Review Article

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Review of Liquid Chromatography Mass Spectroscopy

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Abstract

The combination of HPLC with Mass Spectrometry (HPLC-MS) or Tandem Mass Spectrometry (HPLC-MS/MS) is one of the most advanced and widely used technique for both qualitative as well as quantitative analysis of various diverse types of compounds including drugs, pesticides, micronutrients as well as naturally occurring toxic substances. This leads to the fact that many of the practitioners should be well versed with this technique. This review paper focusses on introduction, instrumentation as well as precautions that should be taken for using it. A wide variety of applications are also discussed along with advancements for making it time as well as cost-efficient.

Keywords: Mass Analyzer; Ionization Technique; MS Detector etc.

History

In1897, Modern mass spectroscopy is credited to the cathode-ray experiment of JJ Thomson of Manchester England. In 1953 Wolfgang Paul's invent the quadrupole and quadrupole ion trap and earned Nobel Prize in physics. In 1968, Malcolm Dole developed electrospray ionization (ESI). In 1974, Atmospheric pressure chemical ionization (APCI) was developed by Horning. In 1983, Vestal and Blakely's work with heating a liquid stream known as thermospray was developed. The high-performance liquid chromatograph (HPLC) has been the laboratory tool of choice for separating, analyzing, and purifying mixtures of organic compounds since the 1970s.

Introduction

LCMS is a very popular, advance and highly sensitive technique, which is formed by the combination of the high-performance liquid chromatography and Mass spectroscopy. LC-MS acquire properties from both the techniques like from HPLC it acquire property of separation along with this MS gives the superiority in identification of compounds. It creates an ideal analytical technique for the laboratory/industry because individual techniques has limited capabilities. LC-MS possess superiority over other technique like GC-MS, due to HPLC column can separate almost any mixture that can be dissolved & MS provide a molecular weight for each peak component and structural identity of the individual components with high molecular specificity and detection sensitivity. An LC/MS/MS system can fragment the parent ion into the daughter ions and produce a characteristic fragmentation pattern. The characteristic fragment pattern is specific for particular ion and used for identification and quantitation. This characteristic fragmentation pattern can be identified by comparison with fragmentation patterns available in database. The MS system require very less amount of effluent from the total amount coming out from HPLC column, and rest of effluent can be go to detector or for preparative sample recovery. Chromatographic technique are mainly applied for separation of complex mixture of compounds, followed by identification. In all the chromatographic technique

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(i.e. HPLC) the identification is based on the comparison of Retention time of analyte of interest with its known standard. This type of identification is very effective in qualitative identification, when standard of analyte is available. But when it comes to the point of Quantitative identification or standard of compound is not available or in case of identical compound or compounds having same retention time or separation is not fully efficient, analyst cannot say that compound under analysis are same or different. While Mass spectrometry gives advantage over these condition because ion generated from the compound, give a particular spectrum even these compound have similar RT.The combination of separation capabilities of HPLC, allows introduce the pure sample into the MS; with the capabilities of identification MS identifies analyte even present in trace quantities.

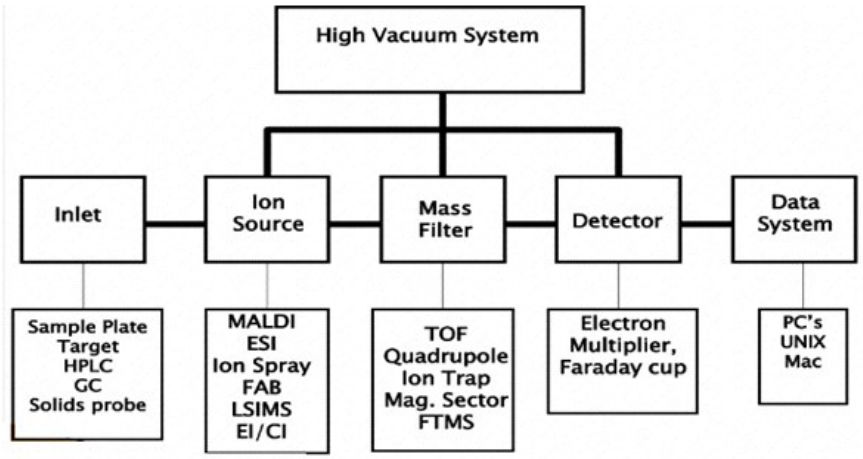

Fig. 1: Flow chart of Mass spectrometer

Tandem mass spectrometry (MS/MS): In this two MS systems are joined togeather in series, Work of first MS system to analyze the samle on the basis ions formed, while second system take ions from first system and ionize into daughter ions, with the analysis of daughter ions, it is possible to determine the structure of the compound as well as it increase the selectivity of instrument for the identification purpose.

Chromatography

According to IUPAC definition of chromatography

'Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (the stationary phase), while the other (the mobile phase) moves in a definite direction. A mobile phase is described as "a fluid which percolates through oralong the stationary bed in a definite direction". It may be a liquid, a gas or asupercritical fluid, while the stationary phase may be a solid, a gel or a liquid.If a liquid, it may be distributed on a solid, which may or may not contributeto the separation process.'

Retention time

It is defined as the time required for an analyte to travels through the column or total time taken since the introduction to the time take till detection. RT varies from the compound to the compound and directly depends upon the interaction of the compound with the mobile as well as stationary phase into the column. For example if a compound interact more with the mobile phase it stays more into the mobile phase, and elute first, detect first, vice versa.

HPLC: HPLC consists of many parts

1. *Pump*: There are different type of pumps are available for the HPLC system, one of the most popular pump is reciprocating pump. The choice of the pump depends upon the available LC-MS interface and diameter of the HPLC column, should have flow rate 10µl/min to 2 ml/min. Pump in the LC-MS system must be pulse free (i.e. it must deliver mobile phase with the constant flow rate).
2. *Sample injection*: Loop injector (or valve injector) can be used. For quantitative determination sample should be introduce along with the internal standard to injection loop.
3. *Mobile phase*: HPLC requires mobile phase which dissolve the analyte completely. There are different types of chromatography are available but usually reverse phase chromatography is used (i.e. mobile phase is more polar than stationary phase). There are two types of elution with mobile phase first is Isocratic elution (mobile phase used have constant composition), another is Gradient elution (composition of the mobile phase change with the time). A range of buffers are also used with the mobile phase like inorganic & involatiles (potassium and sodium phosphate) and organic and volatiles (ammonium acetate). The pH of mobile phase

should be in considerable range, so it will not degrade the stationary phase in the column.

4. *Stationary phase*: The most commonly used stationary phase in the columns is modified silica. The polarity is determined with the chemical modification. The most common stationary phase is C18 alkyl group bonded on the surface of the silica.
5. *Detector*: There are number of detectors available for HPLC, including the Fluorescence, electrochemical and refractive index detector. UV detector mainly monitors the activity of solute, and Refractive index detector measure the change in the property of the mobile phase. In LC-MS these detectors are used as a side detector along with the MS.

The time taken for an analyte to elute from a chromatographic column with a particular mobile phase is termed as Retention time (tan), depend upon the column length and mobile phase flow rate.

Mass Spectrum

The mass spectrum produced should provide unambiguous molecular weight information, structural information that should be reproducible, interpretable and amenable to library matching from the wide range of compounds including biomolecules. The mass spectrum is the plot of mass to charge ratio of positively charged ions against their relative abundance. The m/z ratio is taken along with the abscissa, while relative abundance is taken on ordinate. The most intense peak of the mass spectrum is called the base peak. Base peak is the highest peak it is assigned a relative intensity of 100%. The ion formed from a molecule by removal of one electron of lowest ionization potential is known as molecular ion. The molecular ion is detected as mass to charge ratio that corresponds to the molecular weight of molecule. The molecular ion peak gives the molecular weight of compound. The molecular ion peak id the highest mass number except isotope peak. The ions produced from the molecular ion by the cleavage of bonds are called Fragment ions. They have lower masses and used as building blocks to reconstruct the molecular structure. Fragmentation of molecular ion cleavage bond occurs in heterolytic and hemolytic cleavage. Mass spectrum of molecule shows sharp peaks at m/z integrals. But some show diffuse, broad low intensity peaks at non integral m/z values these are called metastable ions.

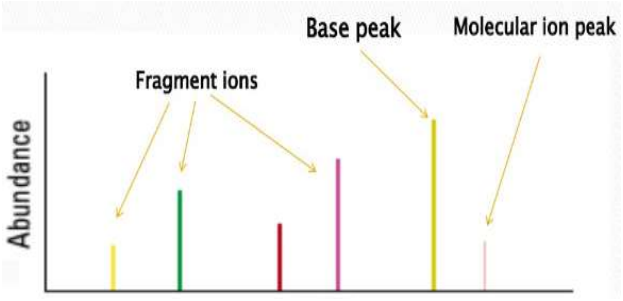

Fig. 2: Show different peak in mass spectrum

Ion Interface Source

HPLC is a method for separating a complex mixture in to its individual components. High sensitivity of mass spectrometry provides the information for identification of compounds or structural elucidation of compound. The HPLC and MS are connected together with an interface which is first introduced in late 1960s. An interface is a mechanical device connects both the instruments. The interface provide quantitative information with reproducibility better than 10% with low limits of detection and have a linear response over a wide range of sample sizes (low picograms to µg). An interface is an interlinking device between LC and MS which efficiently transfers the separated components from the LC column into the MS analyzer. The interface is a very important part of LCMS technique because fundamentally HPLC and MS techniques are incompatible due to LC operates on high pressured mobile phase, in opposite to this MS operates under high vacuum (~ 10−6 torr), so it is not possible to send elute from LC column to the MS system. The interface is a simple mechanical device, which separate the mobile phase from the elute, and transfer solute into the MS system. Ideal interface should follow these conditions

- The interface should cause no reduction in chromatographic performance, useful in analysis of complex multi- component mixture, and do not interfere with vacuum system of MS (i.e. ionization mode, resolution).
- The interface should be reliable, easy to operate, simple and inexpensive and also gives low chemical background (i.e. minimize the possibilities of interference with the analyte or any kind of chemical modification).
- The interface should transfer high quality of sample into the MS. Interface preferred with inbuilt ionization source, important in case trace/polar/labile compound analysis.

- The interface should be compatible with the all chromatographic conditions like flow rate (20 nl/min–2 ml/min), solvent (organic to aqueous, gradient elution), organic and inorganic, volatile and non-volatile buffers.

Direct liquid introduction: The simplest way to introduce HPLC effluent into the mass spectrometer is to split the flow, so that the amount of the solvent enters is remain very less. Vacuum inside MS system remove the solvent leaving analyte behind in gaseous phase (Fig. 3).

Moving wire or Belt interface: The moving-belt interface separates connect the LC system to MS system with a moving belt which uses to transport the analytes from one to the other.The mobile phase of the LC isdeposited on a band and evaporated. Theanalytes remain on the continuously cyclingbelt and are transported from atmosphericpressure into the vacuum of the ion sourcethrough two differentially pumped vacuumlocks. A heater in the ion source evaporatesthe sample from the belt allowing MSanalysis. Most moving-beltanalyses deal with volatile analytes using CI/EI, and formed ions (Fig. 4).

Thermospray: M. Vestal, his co-workers first develop the Thermospray interface. In this system a spray of super-heated mist containing small liquid droplets are produced from the liquid

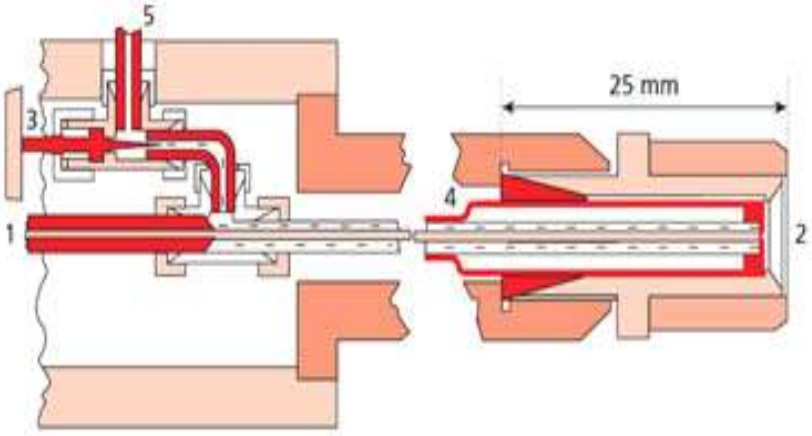

Fig. 3: Direct Liquid Introduction interface

The DLI interface. 1. Connection to LC column, 2. Diaphragm 5 µm opening to MS, 3. Needle valve, 4 .Cooling region, 5. UV detector or waste.).

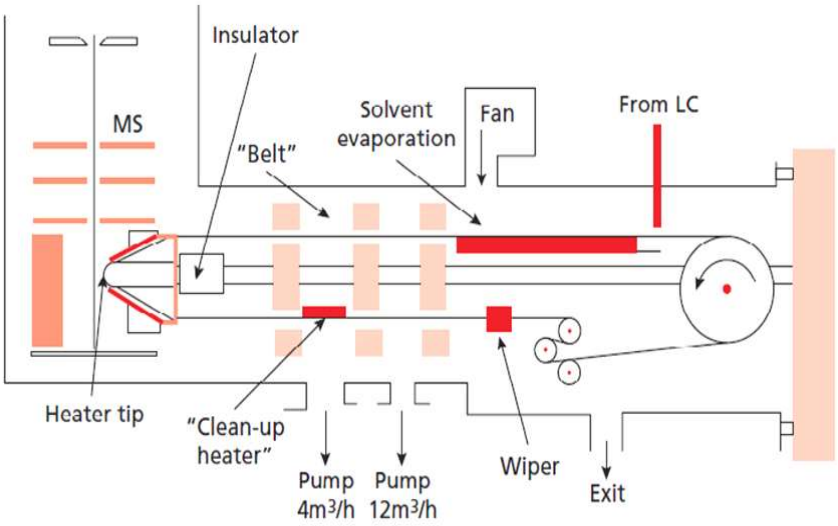

Fig. 4: Moving belt interface

(solute + solvent) coming out from LC column, by passing through the electrically heated capillary, which directly opens into the MS ion source The dropletsfurthervaporized as they collide against the wall of the heated ion source. The ion source is equipped with a mechanical pump which evacuate the excess solvent vapors. The analyte ions enter into the MS through a sampling cone, according to need of analysis sometimes an electric field (repeller or accelerating electrode) is applied. In TSP there are two methods for ionization of the analyte, one without an external ionization source (i.e. so called Real thermospray) and another with an external ionization. The real thermospray use volatile buffers, often ammonium acetate (part of LC effluent). The negative charged buffer ions (like NH_4 & CH_3COO) act as ions of the chemical ionization reagents to form protonated and deprotonated ions respectively. The relative basicity or acidity of analyte with buffer determines which types of ions formed (Fig. 5).

The ionization process takes place both in the gas as well as droplet form. Ions formed in the liquid phase are subsequently transfer into the gaseous phase either by evaporation of the solvent or by evaporation of ions. In cases where no buffer is used, or if a higher percentage of organic modifier are employed, an external ionization method is used. The TSP source, a discharge electrode or an electron impact filament can be used to generate plasma of ions in the mobile phase. These modes such as filament-on and discharge-on, is used to produce solvent mediated Chemical ionization spectra. TSP is a high temperature technique, but it is very useful for analysis of thermolabile compounds such as DNA, and equally important for non- volatile compound (without pyrolysis) analysis. One of the major advantage of TSP over other LC–MS interfaces is its ability to handle the high flow-rates delivered by LC (up to 2 mL/min).

Particle Beam Interface

This interface is also known asMAGIC (monodisperse aerosol generation interface for chromatography) interface, it provides the possibility of using EI/CI without any mechanical method for solvent evaporation (like moving belt in moving belt interface). In this interface LC effluent is forced to pass through a small nebulizer with He gas to form a stream of uniform droplets. These droplets reached in the desolvation chamber and

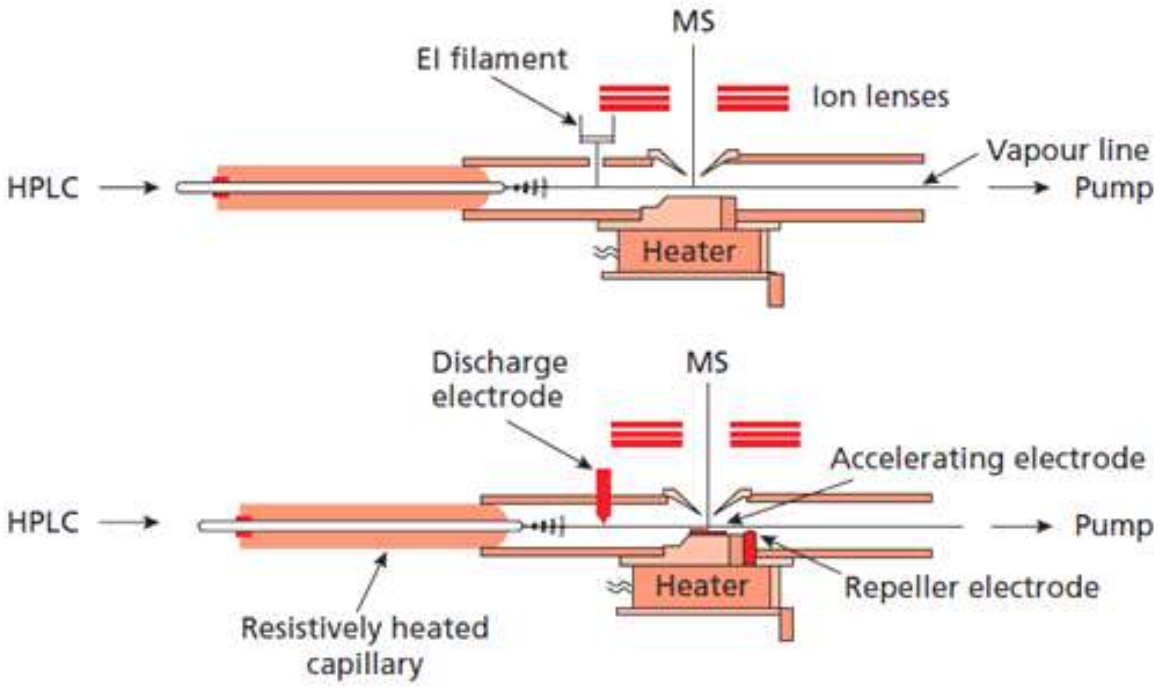

Fig. 5: Thermospray interface. (a) Configuration for 'real-TSP-ionization' (filament off). (b) Configuration with discharge electrode for external ionization and repeller electrode

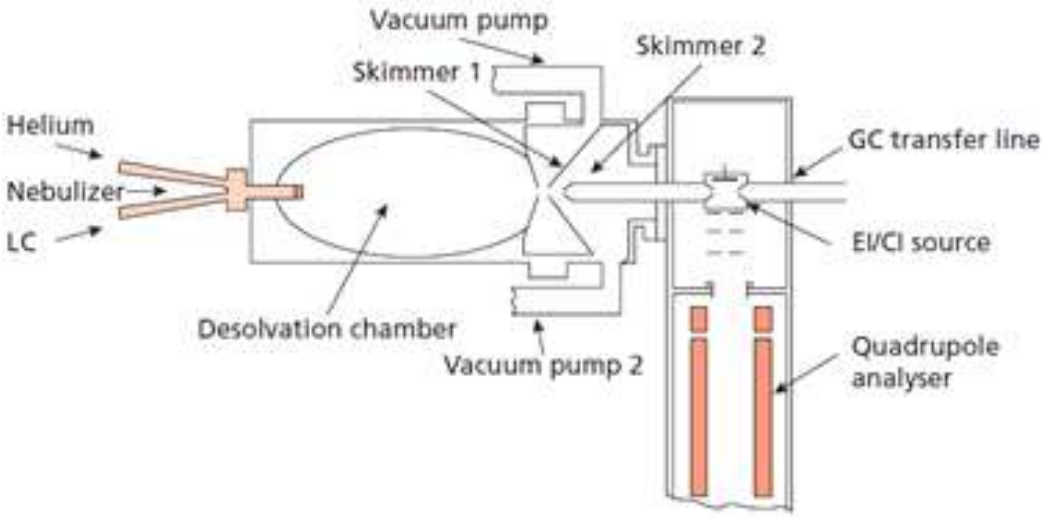

Fig. 6: Particle Beam or MAGIC interface

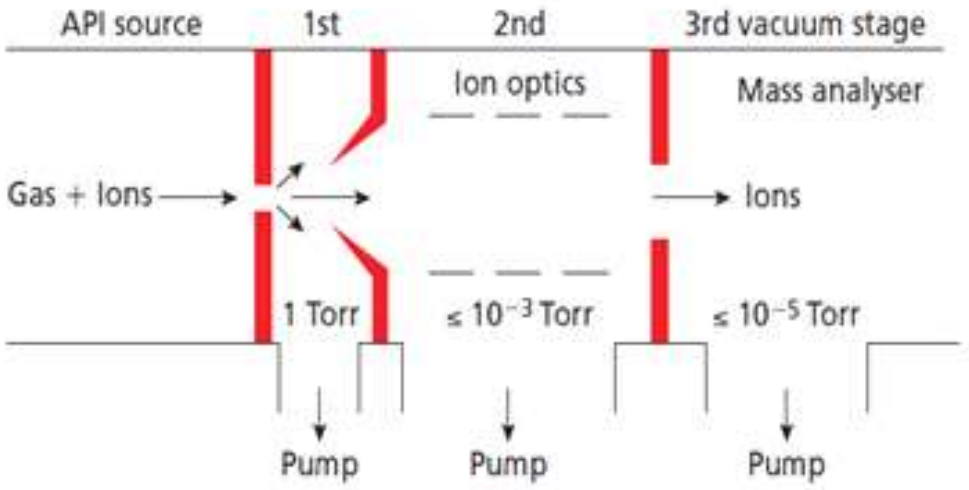

Fig. 7: Atmospheric Pressure Ionization interface

evaporate as solid particle. A differently pumped momentum separator, separate these particles from the gas and transport into the MS source. The particle beam interface optimum flow rate is 0.1-0.5 ml/min (Fig. 6).

Atmospheric Pressure Ionization (API): The increase in the application of LC-MS is the result of introduction of API in commercial market. API includes all the ionization technique in which ions are formed at the atmospheric pressure. Today under API we use two major techniques, 1). Electronspray (ES), 2). Atmospheric pressure chemical ionization (APCI). The ions formed in source at atmospheric pressure are pushed into the vacuum of the analyser. To the way of MS analyser ions are pass through one or more differently pumped stages, which are separated by the skimmers. The ions are focus and transport through the narrow opening of skimmers with the help of Electric field or other ion optics.The advantages of API are 1). API is best technique for analysis of polar, thermally labile, non-volatiles compounds. 2). API is capable in handling the volume of liquid used in LC system. 3). API-MS systems are sensitive and give better limit of detection than any other systems. 4). API is very tough and easy to use (Fig. 7).

Electrospray (ES) or Electrospray ionization (ESI): ESI is most advance method among all the API methods. Zeleny (1917) was first describes phenomena of electrospray, he was explain when

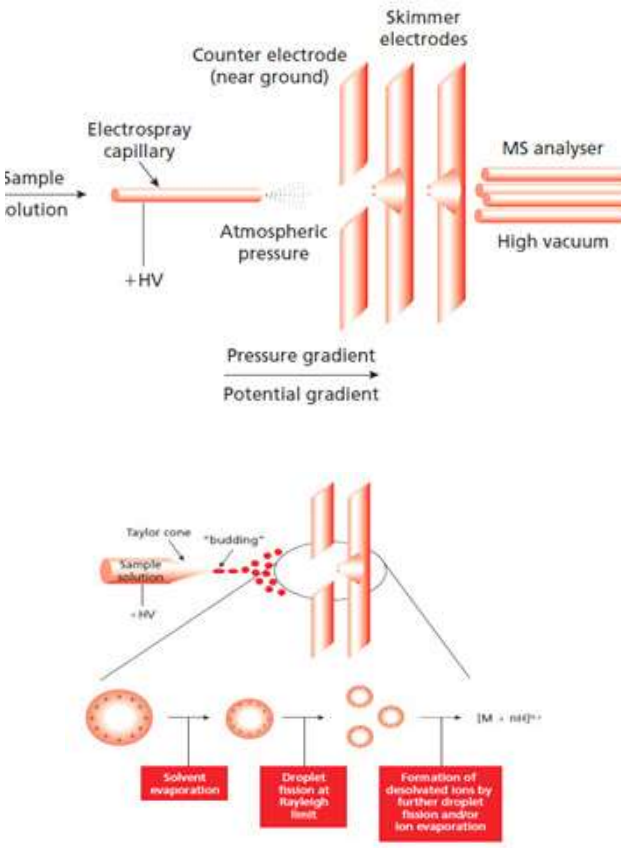

Fig. 8: Electrospray ionization

a high electrical potential applied to a capillary causes the solvent to break into the fine threads disintegrate into the small droplets. Now this becomes the basis of the different commercial ES ion sources.

The ESI source consists of two electrodes, 1). ES capillary, 2).Atmospheric aperture plate of the MS as the counter electrode.Both the electrodes keep at the potential difference of about 3 kV(by applying high voltage). The ions of the same polarity migrate towards the liquid at the tip of the capillary under the influence of applied electric field, from where the liquid is comes out from the side where the capillary forming a "Taylor cone". The buildup (excess) of ion of one polarity at the surface of the liquid droplet, it is the point where coulombic force develops at sufficient strength to overcome the surface tension of the liquid droplets (1 µm) comes out from the capillary. The droplets shrink by the solvent evaporation which again cause disintegration and leads to form small charged droplets (3-10 nm), which are capable producing gas phase ions. The ions formed are passing through a set of skimmer electrodes and finally analyze in the MS system. A gas curtain is placed to avoid entry of the neutral molecules, large particle into the MS system, as well as reduce detrimental effect of deposits on the electrodes and skimmers. The electrodes are set up such that the ions must round angle one or two times may be more guided by the electric field. The spraying an angle (generally 90 degree)to the skimmers increase the sensitivity of the interface. ESI shows optimum performance at flow rate nl/ml. ESI method is very soft method, because ESI allows to analyze highly thermolabile compound by converting them into gas phase without altering their structures, e.g. study if protein folding status, non-covalent bonding, DNA duplexes etc. ESI spectra in first instance contain only give weight information of the ions, for more structure information is need to use tandem MS. (Fig. 8)

Atmospheric Pressure Chemical Ionization (APCI): In APCI the effluent from the LC is sprayed and rapidly evaporated by a coaxial nitrogen stream and heating the nebulizer to high temperature (350-500°C). Such high temperature may cause degradation of the analyte but high flow rate and coaxial nitrogen flow protect molecule from the breakdown. Ions present in the effluent directly goes into the gas phase, along with this additional ionization achieved using a corona discharge (3-6 kV) in the spray. The corona discharge ionizes the analyte molecule as well as the solvent molecules. The solvent ions react with the analyte in gas phase. In the positive ion mode protonated molecules and adducts are formed; in negative ion mode ions are formed by the deprotonation, combination with anions or electron-capture. In APCI the process of the solvent evaporation and ion formation are separate, which allow the use of solvents that are unfavorable for the ion formation (like low polarity solvents). APCI techniques shows optimum performance at the high flow rate (1 ml/min), lower flow rate can be used. APCI works on the high to medium molecular weight, low polarity molecules,

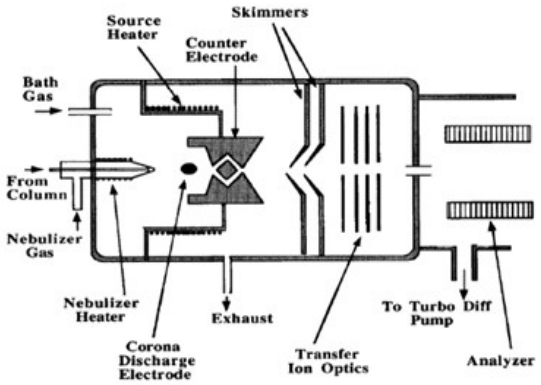

Fig. 9: APCI

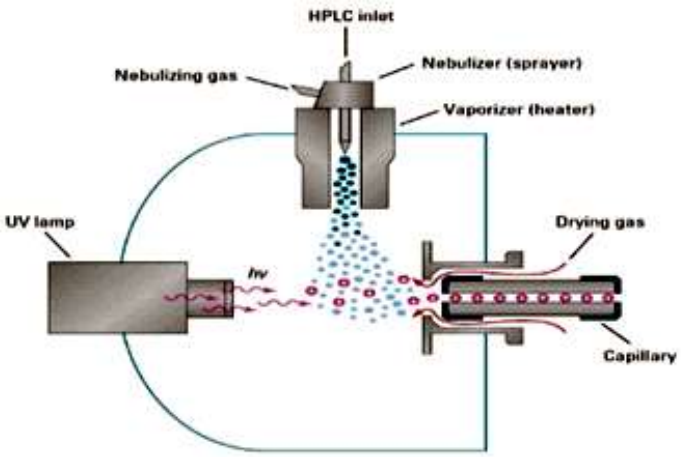

Fig. 10: APPI

like pesticide, drugs, steroids etc. This method is not favorable for too thermolabile molecules. The ionization efficiency of APCI is better than the standard Chemical ionization method (CI), because it occur at high pressure which increase the collision frequency.

Atmospheric pressure photo ionization (APPI): APPI is relatively new technique. Here a discharge UV lamp is placed which generate photons in a narrow range of ionization energies. It shows its ionization for highly non polar compounds and low flow rates (<11m/min) (Fig. 10).

Continuous-Flow Fast AtomBombardment (FAB): Dynamic FAB or continuous flow FAB is the modification of the liquid on the FAB target. This liquid is a high boiling solvent (like glycerol, thioglycerol or nitrobenzyl alcohol), added to the LC effluent and transported through a capillary to the probe tip. The fast atoms (usually Ar, Xe etc.) bombard the sample and ions are sputtered out of the solution and into the gas phase, and sucked in the MS system. Along with the sample some of the matrix is ionizes, and give a low m/z background in spectra. This phenomenon is morepronounced in static FAB. The greatest advantage of cf-FAB, it allows to analyse the highly polar thermolabile, often involatile compounds like surfactants, DNA

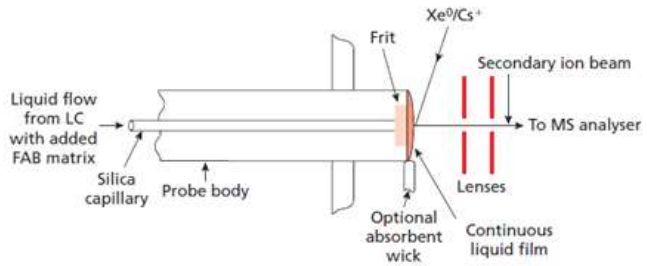

Fig. 11: cf-Fast atom bombardment

adducts, pharmaceuticals etc. To avoid compromise of chromatographic column performance in LC-MS, should be given attention to the addition of the matrix to LC eluent. So Liquid should be introduced on post-column, or in coaxial setup to get better chromatographic performance (Fig. 11).

Mass Analysers: They deflect ions down curved tubes in a magnetic fields based on their kinetic energy determined by the mass, charge and velocity. The magnetic field is scanned to measure different ions. The properties of the mass analyzer are as following:

1. *Sensitivity*: ability to respond by mass analyser on particular ion or m/z ratio.
2. *Resolution*: ability of mass analyzer to separate adjacent ions.
3. *Mass accuracy*: ability of mass analyzer to determine the mass of an ion close to its true mass.
4. *Mass range*: defined as the lower and the upper limits of m/z value record by the mass analyser.
5. *Scan speed*: rate to give mass spectrum of a molecule in terms of mass units per unit time.

Types of Mass Analyzer

1. *Magnetic Sector Mass analyser:* The ions formed in the ionization process have passed through a curved tube; a magnet is placed outside the tube. The ions in the tube accelerated with voltage of 4-8 kV under the analyzer magnetic field. The radius of curvature of the tube in a Magnetic sector mass analyser is a function of m/z. The magnetic force deflects the ions toward the deflector. Lighter ion (low m/z ratio) deflects too much while heavier ion (high m/z ratio) deflect to less, so only ion which having right m/z ratioand pass through the tube. By varying either the magnetic field (H) or the accelerating voltage (V), ions of different m/z are separated. The mass analyser should be maintained on vacuum.

   Theory: When voltage V applied on an ion in the electric field, it start accelerating, so energy of the ion is zV, So as we know potential energy = kinetic energy

   $zV = 1/2mv2$, $v2 = 2zV/m$

   When all charged particles have been

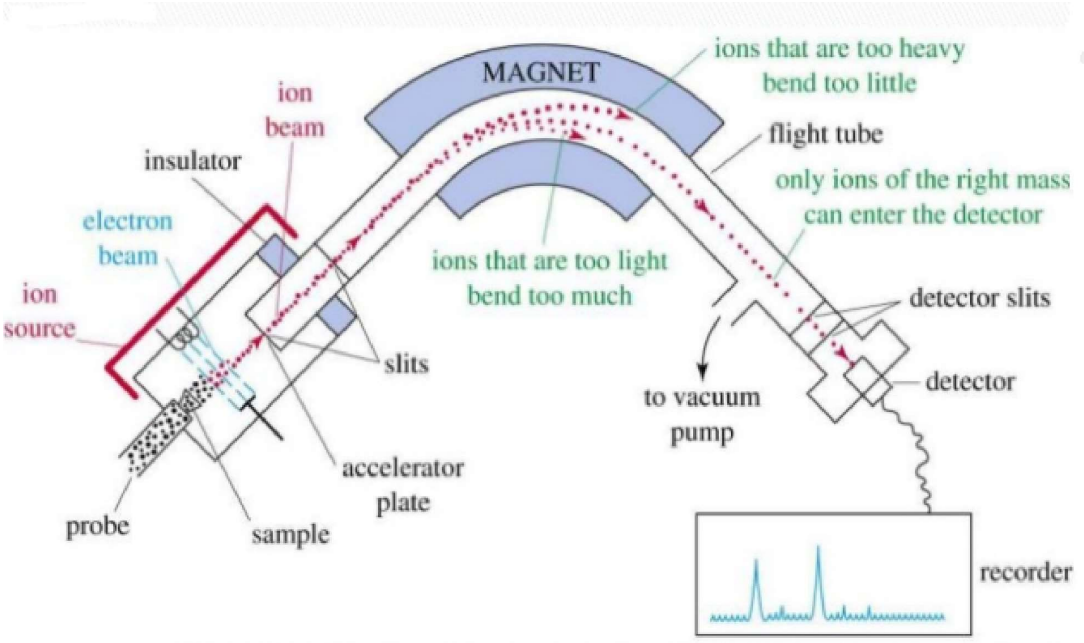

Fig. 12: Magnetic sector mass analyser

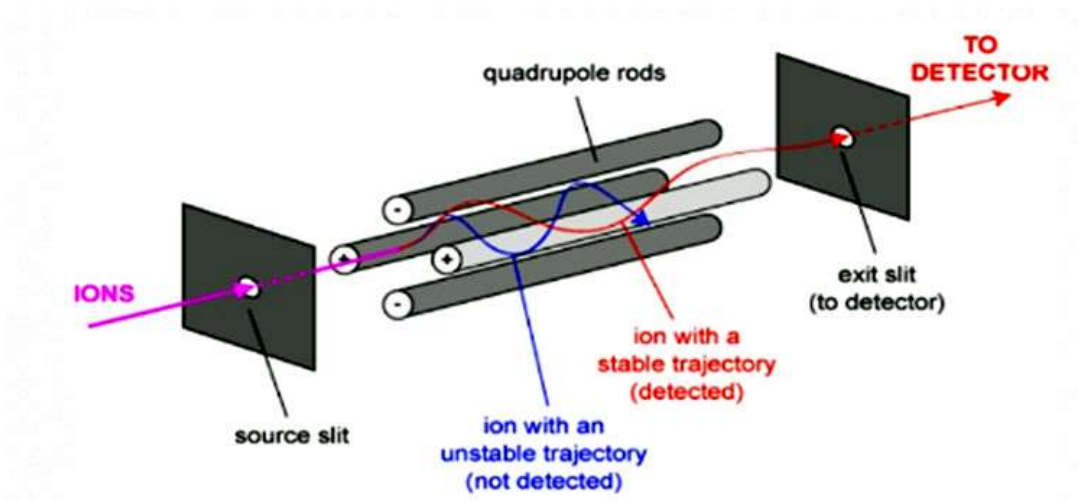

Fig. 13: Quadrupole mass analyser

accelerated by an applied voltage, they enter into magnetic field H, r is the radius of the path. Then attractive force is HzV, and balancing force of particle is mv2/r (Centripetal = Centrifugal). So, HzV = mv2/r (i.e. add value of v2from above equation H2 z2 = m2 (2zV/m)/r2).(where, v = velocity of particle, M = mass, Z = charge on an electron, V = Acceleration voltage). (Fig. 12)

m/z = H2r2/2v

2. *Quadrupole Mass analyser*: The quadrupole mass analyser is one of the most common mass analyser because of its low cost, smaller size, easy to handle, low scan time (<100 ms), more rugged than magnetic sector mass analyser. Quadrupole mass analyser is also called mass filter analyser. Quadrupole mass analyser consist of a set of four metal rods are arranged parallel to each other. The rods are keep on the constant voltage (DC current) and a radio frequency oscillating voltage (AC current) to generate oscillating electrostatic field in between the rods. The opposite rods always have potential of same sign. The ions are accelerated by a potential of 5-15 V and injected into the area between 4 rods. Ions travel in quadrupole axis with cork screw type of trajectory. On a fixed value of oscillating electrostatic field, only a particular value m/z

ion able to travel straight upto detector in between the rods. Other ions (nonresonant ions) collide with the rods and are lost before reaches upto the detector. The continuous change in the voltage, select the ions of different m/z ratio to reach upto detector. Quadrupole mass analyser can record 2-8 spectra per second, upto the range of 4000 m/z units, and worked under the constant resolution (Fig. 13).

3. *Ion trap Mass analyser*: The ion trap mass analyser operates by similar principles where it consists of circular rings electrode, plus two end caps that form a chamber. Here AC orDC power along RF potential is applied between the cups and the ring electrode. There the ions entering into the chamber are trapped by electromagnetic field and they oscillate in concentric trajectories. This process is called resonant ejection (Fig. 14).

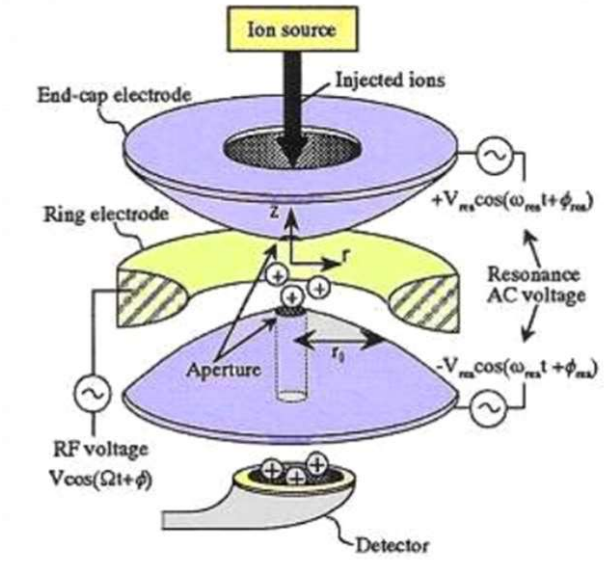

Fig. 14: Time of Flight mass analyser

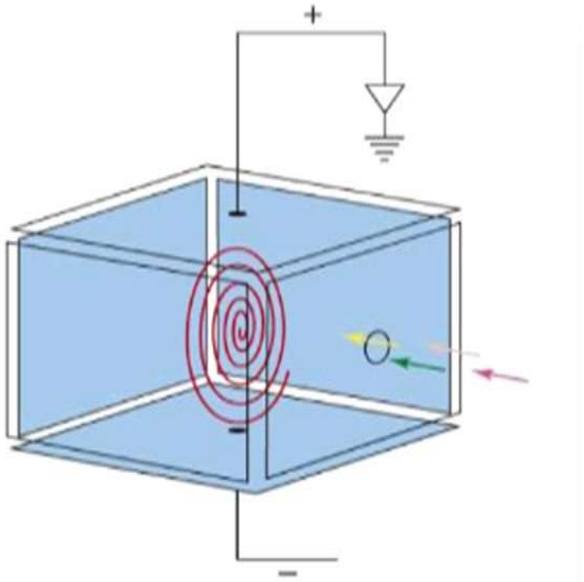

Fig. 16: FT-ICR mass analyser

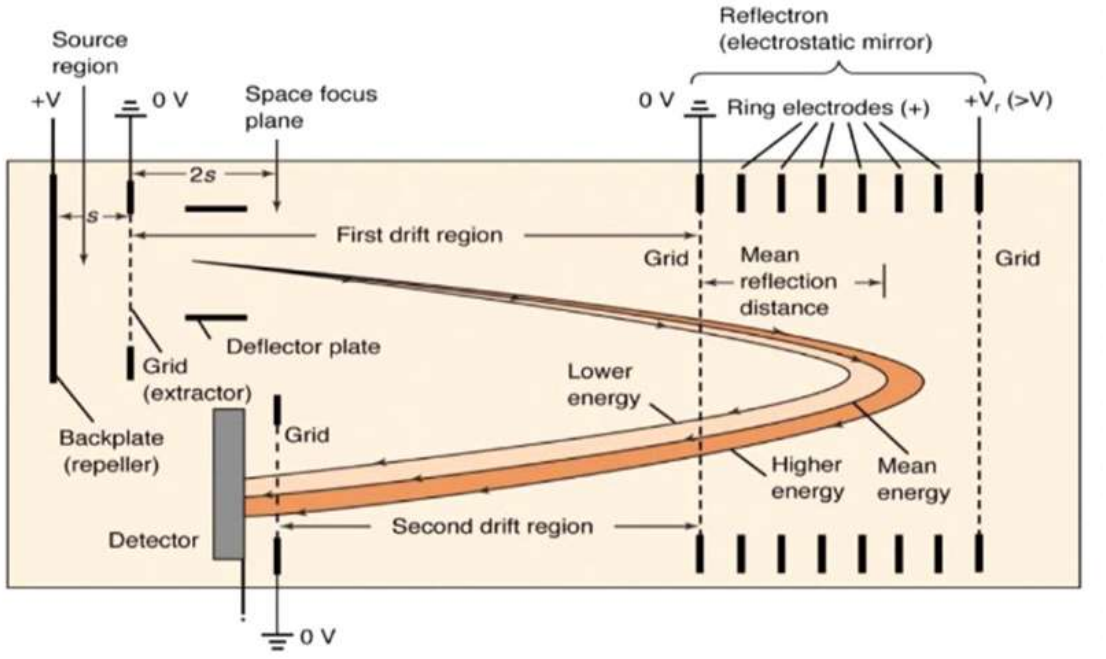

Fig. 15: Time of Flight mass analyser

4. *Time of Flight MS:* TOF mass analyser is based on the simple idea that the velocities of the two ions are created by uniform electromagnetic force applied to all the ions at same time, causing them to accelerate down a flight tube. Lighter ions travel faster and strike the detector first so that the m/z ratio of ions is detected (Fig. 15 and 16).

Detectors

1. Photographic plate

It is used as it is capable of higher resolution and speeder electronic device ie it can detect ions of all the masses and provide a reverse geometry analyzer.

2. Faraday cup

It is a metal cup into which all the ions are directed and the signal produced is very stable and reproducible. It is used on spectrometers where quantitative data is very important.

3. Electron multiplier

In this the current can be measured so accurately by just one ion strikes the detector can be measured ie when an ion strikes the surface of the electron multiplier two electrons are ejected. This process continuous until the end of electro multiplier is reached and electric current is analysed and recorded with electron multiplier surface. Equation describe is 2n. Where n = no of collisions with the electron multiplier surface.

Conclusion

1. *Data handling*: All the MS now employ computer control of same functions and also use a computerized display and output. The amount of data generated even by a fairly modest MS is very large indeed, a single run may store data for upto 100 fragments from each type of molecule and if, LCMS analyze is being performed, a complete mass spectrum is generated and stored every sec for upto 90 min.
2. *Applications*: Molecular weight determination. This technique widely used in laboratories, educational institute and industries to analyze different type of compounds like organic and inorganic biochemical compounds having environmental and biological origin, pharmaceutical, biotechnology, food processing, environment monitoring, cosmetic and agrochemical. This tandem technique can be used to analyze pure as well as impure samples. Differentiation of similar octapeptides: The spectra of octapeptides whose m/z ratio differ only by 1m/z.
3. *Advantage of Linking Hplc with Ms*: The coupling of these two techniques is advantageous in many respects, like convenient, speed analysis of multicomponent mixture, reduce possibility of sample loss, carryout quantization using isotopically labeled internal standard, peak purity.
4. *Problems in combing Hplc and Ms:* HPLC and MS is fundamentally different techniques, because HPLC use liquid mobile phase under pressure with typical flow rate 1 ml/min, While MS operate under the vacuum (10-6 torr). So we cannot simply send elute into the MS system, here we need interface. For in-volatile/ thermally labile compounds EI & CI ionization techniques are not suitable. There should be cautious about some factors limiting performance of the LCMS, like Analyzer signal swamping by the elution solvent, Solvent composition changing in gradient elution, Buffer use for pH control, Ionization of neutral peak components, separation the solvent from the eluent. If the HPLC elute directly introduce into the MS, which cause overwhelming of MS by the signal from the solvent, also buried the solute signal under the solvents signal.

S. No.	HPLC	MS
1.	Liquid phase operation	Vacuum operation
2.	20-25 degree	200-300 degree calcius
3.	No mass range limit	Upto 4000Da for quadrapole
4.	Inorganic buffers	Volatile Buffers
5.	1 ml/min eluent flow is equivalent to500 ml/ min of gas	Accept 10 ml/ min gas flow

Reference

1. Pharmaceutical analysis – Atest book for pharmacy students & chemical application by David G. Watson, pp.206-213.
2. Instrumental analysis by- Skoog, Holler, Crouch, Page no. 607-631, Chapt. 20, page no. 511-524.

3. Spectroscopy by Pavia, Lampman, Kriz, Vyvyam, pp.401-417.
4. Elementary Organic spectroscopy principle & chemical applications by Y.R. Sharma, pp.280-289.
5. Instrumental methods of chemical analysis. By B.K. Sharma, pp.c286-c291.
6. Instrumental methods of chemical analysis by Gurdeep R. Chatwal, Sham K. Anand, pp.20272-20286.
7. Organic spectroscopy principles & Applications, By Jag Mohan, pp.443-46.
8. Vogel's text book of quantitative chemical analysis by J.Mendhan, R C Denny, J.D. Barnes, M. Thomas, B. Siva Sankar, pp.720-741.
9. Agilent technologies, pp.6-30.
10. LC/MS: A Practical User's Guide By Marvin C. McMaster, pp.51-84.
11. Liquid Chromatography-Mass Spectrometry, Third Edition By Wilfried M.A. Niessen.
12. W. Paul & H. Steinwedel; Zeitschriftfür Natur forschung, 8A; 1953.p.448.
13. W. Paul; Agewandte Chemie - International Edition, 29; 1990.p.739.
14. G.C. Stafford et al.; International Journal of Mass Spectrometry and Ion Processes, 60; 1984.p.85 and Analytical Chemistry, 59; 198.p.1677.
15. Quadrupole and Ion Trap Mass Analysersand an introduction to Resolution, by ThermoFinnigan.
16. Philip E. Miller' and M. Bonner (Denton University of Arizona, Tucson, AZ 85721), The Quadrupole Mass Filter: Basic Operating Concepts.