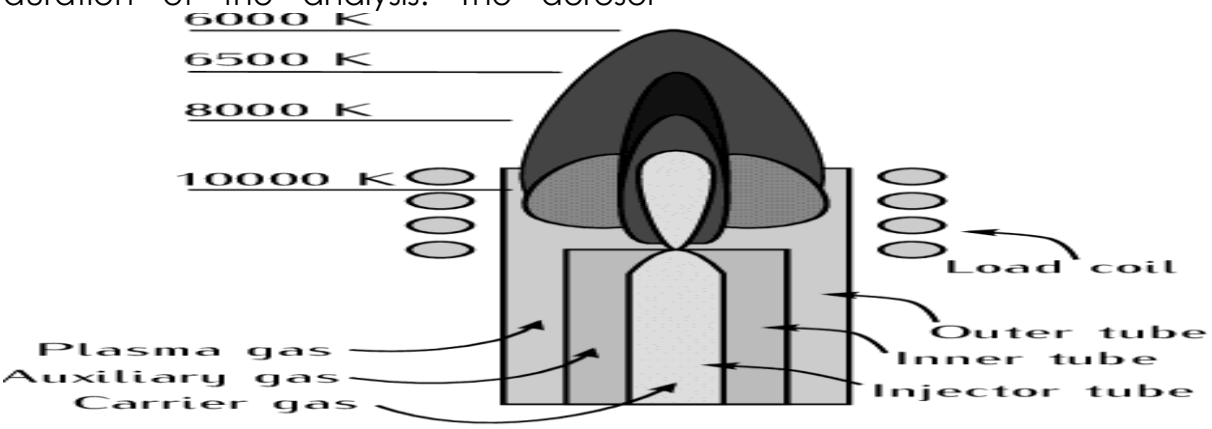

Fig11-ICP Torch

INTERFACE:2

Generally speaking, the interface can be described as the point at which sample from the ICP portion of the instrument is introduced to the mass spectrometry (MS) portion of the instrument. The interface portion of the instrument serves to allow the ICP and MS portions to be coupled. The first component the sample matrix confronts after ionization in the ICP torch is the sampler cone. This is a water-cooled cone with a small orifice, allowing for the hot plasma gas to enter a depressurizing chamber. In this chamber, rapid cooling, and thus rapid expansion, of the gas occurs. A fraction of this gas then passes through a skimmer cone, and into a chamber that is maintained at a vacuum of that of the MS This two-pressure reduction allows the ionic gas to enter the MS at proper temperature and pressure

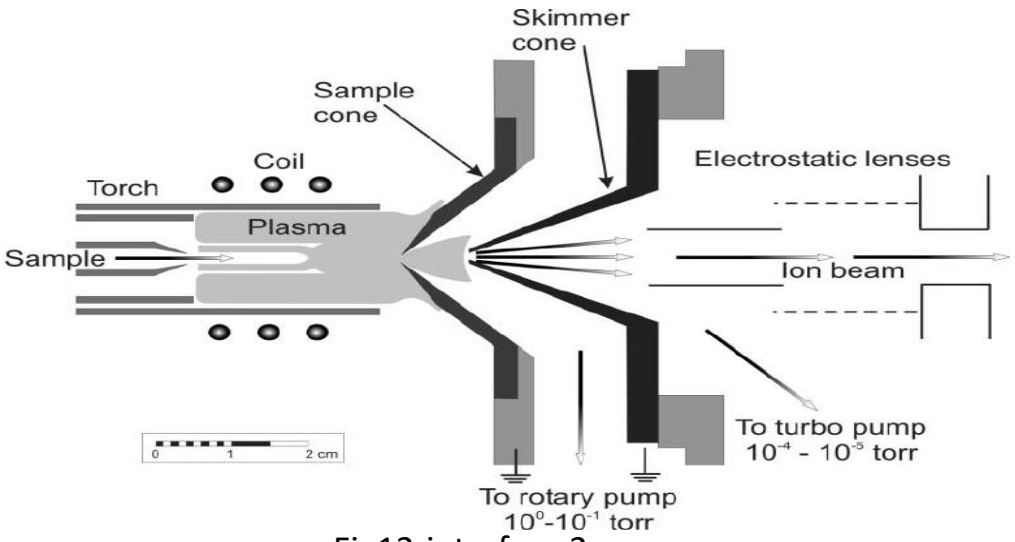

Fig12-interface 2

Mass Spectrometry

After passing through the sample and skimmer cones, the ion stream is focused into the quadrupole region by single ion lenses. Ions generated in plasma are nearly all positively charged and have a tendency to repel each other. The ions pass through a charged metallic cylinder which keeps the ion beam from diverging. The quadrupole mass spectrometer is the most common type of mass spectrometer used in atomic mass spectroscopy. Four parallel cylindrical rods serve as electrodes; opposite rods are connected electrically with one pair connected to the positive side of a variable direct current (dc) source, and the other to the negative terminal. Variable radiofrequency alternating current (ac) voltages 180° out of phase are applied to each pair of rods. Ions are accelerated into the space between the rods by a potential of 5 to 10 V while the ac and dc voltage son the rods are increased simultaneously but with a constant ratio. The quadrupole is a mass filter which only allows ions having a limited range of m/z values to reach the transducer. Ions that are not of the correct m/z collide with the rods or exit the path between the rods and are pumped out of the system. Quadrupole instruments easily resolve ions that differ in mass by one unit.

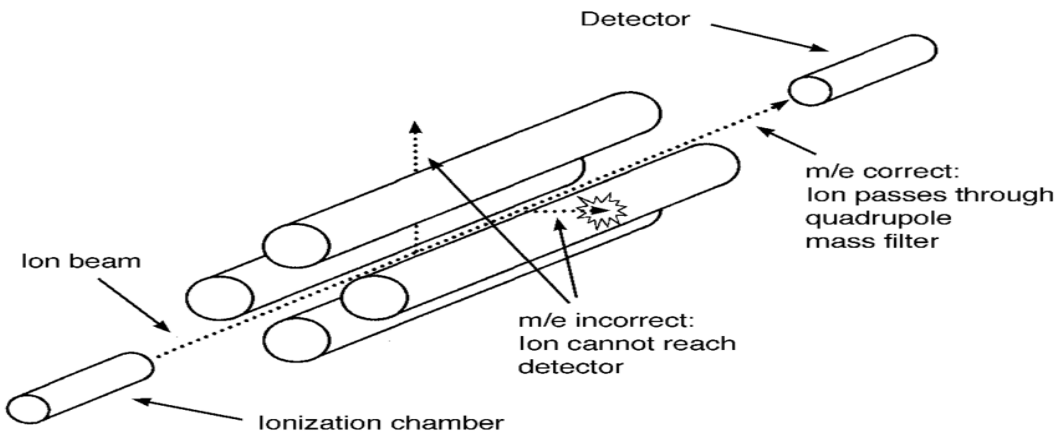

Fig13 -Quadrupole

Detector:-

The most common detector used for ICP-MS is an electron multiplier (EM) The operation of electron multipliers is fundamentally based on the concepts of "dynodes" and "secondary emission". A "dynode" is simply an electrode in the vacuum that emits electrons when an ion or electron with sufficient kinetic energy slams into it. This process of emitting electrons is termed "secondary emission".

Electron multipliers essentially string together a series of dynodes so that the process of secondary emission happens repeatedly, amplifying the number of electrons exponentially at each step along the way. There are two common geometries for an electron multiplier: the continuous dynode, and discrete dynode. Ions that exit the mass analyzer enter the detector. The Elan DRC-e is equipped with a dual-mode detector. The ions exiting the quadrupole produce an amplified signal that is processed by the detection electronics and sent to the computer for data processing. The detector is comprised of 26 dynodes.

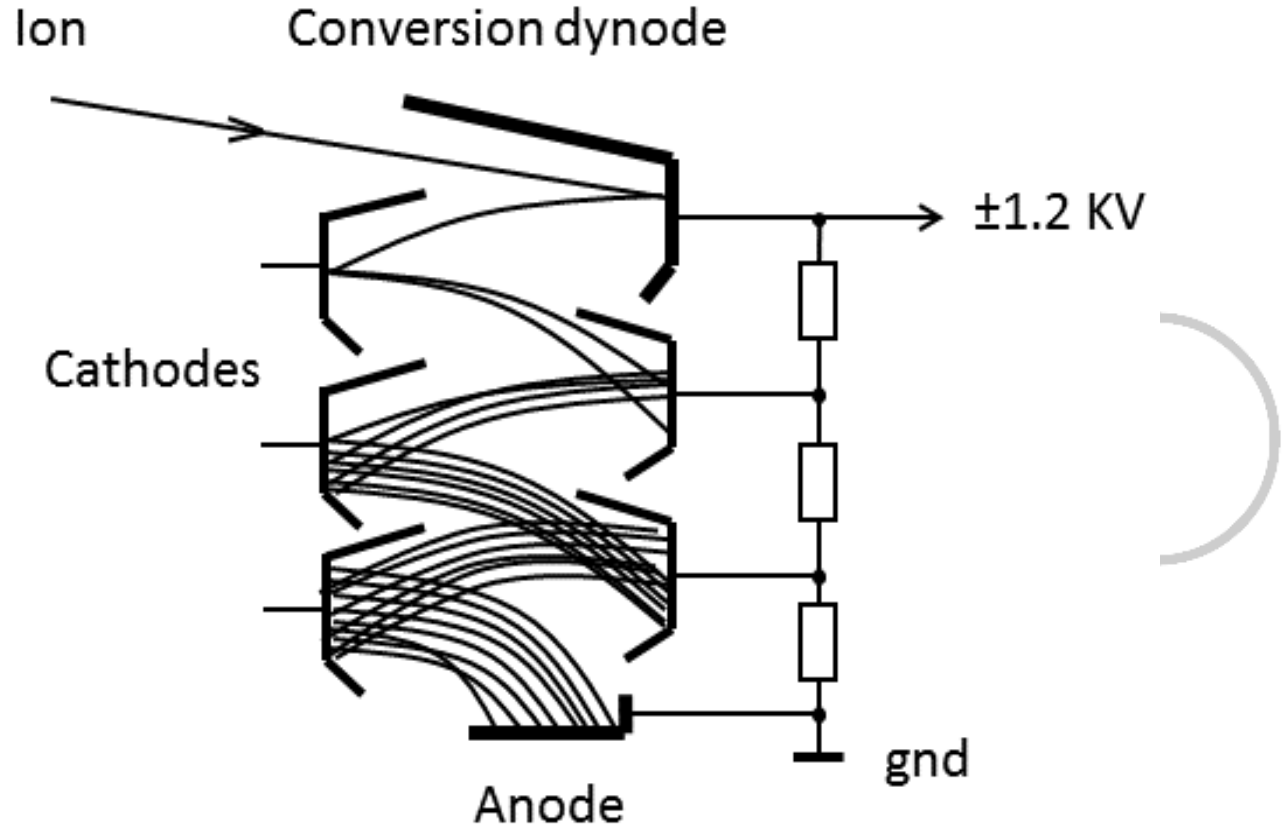

Fig14-electron multiplier tube

APPLICATIONS

- Screening for Cd-Phyto chelation in Biological Tissues.
- Metal Complexed with Cell Wall Polysaccharides: Focus on Speciation of Boron and Metal Complexes with Dimers of RG-II.
- Determination of Ceruloplasmin in Human Serum.
- The Analysis of Therapeutic Metal Complexes and Their

Biomolecular Interactions.

- Analytical methodologies for the determination of cisplatin
- Size exclusion chromatography (SEC) was coupled online to inductively coupled plasma mass spectrometry (ICP-MS) for speciation study of trace metals in cancerous thyroid tissues in comparison to healthy thyroids aimed at the estimation of changes in metalloprotein speciation in pathological tissue.

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