Spectroscopic Techniques for Pharmaceutical and Biopharmaceutical Industries
Professor Shashank Deep
Department of Chemistry
Indian Institute of Technology Delhi
Lecture 34
Mass Spectroscopy

Hello students, welcome back to the lecture 34 of this course. In this lecture I will discuss about mass spectroscopy.

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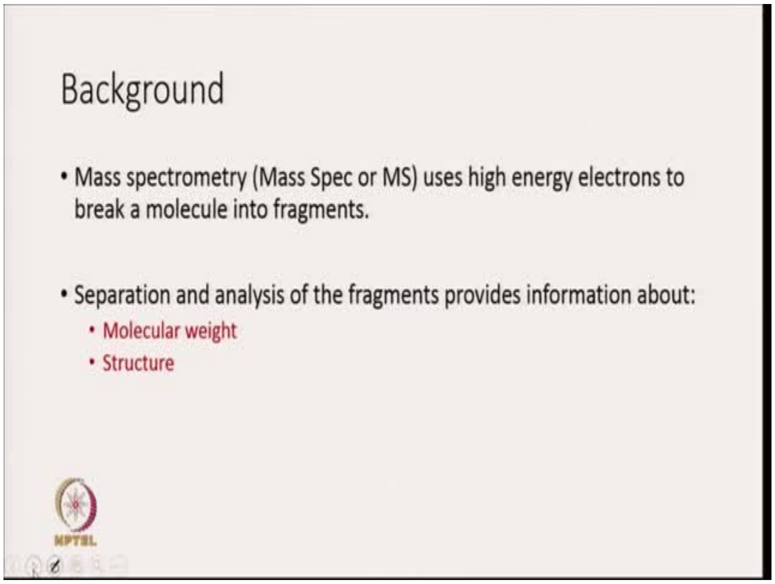

Background

- Mass spectrometry (Mass Spec or MS) uses high energy electrons to break a molecule into fragments.
- Separation and analysis of the fragments provides information about:
  - Molecular weight
  - Structure

Mass spectrometry uses high-energy electron to break a molecule into fragments and when you break a molecule into fragments and you analyse the fragments it can provide information about molecular weight and the structure.

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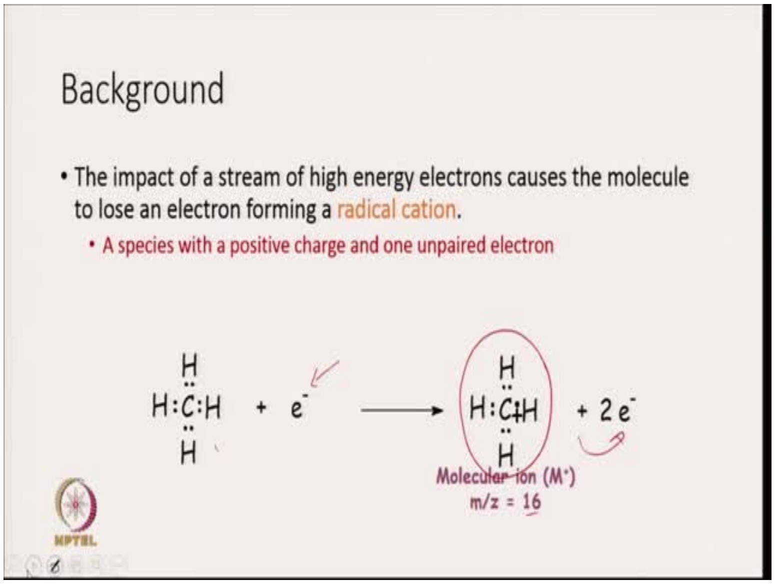

Background

- The impact of a stream of high energy electrons causes the molecule to lose an electron forming a radical cation.
  - A species with a positive charge and one unpaired electron

The impact of a stream of high-energy electrons causes and the molecule to lose an electron forming a radical cation. So when high-energy electrons is bombarded on molecule, molecule lose an electron and forms a radical cation. Radical cation is a species with a positive charge and one unpaired electron. So here is an example of CH4 molecule getting bombarded with electron, so this electron takes out one more electron and generates a radical cation whose molecular weight is 16 or m by z ratio is 16 and you get a peek at 16 corresponding to this radical cation.

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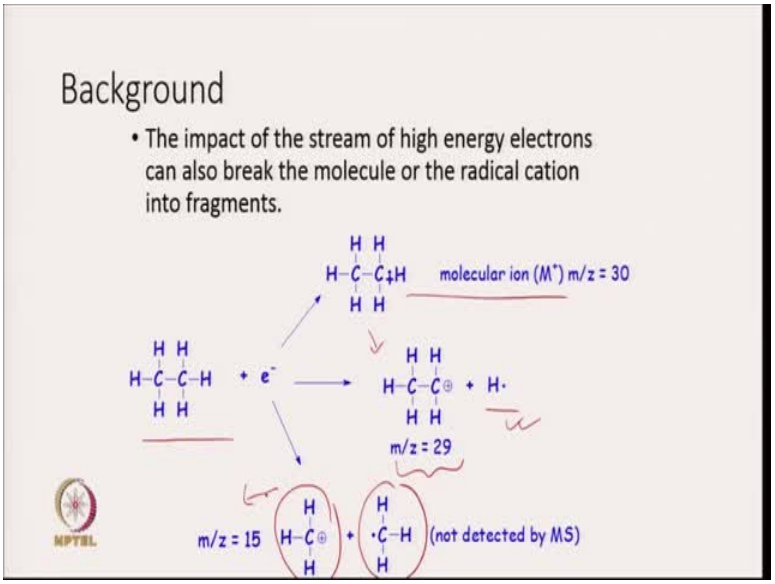

Background

- The impact of the stream of high energy electrons can also break the molecule or the radical cation into fragments.

The high-energy electron can not only remove electron, it can also break molecule or the radical cation into fragments. So suppose, if you bombard ethane with electron molecular ion with m by z ratio is equal to 30 (())(2:15). Apart from that this radical cation can lose a hydrogen radical and a species with molecule weight 29 m by z ratio 29 will also be formed, the radicals are not detected in mass spectroscopy. This molecule can also break into CH3 cation and CH3 radical. Radical is again not detected by mass spectrometry, but this positive ion will be detected and a peak value of observed at 15, at 15.

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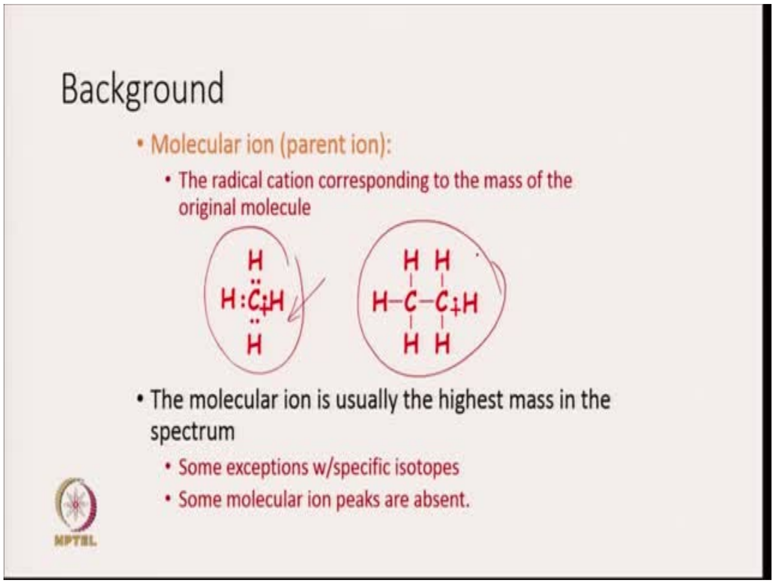

Background

- Molecular ion (parent ion):
  - The radical cation corresponding to the mass of the original molecule
- The molecular ion is usually the highest mass in the spectrum
  - Some exceptions w/specific isotopes
  - Some molecular ion peaks are absent.

Now, there are certain terms which we should be familiar if we are dealing with mass spectrometer. One is your molecular ion or parent ion peak. So this basically corresponds to radical cation having mass of original molecule. For example, if methane is bombarded electron, one electron is removed and the left one is your molecular ion. Similarly, in case of ethane this is your radical cation which is basically your molecular ion. Molecular ion is usually the highest mass in the spectrum. Exceptions are when there are isotopes of higher molecular weight is present and in some cases molecular iron peaks are absent.

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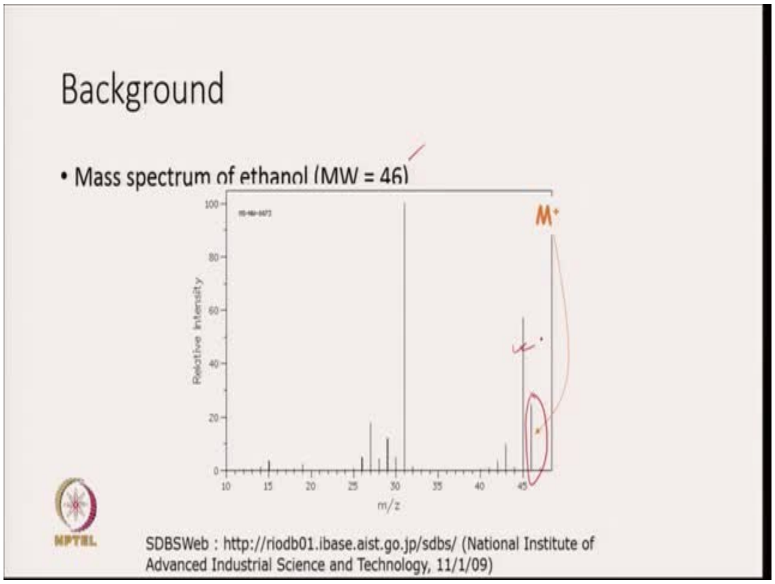

Background

- Mass spectrum of ethanol (MW = 46)

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

So this is your mass spectrum of ethanol which has molecular weight of 46. So now you can see that M plus ion peak is here. M plus ion peak is here and you can get a peak at minus 1.

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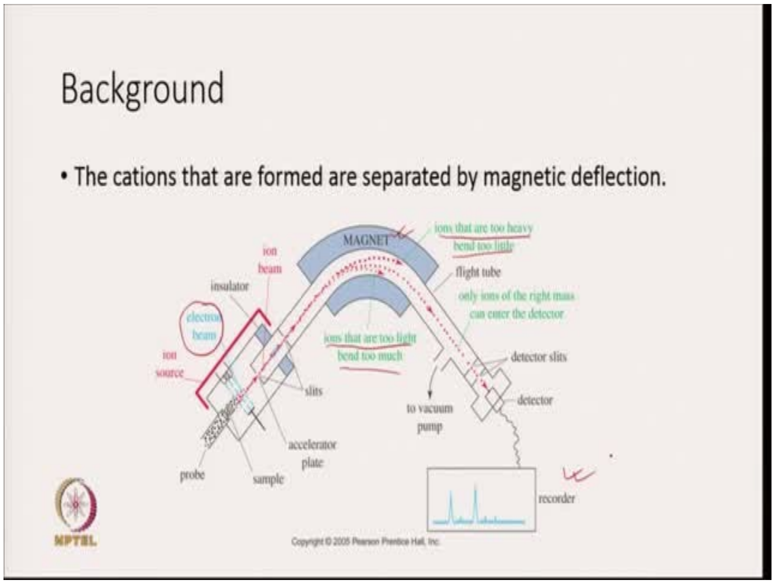

Background

- The cations that are formed are separated by magnetic deflection.

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So the cations that formed are separated by a magnetic deflection. So, what happens that these molecules are bombarded with electron beam here and then ions are produced, this ion beam gets deflected here in the magnet. The ions that are too heavy bend too little, whereas ions that are so light bend too much. And then only ions of right mass can enter the detector once it enters the detector the peak is recorded. The peak corresponding to that ion is recorded.

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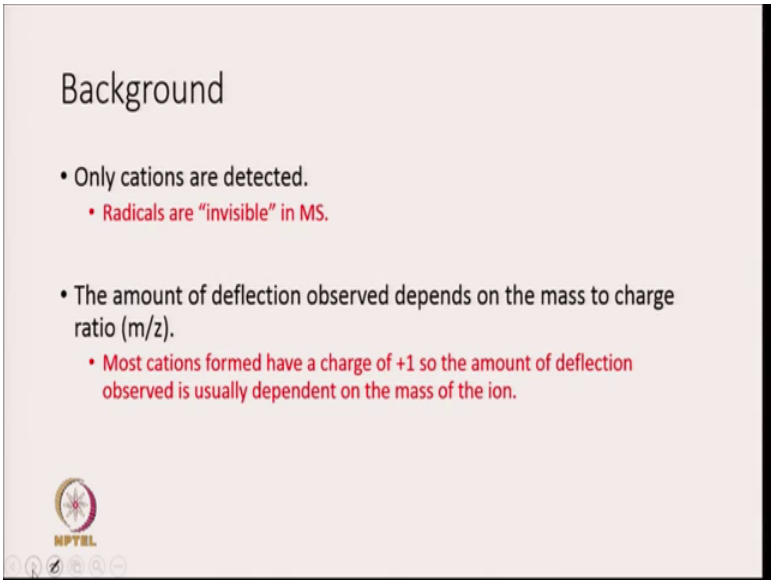

Background

- Only cations are detected.
  - Radicals are "invisible" in MS.
- The amount of deflection observed depends on the mass to charge ratio (m/z).
  - Most cations formed have a charge of +1 so the amount of deflection observed is usually dependent on the mass of the ion.

So in mass spectrometer only cations are detected, radicals are invisible in mass spectrometer. And the amount of deflection of observed depends on mass to charge ratio m by z ratio. Most cation form has a charge of plus 1, so amount of deflection of observed is usually dependent on the mass of the ion. Usually dependent on the mass of the ion.

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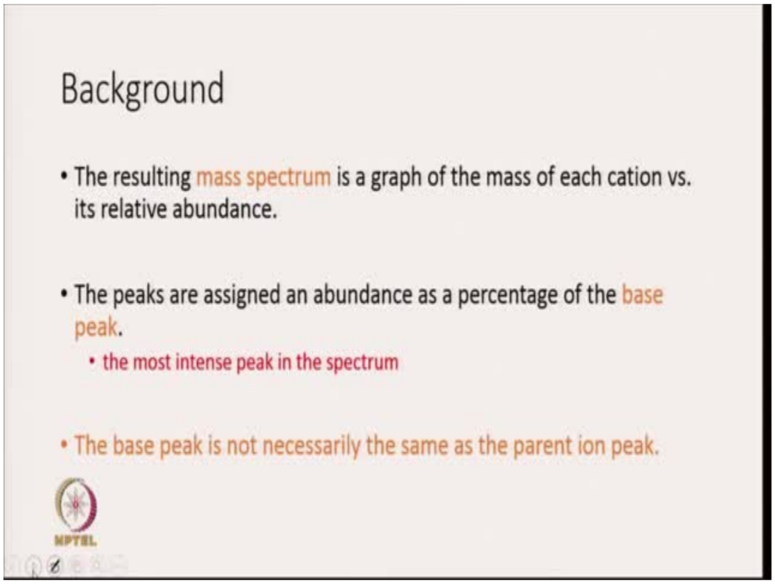

Background

- The resulting mass spectrum is a graph of the mass of each cation vs. its relative abundance.
- The peaks are assigned an abundance as a percentage of the base peak.
  - the most intense peak in the spectrum
- The base peak is not necessarily the same as the parent ion peak.

The resulting mass spectrum is a graph of mass of each cation versus its relative abundance. So basically you are plotting relative abundance versus mass of each cation. The peaks are assigned as an abundance as a percentage of base peak. So base peak is considered to be of 100 percent and then abundance is assigned for each peak which is basically expressed as a percentage of a base peak. Base peak is not the same as molecular ion peak, base peak is the most intense peak in the spectrum. So base peak is not necessarily the same as parent ion peak.

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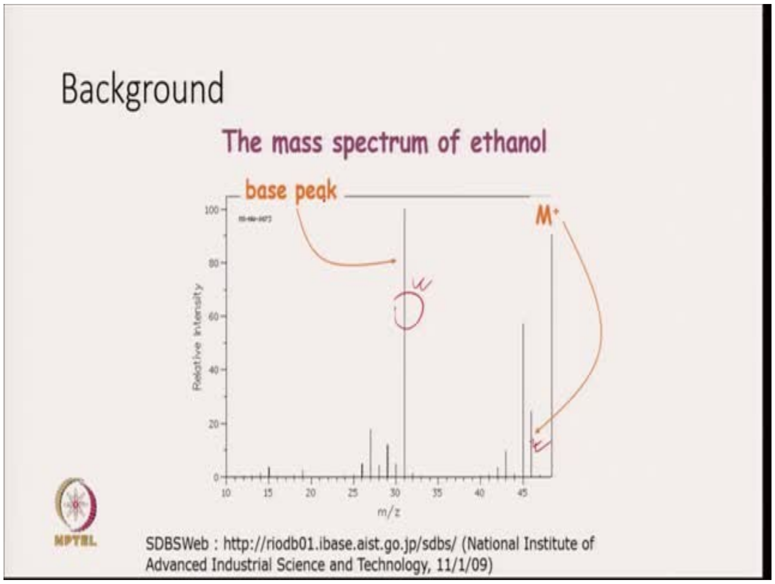

Background

The mass spectrum of ethanol

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

So, now again let us go back to mass spectrum of ethanol. Here you can see that this is the molecular ion peak which corresponds to molecular weight of ethanol and it comes at 46 m by z ratio. But this is less intense in comparison to this peak, this peak is most intense one and hence called base peak, whereas this one is molecular ion peak. And then you assign a abundance with respect to this base peak and here you can see this has been assigned 100, so this is your relative intensity and other peaks are assigned relative intensity as a percentage of base peak.

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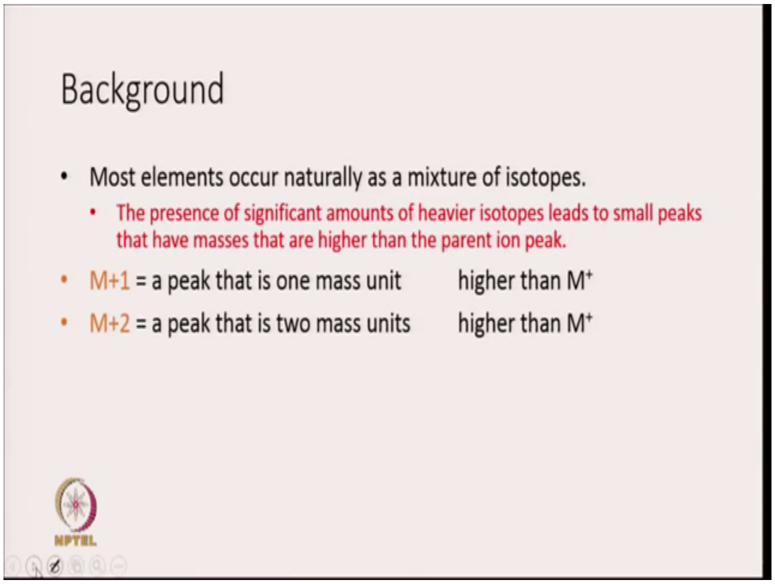

Background

- Most elements occur naturally as a mixture of isotopes.
  - The presence of significant amounts of heavier isotopes leads to small peaks that have masses that are higher than the parent ion peak.
- M+1 = a peak that is one mass unit higher than M^+
- M+2 = a peak that is two mass units higher than M^+

Most elements occur naturally as mixtures of isotopes. So the presence of significant amounts of heavier isotopes leads to smaller peaks that have masses that are higher than the parent ion peak. For example, you can also see a peak with m by z ratio M plus 1, so a peak that is one mass unit higher than M plus, you can also see, you can also see a peak that is two mass units higher than M plus.

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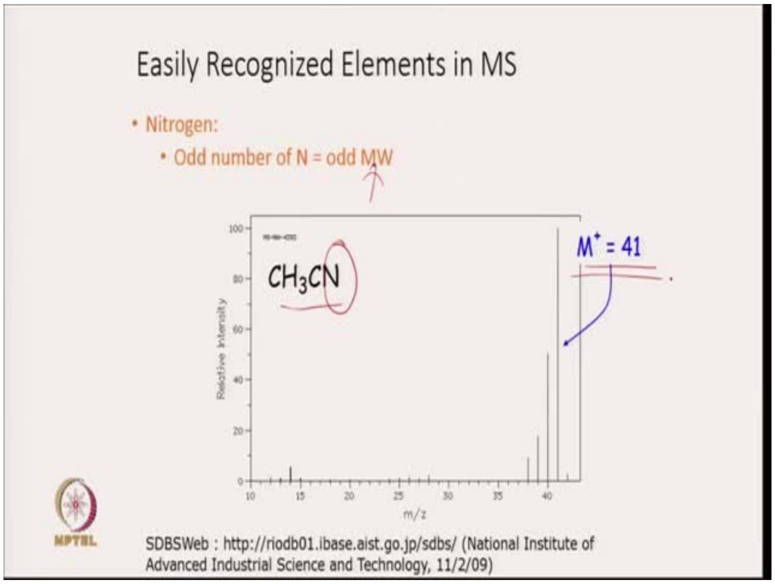

Easily Recognized Elements in MS

- Nitrogen:
  - Odd number of N = odd MW

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/2/09)

Now there are certain very recognized elements in mass spectrometer which can give you an idea about what kind of structure that molecule has. For example, if there is odd number of nitrogen then you will observe a odd molecular weight peak. So, in this case you can see there is only one nitrogen and you can see a molecular ion peak which is equal to 41 which is an odd number so all number of molecular ion peak will basically show you that the molecule may have odd number of nitrogen.

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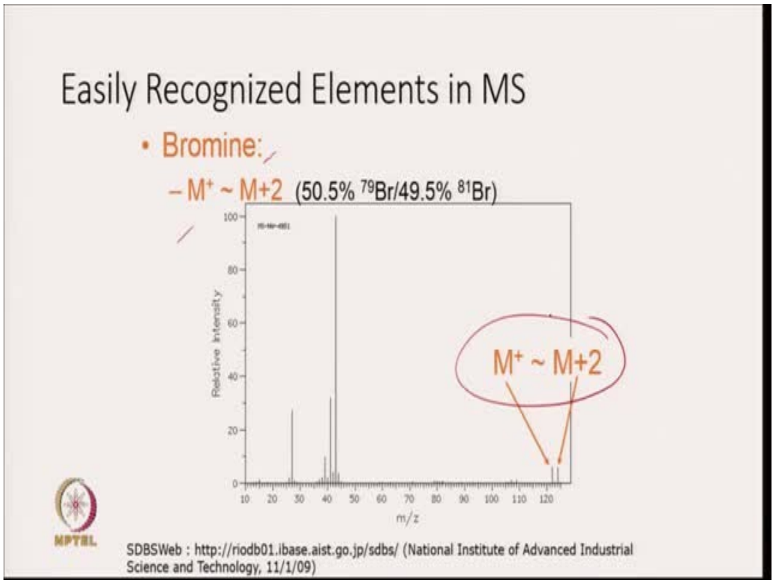

Easily Recognized Elements in MS

- Bromine:
  - $M^+ \sim M+2$ (50.5% ^{79}Br/49.5% ^{81}Br)

$M^+ \sim M+2$

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

This is a very important you know element in mass spectrometer. If suppose, your molecule has bromine then it is M plus and M plus 2 peak will be of equal intensity, M plus and M plus 2 peak will be of equal intensity and presence of equal intensity M plus and M plus 2 peak will suggest that molecule has bromine atom.

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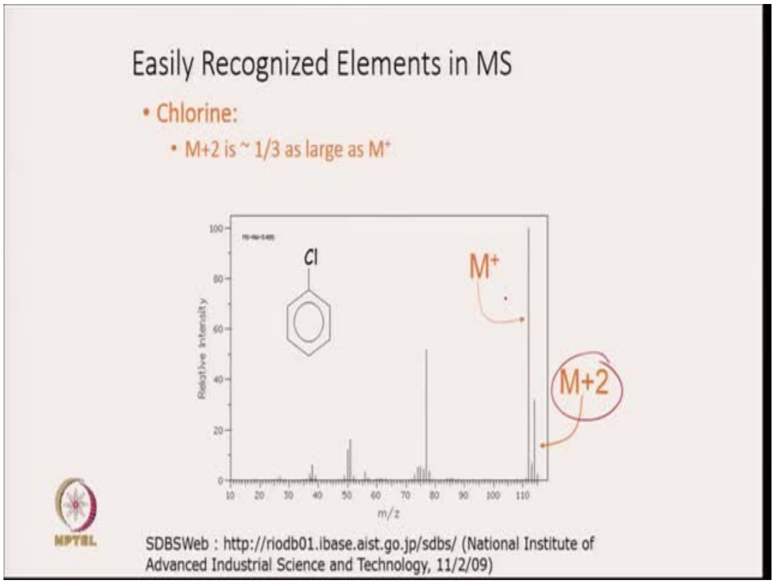

Easily Recognized Elements in MS

- Chlorine:
  - M+2 is ~ 1/3 as large as M^+

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/2/09)

Similarly, if molecule has chlorine atom then M plus 2 is one by third as large as M plus ion. So M plus 2 you can see here, M plus 2 is one by third of M plus ion and that can tell you that chloro group is present in in the molecule.

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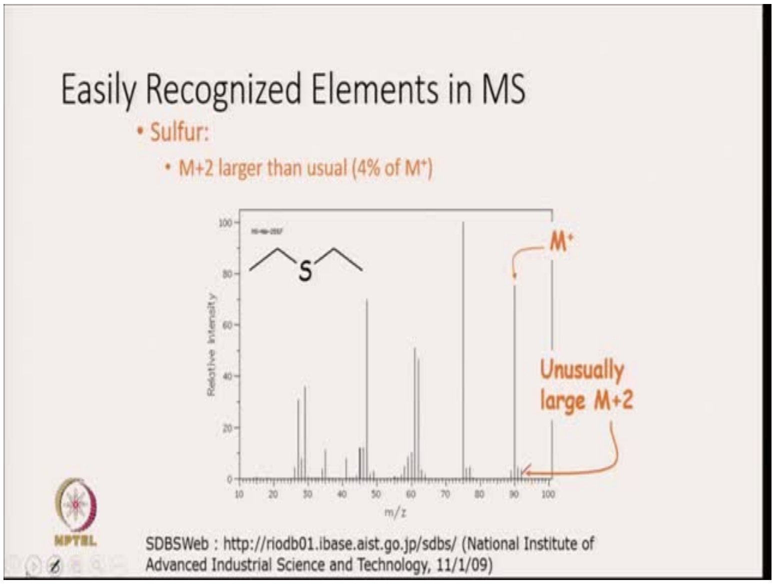

Easily Recognized Elements in MS

- Sulfur:
  - M+2 larger than usual (4% of M^+)

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

If the molecule has a Sulphur atom then you can see M plus 2 larger than usual it is almost 4 percent of M plus ion, so here you see that M plus and this is M plus 2 peak and M plus 2 peak is a 4 percent of M plus peak. So M plus 2 peak is generally larger than usual, larger in comparison to whatever you have observed and that is almost 4 percent of M plus peak.

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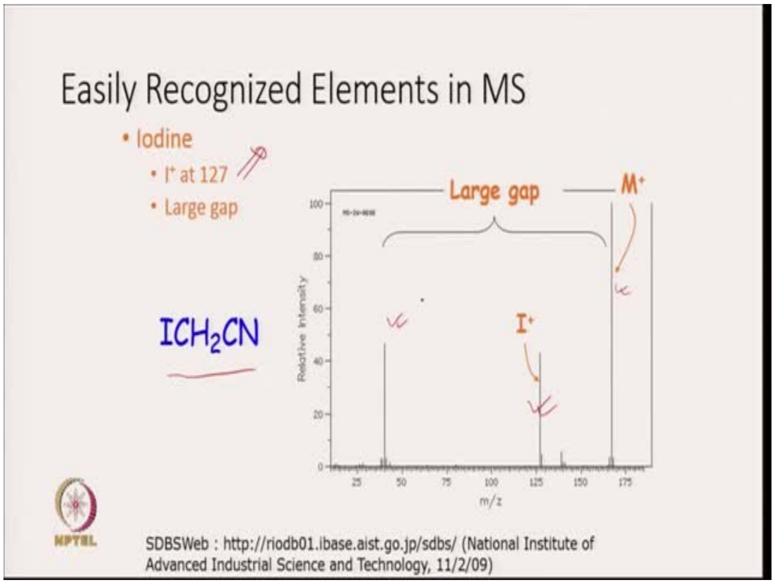

Easily Recognized Elements in MS

- Iodine
  - I^+ at 127
  - Large gap

ICH_2CN

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/2/09)

If a molecule has iodine, if a molecule has iodine then you can see a peak at 127 m by z ratio 127 and you can see it here that this molecule ICH2CN has one peak at 127 that drop, that suggests that your molecule has probably iodine and then you will also observe a large gap between M plus and the next peak, M plus and the next higher molecular peak. So, if there is a large gap it means I is getting detached from the molecule and rest of the molecule which is as cation form has lower molecular weight.

So this is about looking at the different kind of peak and getting information about particular element for example the presence of chloro group, bromo group, iodo group can be you know estimated, can be estimated from looking at the different peaks for example M plus 1 peak, M plus 2 peak, but we can also look at the fragmentation pattern to know the structure of the, structure of the molecule.

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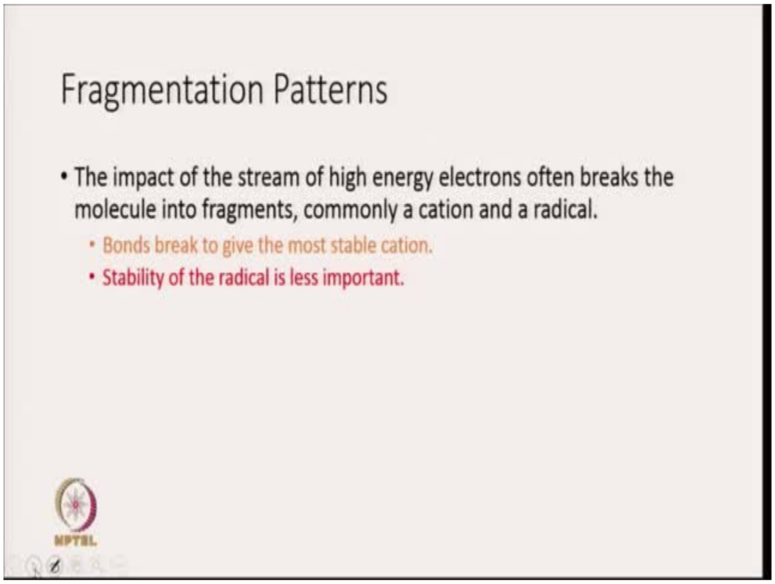

Fragmentation Patterns

- The impact of the stream of high energy electrons often breaks the molecule into fragments, commonly a cation and a radical.
  - Bonds break to give the most stable cation.
  - Stability of the radical is less important.

So when beam of high energy electrons is bombarded on the molecule, the molecule may break into fragments, one fragment will exist as a cation, whereas another fragment will exist as a radical. So bonds break to give most stable cation. So generally, if there is a breaking of the bond then most stable cation is form. Stability of radical is less important.

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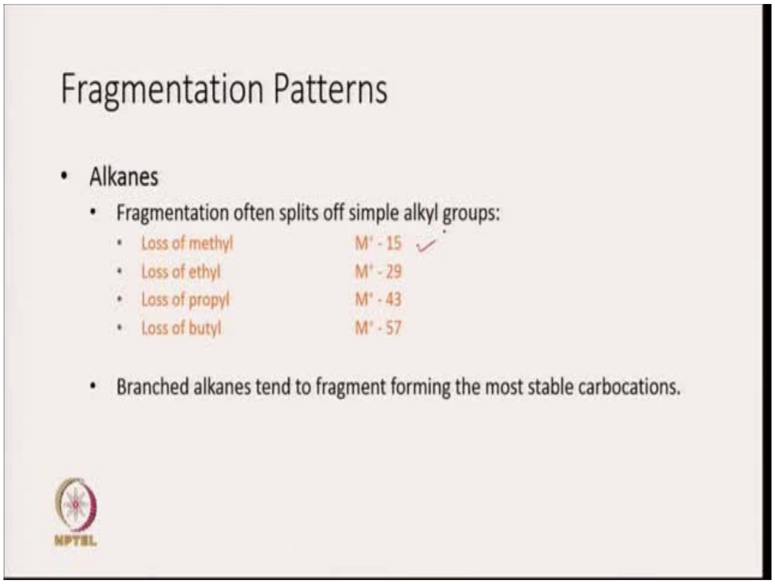

Fragmentation Patterns

- Alkanes
  - Fragmentation often splits off simple alkyl groups:
    - Loss of methyl $M^+ - 15$ ✓
    - Loss of ethyl $M^+ - 29$
    - Loss of propyl $M^+ - 43$
    - Loss of butyl $M^+ - 57$
  - Branched alkanes tend to fragment forming the most stable carbocations.

So, if you look at alkanes mass spectra of an alkane. The fragmentation often splits of simple alkyl group. For example, there can be loss of methyl group, loss of ethyl group, loss of propyl group and loss of butyl group. And so the peaks will be obtained at the molecular weight minus

15 the mass of M plus ion minus 29, mass of M plus minus 43, mass of M plus minus 57. So the peak, the peaks which will be obtained will be 15 molecular, 15 unit smaller than a molecular weight, 29 units smaller than molecular weight, 43 units smaller than molecular weight, and 57 units smaller than molecular weight. Branched alkanes tend to fragment forming the most stable carbocations. Most stable carbocations.

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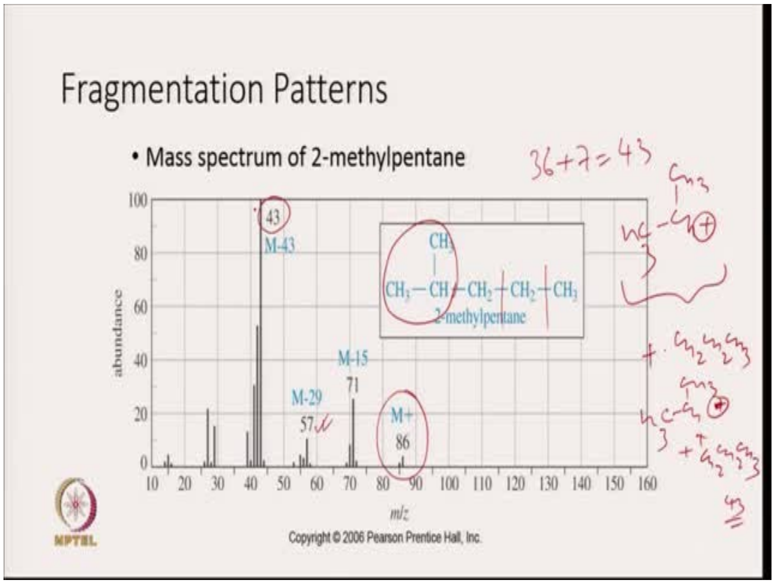

Fragmentation Patterns

- Mass spectrum of 2-methylpentane

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Now look at the fragmentation pattern of to methylpentane. So M plus is obtained at 86, so 86 is its molecular weight. Now, if it loses a methyl group then you will get the peak at minus 15 if it loses CH2CH3 then 15 plus 14 is 29, so this peak can be assigned to M minus 29 and if it loses this CH3CH3 then so you see this is 12 into 3 this is 36 plus 3 plus 3 plus 1 7 43 so if this molecule fragment giving this cation, this cation, this cation plus CH2CH2CH3 radical you will get a peak at M at 43 peak at 43, whereas if you get this CHCH3 plus sorry radical plus CH2 plus CH2CH3 so in this case you have your 36 plus 7 43. This is also 43. So in both case you will get a peak at M minus 43 and this is basically equal to 43, this is basically equal to 43.

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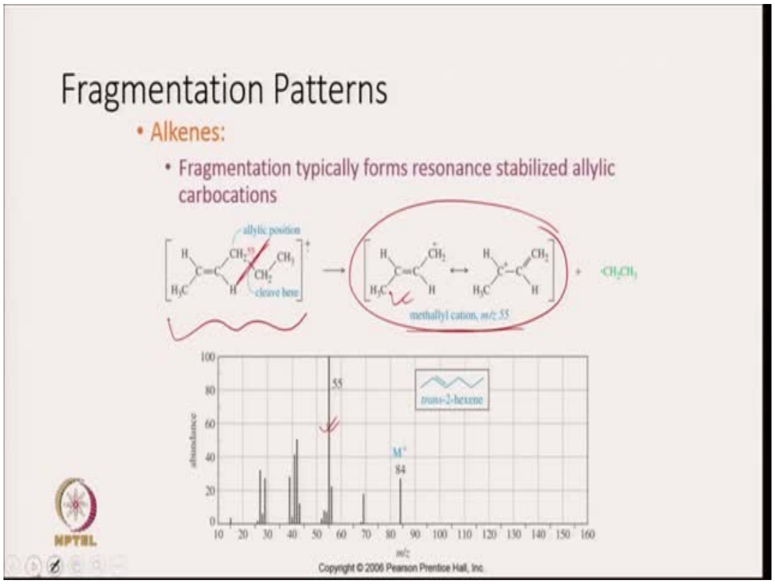

Fragmentation Patterns

- Alkenes:
  - Fragmentation typically forms resonance stabilized allylic carbocations

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This is the fragmentation pattern for alkenes. A fragmentation generally forms resonance stabilized allylic carbocation, so if you look at this case allylic position at this one. So the bond breakage will happen at this position, the fragmentation will take place at this position giving you this cation. Since this is resonance stabilized, so this will be formed in greater amount and so this molecule this species will corresponds to the base peak. This molecule will correspond to a base peak size of this species is 55. So you are seeing a most prominent peak at the position 55 and that is basically your base peak.

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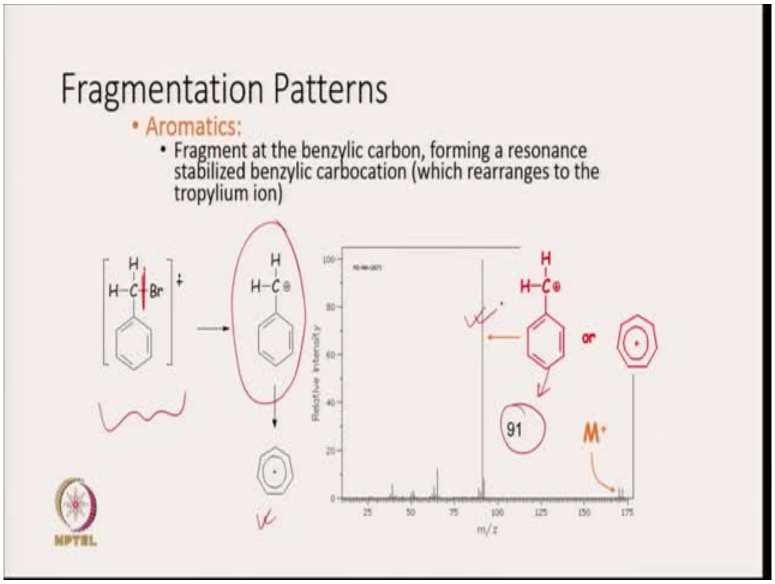

Fragmentation Patterns

- Aromatics:
  - Fragment at the benzylic carbon, forming a resonance stabilized benzylic carbocation (which rearranges to the tropylium ion)

Similarly in aromatics the fragmentation can take place at benzylic carbon. Fragmentation will take place benzylic carbon forming a resonance stabilized benzylic carbocation. So you can see this, this is your benzylic position and the fragmentation will take place at this position so that a benzylic carbocation is formed and that can go to tropylium carbocation and this has a molecular weight of 91 and so most of the aromatic compound will give a peak corresponding to 91 if there is CH2 group attached, CH2 group attached and that is basically because of benzylic carbon and this is you know the percentage of this will be more because here plus is resonance stabilized and so this will be as a base peak.

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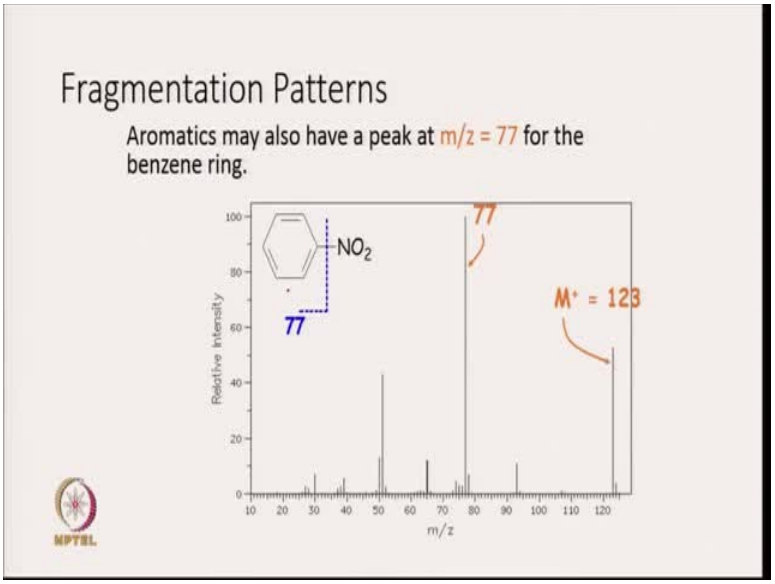

Fragmentation Patterns

Aromatics may also have a peak at m/z = 77 for the benzene ring.

Aromatics may also have peak at m by z is equal to 77 for the benzene ring so suppose your nitro compound is there this is nitro benzene so breaking will take place at this position and you expect a peak as 77 so if there is no CH3 group attached to benzene then this will be your base peak, this will be your base peak.

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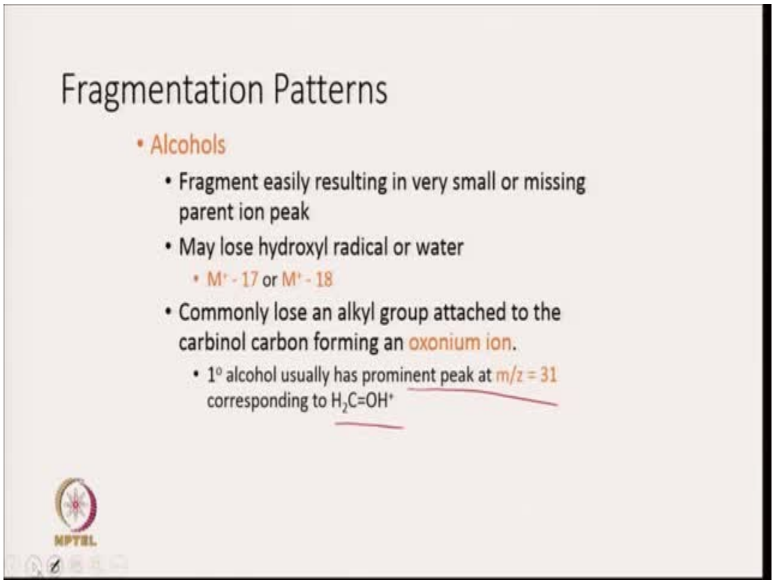

Fragmentation Patterns

- Alcohols
  - Fragment easily resulting in very small or missing parent ion peak
  - May lose hydroxyl radical or water
    - $M^+ - 17$ or $M^+ - 18$
  - Commonly lose an alkyl group attached to the carbinol carbon forming an oxonium ion.
    - 1° alcohol usually has prominent peak at $m/z = 31$ corresponding to $H_2C{=}OH^+$

Now, if you go to alcohols, fragments can easily result into very small or missing parent ion peak. You may not see parent ion peak or very small ion peak you can get M plus minus 17 because of loss of hydroxyl radical or you can see M plus minus 18 peak which is because of loss of H2O. Commonly it loses an alkyl group attached to carbinol carbon forming an oxonium ion, oxonium ion and 1 degree alcohol usually have prominent peak at m by z is equal to 31 corresponding to CH2 double bond OH plus.

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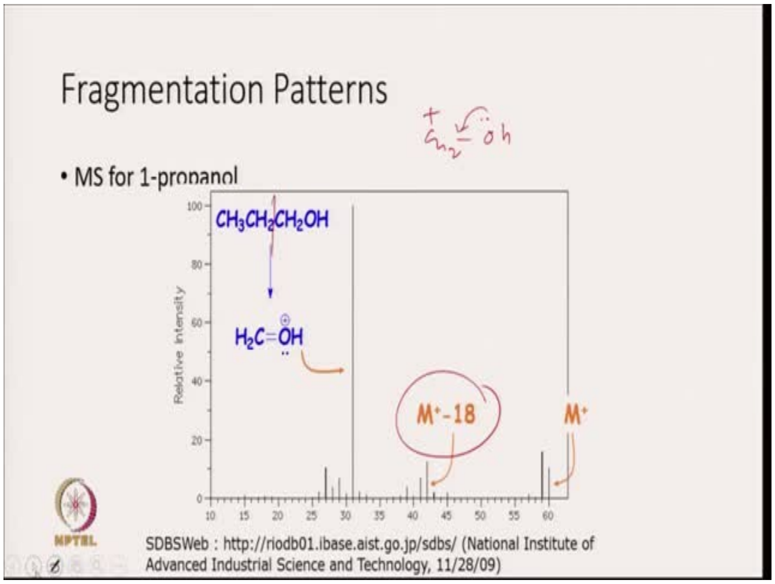

Fragmentation Patterns

- MS for 1-propanol

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

This is mass spectrum of 1 propanol, 1 propanol and it can break here giving you CH2 plus OH and here you are plus charge is resonance stabilize and so it will act as a base peak with maximum intensity. Apart from that you can get M plus minus 18 peak this is because of loss of water molecule and then M plus peak is very small, M plus peak is very small in case of alcohols.

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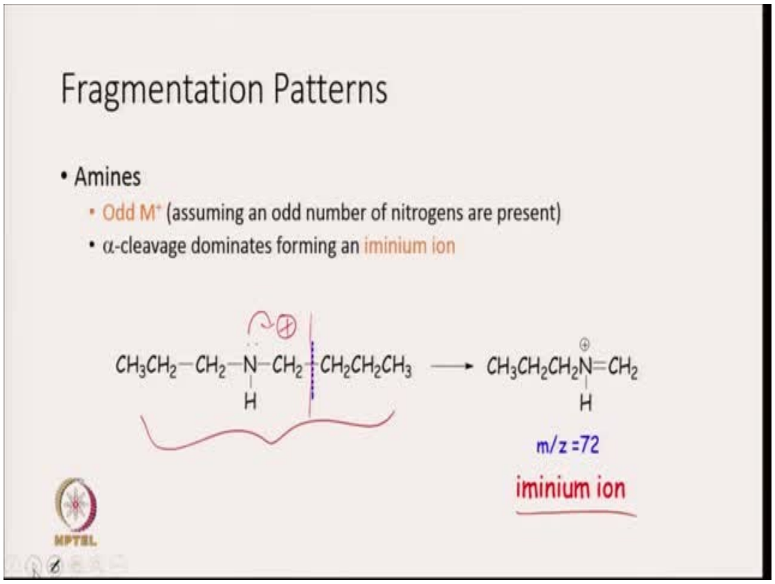

Fragmentation Patterns

- Amines
  - Odd M^+ (assuming an odd number of nitrogens are present)
  - α-cleavage dominates forming an iminium ion

$CH_3CH_2-CH_2-N(H)-CH_2 \vdots CH_2CH_2CH_3 \longrightarrow CH_3CH_2CH_2\overset{\oplus}{N}(H)=CH_2$

m/z =72

iminium ion

For amines if there is odd number of nitrogen and then you will get a M plus peak which is odd again here Alpha cleavage will dominate, since Alpha cleavage gives you a iminium ion and in which plus charges resonance stabilized, so take this case of an amine, the break will take place at this position and so that your plus charge will be here and this will be resonance stabilized. So plus charge will come here and then this will get resonance stabilized. And you will get iminium ion at m by z value of 72, m by z value of 72.

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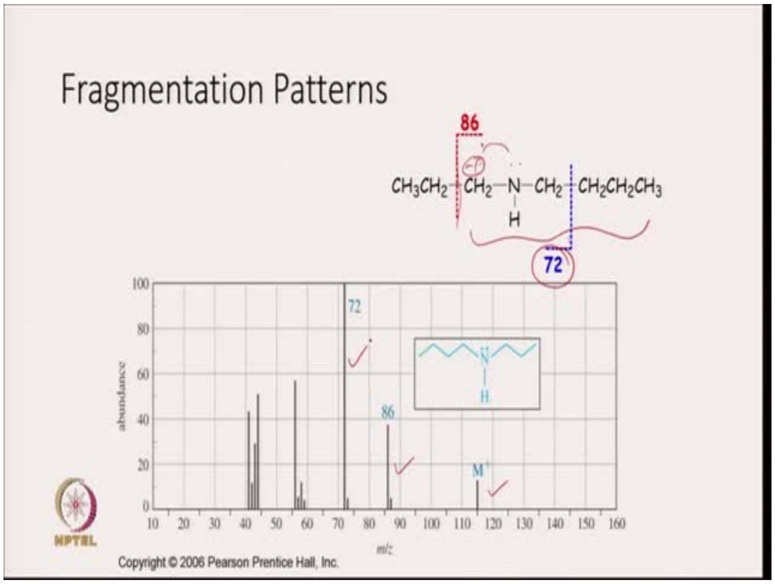
Fragmentation Patterns

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Now look at the fragmentation pattern, so if there is a breakage here then you will get a peak at 72, there is another cleavage possible at the Alpha, again Alpha position here you see if it breaks at this position then again this plus charge will be resonance stabilized and this whole mass will correspond to 86, this whole mass will correspond to 86 and thus if you are if you go for mass spectrum this you will see a peak at M plus, you will see a peak at 86 and you will see a peak at 72.

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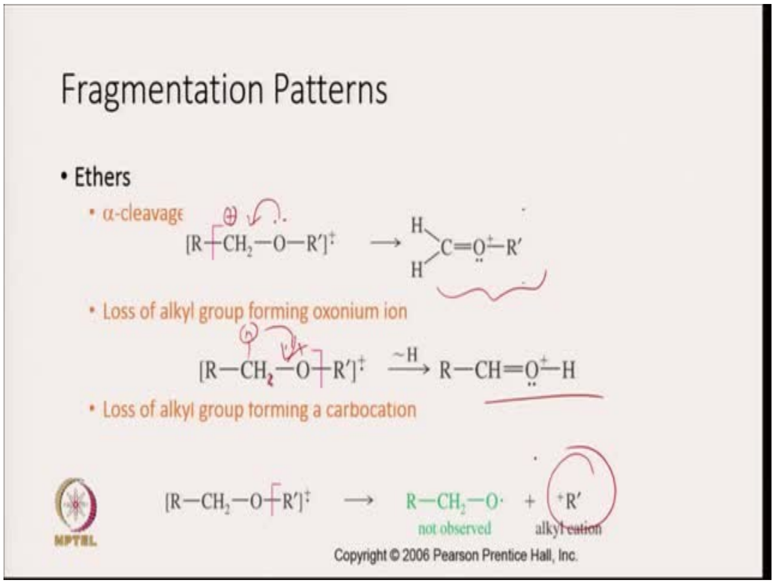
Fragmentation Patterns

- Ethers
  - α-cleavage

$$[R-CH_2-O-R']^{+\cdot} \longrightarrow H_2C=\ddot{O}^{+}-R'$$

  - Loss of alkyl group forming oxonium ion

$$[R-CH_2-O-R']^{+\cdot} \xrightarrow{\sim H} R-CH=\ddot{O}^{+}-H$$

  - Loss of alkyl group forming a carbocation

$$[R-CH_2-O-R']^{+\cdot} \longrightarrow \underset{\text{not observed}}{R-CH_2-O\cdot} + \underset{\text{alkyl cation}}{{}^{+}R'}$$

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Now, let us look at ethers again here Alpha cleavage will be more predominant, since Alpha cleavage will give you a positive charge which is your resonance stabilize with the lone pair of on the oxygen. So, if it breaks your then there will be plus charge here and this will be resonance stabilized and you will get this ion. Loss of alkyl forming an oxonium ion and so if there is a loss here then O plus there will be plus here and then there will be loss of one H here and then you will get this oxonium ion. Loss of alkyl group can also happen where your plus charge is on alkyl group, plus charges on alkyl group and that is how where carbocation can get formed.

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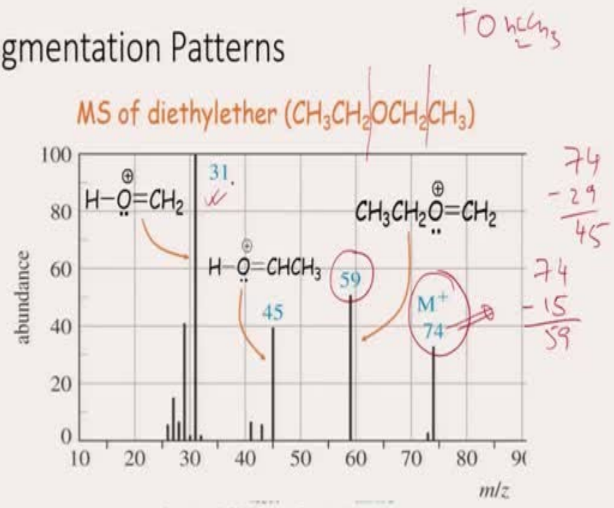
Fragmentation Patterns

MS of diethylether ($CH_3CH_2OCH_2CH_3$)



Now, let us look at the fragmentation pattern of diethylether and this is mass spectrum of diethylether. So a peak will be formed at M plus, this is the molecular ion peak and this corresponds to your 74, this corresponds to 74 and if suppose there is a loss of this CH3 (right am I right), loss of this CH3 means 74 minus 15 and that will be 59 and so you get a peak at 59 so this is because of Alpha cleavage and plus charge is on CH2 plus which is in the resonance is stabilized due to lone pair on oxygen. The next one will be because of loss of, because of loss of this one CH3 CH2 means we are reviewing 29 the 74 minus 29 is 45 so basically you have CH3 CH2 and O plus, and so you will get a peak at 45. And then you can also get CH2OH and that will, there will be a peak at 31, there will be a peak at 31.

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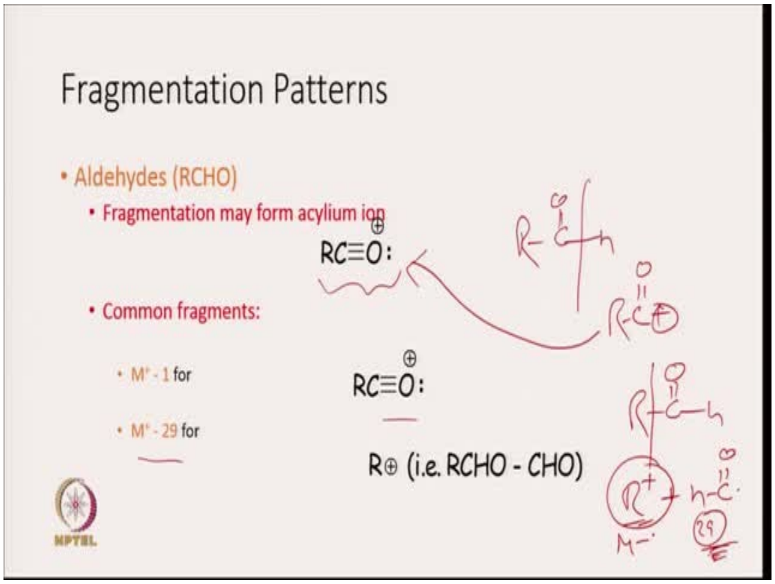

Fragmentation Patterns

- Aldehydes (RCHO)
  - Fragmentation may form acylium ion $RC\equiv\overset{\oplus}{O}:$
  - Common fragments:
    - $M^+ - 1$ for $RC\equiv\overset{\oplus}{O}:$
    - $M^+ - 29$ for $R\oplus$ (i.e. RCHO - CHO)

Now, let us look at the fragmentation pattern of the aldehyde. The fragmentation may form acylium ion. So H radical will be removed giving you acylium ion. So, if you take RCO H the break is can happen here giving you RCO plus which will be resonance stabilized, so common fragments are M plus minus 1 for this and then you can also get fragment due to breakage here so are plus plus HCO radical. So R plus will give you peak at your M plus minus 29 because this corresponds to 12 plus 16 28 1 29. So this corresponds to 29, this corresponds to 29 and this corresponds to M minus 29.

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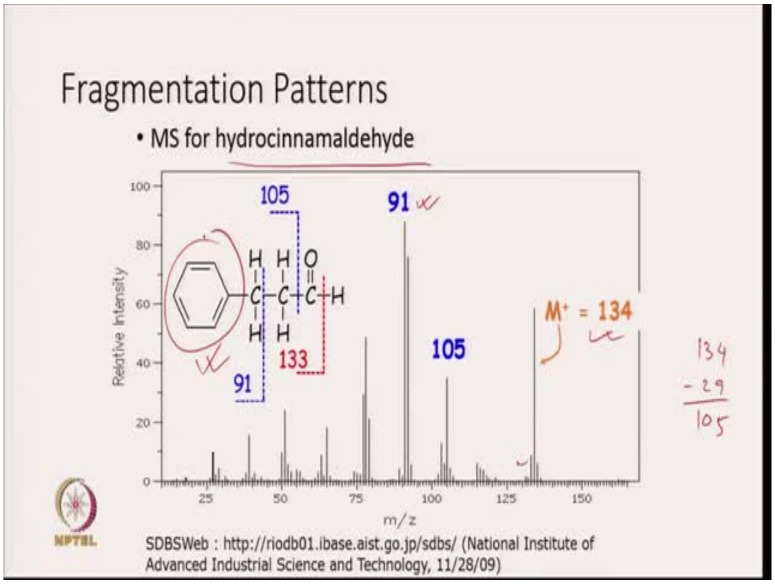

Fragmentation Patterns

- MS for hydrocinnamaldehyde

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

Now, let us look at the fragmentation pattern in hydrocinnamaldehyde and this is the formula of hydrocinnamaldehyde. So you will get peak at 134 which is your molecular ion peak, hydrogen can be lost and that is how you can get a peak at 133 then if there is a loss of this COH group then you get 105 because this corresponds to M minus 29 and if you take minus 29 then this comes out to be 105. Then if there is a break here then you will get a peak at 91, you will get a peak at 91 and then your peak at 77 can be observed because of this ring.

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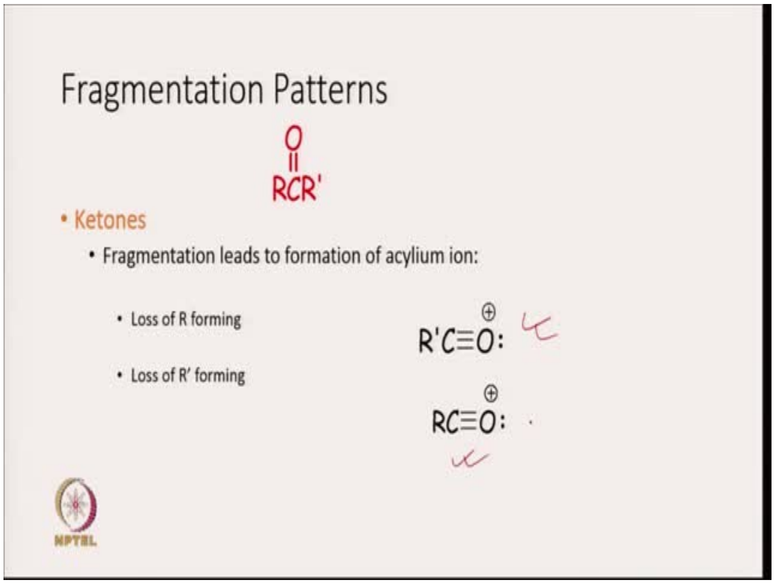

Fragmentation Patterns

$R\overset{\overset{O}{\|}}{C}R'$

- Ketones
  - Fragmentation leads to formation of acylium ion:
    - Loss of R forming $R'C\equiv\overset{\oplus}{O}:$ ✓
    - Loss of R' forming $RC\equiv\overset{\oplus}{O}:$ ✓

Ketones fragments leads to formation of acylium ion, so you can get this or you can get this one, so two different kind of fragmentation is possible, two different kind of fragmentation is possible. And that is you expecting the mass spectrum of your ketones.

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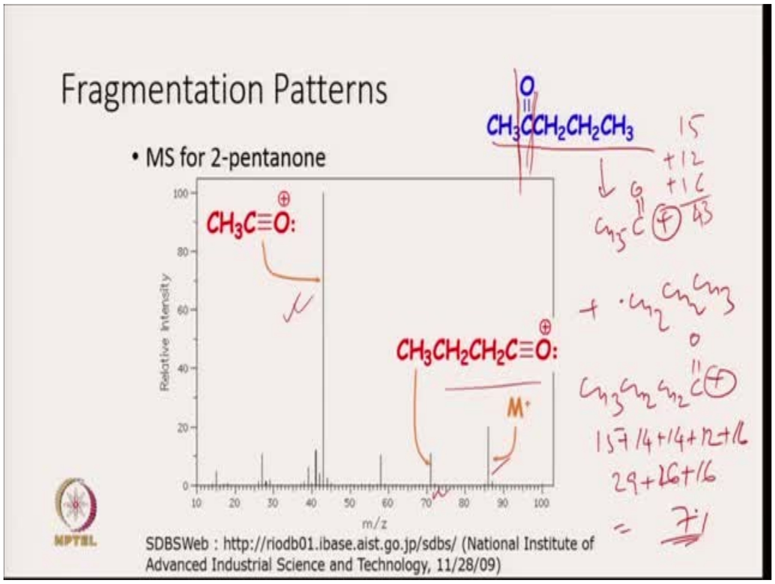

Fragmentation Patterns

- MS for 2-pentanone

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

Here is mass spectrum of two pentanone. Here is the mass spectrum of two pentanone and this is the formula for two pentanone, this is formula for two pentanone CH3 CH2 CH2 CO CH3 and there can be fragments formed from this fragmentation leading to CH3 CO plus group plus CH2 CH2 CH3 radical and when this results then you have mass at your 43 43 so this corresponds to 15 plus 15 plus 12 plus plus 16 and this is 853 and your 43 right this is 43.

So you will get a peak at 43 then there will be peak at this position so if this CH3 breaks out then you have CH3 CH2 CH2 CO plus and this is 15 plus 14 plus 14 plus 12 plus 16 and this is 29 plus 26 plus 16 and 6 6 12 9 1 21 2 8 1 3 2 5 2 7 so peak at 71 so you will see your peak at 71 so you will see your peak at 71 is formed. And this is your molecular ion peak ketone fragments will be formed and ketones breaks from two sides of CO group two sides of CO group. So if it breaks here then there will be your peak at 43 whereas if there is a break here then you will get mass peak around 71.

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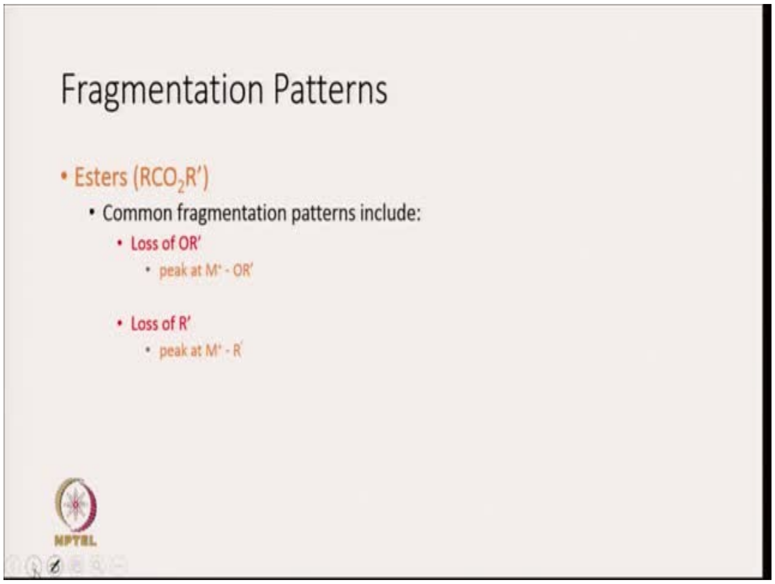

Fragmentation Patterns

- Esters (RCO_2R')
  - Common fragmentation patterns include:
    - Loss of OR'
      - peak at M^+ - OR'
    - Loss of R'
      - peak at M^+ - R'

Now, look at the fragmentation pattern of Ester. Ester, common fragments of Ester includes a fragment resulting from loss of OR dash group and so a peak is expected to be observed at the molecular mass minus OR dash, mass of OR dash group. Another fragment which can result from Ester is due to loss of R dash group and in that case the observed peak will be at the molecular mass minus mass of R dash group.

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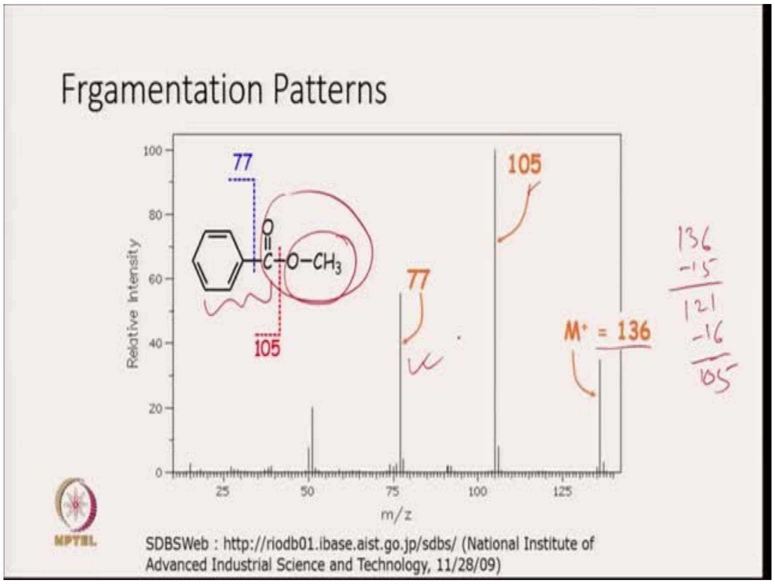

Frgamentation Patterns

SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/28/09)

And here is one example of fragmentation pattern resulting from, fragmentation pattern resulting from an ester so if this is CO there is CH3O groups leafs are giving cations having CH6H5CO plus structure that will give you peak at 105. And molecular mass peak will be at 136 so 136 minus 15 is 121 and this is minus 16 so 15 due to CH3 and there is a loss of oxygen also so this is 105. So you will see a peak at 105 and that will be a base peak. Another peak may result from the loss of this whole thing, this whole group and that will be because of this ring and that is expected to be at 77, this is expected to be at 77.

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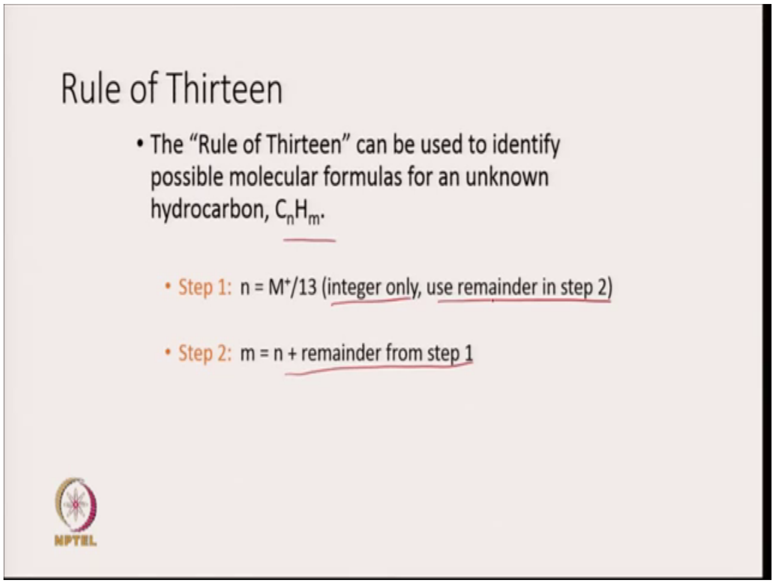

Rule of Thirteen

- The "Rule of Thirteen" can be used to identify possible molecular formulas for an unknown hydrocarbon, C_nH_m.
  - Step 1: $n = M^+/13$ (integer only, use remainder in step 2)
  - Step 2: $m = n$ + remainder from step 1

Now, there is another thing which you keep in mind this will help you in deriving the molecular formula that is known as rule of 13, that is known as rule of 13. The rule of 13 can be used to identify for simple molecular formulas for an unknown hydrocarbon. For example, if you have CNHM. N corresponds to the subscript with carbon and M subscript of hydrogen it means there N carbon's in the molecule and there are M hydrogen in the molecule.

So first thing is that you divide the molecular mass of the hydrocarbon by 13 and that is why this is known as rule of 13. It will give you integer and the remainder we are going to utilize in a step 2. So, N is simply M plus divided by 13 and M is N plus remainder from step 1. So whatever remainder you got in the step 1, just add to N value and that will give you the M value.

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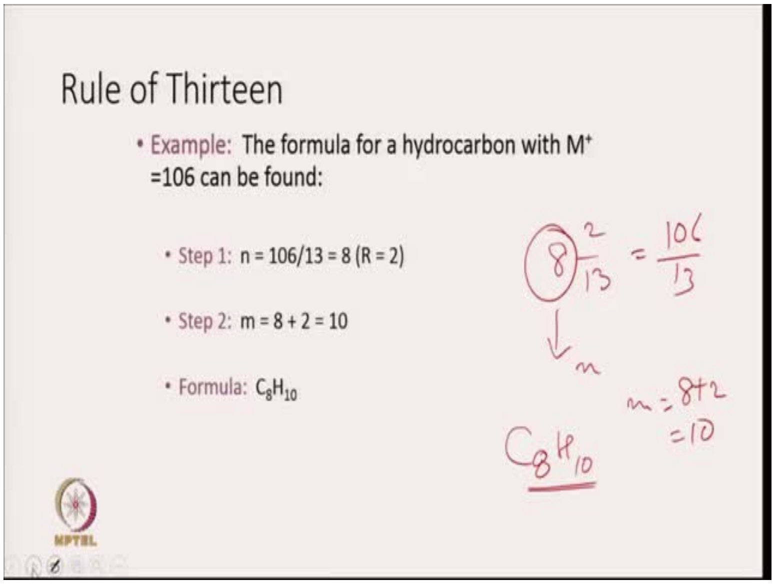

Rule of Thirteen

- **Example:** The formula for a hydrocarbon with M^+ =106 can be found:
  - Step 1: n = 106/13 = 8 (R = 2)
  - Step 2: m = 8 + 2 = 10
  - Formula: C_8H_{10}

For example, suppose I have a hydrocarbon with formula, with molecular mass 106 and that is what you get from suppose a mass spectrum. Now you want to know that what will be the formula of hydrocarbon and what we need to do is, we take 106 and divide by 13 and that is going to give you the integer 8 and the remainder will be 2.

Integer 8 and remainder will be 2 and so this is basically like so 8 2 by 13 equal to 106 by 13. So this will give you the value of n and m will be n plus remainder. So remainder is 2, so 8 plus 2 10. So this is your formula for hydrocarbon will be C8H10. And once you know that then you can know how many ion saturation is there and that can give you an idea of the structure of the molecular species.

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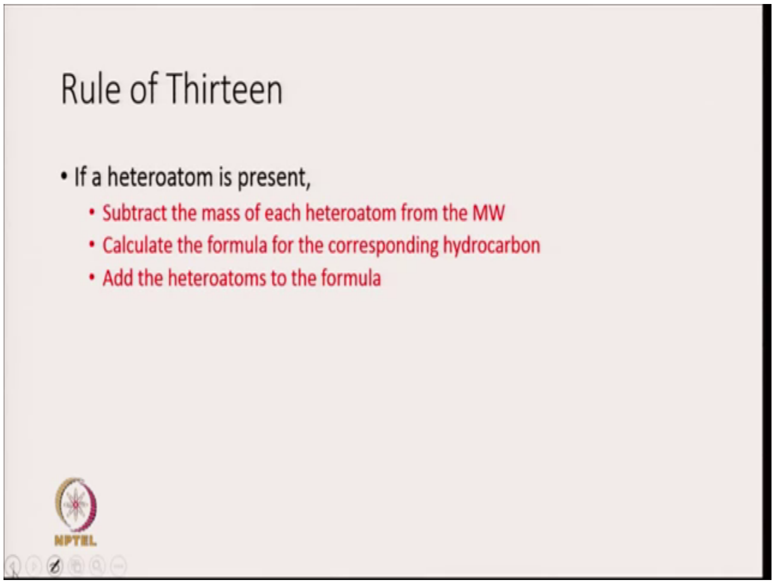

Rule of Thirteen

- If a heteroatom is present,
  - Subtract the mass of each heteroatom from the MW
  - Calculate the formula for the corresponding hydrocarbon
  - Add the heteroatoms to the formula

Now, suppose a heteroatom is also present in the hydrocarbon. For example, in alcohol oxygen is there, amine nitrogen is there then what you need to do is subtract the mass of each heteroatom from the molecular weight. So, if you expect that there is one oxygen atom then you simply subtract the mass of each heteroatom, then calculate the formula for the corresponding hydrocarbon. Add the heteroatoms to the formula, add the heteroatom is to the formula. From that you can know what is the formula of the compound. So once you apply rule of 13 you know the formula.

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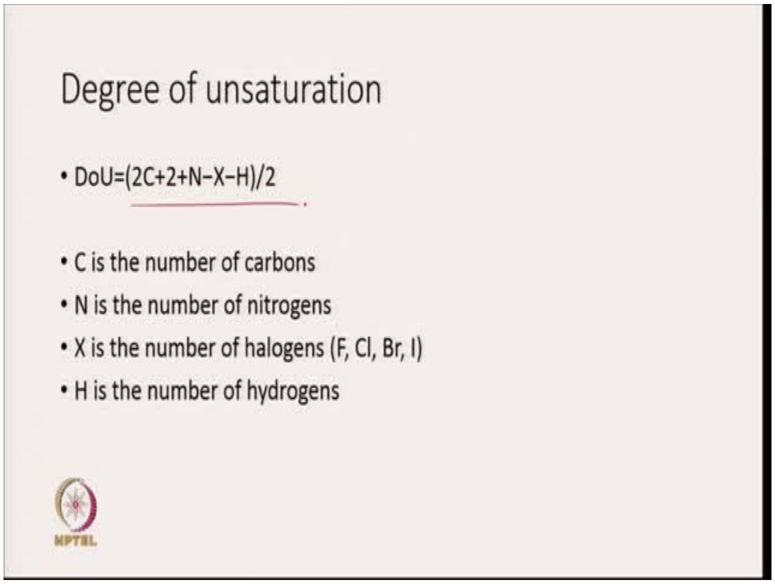

Degree of unsaturation

- DoU=(2C+2+N−X−H)/2

- C is the number of carbons
- N is the number of nitrogens
- X is the number of halogens (F, Cl, Br, I)
- H is the number of hydrogens

Now next step is to get the degree of unsaturation. And degree of unsaturation is given by this equation, 2 into carbon C is basically number of carbon. 2 into number of carbon plus 2 plus number of nitrogen which is given by N minus number of halogens which may be either Floro group or chloro group or bromo group or iodo group. And then you subtract that with the number of hydrogens and then divide by 2 that will give you degree of unsaturation. So just by looking at molecular mass you can know the formula and once you know the formula you can calculate degree of unsaturation, you can calculate degree of unsaturation.

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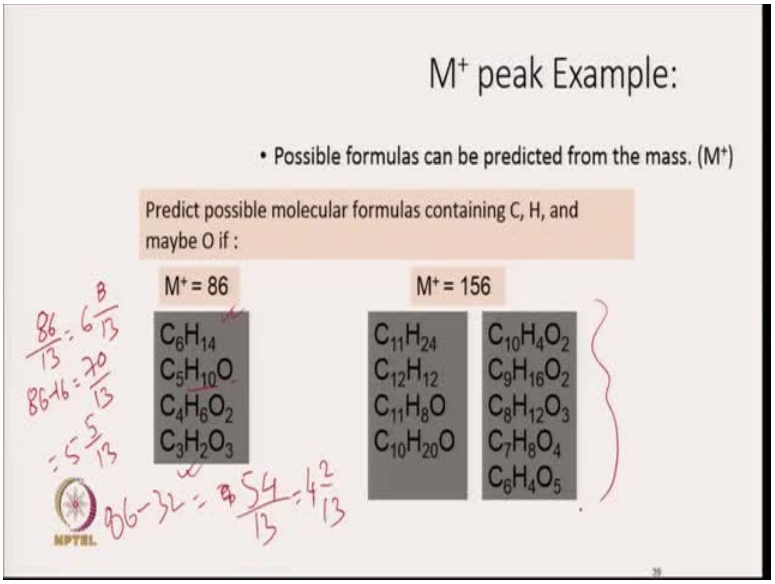

M^+ peak Example:

- Possible formulas can be predicted from the mass. (M^+)

Predict possible molecular formulas containing C, H, and maybe O if :

$M^+ = 86$

C_6H_{14}
$C_5H_{10}O$
$C_4H_6O_2$
$C_3H_2O_3$

$M^+ = 156$

$C_{11}H_{24}$
$C_{12}H_{12}$
$C_{11}H_8O$
$C_{10}H_{20}O$

$C_{10}H_4O_2$
$C_9H_{16}O_2$
$C_8H_{12}O_3$
$C_7H_8O_4$
$C_6H_4O_5$

$\frac{86}{13} = 6\frac{8}{13}$

$86-16 = \frac{70}{13}$

$= 5\frac{5}{13}$

$86-32 = \frac{54}{13} = 4\frac{2}{13}$

Okay, let us see and see whether we can predict the formula from the mass. So, if you know suppose your molecule has carbon, hydrogen and oxygen, carbon, hydrogen or maybe oxygen then if you have a M plus equal to 86 then your formula can be C6 H 14, C5H 10 O, C4H6O2, C3 H2 O3, and this you can get from so suppose 86 divided by your 13 and that will give you 6. So 13 into 6 is 78 plus 8 by 13. So formula will be C6 and H6 plus 8 14. Now there is possibility that one oxygen can also be present in that case you will write 86 minus 16 is equal to 70 divided by 13 and that will give you 5, 5 by 13, 5 5 by 13. And so your formula will be C5 and 5 plus 5 10 so H10 and 1 oxygen.

If suppose, you expect that there is 2 oxygen then you have 86 minus 32 it means you have (7 sorry) you have 4 and 554 and then you divide by 13 and then you will get 4 2 by 13 and so C, number of C will be 4, number of H will be 4 plus 2 6 and then O2. And similarly you can get this formula. So these are the formula which you can guess just from the molecular weight. And if M plus is 156 then you have these many possibilities.

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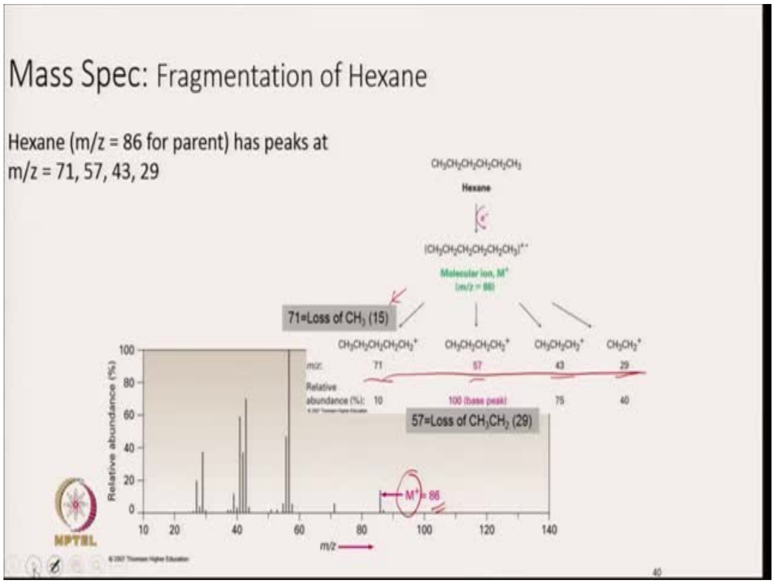


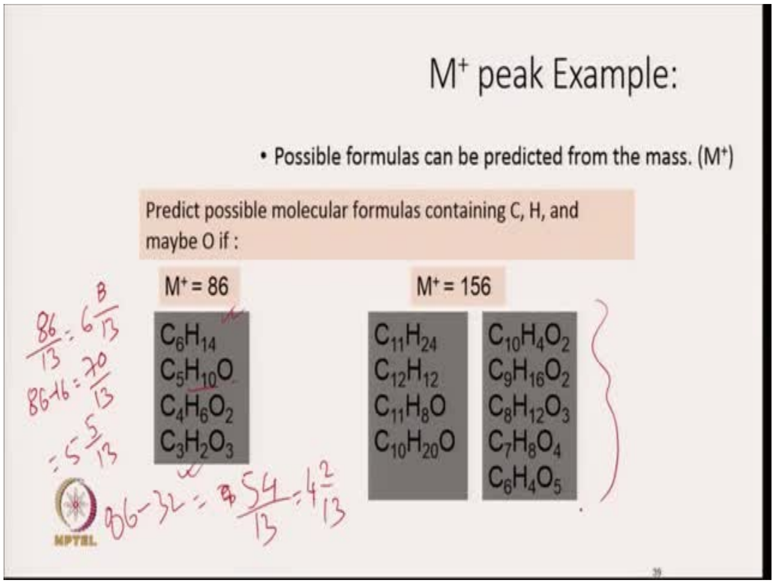
Mass Spec: Fragmentation of Hexane

Hexane (m/z = 86 for parent) has peaks at m/z = 71, 57, 43, 29

71=Loss of CH_3 (15)

57=Loss of CH_3CH_2 (29)

	$CH_3CH_2CH_2CH_2CH_2^+$	$CH_3CH_2CH_2CH_2^+$	$CH_3CH_2CH_2^+$	$CH_3CH_2^+$
m/z:	71	57	43	29
Relative abundance (%):	10	100 (base peak)	75	40



M^+ peak Example:

- Possible formulas can be predicted from the mass. (M^+)

Predict possible molecular formulas containing C, H, and maybe O if :

$M^+ = 86$	$M^+ = 156$	
C_6H_{14}	$C_{11}H_{24}$	$C_{10}H_4O_2$
$C_5H_{10}O$	$C_{12}H_{12}$	$C_9H_{16}O_2$
$C_4H_6O_2$	$C_{11}H_8O$	$C_8H_{12}O_3$
$C_3H_2O_3$	$C_{10}H_{20}O$	$C_7H_8O_4$
		$C_6H_4O_5$

$\frac{86}{13} = 6\frac{8}{13}$

$86-16 = \frac{70}{13} = 5\frac{5}{13}$

$86-32 = \frac{54}{13} = 4\frac{2}{13}$

And if suppose we have hexane, so here you see this is hexane, so suppose what we are getting is if we have a hexane then what do we expect that there will be peak corresponding to M plus equal to 86 and then the first loss of methyl group result into loss of mass by 15. And then you will get 71 m by z value. The second loss of CH2CH3 will result into loss of CH2CH3 which is 29 and if you subtract it as 6 by 29 then you will get 57 and 57 minus 14 is 43 and 43 minus 14 is

29. So these peaks you expect and here you see that you have got peak at 86. You have got a peak at 57, you have got a peak at 43 and you have got a peak at 29.

And if you get this spectrum then you are sure that you have a hexane, you have a hexane. And so by combining your M plus information plus from M plus peak and looking at the spectrum you can know what structure you have got.

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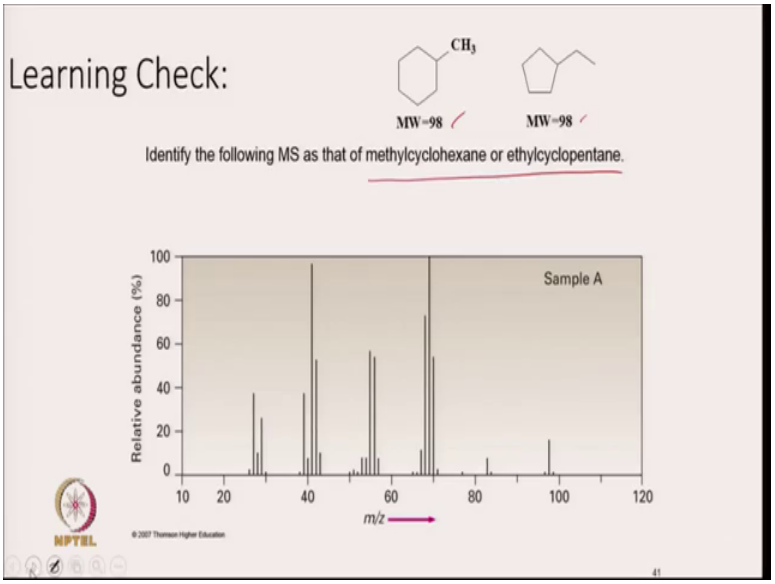

Learning Check:

Identify the following MS as that of methylcyclohexane or ethylcyclopentane.

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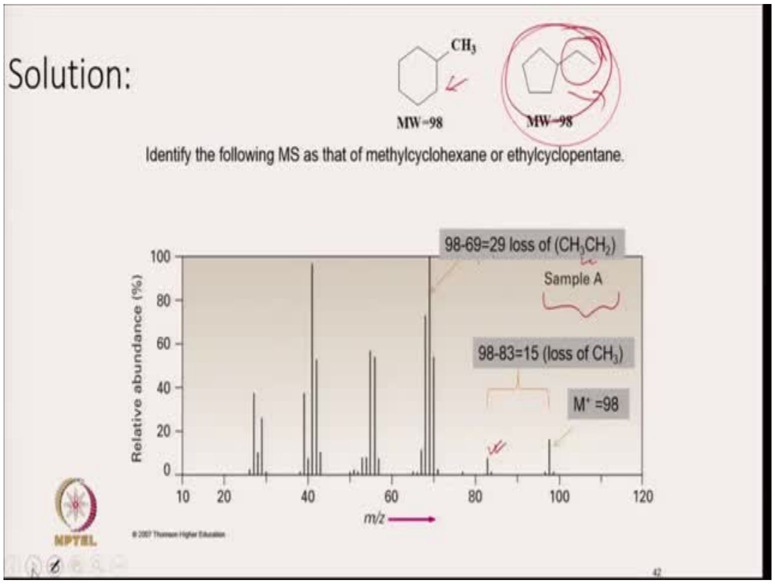

Solution:

Identify the following MS as that of methylcyclohexane or ethylcyclopentane.

98-69=29 loss of (CH$_3$CH$_2$)

98-83=15 (loss of CH$_3$)

M$^+$ =98

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Now, we can also distinguish between two different isomer. For example, methylcyclohexane or ethylcyclopentane both have same molecular weight 98, 98. So they cannot be distinguished by

M plus ion peak but it is fragmentation pattern is going to be the different and that is how you can differentiate between these 2 molecules. Suppose you have a, you have Sample A and you want to know whether this Sample A is basically this compound or this compound. And this is spectrum of Sample A and then you look at this peak there are these 3 peaks and they are very characteristic.

This is M plus peak which is equal to 98 so this is molecular weight of 98 and then if there is a loss of CH3 then you can get a peak at your 83 peak at 83 that is what you see this is because of loss of CH3. And if there is loss of CH3 CH2 group then there will be loss of 29 and so 98 minus 69 gives you a loss of 29 that is a base peak and that can only be possible from this compound, only can be possible from this compound because this loss of CH3 CH2 is more prominent in this compound and so base peak at 69 is due to this compound.

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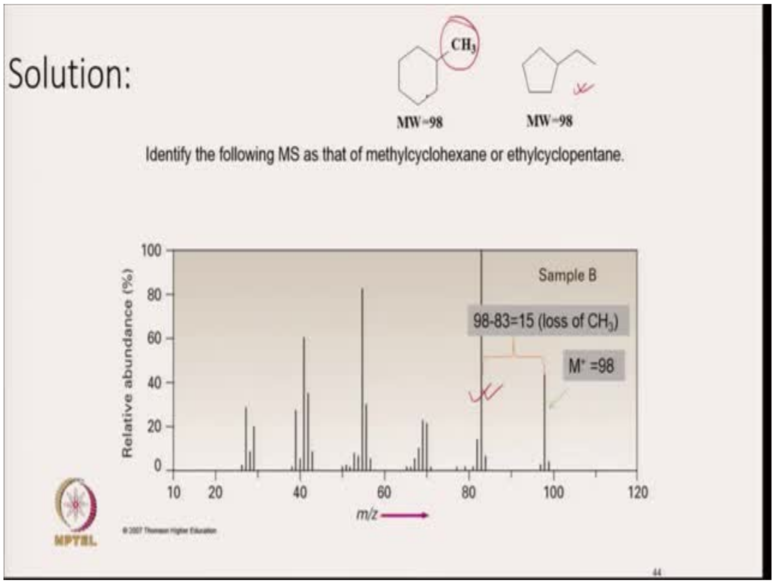

Solution:

Identify the following MS as that of methylcyclohexane or ethylcyclopentane.

98-83=15 (loss of CH_3)

M^+ =98

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And now look at Sample B. How does the fragmentation pattern of this looks like, here M plus is equal to 98 and now here the base peak will be due to loss of CH3, due to loss of CH3. And that is why you are seeing a big peak here and so just by looking at the structure you expect a base peak in this due to loss of ethyl group whereas base peak in this due to loss of methyl group and so just looking at their mass spectrum you can distinguish between these two compounds, these two compounds.

So I will stop here. I suppose that have given enough clue to you, how to use mass spectroscopy, how to use mass spectroscopy for the structure determination of organic compound and this is my last lecture, so I believe that I have given a very good flavor for you of different kind of spectroscopy, I have started with the theory part and I have tried to go into lot of application corresponding to each spectroscopy, we not only looked at the spectroscopy, we also have looked into, we also have looked into microscopy and if you understand the principle it is not very difficult to use spectroscopy for a structure elucidation and other kind of application.

Application is not only for the elucidation of a structure, the application of spectroscopy include your analysis, include your qualitative determination, include quantitative determination, includes your applications in environment, application in forensic science, application and pharmaceuticals, biopharmaceuticals, application in cosmology. So there are lot of applications and your potential of a spectroscopy and microscopy is vast and so it is very difficult to give you all the, all different kind of application which can be done using a spectroscopy, but I have given a good flavor of each of the topic and so I believe that you must have enjoyed this course, thank you, thank you very much.