Application of Spectroscopic Methods in Molecular Structure Determination

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Lecture – 17
Mass Spectrometry

Hello, welcome to module 17 of the course on Application of Spectroscopic Methods in Molecular Structure Determination. In this module, we will continue with mass spectrometry and look into some aspects of electron impact ionization mass spectrometry and chemical ionization mass spectrometry. These are the two most widely used mass spectrometric techniques for structural elucidation of simple organic molecules with a molecular wide range of something like 800 to 1,000 or so.

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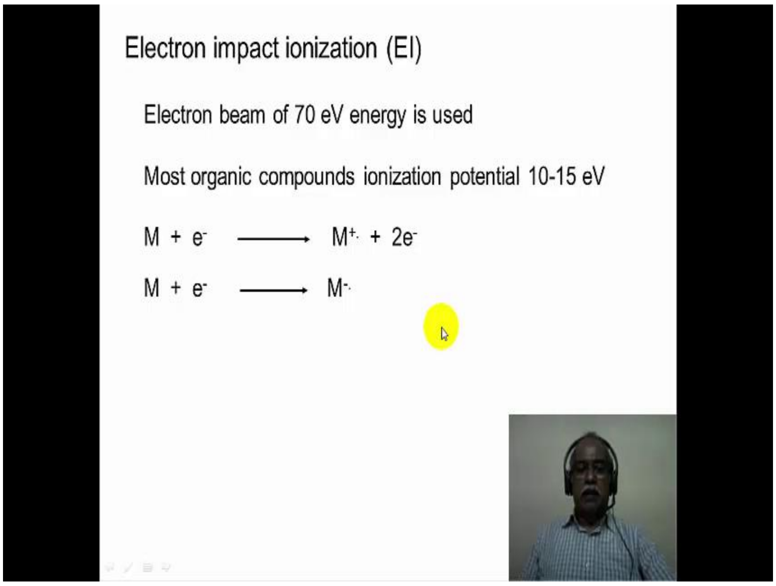

Electron impact ionization (EI)

Electron beam of 70 eV energy is used

Most organic compounds ionization potential 10-15 eV

$M + e^- \longrightarrow M^{+\cdot} + 2e^-$

$M + e^- \longrightarrow M^{-\cdot}$

In the electron impact ionization mass spectrometry, high-energy electron beams where the electronic energy is about 70 electron volt is used. Most organic compounds has ionization potential in the range of ten to fifteen electron volt. So, the use of the high energy electron beam essentially ensures that all the molecules which come in contact with such a beam is essentially ionized using these two mechanisms that are shown here. The molecule can either knock of an electron from it is valential by the high-energy electron to produce the cation radical or the electron can be added to the molecule producing an anion radical. So, mass spectrometry can be done either in the positive ion mode detecting the cation radical species is that are produced, or in the negative ion mode detecting the anion species that are produced during the course of the ionization.

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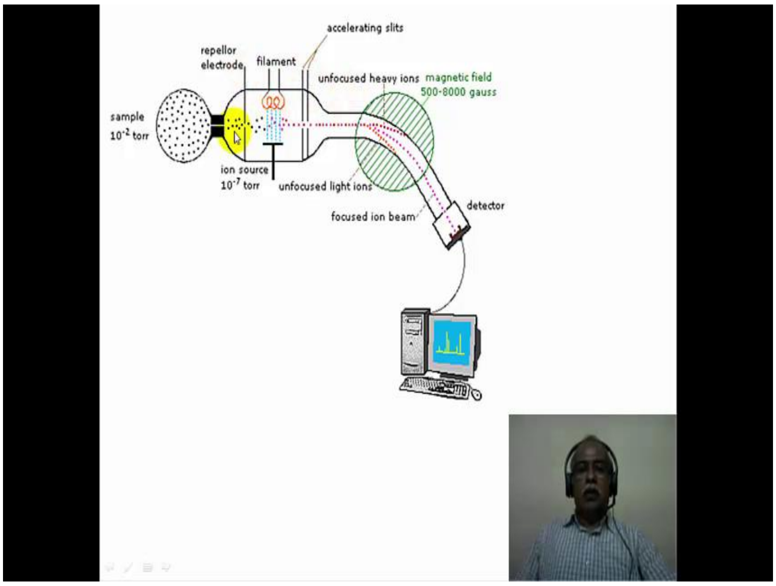

This is a simple diagrammatic representation of a mass spectrometer consisting of a sample chamber in which sample is produced in the vapor phase and then ionization chamber and then an acceleration zone and finally, a magnetic sector analyzer and a detector which is connected to the computer. Now in the sampled chamber essentially sample is introduced and it is brought to the gas phase by applying a vacuum of 10 to the power minus 2 or so. Most organic compounds are volatile in nature therefore, it is possible to bring them in the gaseous state those molecules, which are not volatile is normally heated in this sample chamber and brought sufficient vapor pressure is brought into the gas phase, so that it can be pumped into the ionization chamber.

The ionization chamber is maintained at the much higher vacuum of 10 to the power minus 7 torr up to the power minus 6 torr in that particular range. And this essentially consist of a cathode ray tube kind of an arrangement, where you have an electrode there are two electrodes one of them is a heated filament which produces the electrons of high energy which gets subtracted to the negative electrode of the combination of electrodes that you have here. The sample essentially passes through a slit through this electrode here, and this sample is brought into under the stream of electron which are having high-energy and this is a process by which the ionization take place and there this equations represent chemistry that is taking place in this region of the ionization chamber of the mass spectrometer.

Now there are two electrodes placed here this is called a repellor electrode. This essentially repelles depending upon the voltage bias of this electrode if it is a positively biased voltage of the electrode, then the positive ions will get repelled; if it is a negatively biased electrode, then the negative ions will get repelled. Essentially getting attracted to the opposite electrode on the other side of the ionization chamber. So, in the process, the ions get accelerated towards the electrode of opposite bias polarity and they get essentially into the acceleration zone. The acceleration is done by applying certain voltage, so that the ions get attracted to that particular electrode through a small orifices in the electrode the ions that are produced come into the analyzer which is the magnetic sector analyzer.

The magnetic sector analyzer essentially is a bent tube; and in this magnetic sector analyzer, the ions take a curvature in terms of the trajectory of the ion, it is a curvature and those ions which have the curvature match to with the curvature of this tube - analyzer tube essentially reach the detector. Those heavy ions which have a larger curvature for example, they get heat on the walls in this particular side and the lighter ions which have the lower curvature, they get heat in the wall in the other side of the tube. So, only a ions which have the m by z the ratio which has the which matches with the curvature of the ionization tube sorry the analyzer tube is essentially reaches the detector and it gets detected in the detector and fed into the computer to record a mass spectrum. Now we will look into the mechanism of the segregation or the analysis of the ions separation of the ions based on their m by z value in the next slide.

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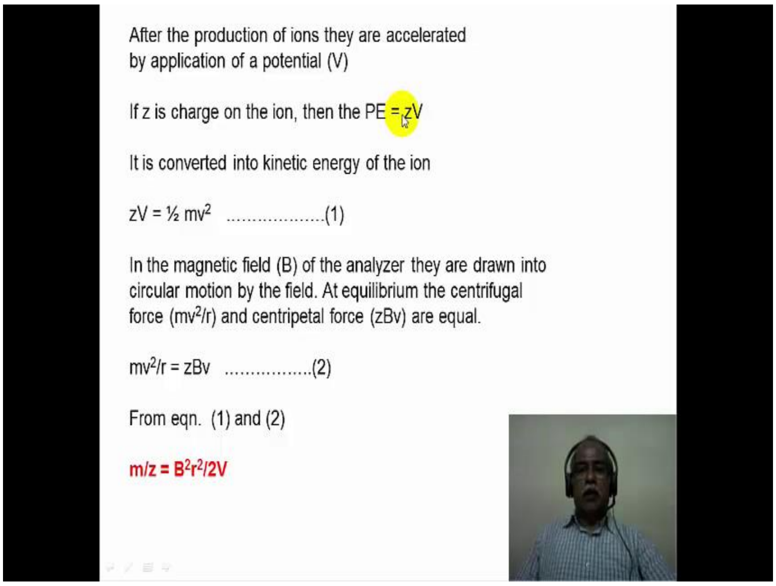

After the production of ions they are accelerated by application of a potential (V)

If z is charge on the ion, then the PE = zV

It is converted into kinetic energy of the ion

$zV = \frac{1}{2} mv^2$ ....(1)

In the magnetic field (B) of the analyzer they are drawn into circular motion by the field. At equilibrium the centrifugal force (mv^2/r) and centripetal force (zBv) are equal.

$mv^2/r = zBv$ ....(2)

From eqn. (1) and (2)

$m/z = B^2r^2/2V$

Now after the production of ions, they are accelerated by application of a potential volt V; in other words, the ions are produced here and there is a potential of volt V is applied between these two electrodes, so that the ions that are produced get accelerated towards that particular electrode. So, the potential energy of the ions that are produced under the voltage of V - acceleration voltage of V essentially corresponds to the charged times the potential which is z V. Now it is converted into the kinetic energy of the ion, during the course of the acceleration. The potential energy that are produced in the ions that are produced here with certain potential energy gets transferred into the kinetic energy. So, the z V actually becomes half m v square during the process of acceleration half m v square is the kinetic energy; m is the mass of the ion and v is the velocity of the ion under the voltage of V which is a acceleration voltage. Capital V and small v are distinguished in this case; capital v corresponds to the applied potential which is acceleration potential small v is the velocity of the ions that is produced.

In the magnetic field of analyzer, they are drawn into circular motion by the field; this is what I refer to having a curvature in terms of the motion of the ions in a magnetic sector. It takes a curvature; the curvature radius depends on the mass of the ion master charged ratio of the ion. At equilibrium, the centrifugal force and the centripetal force forces are equal acting on this particular ion. In other words, when it is passing through this particular circular pathway, the centripetal force and the centrifugal force have to be equal in order for the ion to survive and go through the radius of curvature of this analyzer and that is what is represented here. The centrifugal force is m v square by r where r is the radius of curvature of that particular ion of mass m and the centripetal force is essentially z B v. The centripetal force is essentially the charged times the magnetic field strength times the velocity of the ion produced under this condition. So, when the centripetal and centrifugal forces match each other, there are equal and this represented in equation-two.

If we combine equation-one and equation-two, essentially you get this equation where the master charged ratio of the ion of mass m and charged z corresponds to the square of the magnetic field strength square of the radius of curvature of the tube - analyzer tube and 2 times the voltage divided by the 2 times the voltage of the acceleration voltage. So, essentially in a mass spectrometer there is no one weighing the masses using a balance. In fact, the masses are segregated by the application of suitable magnetic field and the acceleration voltage. By tuning the acceleration voltage and the magnetic field strength, one can bring a specific ion into the curvature r which matches the spectrometer curvature; only those ions which match the spectrometer curvature will go through this passage and reach the detector. The rest of the ions which do not have the radius of curvature corresponding to the radius of the spectrometer essentially will get destroyed by hitting the walls of the walls of the analyzer tube in a spectrometer.

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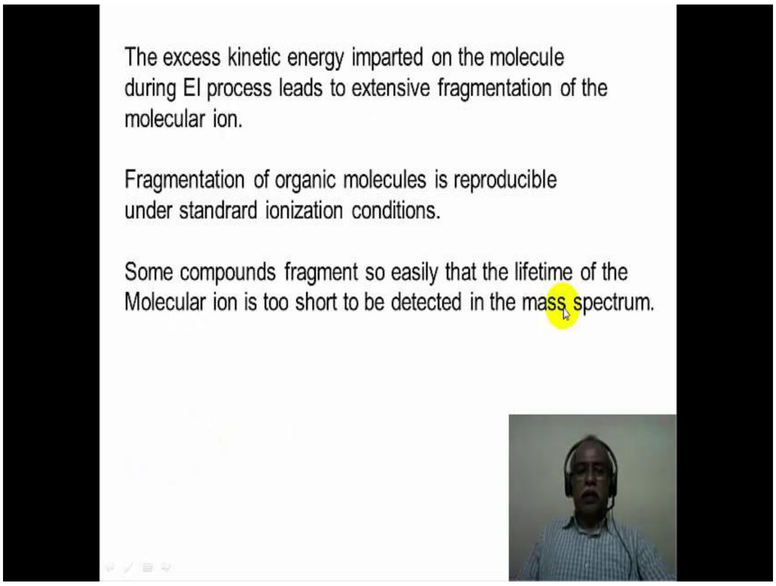

The excess kinetic energy imparted on the molecule during EI process leads to extensive fragmentation of the molecular ion.

Fragmentation of organic molecules is reproducible under standrard ionization conditions.

Some compounds fragment so easily that the lifetime of the Molecular ion is too short to be detected in the mass spectrum.

So, only a fraction of the ions will essentially pass through the particular radius of curvature, which is tuned by the magnetic field strength and the acceleration voltage. Now during the bombardment of the molecules with the 70 electron volt electron beam, there is a large excess of kinetic energy that is imparted on the molecule. The EI process leads to extensive fragmentation of the ion as a result of the excess kinetic energy that is given to the molecule.

In other words, more than the ionization potential energy is supplied as a result of that the excess energy gets the molecule into higher vibrational state; from the higher vibrational state of course, they undergo extensive fragmentation by breaking of bonds. The fragmentation of an organic molecule is not a bad thing actually; it is reproducible under standard ionization condition. So, it is acting like a fingerprint of that particular molecule so as long as the standard ionization conditions are maintained in a spectrometer.

If there are two spectrometers which are both electron impact ionization mode as long as they are operated under standard conditions of 70 electron volt; the spectra that is produced on the fragmentation that is produced by the molecule will essentially look same. So, as a result of that one can have a library of fragmentation pattern of various types of compounds because the fragmentation process is essentially a reproducible process under standard set of conditions. Now some fragments some compounds fragments so easily that their lifetime is so short the molecular ion is never detected in a mass spectrum. In other words, it is not always necessary that mass spectrum will give you the information regarding the molecular weight of the compound, sometime it is not possible to have the molecular weight of the compound detected, because the corresponding iron is too short leave to be detected in a mass spectrometer.

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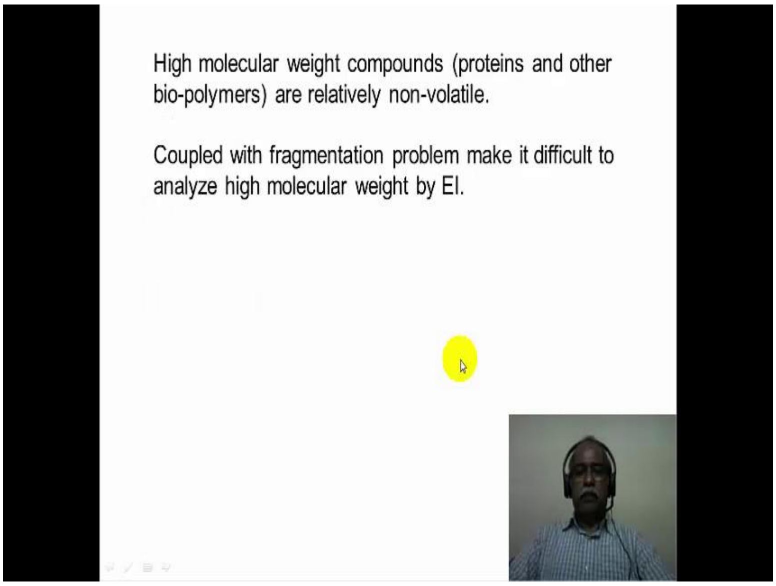

High molecular weight compounds (proteins and other bio-polymers) are relatively non-volatile.

Coupled with fragmentation problem make it difficult to analyze high molecular weight by EI.

The high molecular weight compounds such as proteins and biopolymers are relatively non-volatile; in addition to that it is difficult to keep the molecular ion from fragmentation. So, the fragmentation process make it difficult for the analysis of high molecular weight compounds by electron impact ionization this is where the electro spray ionization mass spectrometry and the maldi mass spectrometry come in handy because under those conditions which are considered to be softer ionization technique the ions that are produced do not undergo eccentric fragmentation.

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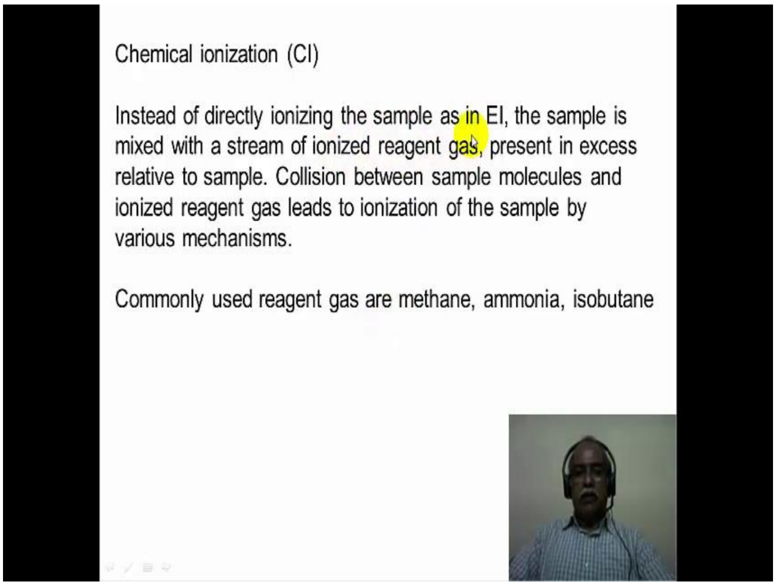

Chemical ionization (CI)

Instead of directly ionizing the sample as in EI, the sample is mixed with a stream of ionized reagent gas, present in excess relative to sample. Collision between sample molecules and ionized reagent gas leads to ionization of the sample by various mechanisms.

Commonly used reagent gas are methane, ammonia, isobutane

Now, let us come to the chemical ionization process. This is a relatively speaking a softer ionization technique. In case the electron impact ionization mass spectrum does not give the molecular ion peak, one can rely on the chemical ionization mass spectrometry to detect the molecular ion peak. How does it work, it is fairly simple instead of directly bombarding the molecules with high energy electron, a stream of ionized gas is used in other words you have a reagent gas which gets ionized by bombardment of this high energy electrons first. And the ionized gas molecules in tern react with the substrate and buy a simple collisional process between the sample molecule and ionized gas ionization takes place by various mechanism. The commonly used reagents for the ionization in other words the reagent gas which gets ionized commonly are the methane, ammonia and isobutane.

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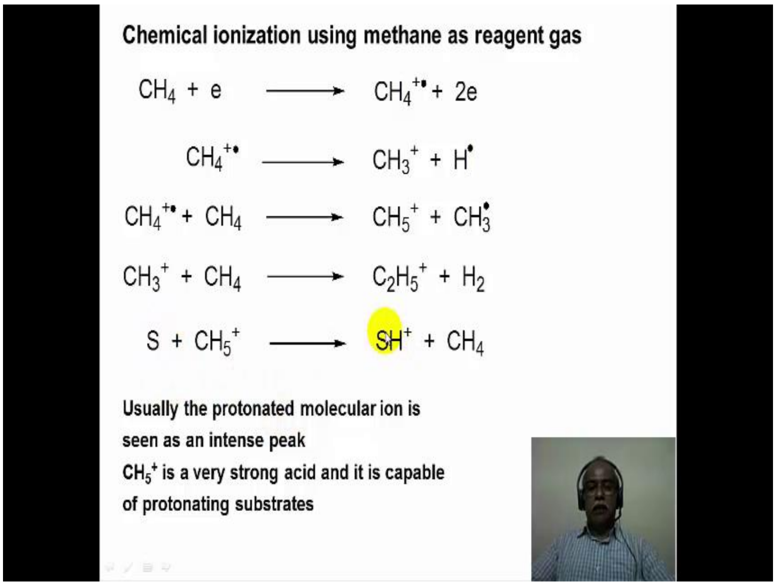

Chemical ionization using methane as reagent gas

$$CH_4 + e \longrightarrow CH_4^{+\bullet} + 2e$$

$$CH_4^{+\bullet} \longrightarrow CH_3^{+} + H^{\bullet}$$

$$CH_4^{+\bullet} + CH_4 \longrightarrow CH_5^{+} + CH_3^{\bullet}$$

$$CH_3^{+} + CH_4 \longrightarrow C_2H_5^{+} + H_2$$

$$S + CH_5^{+} \longrightarrow SH^{+} + CH_4$$

Usually the protonated molecular ion is seen as an intense peak

CH_5^+ is a very strong acid and it is capable of protonating substrates

When methane is used as a reagent gas the following chemical reaction take place in the ionization chamber. Essentially the concentration of methane is much higher than the substrate. So, the electron beam interacts with the methane producing the methane and cation radical initially by the ionization of methane molecule interacting with seventy electron volt electron beams. The methane cation radical that is produced, looses a hydrogen atom to produce a methyl cation or it reacts with another molecule of methane in a bimolecular gas phase reaction to produce the methonium ion which is an of oxonium kind of an ion this is a hyper valent carbon with a positive charged.

So, it is CH 5 plus is what is produced. It is a very strong acid, because it can donate a proton very readily to the substrate and that is a reaction that takes place essentially with the substrate the substrate gets protonated and in the process it gets ionized. It is not by the removal of an electron from the substrate it gets ionized, it is by addition of a proton it gets a positive charge. Usually the protonated molecular ion is what is seen as the intense peak in the methane reagent gas must chemical ionization mass spectrometry. CH 5 plus as i mentioned is a very strong acid and it is capable of protonating the Lewis basic sides of the substrate and there by producing the protonated substrate molecule

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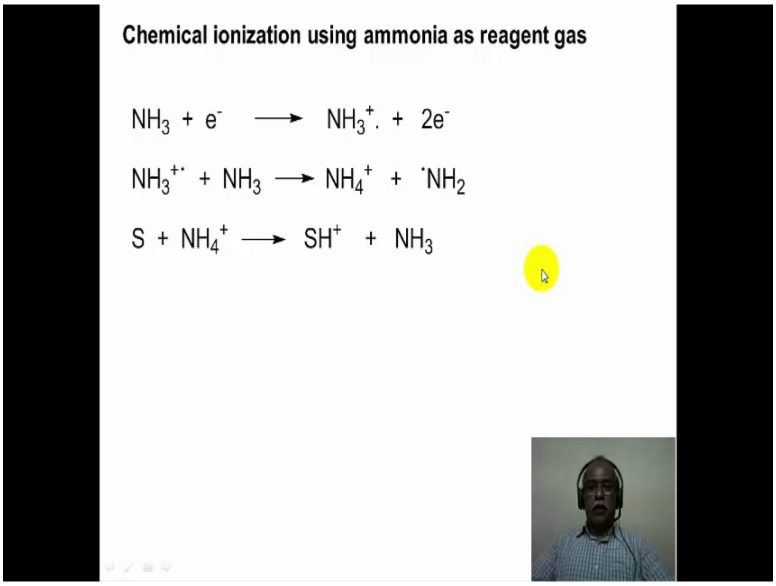

Chemical ionization using ammonia as reagent gas

$NH_3 + e^- \longrightarrow NH_3^{+\cdot} + 2e^-$

$NH_3^{+\cdot} + NH_3 \longrightarrow NH_4^+ + {}^{\cdot}NH_2$

$S + NH_4^+ \longrightarrow SH^+ + NH_3$

When ammonia is used as a reagent gas, the following reaction take place in the mass spectrometer. Ammonia gets ionized to the ammonia cation radical; ammonia cation radical reacts with another molecule of ammonia in the gas phase to produce an ammonium ion. The ammonium ion can either a transfer a hydrogen proton to the substrate producing the protonated substrate or an adduct can be formed between ammonium ion and the substrate producing a mass which is 18 units more than the substrate mod because of the adduct formation. We will see some examples of the chemical ionization mass spectrum recorded using ammonia as a reagent gas soon.

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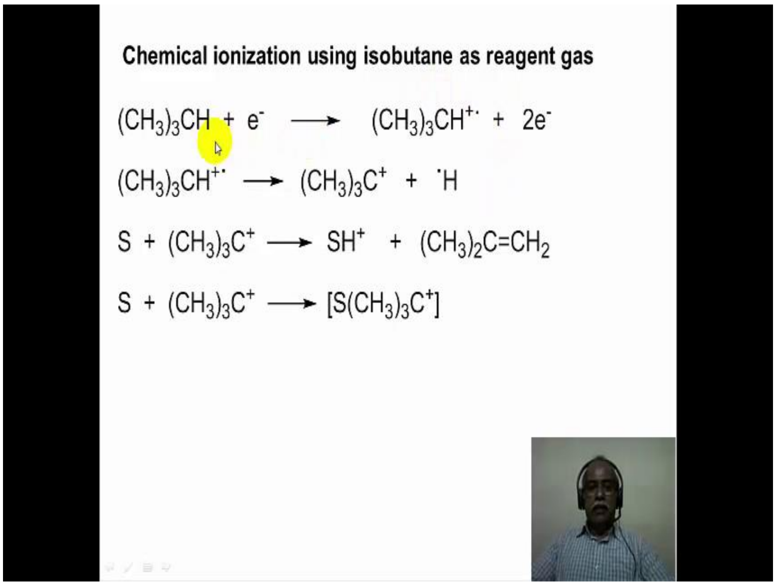

Chemical ionization using isobutane as reagent gas

$(CH_3)_3CH + e^- \longrightarrow (CH_3)_3CH^{+\cdot} + 2e^-$

$(CH_3)_3CH^{+\cdot} \longrightarrow (CH_3)_3C^+ + {}^{\cdot}H$

$S + (CH_3)_3C^+ \longrightarrow SH^+ + (CH_3)_2C{=}CH_2$

$S + (CH_3)_3C^+ \longrightarrow [S(CH_3)_3C^+]$

Isobutane can be used as a reagent gas. Essentially isobutane gets ionized in the form of isobutane cation radical. The isobutane cation radical loses a hydrogen to produce a tertiary butyl cation, this tertiary butyl cation essentially donates a hydrogen proton to the substrate producing the protonated substrate which is an ion that is detected. Alternatively, the tertiary butyl cation itself can form an adduct with the substrate producing the tertiary butylated substrate essentially this is an ion which is detected in the mass spectrometer.

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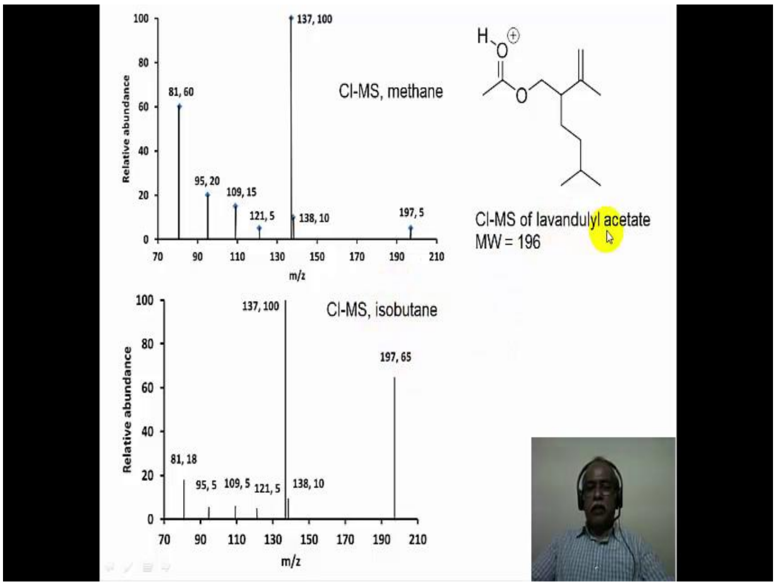

CI-MS, methane

CI-MS of lavandulyl acetate
MW = 196

CI-MS, isobutane

These are some examples of chemical ionization mass spectrum of methane as the reagent gas. The compound is lavandulyl acetate, this is a essentially ingredient of the lavender flower, the fragrance responsible for the lavender flower. And the molecular weight of this compound is 196; what is registered in the mass spectrum is 197 as the molecular ion peak this is essentially because of the protonation the proton adds one mass unit more to the molecular weight. So, the protonated species is what is registered in the methane as a reagent gas in the chemical ionization mass spectrum. When isobutane is used for example, isobutane the protonated species is much more abundant than the methane as the reagent gas and the chemical ionization mass spectrum using isobutane as reagent gas essentially gives the protonated species of the lavandulyl acetate.

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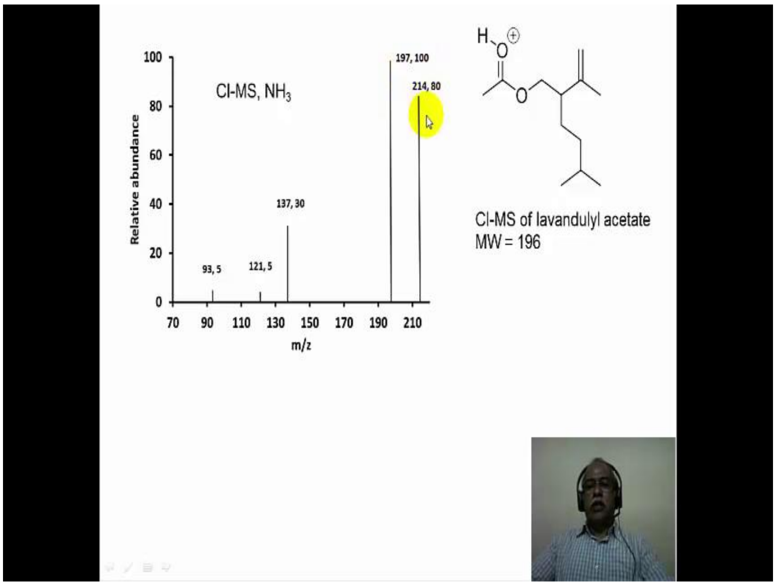

CI-MS of lavandulyl acetate
MW = 196

Now, when the compound is used for chemical ionization mass spectrum using ammonia as the reagent gas, there are two peaks formed. This is a protonated species which is the base peak 197 mass unit 100 percent relative abundance for example. This is about eighteen mass units more than the molecular ion peak molecular ion is a 196, if we add 18, you will get 214, so that essentially corresponds to the adduct with ammonium ion substrate plus ammonium ion would give this particular peak 214 as the mass unit of this particular peak. So, this is what I mentioned. When I said in this particular case ammonium ion can either donate proton or it can actually form an adduct and this is what is seen it is donating both a proton as well as forming an adduct when the ammonia is used as the reagent gas in the chemical ionization mass spectrum of lavandulyl acetate.

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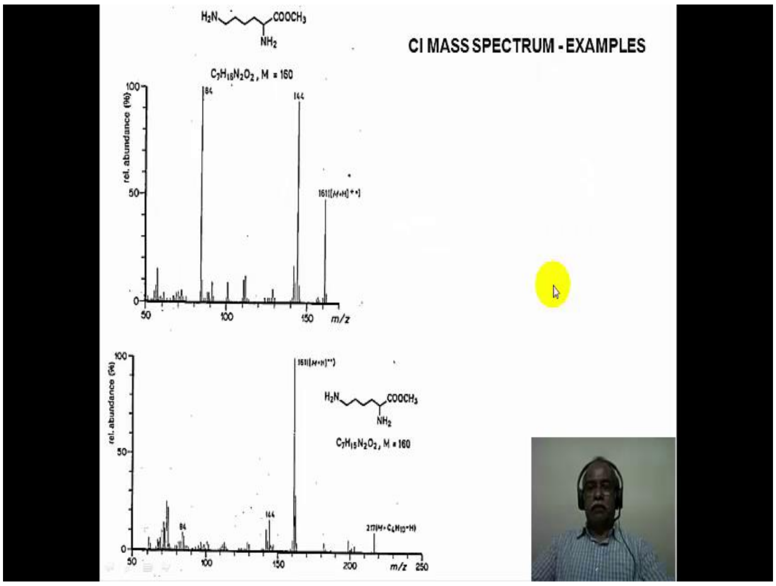

CI MASS SPECTRUM - EXAMPLES

These are some mass spectral studies have chemical ionization mass spectral studies. This particular amino acid does not give a molecular ion peak when electron impact ionization is used for example. So, when methane as used as a reagent gas and the chemical ionization mass spectrum is done, it is gives very clearly the molecular ion peak. The molecular weight is 160, what you register is 161 corresponding to M plus hydrogen cation radical. In other words, this cannot be a cation radical this is the protonated species of this particular molecule M plus h plus is what is registered in this particular case. Now when isobutane is used as the reagent gas, it is not only the protonated species that is formed; in addition to that the tertiary butylated substrate is also formed. And this is corresponding to the molecular weight plus the tertiary butyl cation weight is also added to this 217 corresponds to the substrate plus tertiary butyl cation adduct is what is registered in this particular case.

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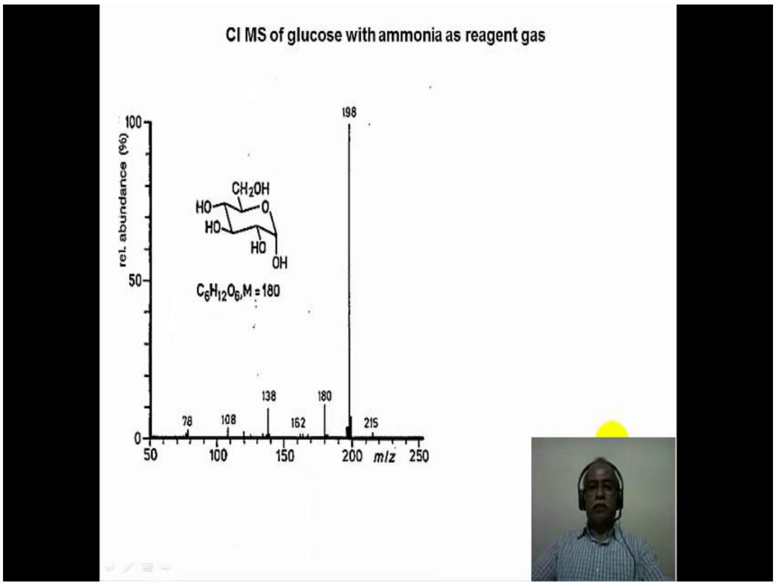

CI MS of glucose with ammonia as reagent gas

Now, molecule like glucose for example, undergoes extensive fragmentation in electron impact ionization mass spectrometry. This is very typical of many sugar derivatives particularly when the sugar derivative has free hydroxy functional group without any protecting groups protecting the hydroxy functional group. So, in order to get the molecular ion peak of glucose, this was done using the chemical ionization mass spectrometry using ammonia as the reagent gas. So, what you see is 18 mass units more than the molecular weight corresponds to the ammonium ion adduct of the glucose is what is registered as the molecular ion peak in this particular case. It is a very small amount of the 180 peak is also formed in this process, but the essentially the molecule plus the ammonium ion the adduct is what is registered as the base peak in this particular case.

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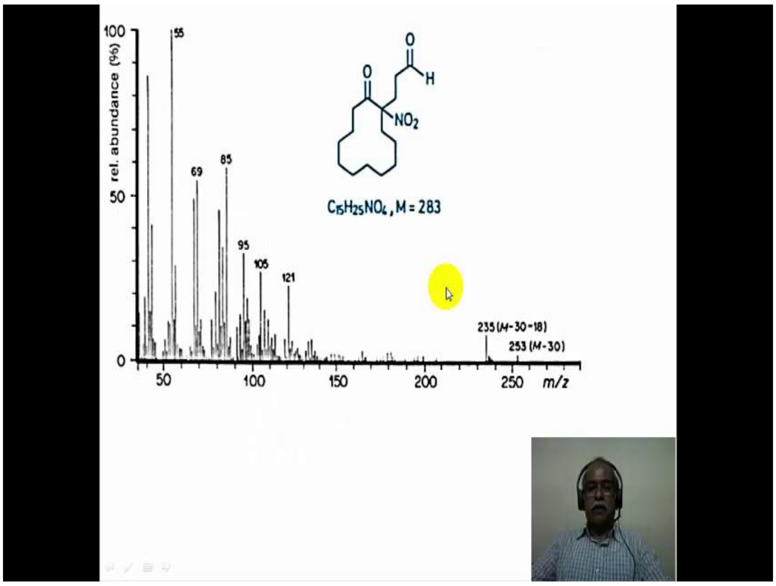

This is a tri functional molecule; there is a ketone functional group, there is a nitro functional group and there is an aldehyde functional group. Aliphatic nitro compounds typically tend to lose the anode two group during the course of electron impact ionization mass spectrum. In fact, the molecular weight is 283, you do not see any peak surround 280, 283 region indicating that the molecular ion is very short leave to be registered in the electron impact ionization mass spectrum. What is registered is molecular ion minus 30 mass unit, molecular ion minus 48 mass unit which all corresponds to the loss of for example, NO 2 group or NO as the group or HNO 2 as a group, these are the loses that takes place during the fragmentation process. And as a result of that the molecular ion is not detected in the mass spectrum of this particular compound.

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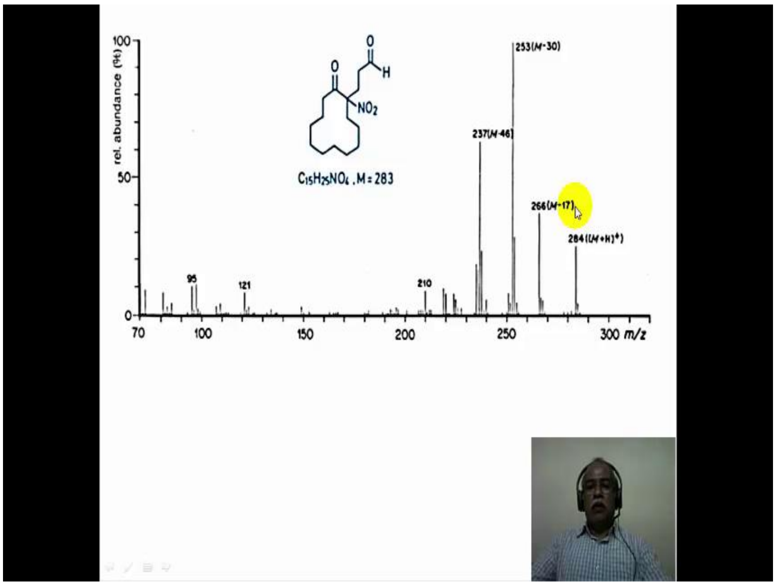

However, when this is carried out using methane as a reagent gas and a chemical ionization mass spectrum is registered, you very clearly see the molecular ion peak at 284, which is essentially the molecular weight plus one proton this is a protonated species is what is registered. So, one can use the chemical ionization mass spectrometry technique, whenever the electron impact ionization fails to produce the molecular ion peak of that particular compound.

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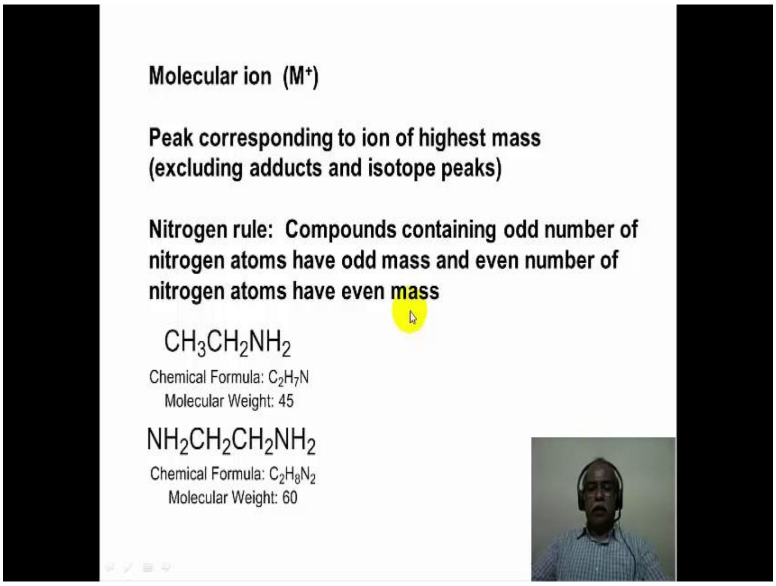

Molecular ion (M^+)

Peak corresponding to ion of highest mass (excluding adducts and isotope peaks)

Nitrogen rule: Compounds containing odd number of nitrogen atoms have odd mass and even number of nitrogen atoms have even mass

$CH_3CH_2NH_2$

Chemical Formula: C_2H_7N

Molecular Weight: 45

$NH_2CH_2CH_2NH_2$

Chemical Formula: $C_2H_8N_2$

Molecular Weight: 60

Now, what are the characteristics of a molecular ion peak, usually the peak corresponding to the highest massed charge ratio in m by z ratio as long as it is not forming any adducts and isotope peaks are not present that is the ion that is produced with the highest mass would correspond to the molecular ion. The something called the nitrogen rule it is fairly simple to understand. Whenever an organic molecule contains an odd number of nitrogen, the molecular weight also will be odd because nitrogen is a trivalent element. So, as a result of that molecular ion is an odd number. When there are even number of nitrogen present of course, the molecular weight will become even number. So, this is what is known as the nitrogen rule, compounds containing odd number of nitrogen atoms have odd mass and even number of nitrogen has an even mass.

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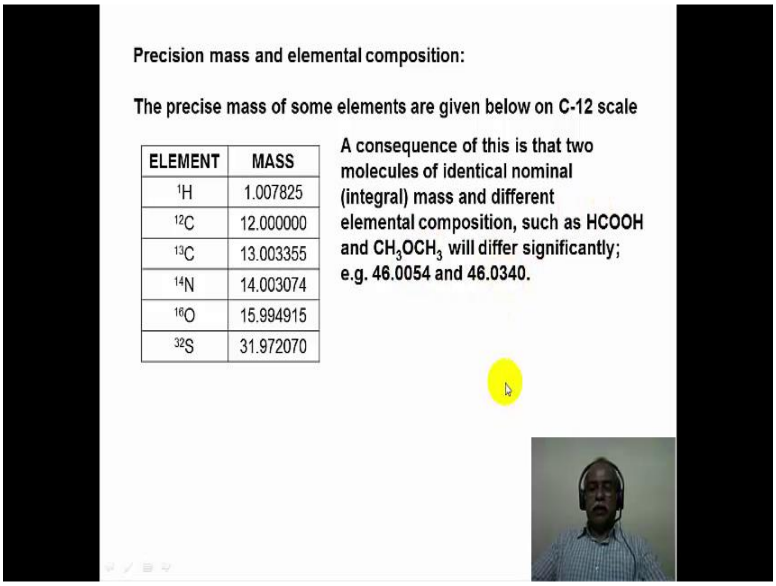

Precision mass and elemental composition:

The precise mass of some elements are given below on C-12 scale

ELEMENT	MASS
^{1}H	1.007825
^{12}C	12.000000
^{13}C	13.003355
^{14}N	14.003074
^{16}O	15.994915
^{32}S	31.972070

A consequence of this is that two molecules of identical nominal (integral) mass and different elemental composition, such as HCOOH and CH_3OCH_3 will differ significantly; e.g. 46.0054 and 46.0340.

There are two parameters, which one can derive from a mass spectrum; one is precision mass, which is extremely valuable, because it gives information regarding the elemental composition; the second one is the isotope peaks which also gives important information regarding the elemental composition. We will see these two aspects in a minute. First we will talk about the precision mass when the mass of atoms are calibrated atomic masses are calibrated using carbon-12 as a standard; in other words you make carbon-12 as the 12.00000 as the standard for the atomic weights scale. Then carbon-13 is 13.003355, this fractional weight is extremely important. Hydrogen is 1.007825 for example, when carbon-12 is used as a standard. Likewise, every element has an isotope with a fractional atomic weight when carbon-12 is taken as a standard. This fractional atomic weight is what is responsible for the precision mass. Suppose mass spectrometer is capable of detecting the mass are capable of analyzing the masses to 4 or 5 decimal place accuracy then it will make a difference in terms of the molecular composition of the compound depending upon the fractional weight that one observes.

Let us take an example. The consequence of this is that I mean the consequence of the fractional weight is that two molecules with identical nominal mass, in other words, unit mass resolution mass for example, and different elemental composition such as in the case of formic acid and dimethyl ether they will differ significantly in the fractional weight. If you take formic acid, there is one carbon let us say that is carbon 12 and there are two hydrogen the two hydrogen would correspond 2 times 1.007825 and two oxygen would correspond to 2 times 15.994915. If you add up all these numbers what one would get is 46.0054. And if you do the same thing for dimethyl ether the number that one gets is 46.0340. Although the molecular weight are same which is 46 in unit resolution if you go for high resolution mass spectrum the fractional weights that you see here essentially differentiate these two molecule in terms of the molecular composition of the molecules.

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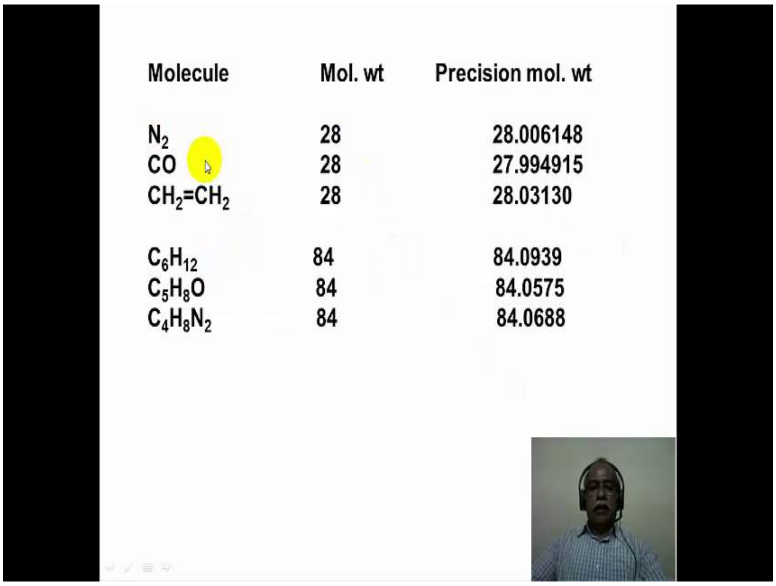

Molecule	Mol. wt	Precision mol. wt
N_2	28	28.006148
CO	28	27.994915
$CH_2=CH_2$	28	28.03130
C_6H_{12}	84	84.0939
C_5H_8O	84	84.0575
$C_4H_8N_2$	84	84.0688

This is better illustrated in this particular table. Nitrogen, carbon monoxide and ethylene all of them have the same unit mass in terms of unit resolution mass it is 28 each. However, if you look at the precision mass the fractional molecular weight that you see for each one of them is very different. So, if the m mass spectrum is capable of for example, detecting the masses 28.006148, it would essentially correspond only a nitrogen molecule, it will not correspond to either carbon monoxide or ethylene. In spite of the fact they have the same atom molecular weight of the compounds are essentially same. This is again illustrated for another fairly medium size molecule for example, with the molecular weight of 84 and their fractional weights are entirely different depending upon the molecular composition of the compound. So, if mass spectrum can measure the fractional weights accurately to 4 decimal places that will essentially lead to the calculation of the molecular formula from the fractional weight. So, the high resolution mass spectrometer is an extremely valuable tool, it essentially gives you the molecular composition very nicely from the fractional molecular weights that can be determined using a mass spectrometer.

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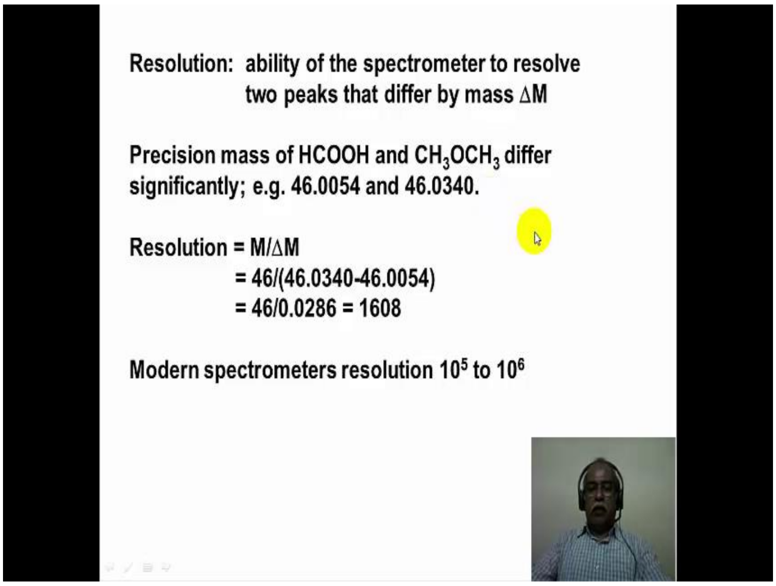

Resolution: ability of the spectrometer to resolve two peaks that differ by mass ΔM

Precision mass of HCOOH and CH_3OCH_3 differ significantly; e.g. 46.0054 and 46.0340.

$$\text{Resolution} = M/\Delta M = 46/(46.0340-46.0054) = 46/0.0286 = 1608$$

Modern spectrometers resolution 10^5 to 10^6

Before we go on, let us define what is the resolution of a mass spectrometer. How do we define the definition is the ability of the spectrometer to resolve two peaks that differ by a mass difference of delta M. Let us say are there is a mass of m 1 and m 2, m 1 minus m 2 is essentially the delta M. The precision mass of formic acid and dimethyl ether differs significantly. In other words, if we take the difference between these two it corresponds to 0.286 that is the difference between the accurate mass of the formic acid and dimethyl ether. Now the resolution is defined as M divided by delta M, M is essentially 46 the unit resolution mass is 46and the difference between these two fractional mass is essentially 0.086. So, as long as the mass spectrometer has resolving power of one 1600 or so, it will be able to differentiate between this mass and this mass. This is essentially what constitutes a high resolution mass spectrometer.

Now 1600 is a very, very small number for the resolution because mass spectrometers which are modern mass spectrometers which are available in the market now they can typically go to 10,000, 50,000 or about 1 million resolution in terms of the mass resolution that one can obtained using this modern mass spectrometer. So, even high molecular weight compounds can be fairly easily treated for high resolution mass and the precision mass can be used for the calculation of the elemental composition of this one. There are computer programs available nowadays. Where you plug in the high resolution mass and the elements that are present in the compound that will essentially give you the molecular composition; the various possibilities of molecular compositions of that particular molecule that we are dealing with.

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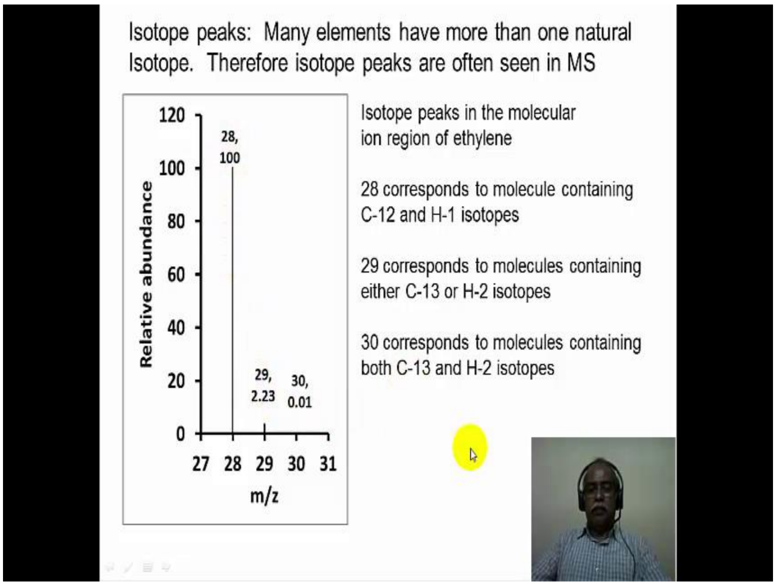

Isotope peaks: Many elements have more than one natural Isotope. Therefore isotope peaks are often seen in MS

Isotope peaks in the molecular ion region of ethylene

28 corresponds to molecule containing C-12 and H-1 isotopes

29 corresponds to molecules containing either C-13 or H-2 isotopes

30 corresponds to molecules containing both C-13 and H-2 isotopes

The second aspect that is important aspect is the isotope peaks. Many elements have more than one natural isotope; this is extremely important. Therefore, the isotope peaks are also seen in the mass spectrum. You remember the isotope separation by J J Thompson of the neon isotopes 20 and 22, essentially you register both the isotopes 20 as well as 22 in the mass spectrum. Similarly, if you take for example, a carbon mass spectrum, you will register both carbon-12 as well as carbon-13, because carbon-13 has a finite abundance in nature. Now let us take the simple example of ethylene; ethylene molecular weight is 28, this is a low resolution molecular weight is what we are talking about in this discussion. The focus is on the isotope peaks and not on the high resolution mass. So, molecular weight of 28 is registered in the m by z value of 28. In addition to that there are peaks at 29 as well as 30, although the relative intensities are much less than the base peak of the molecular ion peak. So, the M plus 1 which is 29 corresponds to 2.23 percent and the M plus 2 which is thirty corresponds to point zero one percent of the base peak intensity for example.

How can you register 29, if there is a carbon-13 in the molecule or deuterium in the molecule which are naturally available isotopes of carbon 13 and the hydrogen when the ethylene is both C 12 and all the hydrogens are H 1 this is what one registers. If one of the carbon is a carbon-13 one would register a molecular weight of 29 or if one of the hydrogen is a deuterium that will also be registered at 29. Suppose both hydrogen deuterium and carbon-13 are present are two deuterium atoms are present isotopes in ethylene molecule that will be very low abundance because it is extremely small amount it is in naturally natural abundance is so small that the probability of having two of this higher isotopes in a molecule is going to be much lower than the single isotope molecules which are seen here.

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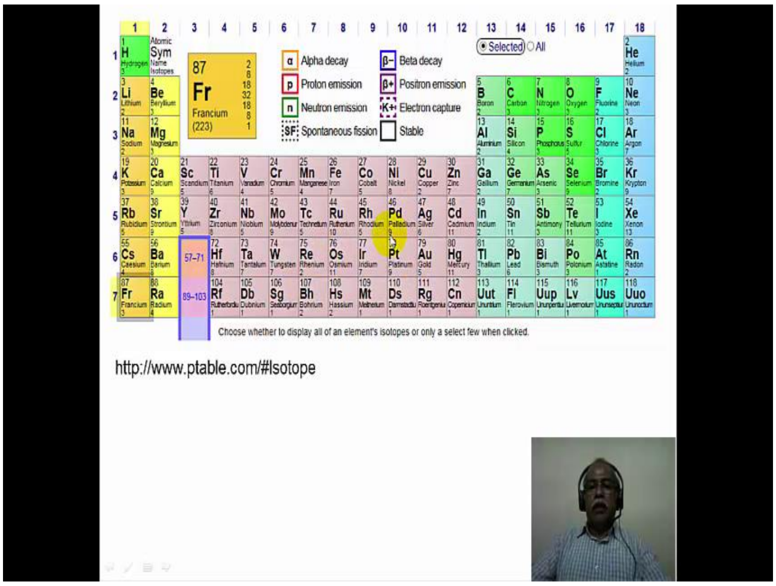

Choose whether to display all of an element's isotopes or only a select few when clicked.

http://www.ptable.com/#Isotope

Now, let me introduced you to an interactive table that is available in the web. This is a website address. If you go to this particular website you will see the periodic table which is shown as the screen shot here for example, and the number that is mentioned in each of the boxes of the bottom is the number of isotopes that is available for this particular element. So, suppose if I want to find out how many isotopes are available for a palladium it says 9 isotopes.

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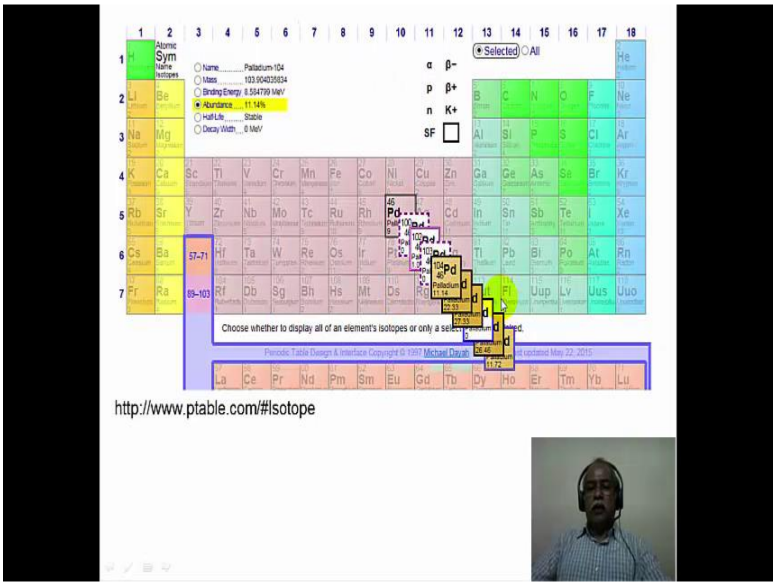

○ Name............ Palladium-104
○ Mass.............. 103.904035834
○ Binding Energy 8.584799 MeV
● Abundance...... 11.14%
○ Half-Life.......... Stable
○ Decay Width..... 0 MeV

● Selected ○ All

Choose whether to display all of an element's isotopes or only a selected...

http://www.ptable.com/#Isotope

And if I want to know what are the nine isotopes, I just click on palladium then all the nine isotopes pop out of the screen in this fashion. Now some of the isotopes have zero abundance, which means that they are not naturally available isotopes it may be synthetic isotope also. And the natural isotopes are given the abundance value for example, 104 palladium has an abundance of 11, 105 has an abundance of 22, 106 has an abundance of 27 and so on. So, essentially you get the information about the available isotope natural abundance of the various available isotopes in the particular atom is what is particular element is what we are seeing in the periodic table. It is a very useful periodic table especially if you want to find out the molecular composition, isotopic abundance, distribution of isotopes and so, on you can easily find out using the periodic table which is inter interactive periodic table.

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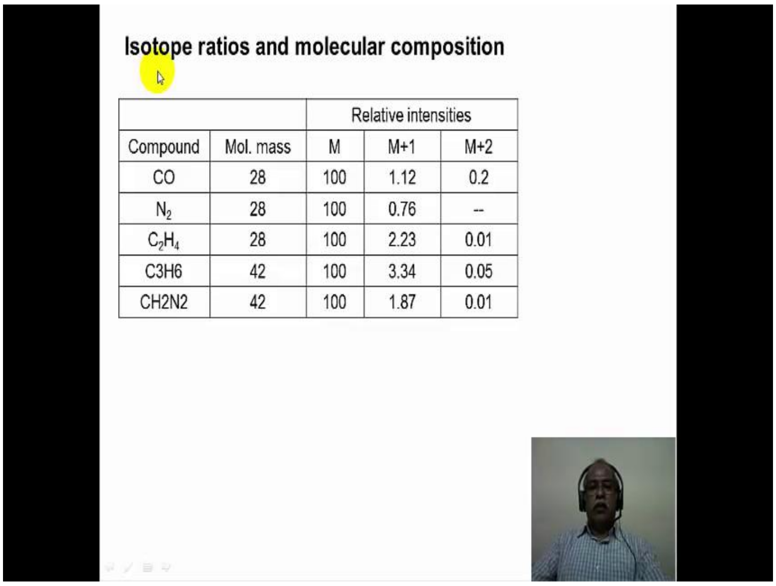

Isotope ratios and molecular composition

		Relative intensities		
Compound	Mol. mass	M	M+1	M+2
CO	28	100	1.12	0.2
N_2	28	100	0.76	--
C_2H_4	28	100	2.23	0.01
C3H6	42	100	3.34	0.05
CH2N2	42	100	1.87	0.01

Now, this gives you the isotope ratios and the molecular composition, because the molecular composition and isotope ratios are interrelated for carbon monoxide for example, if the molecular ion peak is 100 percent then the M plus 1 will be 1.12 and the M plus 2 will be 0.2. On the other hand, if we have nitrogen it will not have an M plus 2 because nitrogen 14 and 15 are the most abundant isotope, 16 is not available, so you do not have a M plus 2. So, if you register the molecular weight of 28, and look at the higher molecular isotopes, isotopomers for example, if it is having an M plus 1 as well as M plus 2 then it is certainly only carbon monoxide. The nitrogen although having a same molecular weight will not have the M plus 2 ion as the isotope peak.

In the case of ethylene and C 6 H 6 and diazomethane kind of molecule which you have the sorry ethylene has a molecular weight of 28 as same as the carbon monoxide and nitrogen that will also have a higher isotope, but the isotope M plus 1 ratio is very different in these two case. So, from the ratios, one can actually calculate the elemental composition of the molecule.

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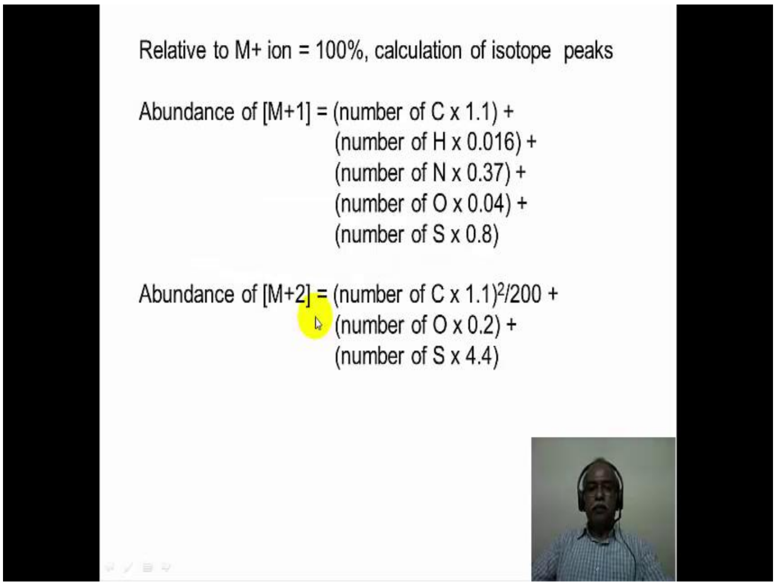

Relative to M+ ion = 100%, calculation of isotope peaks

Abundance of [M+1] = (number of C x 1.1) +
(number of H x 0.016) +
(number of N x 0.37) +
(number of O x 0.04) +
(number of S x 0.8)

Abundance of [M+2] = $(\text{number of C} \times 1.1)^2/200$ +
(number of O x 0.2) +
(number of S x 4.4)

How is it done, suppose if the molecular ion M plus is having a hundred percent abundance, the abundance of the M plus 1 what are the contributing factors that is what is mentioned here. To contribute a M plus 1 if there are n number of carbons in this molecule then the number of carbon times 1.1 which is 1.1 is the carbon-13 abundance. So, 1.1 times the carbon-13 abundance is going to contribute to M plus 1 or it is the number of hydrogen times 0.016 this is a deuterium isotope abundance that will also contribute to M plus 1. So, essentially you can see the contributors corresponding to M plus 1 and the contributors corresponding to M plus 2. In the case of carbon, if it were to be two carbons present in the molecular carbon-13, it will be 1.1 times 1.1 abundance of the carbon thirteen that is why this is given in this to the power two essentially indicating that is a very small fraction of a molecule, which will have to carbon13 simultaneously the molecule and that would essentially contribute to M plus 2.

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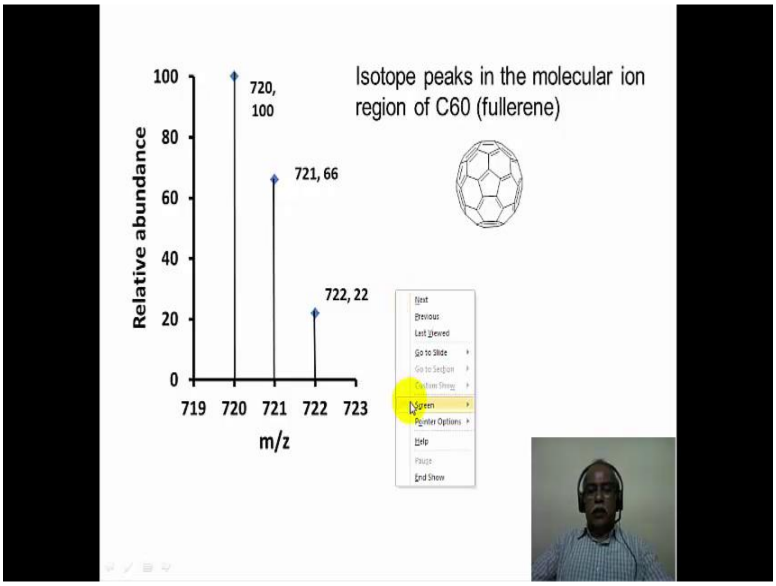

Isotope peaks in the molecular ion region of C60 (fullerene)

Now, let us look at the isotope abundance peak of something like a C 60. If you go back here, C 60 if you want to know what is the M plus 1 ion abundance if the molecular ion is 100 percent, all you need to do is plug in this number 60 here times 1.1 that would correspond to 66. There are no other elements present in the C 60 only carbon is contributing. So, if M plus ion is 100 percent M plus 1 ion should be around 66 percent. Now you can also do the same calculation for 60 times 1.1 times whole square divided by 200 that would turn out to be number 22. In other words, you have a molecular ion peak which is 720 of 100 percent abundance if that is the 100 percent abundance ion then you have a 721 which is the M plus 1 ion of this particular molecule. And the M plus 2 ion of that particular molecule has abundance of 22 in this particular case.

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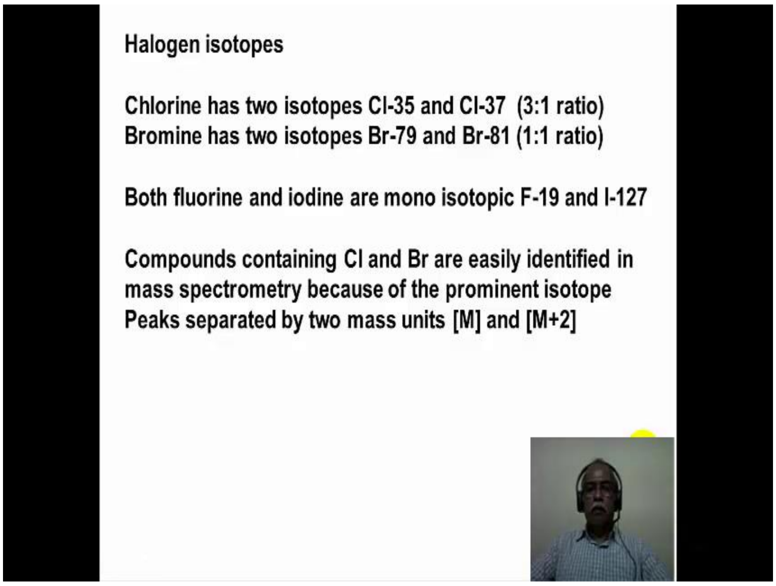

Halogen isotopes

Chlorine has two isotopes Cl-35 and Cl-37 (3:1 ratio)
Bromine has two isotopes Br-79 and Br-81 (1:1 ratio)

Both fluorine and iodine are mono isotopic F-19 and I-127

Compounds containing Cl and Br are easily identified in mass spectrometry because of the prominent isotope Peaks separated by two mass units [M] and [M+2]

Now, let us come to the halogen isotopes. Chlorine and bromine are extremely important they are easily detected in a mass spectrum, because they have two isotope with a mass difference of two units for example, chlorine-35 and chlorine-37 in the intensity ratio natural abundance of about 3:1 is the three times more of c 35 compared to CL-37. Bromine has isotope of 79 and is 81 in equal abundance for example, 50 percent of 79 and 50 percent of 81 is available on earth crust. Whereas, fluorine and iodine are mono isotopic; fluorine exist only in fluorine 19 form, and iodine exist only in iodine 127 form in the natural abundance of the two halogens which are fluorine and iodine in this particular case. Therefore, compounds containing chlorine and bromine are easily identified in the mass spectrometry, because of the prominent isotope peaks which are separated by two mass units namely M and M plus 2.

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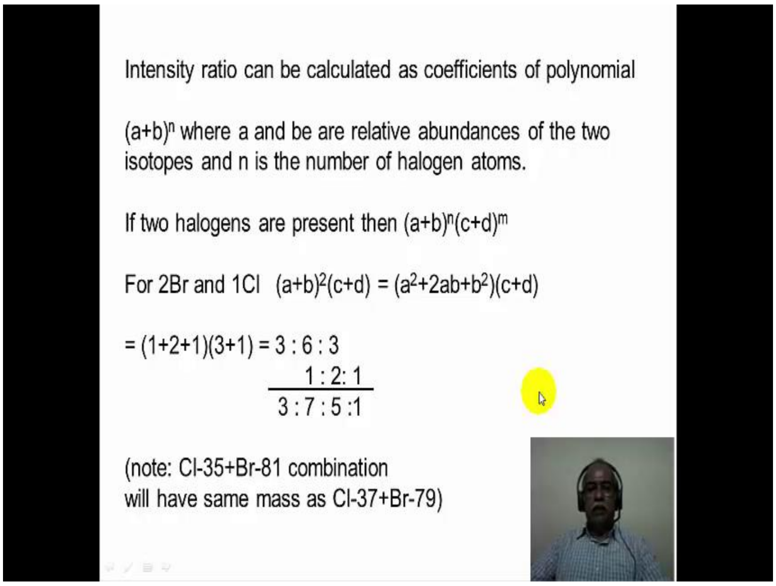

Intensity ratio can be calculated as coefficients of polynomial

$(a+b)^n$ where a and be are relative abundances of the two isotopes and n is the number of halogen atoms.

If two halogens are present then $(a+b)^n(c+d)^m$

For 2Br and 1Cl $(a+b)^2(c+d) = (a^2+2ab+b^2)(c+d)$

$$
\begin{array}{r}
= (1+2+1)(3+1) = 3 : 6 : 3 \\
 1 : 2 : 1 \\
\hline
3 : 7 : 5 : 1
\end{array}
$$

(note: Cl-35+Br-81 combination
will have same mass as Cl-37+Br-79)

Let us have a look at this. The intensity ratios of the isotope peaks can be easily calculated which is essentially coefficient of a binomial which is shown here. Let us say for example, you have a chlorine 35 and chlorine 37; a corresponds to the abundance of chlorine 35 and b corresponds to the abundance of chlorine 35. So, a plus b to the power n where n is the number of such chlorines that are present in a molecule, if it is a carbon tetrachloride n will be 4; if it is hexachlorobenzene, n will be 6; if it is dichloromethane, n will be two for example. So, the abundance of the two isotopes with the n number of halogen atoms can be easily derived from the coefficient of this particular polynomial expansion that you can do.

Suppose if two halogens are present one is bromine another one is chlorine if they are two bromines and one chlorine present let us illustrate the example using this. This is the polynomial expansion that one needs to do. Let us say this is bromine 79 and this is bromine 81, and this is chlorine 35 and chlorine 37, the expansion essentially gives you this a plus b whole to the power two because there are two bromines c plus the whole to the power one because there is one chlorine which relates to this particular expression here. Now if you plug in the abundance has 1:2, 1:3 ratio for the chlorine and 1:1 for the bromines here this is what the coefficients are going to look like.

Let us for example, if it is chlorine 35 and 37 that will be 3:1 ratio; if it is bromine 79 and 81 that will be 1:2:1. In terms of the coefficients of the binomial that you have expanded. Now this essentially corresponds to 3:6:3. When you multiply by three; and when you multiply by one, it is 1:2:1. And this remember the six and one relative intensity correspond to the M plus 2 and this corresponds to the M plus 4, and this corresponds to the M plus 6. In other words, a combination of chlorine 35 and 81 will have essentially the same mass as combination of chlorine 37 and bromine 79 which will be the M plus 2 ions. So, one needs to be careful to add up the corresponding molecular weights of compounds in order to obtain the relative ratios of the isotope peaks that one would see this kind of a situation.

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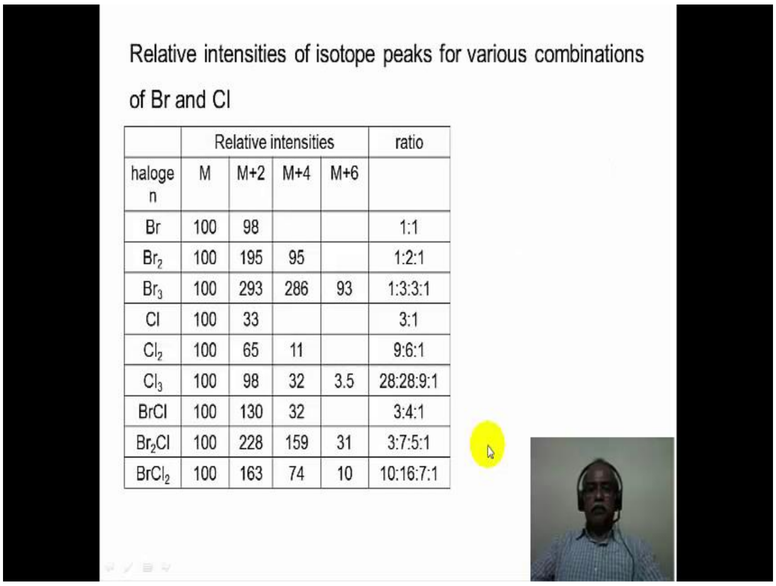

Relative intensities of isotope peaks for various combinations of Br and Cl

	Relative intensities				ratio
halogen	M	M+2	M+4	M+6	
Br	100	98			1:1
Br_2	100	195	95		1:2:1
Br_3	100	293	286	93	1:3:3:1
Cl	100	33			3:1
Cl_2	100	65	11		9:6:1
Cl_3	100	98	32	3.5	28:28:9:1
BrCl	100	130	32		3:4:1
Br_2Cl	100	228	159	31	3:7:5:1
$BrCl_2$	100	163	74	10	10:16:7:1

Using such a procedure, I have calculated the isotope ratio of the various bromine and chlorine containing molecules for example. If there is only one bromine, it will be 1:1 ratio 79 81 will be 1:1. There are two bromines in the molecule the molecular ion peak will be 100 and the ratio of the isotope peaks will be 1:2:1, this is a relative intensities that we are talking about and this is the ratios of the intensity that is given separately here. So, essentially you can see this is a Pascal triangle kind of a thing as far as the bromine is concerned; when it comes to chlorine it is a 3:1 ratio if one only one chlorine is there, there are two chlorines present it will be 9:6:1 and so on. So, one can easily determine the ratios of the isotope peaks of the halogens using this kind of a polynomial expansion and identifying the coefficient of the polynomial.

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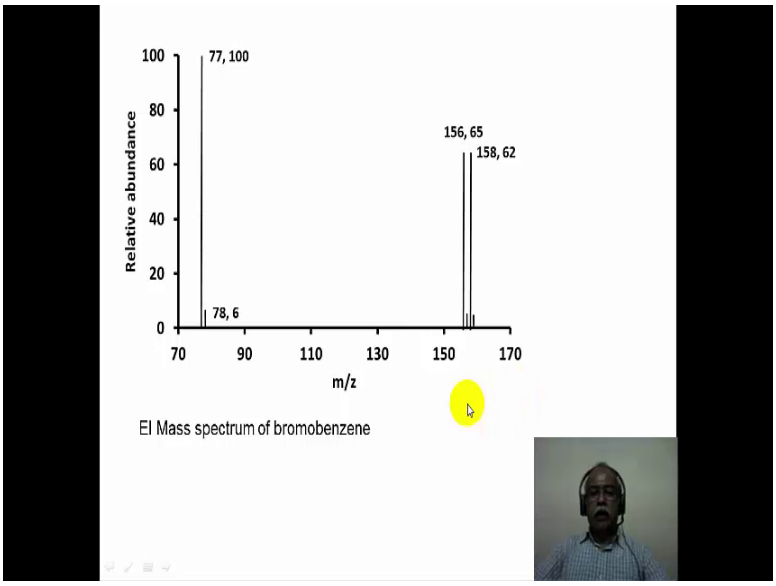

EI Mass spectrum of bromobenzene

Let us look at examples of mass spectra containing bromine containing compounds, and this is the spectrum of bromobenzene. Bromobenzene has one bromine. So, the molecular weight are 156 and 158, if the molecular weight is 156 that would correspond to bromine 79 isotope, 158 would correspond to bromine 81 isotope. And this is appearing in nearly 1:1 ratio has the molecular ion in the molecular ion region. The small peaks that you see here are the M plus 1 and the M plus 3, whereas this is M and M plus 2, the M plus 1 and M plus 3 are essentially coming as isotopes from the carbon and the deuterium component of this molecular formula. And when the molecule looses the bromine by fragmentation, it gives only one peak corresponding to the phenyl cation. Since the phenyl cation does not have any bromine it does not show any M plus 2 peak; however, it shows M plus 1 because of the carbon thirteen and deuterium content of the molecular formula.

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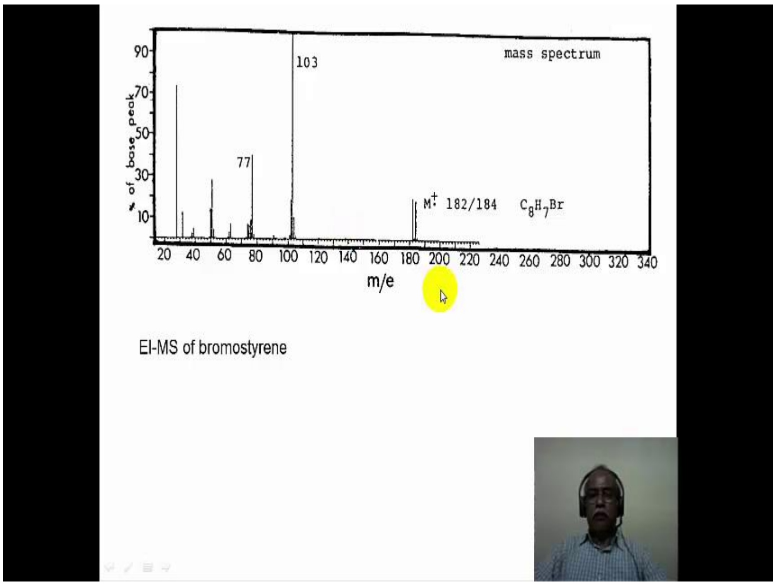

EI-MS of bromostyrene

This is bromostyrene, you cannot tell which bromostyrene we are talking about in terms of the isomer of the bromostyrene. Nevertheless, one can see the molecular ion peak at 182 and 184 corresponding to bromine 79 and bromine 81. When it loses a bromine, you see a single peak of chiral cation which is 103 of molecular weight.

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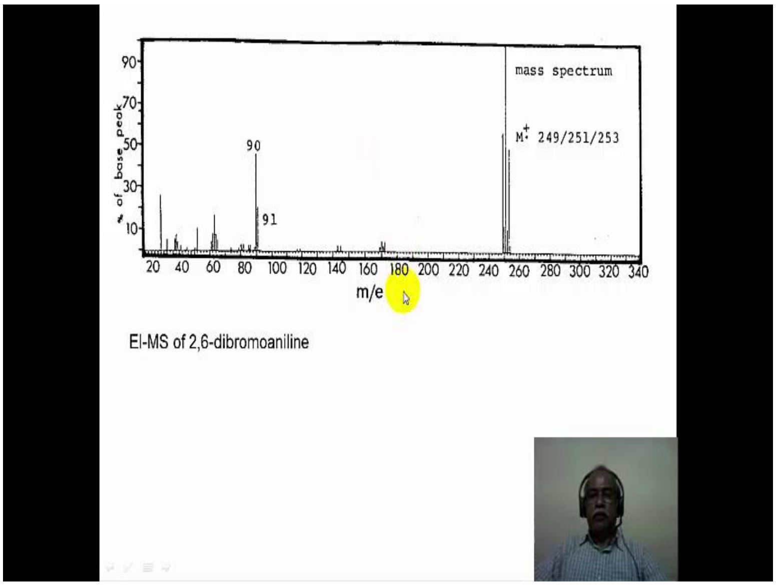

EI-MS of 2,6-dibromoaniline

This is compound containing two bromines for example, 2, 6 dibromoaniline. So, both the bromines can be 79 one bromine can be 79 the other can be 81 or both the bromines can be 81. There are three possibilities in the ratio of 1:2:1 you get the molecular ion peak 249, 251, 253 corresponding to M, M plus 2 and M plus 4 ions of this particular molecule.

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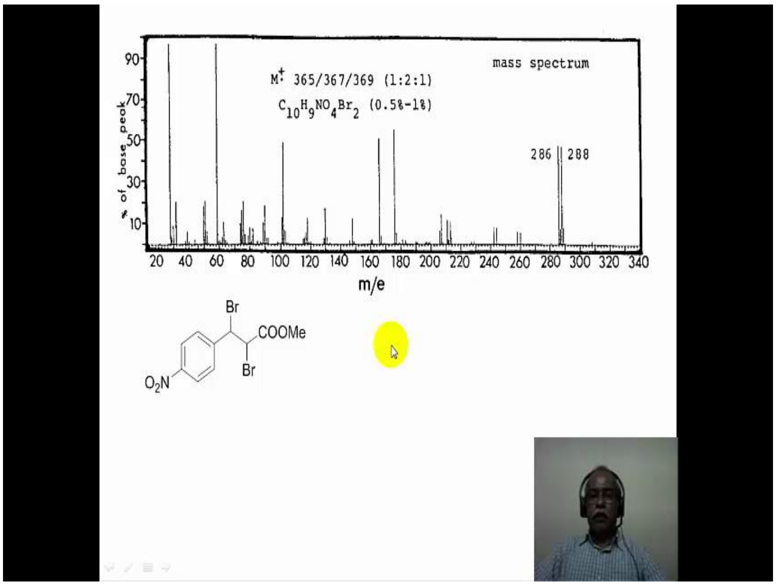

Now, this is another molecule, unfortunately the molecule does not show the molecular ion peak because of extensive fragmentation. A very tiny a little amount of the molecular ion is formed from which the information is extracted as the M plus 365, 367, 369 corresponding to the presence of two bromine in the intensity ratio of 1:2:1. And the loss of one bromine results in the formation of fragmented ion at 286 and 288 corresponding to 79 and 81 bromine isotope. So, the presence of bromine is easily detected in the mass spectrum. If you look carefully at the intensities of ions which are M and M plus 2, mass unit difference should be two and the intensity should correspond to the two bromines being 1:1 ratio in the natural abundance 79 and 81. And this a very valuable tool to identify the presence of chlorine as well as bromine in mass spectra using mass spectrometric technique and this is what is illustrated in this particular example

So, to conclude, let us conclude by saying that we have looked at the basic principle behind the electron impact ionization mass spectrum and the chemical ionization mass spectrum. We also looked at the importance of the precision mass, how it relates to the molecular composition. We also relate to the importance of the isotopic peaks and how it is related to the molecular composition. One can in principle get the molecular composition, if you know the isotope abundance peaks and the calculate it backwards to arrive at the molecular formula in terms of small molecules, it is easy enough to identify the molecular formula from the isotope abundance peak of M plus 1 and M plus 2. Finally, the major isotope abundance peaks of halogens, particularly chlorine and bromine for example, some illustrative examples were shown for the brominated compound. Similarly, one can also show for the brominated compound.

I thank you very much for your attention