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AN OVERVIEW ON FOURIER TRANSFORM MASS SPECTROMETRY

Lakshmidurga K[*1], Ram Mohan Reddy.T[1], Ajitha.A[1], Uma Maheswara Rao.V[1]

Department of Pharmaceutical Analysis & Quality Assurance, CMR College of Pharmacy, JNTU (H) University, Hyderabad, Andhra Pradesh, India.

ABSTRACT

This article provides an introduction to Fourier transform-based mass spectrometry. The key performance characteristics of Fourier transform-based mass spectrometry, mass accuracy and resolution, are presented in the view of how they impact the interpretation of measurements in proteomic applications. The theory and principles of operation of two types of mass analyzer, Fourier transform ion cyclotron resonance and Orbitrap, are described. Major benefits as well as limitations of Fourier transform-based mass spectrometry technology are discussed in the context of practical sample analysis, and illustrated with examples included as figures in this text and in the accompanying slide set. Comparisons highlighting the performance differences between the two mass analyzers are made where deemed useful in assisting the user with choosing the most appropriate technology for an application. Recent developments of these high-performing mass spectrometers are mentioned to provide a future outlook.

Keywords: Mass spectrometry, Orbitrap, Laser desorption, Analyzers.

INTRODUCTION

FTMS was first described by Comisarow and Marshall in 1974 and was first reviewed by Amstar in 1966 and by Marshall *et al*. in 1998.This technique consists of simultaneously exciting all of the ions present in the cyclotron by a rapid scan of a large frequency range within a time spam of about 1micro sec. This induces a trajectory that comes close to the wall perpendicular to the orbit and also puts the ions in phase. This allows the transformation of the complex wave detected as a time dependent function into a frequency dependent intensity function through a Fourier Transform [1].

Fourier transform ion cyclotron resonance mass spectrometry, also known as Fourier transform mass spectrometry, is a type of mass analyzer (or mass spectrometer) for determining the mass-to-charge ratio *(m/z*) of ions based on the cyclotron frequency of the ions in a fixed magnetic field.

FTMS has received considerable attention for its ability to make mass measurements with a combination of resolution and accuracy that is higher than any other mass spectrometers, most recently for bio-molecules ionized by electro-spray ionization (ESI) and matrix assisted laser desorption or ionization (MALDI). It is a versatile instrument that can be adapted to a variety of analytical and physical chemistry measurements. It can be used to obtain high-resolution mass spectra from ions formed by practically every known ionization method, to perform tandem mass spectrometric measurements and to examine ion chemistry and phyto chemistry.

Its versatility follows from the fact that it is an ion trapping instrument, as is the R.F. quadruple mass spectrometer. The FTMS instrument mass analyzes and detects ions using methods which are unique among mass spectrometers [2-4].

NEED OF STUDY

FTMS differs significantly from other mass spectrometry techniques in that the ions are not detected by hitting a detector such as an electron multiplier but only by passing near detection plates.

Corresponding Author:-K. Lakshmidurga **Email:-** lakshmidurgas.k@gmail.com

Advantages

- High mass resolution
- High upper mass limit
- High mass accuracy

Disadvantages

- FTMS is more sensitive to pressure comparing to other instruments

PRINCIPLE

Ion cyclotron resonance

It is a phenomenon related to the movement of ions in a magnetic field. It is used for accelerating ions in a cyclotron, and for measuring the masses of an ionized analyte in mass spectrometry, particularly with Fourier transform ion cyclotron resonance mass spectrometers. It can also be used to follow the kinetics of chemical reactions in a dilute gas mixture, provided these involve charge species.

Fourier Transform

A sound signal whose intensity is measured as a time dependent function is made up of many frequencies superposed one over the other, each with its own intensity. The Fourier transform allows one to find the individual frequencies and their intensities [5].

Theory involved

Principle of a FTMS spectrometer is based on ion containment. Ions are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited (at their resonant cyclotron frequencies) to a larger cyclotron radius by an oscillating electric field orthogonal to the magnetic field. After the excitation field is removed, the ions are rotating at their cyclotron frequency in phase (as a "packet" of ions). These ions induce a charge (detected as an image current) on a pair of electrodes as the packets of ions pass close to them. The resulting signal is called a Free induction decay (FID), transient that consists of a superposition of sine waves. The useful signal is extracted from this data by performing a Fourier transform to give a mass spectrum.

INSTRUMENTATION

All FTMS instruments have in common five main components.

- **Ionizer-** Most widely used ionizer is electron ionizer, photo-ionizer, chemical ionizer

ELECTRON IONIZER

In EI an electron beam of controllable energy and current (70ev & 1A) is directed through the center of ion trap for a specified time period. The interaction between electrons and neutral molecules can result in either ejection if valance electrons to form(+) ve ions or electrons capture to form (–) ve ions.

- **Magnet-** Permanent magnet, electromagnet or a superconducting magnet
- **Permanent magnet:** It have low conducting field strength that limit the performance of an FTMS instrument and only a few systems have been built with these.
- **Electromagnets:** These are limited to field strengths below 2T, although 1T is most common. At these fields, FTMS instruments are capable of achieving high performance for ions of relatively low mass-to-charge ratio.
- **Superconducting:** These magnets are sole-noidal magnets used for FTMS have field strengths ranging from 3 to 9.4 T. The performance of the FTMS instrument improves as the magnetic field strength increases and so the trend is to design instruments with stronger fields using superconducting magnets [6].

ANALYZER CELL

The cell is the heart of the FTMS instrument, where ions are stored, mass analyzed and detected. Several analyzer cell designs have been developed

1. Cubic cell
2. Open ended cylindrical cell

Cubic cell

The cubic cell is the first type of analyzer used for FTMS and in still widely used today. It is composed of six plates arranged in the shape of a cube. This cell is oriented in the magnetic field so that opposing pair of plates is orthogonal to the direction of the magnetic field lines and two pair of plates lie parallel to the field. The plates that are perpendicular to the field are called the trapping plates. One of the two trapping electrodes is visible and can be identified as the plate with a hole through its center. It is common for trapping electrodes of cubic cell to have openings that permit electrons or ions to enter the cell along the magnetic field lines. The four remaining plates are used for ion excitation and ion detection [7].

Open ended cylindrical cell

The open ended cylindrical cell has six electrodes that perform the same functions as those of the cubic cell. This cell is oriented so that the principle axis of the cylinder aligns with the magnetic field. The trapping electrodes in this design are the two cylinders at the ends of the cell. The centre cylinder is divided into four electrodes that function as excitation and detection plates.

FTMS ANALYZER CELLS

ULTRA HIGH VACCUM SYSTEM

The third feature required of FTMS instruments is an ultra-high vaccum system. While all mass spectrometers require vaccum for the analysis and

detection of ions, the performance of the FTMS instrument is more sensitive to pressure than other instruments. High vaccum is required to achieve high resolution. For ultra high resolution, pressures of 10^{-9} to 10^{-10} are required. To achieve these low pressures, cryogenic pumps or turbo molecular pumps are used more frequently than diffusion pumps. Most FTMS instruments vaccum systems are equipped with pulsed valves so that the pressure can be increased for a brief duration [8].

SOPHISTICATED DATA SYSTEM

The fourth feature that is shared by all FTMS instruments is a sophisticated data system. Many of the common components of the data system are similar to those used for FT-NMR. They include a frequency synthesizer, delay pulse generator, broadband R.F. amplifier and a computer to co-ordinate all of the electronic devices during the acquisition of data, as well as to process and analyze the data

INTERFEROMETRE

Light from the source is split into two beams by a half-silvered mirror, one is reflected off a fixed mirror and one off a moving mirror which introduces a time delay—the Fourier transform spectrometer is just a Michelson interferometer with a movable mirror. The beams interfere, allowing the temporal coherence of the light to be measured at each different time delay setting, effectively converting the time domain into a spatial coordinate. By making measurements of the signal at many discrete positions of the moving mirror, the spectrum can be reconstructed using a Fourier transform of the temporal coherence of the light.

WORKING

Event Sequences

A simple experimental sequence is composed of four events; they are as follows.

1. Quench
2. Ion formation
3. Ion excitation
4. Ion detection

Quench

The quench event is used to empty the analyzer cell of any ions that may be present from a previous experiment. This can be accomplished by applying anti-symmetric voltages to the trapping plates [9].

Ion Formation

After a short quench event, a new set of ions is formed in the cell, Ions can either be formed in the cell, for example by electron impact ionization, or be formed outside the cell and transferred into the cell during the ionization event.

Ions are formed by passing controllable energy and current (70ev &1a) through centre of ion trap for a specified time period. The interaction between electrons and neutral molecules can result in either ejection of valence electrons to form +ve ions or capture the electrons to form –ve ions.

Ion Excitation

The next event in the experimental sequence is excitation of the ions cyclotron motion. After ions are formed and trapped in the analyzer cell, they often have only a small amount of kinetic energy, less than 1Ev. The magnetic field is applied to the excitation plates. A sinusoidal voltage is applied to the excitation plates. Ions which are in resonance with the excitation frequency gain kinetic energy and spiral outwards from the centre of the cell into a large cyclotron orbit [10].

Ion Detection

Ions of many masses can be detected simultaneously with FTMS. After the completion of excitation event, ions undergo cyclotron motion with a large radius orbit. An image signal is produced on the detection plates, which are connected to the amplifier. The frequency spectrum is converted to mass spectrum by applying a calibration formula derived from cyclotron equation

TIME DOMAIN AND ITS MASS SPECTRUM

Applications and future prospects of FTMS

Two important features of FTMS that will be widely exploited in the future are the ultra-high mass resolution and the wide mass range of the technique. It should be noted that to a considerable extent, the need for greater mass range and higher mass resolution has been stimulated by the development of specialized ionization techniques such as laser desorption, fast atom bombardment, plasma desorption, and secondary ion (SIMS) methods. As these and other processes for the production of gas phase ions from in-volatile solid samples are developed and refined, FTMS will be exceptionally useful for the mass analysis of these materials. For example, the analysis of biopolymers by mass spectrometry is an area that is growing rapidly at present for ordinary protein and nucleotide sequencing mass spectrometry can be used as an adjunct to traditional biochemical techniques. However, for modified or blocked proteins, for which the conventional edam degradation method often fails mass spectrometry will be important in its own right. Similarly, FTMS could be used to sequence modified nucleotides. The ultra-high mass resolution capabilities of FTMS will also be used in the future for accurate determination of nuclide masses. It is possible that all the known stable nuclides will have their masses reexamined by FTMS [11-14].

FTMS also has the potential of becoming an important tool for determining molecular structure. Traditionally, mass

spectrometry has been rather limited in its ability to determine the structure of an unknown compound unambiguously. Additional structural methods, such as nuclear magnetic resonance or crystallography, are commonly used in conjunction with mass spectrometry to elucidate the identity of a molecule. However, when the amount of sample is severely limited or when the sample is a component in a complex mixture, mass spectrometry is often one of the few analytical techniques that can be used [15-19].

The ability to trap and manipulate ions in the FTMS makes this a potentially powerful tool for structural determination. The FTMS has been described as a "complete chemical laboratory" where reactions can be used to "pick apart" a molecule systematically using sequential CAD, photo-dissociation, chemical reactions, or other techniques. As selective and sensitive processes for these reactions are developed, FTMS has the potential of yielding detailed information on the structure of a molecule which is currently only obtainable using techniques that require considerably larger sample sizes. It should also be noted that reactions of trapped ions with neutrals can be also be devised for the step-wise synthesis of a particular species in the FTMS

As noted earlier, the development of dual celltandem quadrupole- FTMS and external ionization cell has facilitated the coupling of FTMS and chromatographic methods. Advances in interfacing separation techniques with FTMS will be important in the analysis of mixtures, especially where high mass resolution is required. For example, liquid chromatographic introduction of mixtures isolated from biological systems directly into an FTMS for analysis wouldeliminate the need for laborious sample clean up.

The future of FTMS from an instrumental standpoint also shows considerable potential. The performance of any FTMS instrument is dependent upon the performance of the computer, magnet, and the vacuum system. While no substantial improvements are on the horizon for the price/performance ratio of vacuum components, the price/performance ratio of computers and magnets improve each year. It is reasonable to expect that the steadily decreasing price of computers and magnets will lower the cost of FTMS instruments and promote their more widespread application. In addition, with the recent surge of research in higher temperature superconducting magnets, it is possible that much smaller, less expensive, and easier to maintain magnets might be available in the future. The commercial development of small, lower performance FTMS instruments based on lower field magnets or other means of ion trapping are also a possibility in the future

Elemental Composition from Accurate Measurements:

A powerful advantage of FTMS over other mass analyzers is its accurate mass capability. For singly charged ions of <700 D, a unique elemental composition can be assigned directly from the measured mass if 1ppm mass accuracy can be achieved

Detection limit for Biological Analysis

The unique combination afforded by accurate mass measurement, ultra high resolution, and nondestructive detection allows for MS in combination with a very low detection limit makes FTMS extremely attractive for biological analysis.

Fig 1. cyclotron motion in the plane perpendicular to the magnetic field lines. The magnetic field is pointing into the plane of the page. An ion moving to the left experiences a downward force that derives it into a counterclockwise orbit

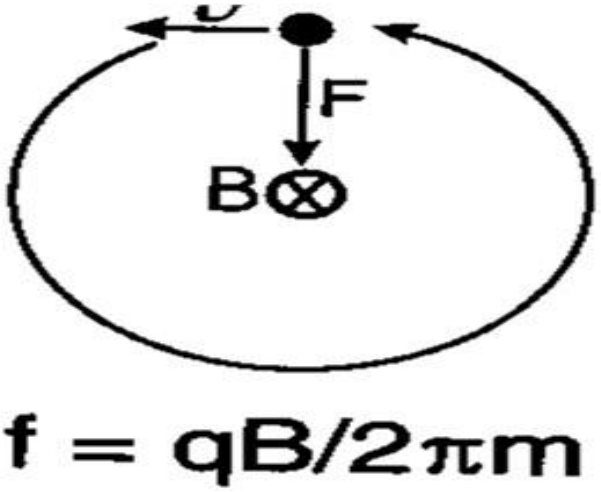


Fig 2. FTMS Instrumentation

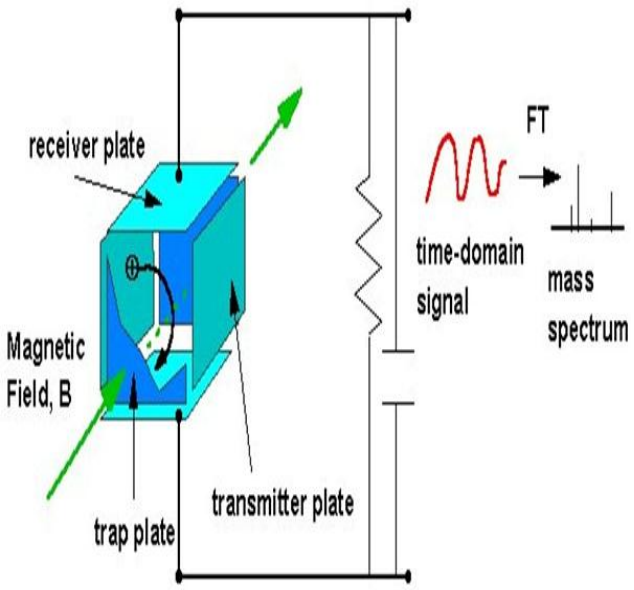


Fig 3. A cubic analyzer cell and a open ended cylindrical cell. The arrow shows the orientation of magnetic field lines.

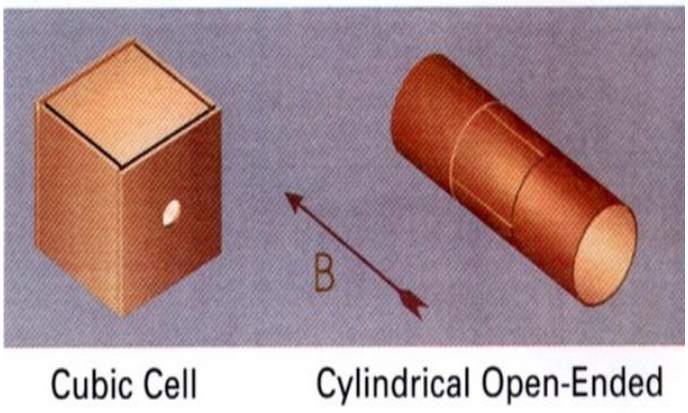


Fig 4. Michelson interferometer- one of the two reflecting mirrors are movable allowing avariable delay to be included in one of the beams

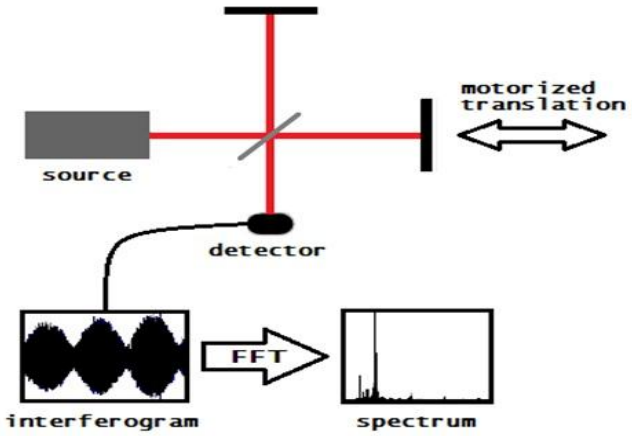


Fig 5. Basic Experimental Sequence

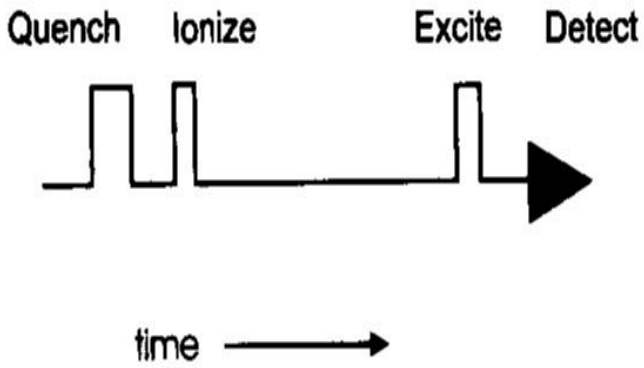


Fig 6. Illustrates Ion Excitation

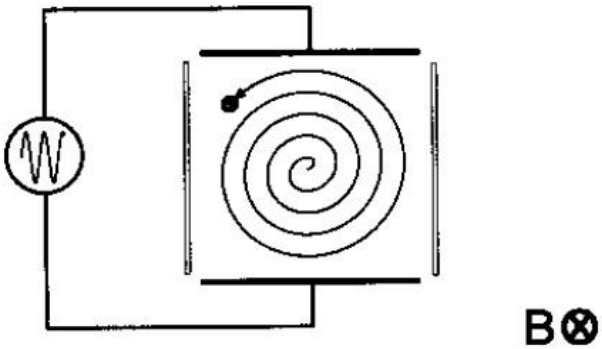


Fig 7. Illustrates Ion Detection

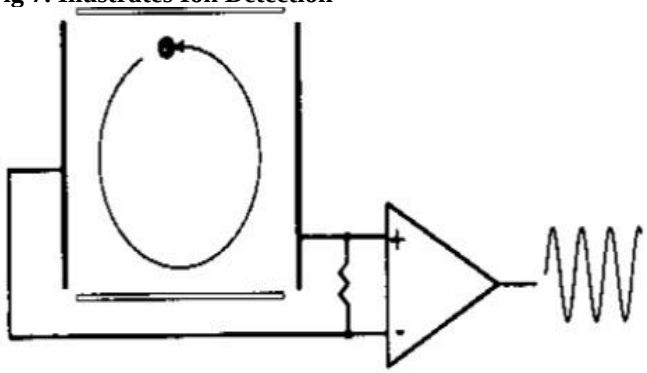
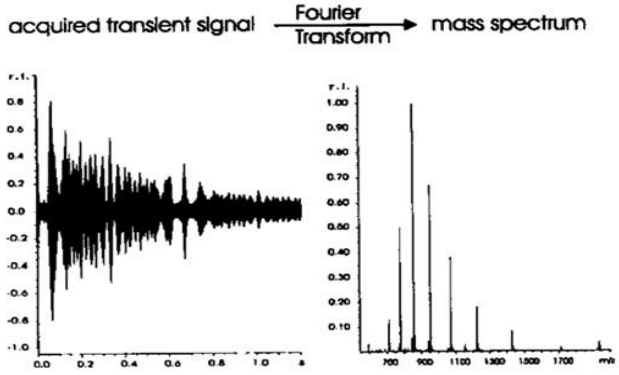


Fig 8. A time domain transient and its corresponding mass spectrum produced by performing Fourier transform



High Mass

The highest mass species carry the greatest number of charges, thereby yielding ions with mass-to-charge ratio especially favourable for FTMS detection even at very high ion mass.

The other applications are:Isotopic Amplification for Unit Mass Accuracy of Bio-macromolecules

- FTMS can be applied in

1. Gas Chromatography Mass Spectrometry
2. Multi-photon Ionization
3. Laser Desorption
4. Secondary Ion Mass Spectrometry

- Useful in studying large macro-molecules such as proteins with multiple charges which can be produced by electro-spray ionization.
- Composition of molecules can be determined based on accurate mass [20-21].

CONCLUSION

Fourier transform mass spectrometry will play an important role in the future because of its unique combination of high mass resolution, high upper mass limit, and multichannel advantage. These features have already found application in gas chromatography-mass spectrometry, multi-photon ionization, laser desorption, and secondary ion mass spectrometry. However, its most notable feature is the ability to store ions. This characteristic, when combined with the others, will allow expeditious study of the interaction of gas-phase ions with both photons (photo-dissociation) and neutral molecules, and the convenient application of this fundamental information for chemical analysis.

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