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Role of Tracer techniques in Animal Nutrition- A review

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Introduction

Tracer will be restricted to meaning a marked form of a substance that is used to determine certain properties of the substance in biological systems. These properties include the exchangeable mass or volume of the substance, its localization, its pathway through chemical reactions, and its transfer rates into, out of, and through components of the system.

Nuclear technologies are used in many areas of livestock research and production. For example, we use isotopic tracer techniques to measure the nutritive value of feedstuff, to determine the nutrient intake or energy balance of animals, and to study the metabolism of nutrients in the animal body. The output of research helps us to formulate balanced diets to achieve efficient growth and production. Isotopic methods are also used to monitor reproductive status, leading to better breeding management. Moreover, nuclear techniques are also used in livestock disease diagnosis.

Some examples of isotopes in animal nutrition techniques are:

1. Stable- (^{15}N) and radio-isotope (^{35}S or ^{32}P) incorporation methods for measuring icrobial mass *in vitro* and *in vivo*, enabling the selection of feeds based on the efficiency of microbial protein production.

2. ^{125}I-labeled bovine serum albumin and ^{14}C-labeled polyethylene glycol assays for measuring tannin in feeds; a method based on the feeding of isotope-labeled protein (^{15}N or ^{125}I) complexed with tannin for ranking different tannins for their abilities to release protein for digestion *in vivo.*

3. ^{14}C-uric acid and ^{14}C-allantoin infusion methods for development of models describing excretion of purine derivatives in urine and microbial protein supply to ruminants, which permit assessment of nutritional status of animals and determination of nutritional quality of feed resources.

4. A ^{15}N isotope dilution technique using ^{15}N-leucine to distinguish feed and endogenous secretions at the ileum, for determination of true digestibility of protein-rich tree leaves and aquatic plants in pigs; feeding of ^{15}N enriched plant material to generate ^{15}N-labeled excreta for research on the fate of excreta N in the environment;

5. ^{15}N, ^{13}C and ^{34}S isotopic methods for nutrient budgeting and for following the nutrient pathways in soil-plant-animal continuum; ^{32}P- or ^{33}P-labeled fertilizers for estimating the efficiency of P utilization in legume leaf production used for livestock feeding;

6. doubly labeled water (^{18}O and ^{2}H labeled) method for estimation of energy expenditures of grazing animals, body composition, basal metabolic rate, and milk output in cows with calves;$NaH^{13}CO3$/

$NaH^{14}CO3$ infusion for estimation of the carbon dioxide production which in turn is used to estimate energy expenditure in free-ranging animals; ^{3}H- or ^{14}C-labelled methane and ^{14}C-labeled volatile fatty acids dilution technique for direct and indirect (using stoichiometry of carbohydrate fermentation) respectively for determination of methane emission from livestock;

7. ^{15}N dilution technique requiring labeling the soil with ^{15}N fertilizer (^{15}N-ammonium sulphate or ^{15}N-urea) for estimation of nitrogen fixation by leguminous trees and pastures, for better management of pastures and efficient integration of cereal crops with the fodder crops.

Energy availability from a feed in the rumen

The rate of formation of volatile fatty acids (VFA), provides one of the most reliable indices of the amount of energy fermented. Isotope dilution technique involving the infusion of VFA labelled with 14C (occasionally with 3H) through a rumen fistula, either singly or as mixture of VFA with samples of rumen fluid being taken at intervals over a period of several hours have been used to measure the extent of energy digestion in the rumen. The *net flux* of VFA (mol/min) through the compartment into which a tracer is infused is calculated as:Net flux of VFA(gC/d) = infusion rate (Bq/ min)/plateau SR (Bq/gC)

A more complete understanding of the rates of production of individual VFA and their interconversions can be obtained by solving multi-compartment models. There is no ideal isotopic tracer for estimating the rates of VFA production. Tracer VFA can be labelled using radioactive or stable isotopes. Rates of net flux of the major VFA (acetate, propionate and butyrate) can be estimated individually using separate tracers for each VFA. Alternatively, one labelled VFAcan be used and a good estimate of the combined rates of net flux of all VFA obtained. Production rates of the individual VFA can be predicted reasonably accurately from their molar concentrations in rumen fluid. As little propionate is metabolized to either acetate or butyrate in the rumen, 14C-labelled sodium propionate is nearest to being an ideal tracer in experiments using only a single labelled VFAbut it is also the most expensive of the three 14C-labelled VFA. As a less expensive alternative, 1-14C, 2-14C or uniformly labelled (U-14C) acetate can be used to determine acetate (or total VFA) net production rate. Acetic acid is the predominant VFA produced by ruminal fermentation and is closely associated with ruminal hydrogen production. Acetate interconverts with butyric acid and this interconversion can cause underestimates of acetate production by 6%. This error can be overcome by making separate injections or infusions of labelled acetate and butyrate on separate days to quantify the interconversion. Procedures for estimating VFA production in ruminants and advantages and disadvantages of the method are described in detail in Hegarty and Nolan (2007). According to these authors, while there is no maximum time for infusions, a 9 h infusion, with sample collection over the final 6 h can give a good estimate of daily production if animals are given their daily ration in equal hourly portions. The 6 h period of sampling is long enough to allow averaging of small variations in VFA production rate that may occur as the substrate supply for the rumen microorganisms varies between meals.

Determination of tannin level and activity

Tannins at high levels produce adverse effects. The radioactive method based on 125I labelled protein (bovine serum albumin) is considered to be the most specific and sensitive method for determining the protein precipitation capacity of tannins (Makkar, 2005c). The 125I based radiolabelled method has been simplified to eliminatethe more difficult procedures of relatively high speed (13,000×*g*) centrifugation andmanipulations of small volumes of radiochemical solutions by binding the radiolabelled protein (125I bovine serum albumin) to tannin immobilized on a paper disk. The tannin–protein complex is formed on the paper disk and the counts, which represent the protein bound to the tannins, are measured by inserting the disk into a gamma counter; the higher the count, the higher protein binding capacity of the tannins. The counts can be converted to the amount of protein bound using a standard curve prepared by using 125I-labelled bovine serum albumin (Henson et al., 2004). Similarly, the *in situ* tannin binding assay method based on 14C-polyethylene glycol (14C-PEG) binding has been simplified by reducing both the amounts of feed sample and 14C-PEG, enabling analysis of a greater number of samples at a lower cost. In addition, a new approach for estimating the level of PEG binding to tannin-containing foliage, which does not require the use of a tannin-free substrate to correct for non-specific binding, has been proposed (Mlambo and Makkar, 2005). Simplification of these assays will enable a large number of laboratories in developing countries to use these methods. These methods are potentially useful in the evaluation and use of tannin-containing feed resource for improving livestock

production. Condensed tannins (CT) are thought to obstruct the digestion of protein in the rumen through formation of tannin–protein complexes. In the rumen, these complexes are considered to be stable; however, they dissociate post-ruminally in response to the extremes of pH that occur there. The low pH in the abomasum as well as the high pH in the small intestine can result in dissociation of these complexes. This understanding forms the logic behind the hypothesis that CT can improve the total availability of protein in forages by reducing the wasteful deamination of protein in the rumen in diets where rumen degradable protein is in excess of microbial requirements (Waghorn and McNabb, 2003; Ben Salem et al., 2005). The protein binding capacity of tannins *in vitro* varies substantially, depending on their nature and structure (Makkar, 2003). That is, on a g/g basis, CT purified from one type of forage can precipitate more protein than another. This raises the possibility that tannin–protein complexes containing particularly astringent CT may not dissociate postruminally as completely as those with less astringent CT. Consequently, some CT may not improve the supply of digestible protein post-ruminally as much as others. A simple *in vivo* method, based on isotopically labelled protein (15N or 125I), that ranks different tannins on their abilities to release protein for digestion has been developed (Andrabi et al., 2005). In this method, CT from four types of the well known Australian native forage tree, Mulga (*Acacia aneura*), and from *Leucaena pallida* were purified and complexed with either 15N lucerne protein or 125I-bovine serum albumin to obtain tannin–protein complex.

The extent of dissociation was determined by estimating the apparent digestibility of plant protein initially in the tannin–protein complex. This was done by comparing disappearance, between the abomasum and faeces, and mouth and faeces of 15N-plant protein in the tannin–protein complex relative to a co-infused indigestible marker, chromium-EDTA (Cr- EDTA). Use of a 15N label was ineffective because of the poor precision associated with the high natural abundance of 15N relative to the levels of enrichment achieved in the TPC. By contrast, *in vivo* rankings of tannins using 125I-labelled protein correlated with the ability of the same tannins to bind protein *in vitro*. Furthermore, a good correlation ($r2 = 0.90$) was found between *in vivo* protein digestibility determined between the rumen and the faeces and digestibility between the abomasum and the ileum (Kariuki, 2004).

In vivo astringency can be assessed simply by dosing the labelled protein–tannin complex orally.

Determination of saponins and other anti-protozoal compounds

A method for determining the bacteriolytic activity of ruminal protozoa *in vitro* has been proposed by Wallace and McPherson (1987). In this method, the ruminal bacterium, *Selenomonas ruminantium*, is labelled with 14C-leucine. The labelled bacteria are then added to ruminal fluid containing protozoa. The degradation of 14C-leucine labelled *S. ruminantium* at different incubation periods is calculated from the acid soluble radioactive label and expressed as a percentage of the total disintegrations per minute present in labelled bacterial suspension. The rate of degradation per hour is calculated as the difference from the linear portion of the degradation curve (normally 0–3 h). The bacteria are broken down by the protozoa at a rate reflecting the activity of protozoa. This method can also be used for quantifying the antiprotozoal activity of a bioactive compound, for example a plant compound. The decrease in the slope (rate of degradation of the labelled bacterium) is a measure of the anti-protozoal activity. This method is useful in screening plants for the presence of compounds having antiproteolytic activity. Rumen bacteria can also be labelled with 15N (Bento et al., 2005) and used for determination of protozoal activity.

True protein digestibility in pigs

True protein digestibility in pigs is measured at the level of the ileum. However, the ileal digesta contains dietary proteins and endogenous proteins, the latter coming from non-reabsorbed digestive secretions and sloughed epithelial cells. The estimation of the true digestibility of dietary proteins and of the endogenous protein losses thus requires a distinction to be made between these two protein sources. The only possibility to achieve this reliably is to resort to the 15N isotope dilution technique (Moughan et al., 1998). One of the two protein sources (dietary protein or digestive secretions) is labelled with 15N and their 15N-enrichment in these protein sources is determined. The dilution of 15N in the total intestinal N content is then measured. The ratio of the enrichment of the intestinal content to that of the labelled protein source gives the proportion of labelled proteins in the intestine. Knowing the flow of total proteins at the end of the small intestine, one can calculate the flow of both protein sources and thus the endogenous protein losses and the true digestibility

of the dietary proteins. In a study conducted in Colombia (Leterme, 2002), the endogenous secretions were labelled by infusing 15N-leucine in blood and true digestibility of protein rich tree leaves and aquatic plants was measured. The labelled amino acid is progressively incorporated in the digestive secretions. The method is based on the assumption that the fraction of free amino acids in blood is the precursor pool of the digestive secretions and as such has the same 15N-enrichment. After 6–8 days, a steady state of 15N-enrichment of the free amino acids in blood, and thus of the digestive secretions, is reached. The ileal digesta is then collected, its 15N-enrichment determined and the proportion of endogenous proteins calculated.

In the second method, the dietary proteins are labelled by applying 15N-labelled fertilizer (for example NH4NO3) to crops. A meal of the crop is prepared in which the labelled ingredient is the sole protein source. Only one labelled meal can be given to the pigs because the dietary 15N in the digestive secretions are recycled quickly (Leterme et al., 1996). The ileal digesta is collected for 12 h after the labelled meal has been given. 15N is analyzed on each sample in order to determine the peak of 15N excretion and, thereafter, the samples with the highest 15N enrichment are analyzed by gas chromatography /combustion/ isotoperatio mass spectrometer (GC/C/IRMS) for determination of the 15N content of every amino acid. The advantage of this method is that all the dietary amino acids are labelled. It is thus possible to make the distinction between the endogenous and dietary amino acids.

The disadvantages are that: (1) it is difficult to label meals (for example soyabean meal), feedstuffs (tree leaves) or animal products (meat meal), (2) only one labelled meal can be provided to the pigs as mentioned previously, and (3) the fast recycling of labelled dietary amino acids in the digestive secretions could lead to overestimation of endogenous nitrogen losses.

Energy expenditure

The doubly labelled water (18O and 2H labelled water) method has potential for measuring energy expenditures over 10–14-day periods in range cattle and draught animals. This method would especially be useful as the available information on maintenance energy metabolism and energy cost for walking in these animals is sparse and confusing. The method could also be used to measure milk intake by calves and thereby calf growth and development, milk output in cows with calves, and body composition. It involves administration of doubly labelled water into the blood or rumen or given orally and then measuring the label at equilibrium phase (when the label has reached plateau) in body fluids such as urine, saliva, milk or blood. The two tracers distribute in body water and the deuterium is eliminated as water. The 18O is eliminated as water and carbon dioxide and its rate of excretion is higher than that of deuterium. The difference between the two elimination rates is therefore a measure of carbon dioxide production over a period of observation (Speakman, 1997). Energy expenditure can be calculated from carbon dioxide production using its respiratory quotient. *Ballevre et al* estimated energy expenditure in dogs and cats using the following formula

- $rCO2 = (N/2) (k2 – k1)$
- where N is the total body water pool
- k2 is the rate of disappearance of ^{18}O
- k1 is the rate of ^{2}H disappearance
- EE (kJ/d) = r CO2 x 22.4(3.7/FQ+1.33) x4.184
- The measured EE for 11 days was 207 , 263 kJ/kg/d for cats and dogs respectively
- These values were 30 and 10% lower, respectively, than NRC recommendations
- The water out put (urine + breath) was 36ml/kg/day for cat and 60 ml for dogs

However its use in livestock research has been limited. The doubly labelled water technique could also be used to understand how maternal energy expenditure affects energy needs and energy transfer to the foetus and how nutritional interventions of the mother affects foetal growth, birth weight and productive and health status of the progeny later in life. The method is particularly useful for field experiments on free ranging animals because, (a) it places no restrictions at all on the animal's activities or movements, (b) the animal does not have to carry any equipment around with it, and (c) the collection and preservation of the samples in the field are very simple; all the complicated analytical procedures are done in the laboratory. Calibration experiments with humans show the doubly labelled water method to be about as accurate as other field measurements. The method is not suitable for short-term measurements, *e.g.*, measuring the immediate energetic response of an animal to stimuli such as work, but it could help characterize livestock breeds for basal metabolic rates. The energy expenditure in free-ranging animals can also be determined byCO2-entry rate technique, in

which the production ofCO2 by the free-ranging animal can be measured using a constant intravenous infusion of NaH13CO3/NaH14CO3. The entry rate of bicarbonate (production of CO2) is calculated from the infusion rate and the dilution of the isotope at equilibrium in saliva. A complete collection of CO2 of expired air is not required. A respiratory quotient value is used, similar to the doubly labelled water method, based on feeding level (or productive status), to estimate heat production. A major disadvantage of both the 13C-bicarbonate and doubly labelled water methods is the high cost of the label. The doubly labelled water gives integrated value over a longer period of time (1–4 weeks) compared to the 13C-bicarbonate method (hours to days). The 13C-bicarbonate method gives an estimate of energy expenditure whereas the doubly labelled water method enables determination of other parameters as well such as body composition. For further information readers are referred to an excellent review on the use of the 13C-bicarbonate method for estimating the energy needs of free-ranging animals (Lachica and Aguilera, 2003). The characterization of local breeds for traits such as basal metabolic rate, energy expenditure for different growth and production parameters, body composition at different stages of growth will aid decision making for the conservation and effective use of these breeds in breeding programs as well for developing effective feeding and animal breed management strategies. Deuterium oxide dilution has also been used for determination of lean body mass, fat content, body composition, total bodywater and milk intake by calves (Worden and Pekins, 1995). Stable isotope labelled water (deuterium oxide) is given to the animal and its concentration in body fluid is generally measured by isotope ratio mass spectrometry (IRMS). Mass spectrometry is expensive and requires specialized skills for operation and maintenance. Infrared spectroscopy is a relatively inexpensive technique and has been validated against IRMS. It can also be used for measuring deuterium oxide in biological fluid samples as accurately as IRMS.

Determination of proteolytic and cellulase activities

Enzyme assays are routinely used to quantify various enzyme activities in pure and mixed cultures. Proteolysis is an important step in the digestibility of proteins and availability of amino acids and peptides for various anabolic processes in animals. Similarly, microbial cellulase is responsible for cellulose degradation in the rumen and the hind gut, providing energy sources in the form of glucose and short chain fatty acids to the animal. The radioactively labelled substrates have been used to study various digestive processes. Plant or animal proteins can be labelled with 14C by reductive methylation, for use as a substrate for proteolytic enzymes. Wallace (1983) labelled casein and other proteins using 14C-formaldehyde in the presence of sodium borohydride. This labelled casein can be used to study the proteolytic activity of rumen microbes and of other biological samples. In this method, determination of acid-soluble radioactivity is taken as the measure of proteolytic activity. Plant proteins can also be labelled by growing the plant in the presence of 14CO2 and used as a substrate. Similarly, growing plants in an atmosphere of 14CO2 or injecting 14C precursors (*e.g.*, 14C-acetate) and isolating and purifying the compound of interest, for example tannins (Makkar, 2003) or saponins (Okumura et al., 2000) containing 14C label, can be used for various degradation and metabolic studies in biological systems. Cellulase assays are generally performed by using carboxymethyl cellulose or acid swollen cellulose and measuring reducing sugar. 14C labelled bacterial cellulose can also be used. The release of radioactivity in the solution from 14C-cellulose as a result of the cellulase enzyme is a measure of the enzyme activity. The use of radiolabelled cellulose makes the assay highly sensitive. The 14C method is limited by potential health hazards and the complexity of handling and disposing of radioactive material. Some researchers have investigated a stable isotope of carbon 13C as a potential tool for studying plant metabolism in ruminants , but the limited amount of plant material that can be labelled and the need for specially designed growth chambers limit the use of this technique. Another stable isotope (15N) has been used to label plant nitrogen fractions (using 15N-enriched N, say in the form of ammonium sulphate as a fertilizer), which then can be used to study ruminal metabolism of feedstuffs.

Gamma irradiation for enhancing nutrient availability

Several novel alternative plants are available that are good sources of protein, carbohydrate and other nutrients for use in livestock diets. But, these unconventional feed resources contain substantial amounts of plant secondary metabolites, which could have both adverse and beneficial effects depending on the level and nature of the plant secondary metabolites. The plant secondary metabolites that are found commonly in these feed resources are: protease inhibitors, tannins, saponins, lectins, phytate, oxalates

and non-starch polysaccharides. At high levels of plant secondary metabolites, the bioavailability of nutrients decreases. Several physical and chemical approaches have been used to remove plant secondary metabolites or inactivate their adverse activities. Ionisation radiation treatment could also be a possible method for inactivating these compounds. Gamma irradiation levels of up to 10 kGy have been found to be effective for inactivating antinutrients such as protease inhibitors, lectin, phytic acid, non-starch polysaccharides and oligosaccharides without altering the nutritional value of food/feed. Higher levels of irradiation, up to 600 kGy can be used to improve rumen degradability of dry matter and crude fibre in crop residues. Commercially available soyameal, widely used in livestock feeds, is generally steam treated to make it safe for livestock feeding. This heat treatment inactivates protease inhibitors and lectin present in soyameal. Itwould be interesting to compare the economics of soyameal treatment by steam and irradiation treatments and the industrial feasibility of using the latter.

Mutation breeding for producing feeds of desired traits

Induced mutations with gamma radiation, electron beam and fast neutrons produce random changes in the nuclear DNA or cytoplasmic organelles. These chromosomal or genomic mutations can be useful to plant breeders for selecting useful mutants of forage plants; for example, plants with high yield, disease resistance, saline/droughtresistance, early maturity, modification of lignin profile to enhance dry matter digestibility,vhigher intake and higher passage rate, higher content of soluble carbohydrates, expression of 'rumen undegadable' protein and containing S-amino acids and 'stay-green' properties. For oil seed crops, the residues of which are fed to livestock, induced mutation could be used to select mutants with lower levels of antinutrients in addition to having proteins of higher nutritive values. The benefits of induced mutation can be compounded using recent molecular techniques, such as restriction fragment length polymorphism, random amplified polymorphic DNA, amplified fragment length polymorphism,DNAamplification fingerprinting, denaturing / temperature gradient gel electrophiresis,high throughput DNA sequencing and microarrays, which are available for characterizing genomes and induced mutations, and enabling the provision of DNAmarkers to assist the selection of desired traits.

Conclusion

Isotopic and nuclear techniques are important tools in animal production research. The principal advantage of stable isotopes over other tracers is that they are not radioactive and thus provide the ease of handling and transport. On the other hand, special precautions and laboratory conditions are required for using radio-isotopes, which could discourage some workers in using them. Although isotopic and nuclear techniques might be more expensive compared to the conventional techniques and probably as expensive as some of the molecular techniques in use, these techniques offer comparative advantages of high specificity, sensitivity and accuracy over non-nuclear techniques. Because of these advantages, isotopic and nuclear techniques help generate accurate data and provide unequivocal answers. These techniques coupled to the use of molecular tools have the potential to revolutionize the understanding of complex biological processes and make the livestock an efficient entity – highly productive with minimum wasteful discharges to the environment – helpingto achieve sustainability of the global food chain.

Mass Spectrometer - Introduction

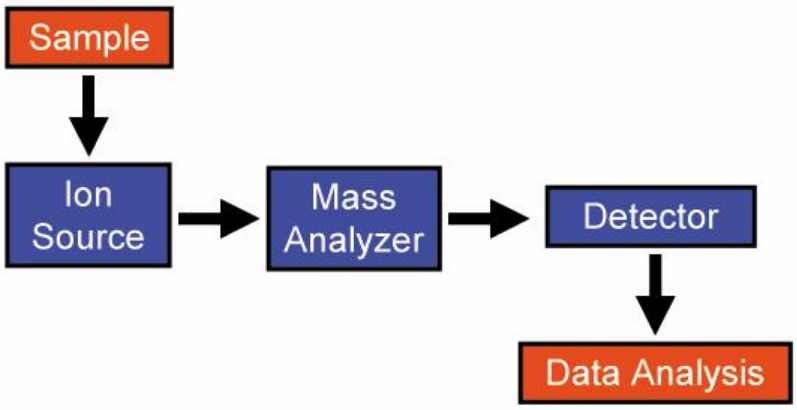
Mass Spectrometer (MS) is a kind of machine which uses an analytical technique to measure the mass-tocharge ratio of ions. This analytical technique is also known as Mass spectrometry. An ion is an atom or group of atoms which have lost or gained one or more electrons, making them negatively or positively charged. Mass spectrometry is an important emerging method for the characterization of proteins. The two primary methods for ionization of whole proteins are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI).As it is an important tool in proteomics, it is essential to understand not only the results, but also the principles of Mass Spectrometer. This report is devoting to provide a simple but clear explanation to the principles of Mass Spectrometer.

Mass spectrometry that are concerned with non-destructive interactions between molecules and electromagnetic radiation. This is because mass spectrometry is the study of the effect of ionising energy on molecules. It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species. Although sample is consumed destructively by the mass spectrometer the technique

is very sensitive and only trace amounts of material are used in the analysis. **A** mass spectrometer converts sample molecules into ions in the gas phase, separates them according to their mass to charge ratio *(rnlz)* and sequentially records the individual ion current intensities at each mass - the mass spectrum.

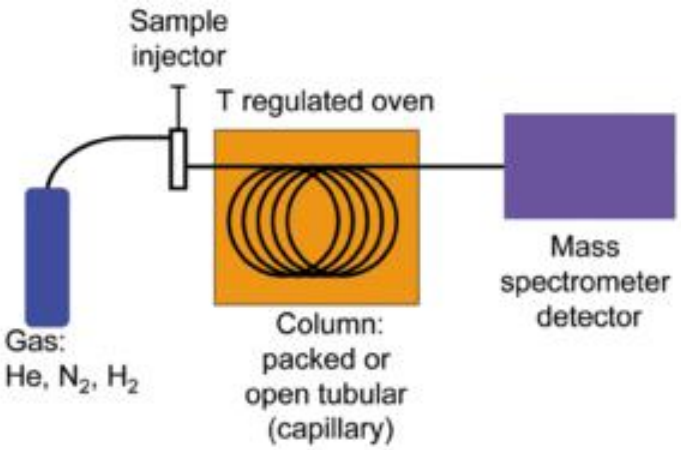
2. General Structure of Mass Spectrometer

Generally, a typical Mass Spectrometer consists of three parts: an ion source, a mass analyzer and a detector. The function of the ion source is to produce ions from the sample. The function of the Mass Analyzer is to separate ions with different mass-to-charge ratios. Then the numbers of different ions are detected by the detector. Finally, the mass spectrum is generated after all the data have been collected.



The output, i.e. mass spectrum, is an intensity vs. m/z (mass-to-charge ratio) graph, from which the Chemists are able to draw clues about the ions.

Gas Chromatography Mass Spectrometry



Gas chromatograph

- The gas chromatograph utilizes a capillary column
- The difference in the chemical properties between different molecules in a mixture will separate the molecules as the sample travels the length of the column.
- The molecules take different amounts of time (called the retention time) to come out of (elute from) the gas chromatograph
- Mass spectrometer capture, ionize, accelerate, deflect, and detect the ionized molecules separately

- Mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio.
- MS analysis requires a pure gaseous sample. The sample inlet is maintained at a high temperature, up to 400° C (752° F), to ensure that the sample stays a gas. Next the specimen enters the ionization chamber. A beam of electrons is accelerated with a high voltage. The specimen molecules are shattered into well-defined fragments upon collision with the high voltage electrons. Each fragment is charged and travels to the accelerator as an individual particle.

  In the acceleration chamber the charged particle's velocity increases due to the influence of an accelerating voltage. For one value of voltage only one mass accelerates sufficiently to reach the detector. The accelerating voltage varies to cover a range of masses so that all fragments reach the detector.
- The charged particles travel in a curved path towards the detector. When an individual charged particle collides with the detector surface, several electrons (also charged particles) emit from the detector surface. Next, these electrons accelerate towards a second surface, generating more electrons, which bombard another surface. Each electron carries a charge. Eventually, multiple collisions with multiple surfaces generate thousands of electrons which emit from the last surface. The result is an amplification of the original charge through a cascade of electrons arriving at the collector. At this point the instrument measures the charge and records the fragment mass as the mass is proportional to the detected charge.
- The MS instrument produces the output by drawing a array of peaks on a chart, the "mass spectrum." Each peak represents a value for a fragment mass. A peak's height increases with the number of fragments detected with one particular mass. As in the case of the GC detectors, a peak may differ in height with the sensitivity of the detector used.

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