

Structural Biology
Prof. Saugata Hazra
Department of Biotechnology
Indian Institute of Technology – Roorkee

Lecture – 28
NMR Sample Preparation and Chemical Shift Related Concepts

Hi everyone, welcome again to the structural biology course, as you all now know, we are going through high resolution structural biology techniques. And now we are in the module of nuclear magnetic resonance spectroscopy. In the last class, I talked about the magnetism, resonance and more importantly about the different components of the hardware. In this new class, I will discuss about different small precautions and methodologies we have to take to perform experimentally the NMR spectroscopy experiments.

So, let us start with that and then we will talk about chemical shift we will talk about some spectra that what are the reason of the spectra to giving different signal in response to 1 nuclei just proton.

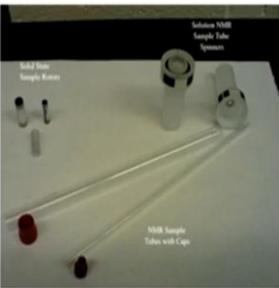
(Refer Slide Time: 01:47)

NMR Sample Holder:

An **NMR tube** is a thin glass walled tube used to contain samples in nuclear magnetic resonance spectroscopy

Typically NMR tubes come in 5 mm diameters but 10 mm and 3 mm samples holders are also known

It is important that the tubes are uniformly thick and well-balanced to ensure that NMR tube spins at a regular rate (i.e., they do not wobble), usually about 20 Hz in the NMR spectrometer



3

So, let us start with NMR sample preparation. So, first what it need is NMR sample holder which is actually a NMR tube. So, NMR tube is a thin glass walled tube used to content sample in nuclear magnetic resonance spectroscopy. If you see in the picture, you see this tubes and caps, typically NMR tubes come in 5 millimeter diameter, but 10 millimeter and 3 millimeters sample

holders are also known, it is important that the tubes are uniformly thick and well balanced to ensure that NMR tubes spin at a regular rate.

For example, they do not wobble usually about 20 hertz in the NMR spectrometer. So, if you see the solid state sample rotors, these are solution NMR sample tubes spinners and NMR sample tubes with caps.

(Refer Slide Time: 02:56)

Preparing Tubes and Caps:

Tubes: NMR tubes are a standard length (7 and 9 inch). When chipped (and reduced in length) they should not be reused as an unbalanced tube will not spin

We have to clean the tubes thoroughly after use

First use the solvent you were using to recover your previous sample, then rinse several times with acetone and finally dry the sample tube laying flat on a layer of kimwipes or placed upside-down on a kimwipe in a beaker or Erlenmeyer flask

Choose the container so the tubes stand vertically. Don't heat the tubes above 50 °C, as the glass might warp

Always store unused, clean tubes uncapped and laying on a flat surface

Caps: Tube caps are disposable and replacements can be easily obtained in bags of 100

So, how we prepare these, the tubes NMR tubes are a standard length 7 and 9 inch when chipped and reduced in length this should not be reused as an unbalanced tube will not spin properly. So, this is a precaution. We have to clean the tubes thoroughly after use cleaning is very important. If you do not clean properly, you will get mixing and different other side effects could be opposite. To clean, you should first use the solvent you were using to recover your previous sample.

Then you have to rinse several time with acetone and finally dry the sample tube lying flat on a layer of Kim wipes which we have in the laboratory mostly or placed upside down on a Kim wipe in a beaker or Erlenmeyer flask which we used to use. Choose the container so the tubes stand vertically, do not heat the tube above 50 degrees centigrade as that could like make harm to the glass.

Always store unused, clean tubes uncapped and laying on a flat surface for the caps because they are so the tubes are not cheap. So, you could not make it use and throw but caps are cheap. So, tube caps are disposable and replacements can be easily obtained in a bag of 100.

(Refer Slide Time: 04:38)

Degassing Samples:

NMR spectra recorded using degassed solvents usually benefit from reduced half-height line-width and thus better Sample to noise ratio

(O₂ gas is paramagnetic!)

There are several ways of degassing your sample:

The best is the freeze-pump-thaw technique,

Placing the sample in a ultrasonic bath works moderately well

Bubbling nitrogen through or over the sample work but not recommended



Degassing is a very important part of sample preparation. The NMR spectra recorded using degassed solvent usually benefit from the reduced half-height line-width and thus better sample to noise ratio. As you know, oxygen gas is paramagnetic so it might influence. There are several ways of degassing one is freeze -pump-thaw technique that is considered as the best one, then placing the sample in an ultrasonic bath which works moderately well or bubbling nitrogen through or over the sample that is the worst one among the 3 techniques.

(Refer Slide Time: 05:25)

NMR Sample Preparation: Quantity

For proton NMR spectra of small organic compounds (up to MW=500) anything between 1 and 20 mg of sample will be fine

Concentrated solutions can be viscous and may result in broad signals

Very dilute samples could be masked by impurities and solvent peaks

Carbon-13 is present at approximately 1.1 % natural abundance

It is intrinsically less sensitive than protons (approx. *six thousand times*)

Please provide as much sample as possible, 50 - 100 mg (or more) is fine

Preparing two samples - one dilute sample for proton NMR and one concentrated sample for carbon NMR is a useful, but unnecessary practice



What quantity we are going to use for making samples so that is very critical. For proton NMR spectra of small organic compounds up to molecular weight 500 Dalton, anything between 1 to 20 milligram of sample would be fine, concentrated solution can be viscous and that may result in broad signals, very dilute sample could be masked by impurities and solvent peaks. Carbon 13 is present at approximately 1.1% we already discussed about the natural abundance.

Carbon 12 is the most abundant and carbon 13 is very less abundant. So, when you are using carbon 13 you know that it is intrinsically less sensitive than protons, we could say approximately 6000 times. So, you have to provide as much sample as possible 50 to 100 milligram or even more is fine. But when we say that we talked about proton we talked about carbon 13 preparing 2 sample, 1 dilute sample for proton NMR and 1 concentrated sample for carbon NMR is useful.

But it will unnecessarily increase your amount of work. So, it is always good to choose in between a moderately high concentrated solution which does not affect the proton, but also is good enough to collect carbon 13.

(Refer Slide Time: 07:11)

NMR Sample Preparation: Quantity

Solvent height (volume) should be uniform, 5 cm or 2 inches equal 0.5 ml

The ends of the sample distort the field homogeneity, shimming on each sample corrects this effect and takes just a minute or so. *shim*

However, vastly different solvent heights (volumes) prevent complete correction and require many minutes shimming to achieve acceptable homogeneity

Samples prepared with too much solvent waste both time and money, and provide poorer S/N.

However, If you have limited amounts of sample (less than 1 mg), using less solvent is permissible

Minimum height: 1 cm, however, this requires special positioning of the sample tube and very intensive shimming



Solvent height should be uniform 5 centimeter or 2 inches which is equal to 0.5 ml. The ends of the sample distort the field homogeneity, shimming on each sample correct this effect and takes just a minute or so. So, what is shimming we are going to check? However, vastly different solvent hides person prevent complete correction and require many minutes shimming to achieve acceptable homogeneity.

Samples prepared with too much solvent waste both time and money and provide poorer signal to noise ratio. However, if you have limited amount of sample which is let us say less than 1 milligram using less solvent is permissible. Minimum height 1 centimeter however, this requires special positioning of the sample tube and very intensive shimming otherwise errors could be happened.

(Refer Slide Time: 08:24)

What is Shimming?

A shim is a device used to adjust the homogeneity of a magnetic field

Shimming is a process that is carried out to correct any inhomogeneities in the applied magnetic field during a nuclear magnetic resonance (NMR) experiment

In modern NMR spectrometers, shimming is done using small electrical circuits having some resistance. When current flows in a wire, a magnetic field is generated. So using these shimming circuits means small magnetic fields can be generated and used to adjust the main applied field and increase its homogeneity

The shimming coils can be in the probe, as it is the field at the sample that needs to be smooth. The coils can adjust the magnetic field in several directions. Typically, an analyst will have to adjust the field in three dimensions whilst measuring a reference peak



So, what is shimming? A shim is a device used to adjust the homogeneity of the magnetic field. Shimming is a process that is carried out to correct any inhomogeneities in the applied magnetic field during a nuclear magnetic resonance experiment. In modern NMR spectrometers shimming is done using small electrical circuits having some resistance when current flows in wire a magnetic field is generated.

So, using these seeming circuits, means small magnetic fields can be generated and used to adjust the main applied field and increase its homogeneity. The shimming coils can be in the probe as it is the field at the sample that needs to be smooth; the coil can adjust the magnetic field in several directions. Typically an analyst will have to adjust the field in 3 dimensions while measuring the reference peak.

(Refer Slide Time: 09:29)

NMR Sample Preparation:



Use clean + dry NMR tubes and caps (tubes can be re-used, caps should not!)

0.5 ml deuterated solvent (i.e. CDCl_3 , C_6D_6 , acetone- d_6 , etc.)

Substrate requirements for routine spectra:
 10 mg for proton NMR
 100 mg for carbon-13 NMR

Min. filling height of tube: 2 inches (5 cm)

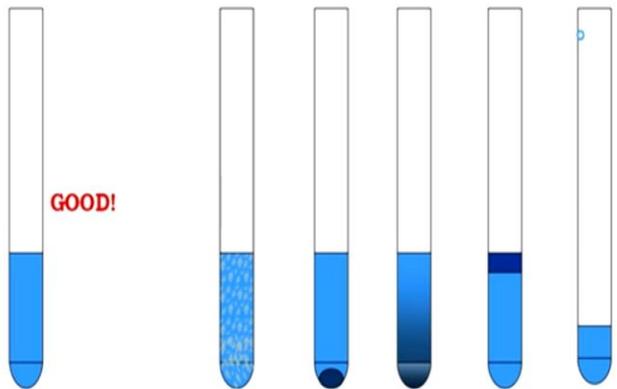
Cleaning of tubes:
 rinse with solvent you were using
 rinse with acetone
 dry in (vacuum-)oven at low temperature

So, sample preparation. This is the tube you have to use clean plus dry NMR tubes and caps, tubes can be reused, caps should not. As I told earlier. 0.5 ml deuterated solvent CDCl_3 , C_6D_6 , acetone- d_6 etcetera are the common solvent. Substrate requirement for routine spectra 10 mg for proton NMR, 100 mg for carbon 13 NMR. The minimum filling height of tube 2 inches which is 5 centimeter. Cleaning up tubes rinse with solvent you were using.

Rinse with acetone many times, dry in vacuum oven at low temperature less than 50 degrees because if you do more than 50 degrees yes you get more drying, but it could affect the glass property which will affect your further experiment.

(Refer Slide Time: 10:34)

NMR Sample Preparation: Bad Samples!



Clean clear solution (GOOD!)

Suspension of opaque solution

Precipitate

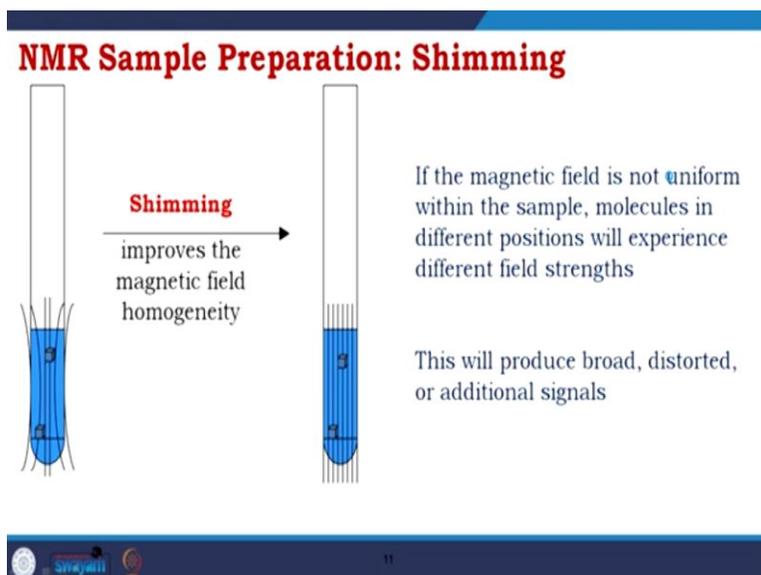
Concentration gradient

Two phases

Not enough solvent

So, in sample preparation, what are good solution if we check this is a clean and clear solution? This is a solution with suspension or the things are opaque. This is a tube with precipitate. This is a tube where we see that development of a concentration gradient. This is a tube where we see a phase develop like there is phase separation, 2 phases are there. This is a tube where we see the solvent is not enough. So, this clean clear solution is always good for sample preparation and all those suspension solution precipitate concentration gradient phase separated and less solvent are considered as bad samples.

(Refer Slide Time: 11:39)



NMR Sample Preparation: Shimming

Shimming
improves the magnetic field homogeneity

If the magnetic field is not uniform within the sample, molecules in different positions will experience different field strengths

This will produce broad, distorted, or additional signals

The diagram illustrates the process of shimming in NMR. It shows two test tubes. The left tube contains a sample with a non-uniform magnetic field, indicated by a distorted blue liquid level and a wavy line representing the field. The right tube shows the same sample after shimming, with a uniform magnetic field and a smooth blue liquid level. An arrow labeled 'Shimming' points from the left tube to the right tube, with the text 'improves the magnetic field homogeneity' below it. To the right of the tubes, text explains that a non-uniform field causes molecules to experience different field strengths, leading to broad, distorted, or additional signals.

Shimming I talked about, what seeming do? Improve the magnetic field homogeneity. So, if the magnetic field is not uniform within the sample, molecules in different positions will experience different field strength and that means, there would be a development of non-homogeneity which will produce broad, distorted or additional signals. So, to correct this you need to do Shimming.

(Refer Slide Time: 12:13)

Good and bad NMR Spectra:

Good and bad NMR spectra are the result of:

Homogeneity of magnetic field

Sample preparation

Choice of solvent

Data acquisition parameters

Processing procedures

Coming to good and bad NMR spectra. So, the good and bad NMR spectra are the result of homogeneity of magnetic field as we have discussed earlier, how you prepare your sample because if some bodies not taking care properly in washing, cleaning and all if you are reusing caps and also that all are coming into sample preparation. Proper sample preparation is a very critical thing for getting good NMR spectra, choice of solvent we have already talked about.

There are standard solvents you should use them so that the sample is soluble. Data acquisition parameters, what are the parameters you are using for data acquisition and processing procedures. How the acquired data is processed.

(Refer Slide Time: 13:09)

NMR spectrum:

NMR spectra is the graphical representation of NMR signal which is analyzed towards understanding the molecular level information

^1H - or Predictable

They are unique, well-resolved, analytically tractable and often highly predictable for small molecules

Different functional groups are obviously distinguishable, and identical functional groups with differing neighboring substituents still give distinguishable signals

NMR has largely replaced traditional wet chemistry tests such as color reagents or typical chromatography for identification

A disadvantage is that a relatively large amount, 2-50 mg, of a purified substance is required, although it may be recovered through a workup

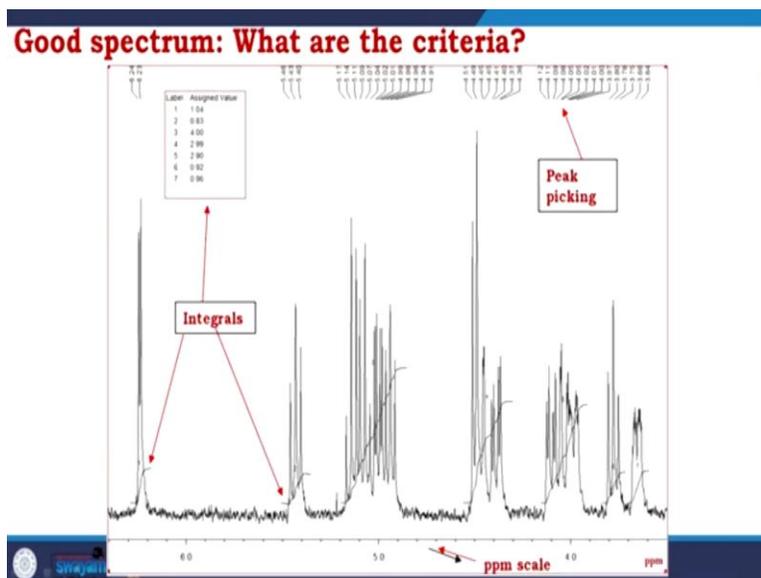
Proper procedure are required to obtain the NMR spectra and depending on several factors spectra could be good or bad

So, NMR spectrum, what is the NMR spectrum? NMR spectrum is the graphical representation of NMR signal which is analyzed towards understanding the molecular level information. The spectrums are unique well result analytically tractable and often highly predictable for small molecules. So, the small molecule if you think they have only few carbon, let us say methyl bromide in these all the 3 hydrogens are same. So, it is very predictable.

But in case of that, if you have a protein molecule, where there are different hydrogens which are in different environment, it is not straightforward. Different functional groups are obviously distinguishable and identical functional groups with differing neighboring substituents still give distinguishable signals. NMR has largely replaced traditional wet chemistry tests such as color reagents or typical chromatography for identification.

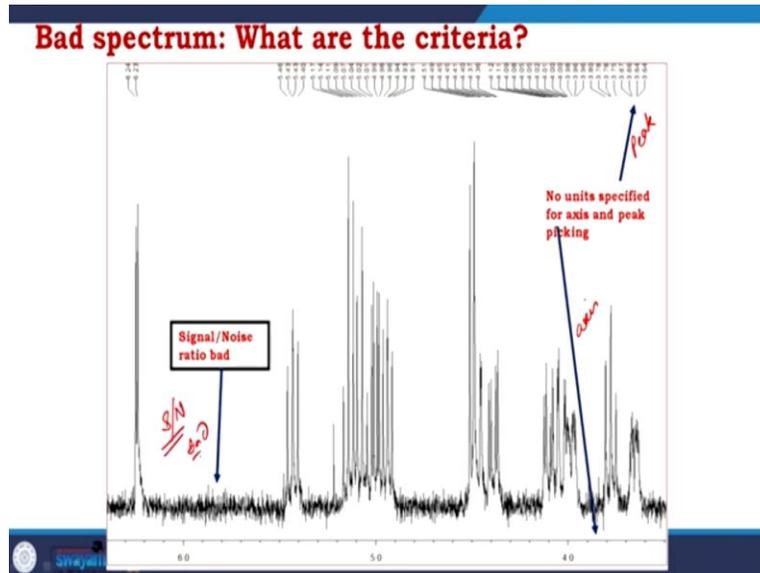
A disadvantage is that a relatively large amount 2 to 50 milligrams of purified substance is required although it may be recovered through it workup as we say, it is a nondestructive technique. Proper procedures are required to obtain the NMR spectra and depending on several factors, spectra could be good or bad.

(Refer Slide Time: 14:51)



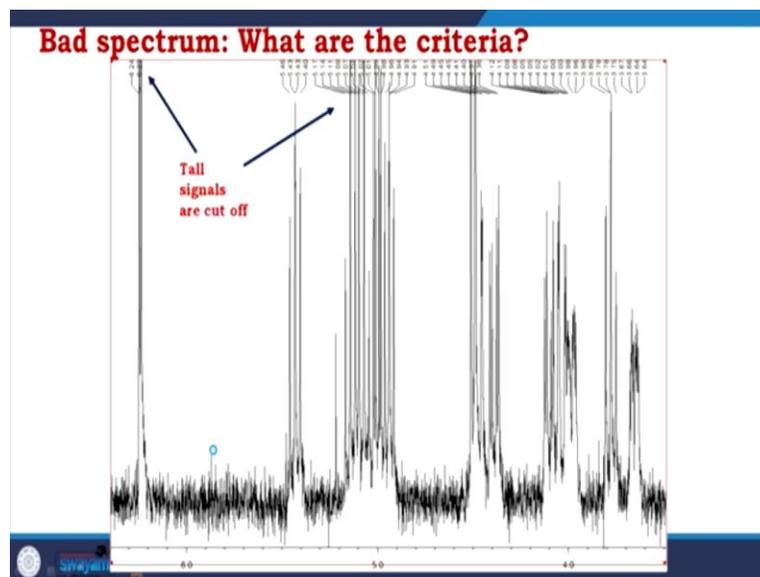
So, a good spectrum, what are the criteria? The criteria is there would be presence of integrals in the assigned value that we could easily get the peak picking.

(Refer Slide Time: 15:08)



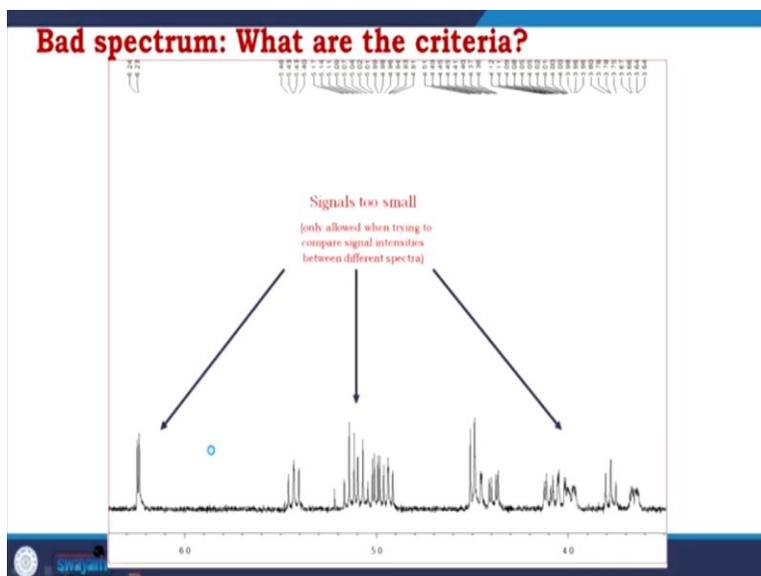
So that is the criteria of a good spectra, we would also try to understand what is it bad spectra. So, in a bad spectra, if you see this is the bad spectra, no unit specified for axis as well as peak picking. Axis, peak picking, the signal to noise that S / N rescue is bad. That is why these are criteria of bad spectrum.

(Refer Slide Time: 15:45)



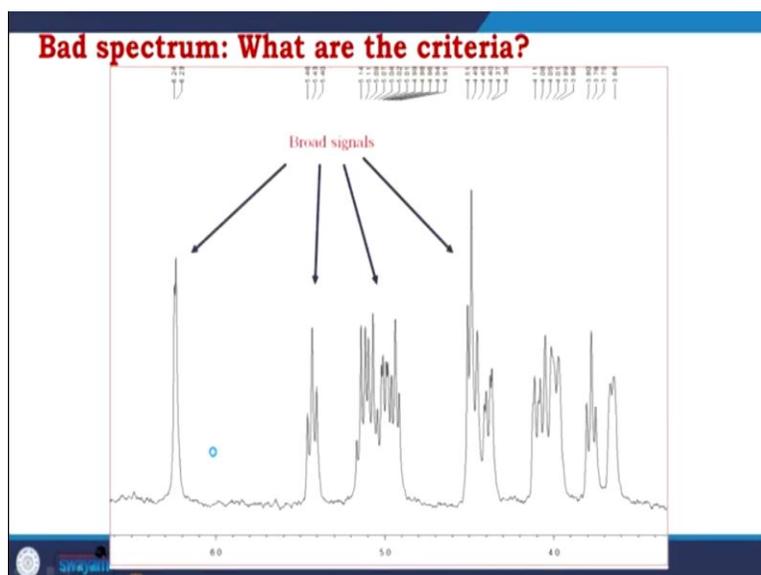
Then if you see, the spectrum is so big that it already cut down, tall signals they are cut off, it is not an example of a good spectrum.

(Refer Slide Time: 16:00)

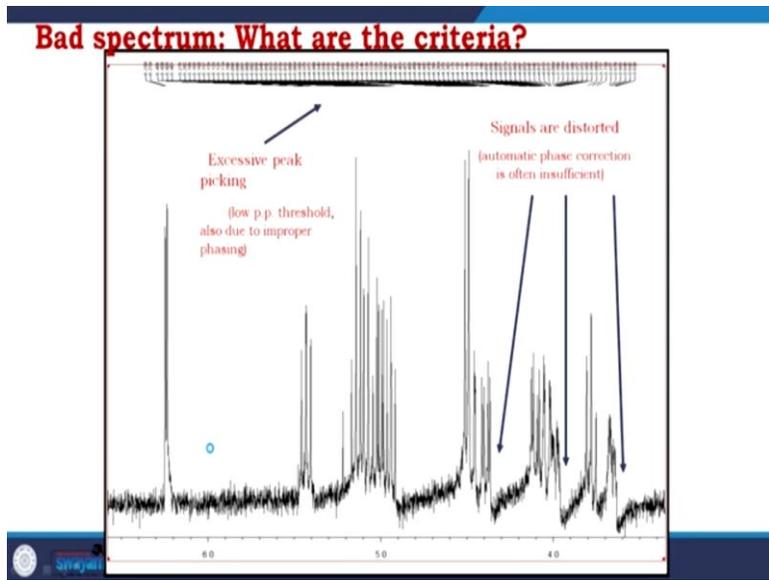


Signals being too small, only allowed when trying to compare signal intensities between different spectra otherwise, it is not allowed and this is also an example to come under bad spectrum.

(Refer Slide Time: 16:20)

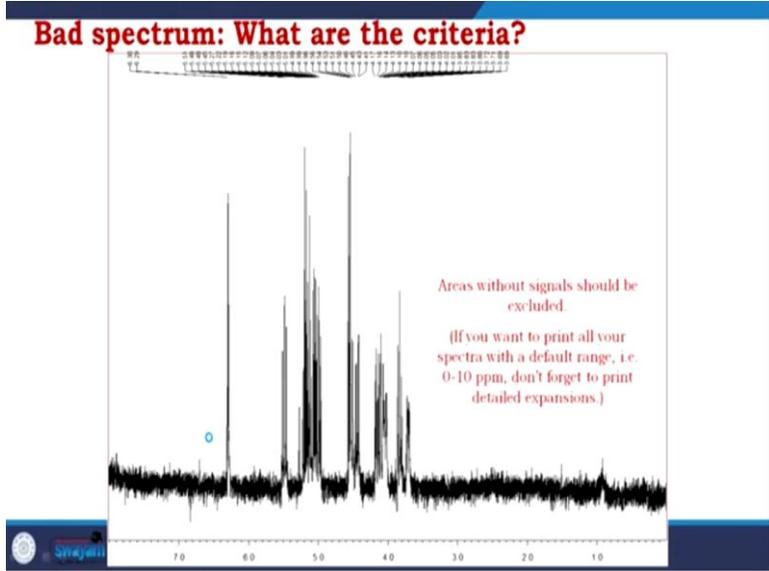


(Refer Slide Time: 16:26)



Broad signals also are not good. Signals are distorted. So, automatic phase correction is often insufficient, we have to take care of that. So that are also example of bad spectrum. And excessive peak picking probably because of when you see here, low PP threshold also due to improper phasing.

(Refer Slide Time: 16:59)



Existence of areas without signal so, it should be excluded an example of bad spectrum, if you want to print all your spectra with a default range 0 to 10 ppm, do not forget to print detail expansion. So, these are few examples up at spectrum.

(Refer Slide Time: 17:22)

The Nature of NMR Absorptions:

The absorption frequency is not the same for all ^1H and ^{13}C nuclei

Nuclei in molecules are surrounded by electrons



Electrons set up tiny local magnetic fields that act in opposition to the applied field, shielding the nucleus from the full effect of the external magnetic field

The effective field actually felt by the nucleus is the applied field reduced by the local shielding effects,

$$B_{\text{effective}} = B_{\text{applied}} - B_{\text{local}}$$

Now, we will talk about the nature of NMR absorptions, because that would help us understanding how the spectra are coming. The absorption frequency is not the same for all ^1H which is proton and ^{13}C nuclei. Nuclei in molecules are surrounded by electrons as we know. So, when we say hydrogen it is surrounded by 1 electron when it is carbon this surrounded by 6 electrons. Electrons set up tiny local magnetic fields that act in opposition to the applied field shielding the nucleus from the full effect of the external magnetic field.

So, we talked about the magnet developed by the nucleons. Now, we also know that the electron setup tiny local magnetic fields and they act in the opposition to the applied field. So, this shielded the nucleus from the full effect of the external magnetic fields that means more number of electrons, more shielding. A carbon is shielded more than the proton. The effective field actually felt by the nucleus is the applied field reduced by the local shielding effects to $B_{\text{effective}}$, equal to B_{applied} minus B_{local} .

(Refer Slide Time: 19:08)

The Nature of NMR Absorptions:

Each chemically distinct nucleus in a molecule has a slightly different electronic environment and consequently a different effective field

Each chemically distinct ^{13}C or ^1H nucleus in a molecule experiences a different effective field and will exhibit a distinct ^{13}C or ^1H NMR signal

In nuclear magnetic resonance (NMR) spectroscopy, the **chemical shift** is the resonant frequency of a nucleus relative to a standard in a magnetic field

Often the position and number of chemical shifts are diagnostic of the structure of a molecule

Chemical shifts are also used to describe signals in other forms of spectroscopy such as photoemission spectroscopy



24

Each chemically distinct nucleus in a molecule has a slightly different electronic environment and consequently a different effective field. Each chemically distinct ^{13}C or ^1H nucleus in a molecule experiences a different effective field and will exhibit a distinct ^{13}C or ^1H NMR signal. In nuclear magnetic resonance spectroscopy the chemical shift is the resonant frequency of a nucleus relative to a standard in a magnetic field.

Often the position and number of chemical shifts are diagnostic of the structure of the molecule. Chemical shifts are also used to describe signals in other forms of spectroscopy such as photoemission spectroscopy.

(Refer Slide Time: 20:05)

Chemical Shift:

Some atomic nuclei possess a magnetic moment (nuclear spin), which gives rise to different energy levels and resonance frequencies in a magnetic field.

The total magnetic field experienced by a nucleus includes local magnetic fields induced by currents of electrons in the molecular orbitals (note that electrons have a magnetic moment themselves).

The electron distribution of the same type of nucleus (e.g. ^1H , ^{13}C , ^{15}N) usually varies according to the local geometry (bonding partners, bond lengths, angles between bonds, and so on), and with it the local magnetic field at each nucleus.

This is reflected in the spin energy levels (and resonance frequencies).

The variations of nuclear magnetic resonance frequencies of the same kind of nucleus, due to variations in the electron distribution, is called the chemical shift.

The size of the chemical shift is given with respect to a reference frequency or reference sample, usually a molecule with a barely distorted electron distribution



25

So, what is chemical shift some atomic nuclei poses a magnetic moment or nuclear spin which give rise to different energy levels and resonance frequency in a magnetic field. The total magnetic field experienced by a nucleus includes local magnetic fields induced by currents of electrons in the molecular orbitals. Now, you could think that the electrons have a magnetic moment themselves which you talk about.

The electron distribution of the same type of nucleus ^1H , ^{13}C , ^{15}N usually varies according to the local geometry like binding patterns, bond lengths, angles between bonds and so on. And with it the local magnetic field at each nucleus. This is reflected in the spin energy levels and resonance frequencies. The variation of nuclear magnetic resonance frequencies of same kind of nucleus due to variation in the electron distribution is called chemical shift. The size of the chemical shift is given with respect to a reference frequency or reference sample usually a molecule with a barely distorted electron distribution.

(Refer Slide Time: 21:29)

Chemical Shift:

$$\text{Chemical shift, } \delta = \frac{\text{frequency of signal} - \text{frequency of reference}}{\text{spectrometer frequency}} \times 10^6$$

An NMR spectrum is a plot of the radio frequency applied against absorption

A signal in the spectrum is referred to as a resonance

The frequency of a signal is known as its chemical shift, delta

The chemical shift in absolute terms is defined by the frequency of the resonance expressed with reference to a standard compound which is defined to be at 0 ppm.

The scale is made more manageable by expressing it in parts per million (ppm) and is independent of the spectrometer frequency

26

So, chemical shift is the frequency of the signal minus frequency of the difference divided by the spectrometer frequency into 10 to the power 6 that is chemical shift or delta. And NMR spectrum is a plot of the radio frequency applied against absorption as we talked about. A signal in the spectrum is referred to as a resonance, we discuss that too. The frequency of a signal is known as its chemical shift or delta.

The chemical shift in absolute term is defined by the frequency of the resonance expressed with the reference to a standard compound which is defined to be at 0 ppm. So, you need to have a compound which is kind of NMR inactive. So, that would work as a difference which is 0 ppm. The scale is made more manageable by expressing it in parts per million or ppm and is independent of spectrometer frequency otherwise, if it would be dependent on then it would be more complex even.

(Refer Slide Time: 22:39)

Larmor frequency:

The operating (or Larmor) frequency ω_0 of a magnet is calculated from the Larmor equation $\omega_0 = \gamma B_0$

What is Larmor frequency?
In physics, **Larmor precession** (named after Joseph Larmor) is the precession of the magnetic moment of an object about an external magnetic field

where B_0 is the actual strength of the magnet in units like Teslas or Gauss, and γ is the gyromagnetic ratio of the nucleus being tested which is in turn calculated from its magnetic moment μ and spin number I with the nuclear magneton μ_N and the Planck constant h ,

$$\gamma = \frac{\mu \mu_N}{hI}$$


The diagram illustrates Larmor precession. A blue sphere represents a nucleus. A vertical green arrow points upwards, representing the external magnetic field B_0 . A black arrow, representing the magnetic moment, is attached to the sphere and precesses around the vertical axis, as shown by dashed circles and arrows. The precession is counter-clockwise when viewed from above.

The operating frequency ω_0 of the magnet is calculated from the Larmor equation. ω_0 is γB_0 , where γ is gyromagnetic ratio and B_0 is the applied magnetic field. What is Larmor frequency? In physics Larmor precession which is named after Joseph Larmor is the precession of the magnetic moment of an object about an external magnetic field, where B_0 is the actual strength of the magnetic units like Teslas or Gauss.

And γ is the gyromagnetic ratio of the nucleus being tested which is in turn calculated from its magnetic moment μ and spin number I with the nuclear magneton μ_N and the Planck constant h . So, γ is $\mu \mu_N / h I$.

(Refer Slide Time: 23:36)

Thus for example, the proton operating frequency for a 1 T magnet is calculated as:

$$\omega_0 = \gamma B_0 = \frac{2.79 \times 5.05 \times 10^{-27} \text{ J/T}}{6.62 \times 10^{-34} \text{ Js} \times \frac{1}{2}} \times 1 \text{ T} = 42.5 \text{ MHz}$$

MRI scanners are often referred to by their field strengths B_0 (eg "a 7 T scanner"), whereas NMR spectrometers are commonly referred to by the corresponding proton Larmor frequency (eg "a 300 MHz spectrometer", which has a B_0 of 7 T).

While chemical shift is referenced in order that the units are equivalent across different field strengths, the actual frequency separation in Hertz scales with field strength (B_0)

As a result, the difference of chemical shift between two signals (ppm) represents a larger number of Hertz on machines that have larger B_0 and therefore the signals are less likely to be overlapping in the resulting spectrum

This increased resolution is a significant advantage for analysis. (Larger field machines are also favoured on account of having intrinsically higher signal arising from the Boltzmann distribution of magnetic spin states)



So, for example, the proton operating frequency for 1 Tesla magnet is calculated as $\omega_0 = \gamma B_0 = \frac{2.79 \times 5.05 \times 10^{-27} \text{ J/T}}{6.62 \times 10^{-34} \text{ Js} \times \frac{1}{2}} \times 1 \text{ T} = 42.5 \text{ MHz}$. So, the MRI scanners are often referred to by their field strength B_0 , whereas, NMR spectrometers are commonly referred to by the corresponding proton larmor frequency.

For example, a 300 megahertz spectrometer which has the B_0 of 7 Tesla. While the chemical shift is reference in order that the units are equivalent across different field strengths the actual frequency separation in Hertz scale with field strength B_0 . As a result, the difference of chemical shift between 2 signals present a larger number of hertz on machines that have largely B_0 and therefore, the signals are less likely to be overlapping in the resulting spectrum they are more separated.

This increases resolution is a significant advantage of analysis. Larger field machines are also favored on account of having intrinsically higher signal arising from the Boltzmann distribution of magnetic spin states. So, this is 1 but the other 1 is larger field machine, more powerful machines.

(Refer Slide Time: 25:24)

Chemical shift δ is usually expressed in parts per million (ppm) by frequency, because it is calculated from:

$$\delta = \frac{\nu_{\text{sample}} - \nu_{\text{ref}}}{\nu_{\text{ref}}}$$

where ν_{sample} is the absolute resonance frequency of the sample and ν_{ref} is the absolute resonance frequency of a standard reference compound, measured in the same applied magnetic field B_0 .

Since the numerator is usually expressed in hertz, and the denominator in megahertz, δ is expressed in ppm

The detected frequencies (in Hz) for ^1H , ^{13}C , and ^{29}Si nuclei are usually referenced against TMS (tetramethylsilane), TSP (Trimethylsilylpropanoic acid), or DSS, which by the definition above have a chemical shift of zero if chosen as the reference

Other standard materials are used for setting the chemical shift for other nuclei.



Coming back to chemical shift, delta is usually expressed in parts per million by frequency because it is calculated from as I talked about, ν_{sample} by $\nu_{\text{reference}}$ divided by $\nu_{\text{reference}}$, where ν_{sample} is the absolute resonance frequency of the sample that $\nu_{\text{reference}}$ is the absolute resonant frequency of a standard reference compound measured in the same applied magnetic field B_0 .

Since the numerator is usually expressed in hertz, then the denominator is megahertz, delta is expressed in ppm. The detected frequency in hertz for protons, ^{13}C and ^{29}Si nuclei usually referenced against TMS tetramethylsilane, TSP tetramethylsilylpropanoic acid or DSS which by the definition, above have a chemical shift of 0 if chosen as the reference. So, TMS, TSP and DSS are chosen as the reference because their value of 0 ppm. Other standard materials are used for setting the chemical shift for other nuclei.

(Refer Slide Time: 26:41)

Thus, an NMR signal observed at a frequency 300 Hz higher than the signal from TMS, where the TMS resonance frequency is 300 MHz, has a chemical shift of:

$$\frac{300 \text{ Hz}}{300 \times 10^6 \text{ Hz}} = 1 \times 10^{-6} = 1 \text{ ppm}$$

Although the absolute resonance frequency depends on the applied magnetic field, the chemical shift is independent of external magnetic field strength

On the other hand, the resolution of NMR will increase with applied magnetic field

Thus, an NMR signal absorb at a frequency 300 hertz higher than the signal from TMS where the TMS resonance frequency is 300 megahertz as a chemical shift of 300 hertz divided by 3 into 10 power 6 hertz equal to 1 into 10 to the power 6 which is 1 ppm. Although the absolute resonance frequency depends on the applied magnetic field, the chemical shift is independent of external magnetic field strength.

So, applied magnetic field is required, because without the applied magnetic fields the NMR experiment is not possible the orientation the alignment is not possible but the value of the chemical shift is independent of the external applied field. So, that means the chemical shift delta value is independent of machine. On the other hand, the resolution of NMR will increase with applied magnetic field.

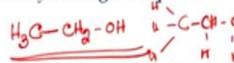
(Refer Slide Time: 27:51)

The electrons around a nucleus will circulate in a magnetic field and create a secondary induced magnetic field



This field opposes the applied field as stipulated by Lenz's law and atoms with higher induced fields (i.e., higher electron density) are therefore called *shielded*, relative to those with lower electron density

The chemical milieu of an atom can influence its electron density through the polar effect



Electron-donating alkyl groups, for example, lead to increased shielding while electron-withdrawing substituents such as nitro groups lead to *deshielding* of the nucleus

Not only substituents cause local induced fields. Bonding electrons can also lead to shielding and deshielding effects

So, the electrons around a nucleus will circulate in a magnetic field and create a secondary induced magnetic field. This field opposes the applied field as stipulated by Lenz law and atoms with higher induce fields are therefore called shielded relative to those with lower electron density, as you could compare between proton and 13 C. The chemical milieu have an account can influence its electron density through a polar effect.

So, we are talking about first just the nuclei, a proton and 13 C. But now we are going to molecule like if we have. Now, here there are 3 types of hydrogens. So, for example, electron donating alkyl groups we will talk about these also in details. Electron donating alkyl groups for example lead to increased shielding while electron withdrawing substituents such as a nitro group lead to deshielding of the nucleus. Not only substituent caused local induced field. Bonding electrons can also lead to shielding and deshielding effects.

(Refer Slide Time: 29:41)

The induced magnetic field:

A striking example of this is the pi bonds in benzene. Circular current through the hyper-conjugated system causes a shielding effect at the molecule's center and a deshielding effect at its edges

Trends in chemical shift are explained based on the degree of shielding or deshielding

Nuclei are found to resonate in a wide range to the left (or more rare to the right) of the internal standard. When a signal is found with a higher chemical shift:

The applied effective magnetic field is lower, if the resonance frequency is fixed (as in old traditional CW spectrometers)

The frequency is higher, when the applied magnetic field is static (normal case in FT spectrometers)

The nucleus is more deshielded

The signal or shift is **downfield** or at **low field** or paramagnetic

Conversely a lower chemical shift is called a **diamagnetic shift**, and is **upfield** and more shielded



Coming to the induced magnetic field, a striking example of this is pi bond in benzene. Circular current through the hyper conjugated system causes a shielding effect at the molecule center and a deshielding effect at its edges. So, both the effect. Trends in chemical shifts are explained based on the degree of shielding and deshielding. Nuclei are found to resonate in a wide range to the left or more rare to the right of the internal standard. When a signal is found with a higher chemical shift.

The applied effective magnetic field is lower. If the resonance frequency is fixed as in old traditional CW spectrometers. The frequency is higher in the applied magnetic field is static which is the normal case in Fourier transform spectrometers. The nucleus is more deshielded the signal or shift is downfield or at low field or paramagnetic. Conversely, a lower chemical shift is called a diamagnetic shift and is upfield and more shielded.

(Refer Slide Time: 30:59)

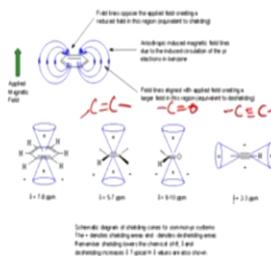
Magnetic Anisotropy:

The word "anisotropic" means "non-uniform". So magnetic anisotropy means that there is a "non-uniform magnetic field"

Electrons in π systems (e.g. aromatics, alkenes, alkynes, carbonyls etc.) interact with the applied field which induces a magnetic field that causes the anisotropy

As a result, the nearby protons will experience 3 fields: the applied field, the shielding field of the valence electrons and the field due to the π system

Depending on the position of the proton in this third field, it can be either shielded (smaller δ) or deshielded (larger δ), which implies that the energy required for, and the frequency of the absorption will change.



Coming to another factor which is called magnetic and anisotropy. The word anisotropy means non-uniform. So, magnetic anisotropy means that there is a non-uniform magnetic field. The electrons in the PI System which we are discussing in the last slide, aromatics, alkenes, alkynes carbonyls. So, aromatics definitely have a conjugated system. Alkenes, alkynes carbonyls also have pi electronic system interact with the applied field which induces a magnetic field that cost the anisotropy.

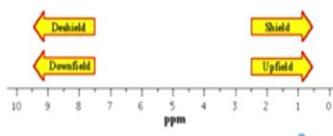
As a result, the nearby protons will experience 3 fields the applied field, the shielding field of the valence electrons that the field due to the PI electronic system. Depending on the position of the proton in this third field, it can be either shielded or deshielded which implies that the energy required for and the frequency of the absorption will change, you will see that in the aromatic you have the conjugated PI system.

So, you have 3 factors field line oppose the applied field creating a reduced field in this region is 1, an isotropic induced magnetic field lines due to the induced circulation of the PI electrons in benzene that field lines which are aligned with the applied field creating a larger field in this region. So, if you look at them, you will see that how the delta values are changing schematic diagram of shielding cones to common PI system.

So, this is benzene system, the chemical shift the value is 7 to 8 ppm, when it is a alkene system delta is 5 to 7, a double bond in a carbonyl, there delta is 9 to 10 and it triple bond between carbon here delta is 2 to 3 ppm.

(Refer Slide Time: 33:24)

It is often convenient to describe the relative positions of the resonances in an NMR spectrum. For example, a peak at a chemical shift, δ , of 10 ppm is said to be downfield or deshielded with respect to a peak at 5 ppm, or if you prefer, the peak at 5 ppm is upfield or shielded with respect to the peak at 10 ppm.



Typically for a field strength of 4.7 T the resonance frequency of a proton will occur around 200MHz and for a carbon, around 50.4MHz. The reference compound is the same for both, tetramethylsilane (Si(CH₃)₄).

34

It is often convenient to describe the relative positions of the resonances in an NMR spectrum. For example, a peak at a chemical shift delta of 10 ppm is said to be downfield or deselected with respect to a peak at 5 ppm. So, what we mean here, it should be relative like, when it is 10 ppm, in respect to 5 ppm, it is deshielded, whereas, the peak at 5 ppm is a upfield or shielded with respect to the peak at 10 ppm It is relative.

Typically for a field strength of 4.7 Tesla, the resonance frequency of a proton will occur around 20 megahertz and for a carbon around 50.4 megahertz. The reference compound is same for both tetramethylsilane and TMS.

(Refer Slide Time: 34:24)

H atom is the one which have help popularizing the use of NMR spectroscopy ^{1H → 90%}

Different types of Hydrogen:
What do we mean by different types of hydrogen?

Terminology
This may seem a trivial concept, but the idea of establishing the number of kinds of hydrogens (or other atoms such as C) is a very important useful concept

Especially in NMR spectroscopy where for example, different types of H (or other atoms) typically each give rise to a different signal. The idea is developed below for H atoms, but can be extended to other types of atom.



H atom is the one which have helped popularizing the use of NMR spectroscopy we have talked several times, if you take any compound you see the presence of a proton and more importantly, proton the ^1H also have a population of 90% making the signal stronger. But, can you imagine that there are different types of hydrogen? Yes, there are different type of hydrogens with the respect to the NMR technology, because you know if as you already have started understanding proton is used everywhere in NMR.

But then a proton is everywhere, how you get the signals differentiated, it is differentiated because NMR could help separate the proton peaks. So, they consider different type of hydrogens, what do you mean by different type of hydrogens. So, first let us go for a terminology which might seem to be a trivial concept but the idea of establishing the number of kinds of hydrogen is a very important useful concept within NMR.

Especially in NMR spectroscopy, where for example, different types of hydrogen or other atoms typically each give rise to a different signal, the idea is developed below like where we are going in the next slide for hydrogen atom but can we extend it to any type of atom but because of its omnipresence, we are more interested in hydrogen.

(Refer Slide Time: 36:08)

$\begin{array}{l} -C=C-H \\ -O-H \end{array}$
 $\begin{array}{l} -C-C-H \\ H \\ H \end{array}$

What do we mean?

A hydrogen atom is "different" to another hydrogen atom if it is not in an identical environment (location) to the other hydrogen

This could mean it is attached to a different type of atom (e.g. compare CH vs OH, or sp³ CH vs sp² CH), or due to the number of adjacent H (e.g. CH₃- vs -CH₂-) or just at a different point in a chain (e.g. compare the H in the methylene (CH₂) groups in CH₃CH₂CH₂OH which has 4 types of H.

36

So, hydrogen atom is different to another hydrogen atom, if it is not in an identical environment, we say environment, we say chemical environment like if you think about, we will talk, if you see, these 3 hydrogen atoms are in similar environment, whereas, these 3 hydrogen are in different to this hydrogen which is in a different environment. So, that is what we are going to investigate. This could mean it is attached with different type of atoms.

Compare CH versus OH, it is connected to carbon or it is connected to oxygen or you know, it is connected to a double bonded carbon and single bonded carbon, triple bonded carbon or due to the number of adjacent hydrogen like 3 hydrogen or just a different point in a chain. We will discuss different type of scenarios here.

(Refer Slide Time: 37:35)

Counting the number of different kinds of H:

There are three methods that can be used to count the number of kinds of H (each achieve the same result). You will probably find it best to master the first of these methods and over time you will find yourself moving towards the 3rd method)

Substitution method (simplest but slowest)

The idea is that you replace each H in the molecule in turn with a "dummy" atom (for example a -Cl) to see if you get a different product (*i.e.* one that will require a different name). Each new product, indicates a different type of H. This idea is related to the [radical chlorination of alkanes](#) where some of each possible product is usually obtained.

For example:

Qu : If you remove one H atom from chloroethane and substitute it for another Cl atom, how many different molecules can you make ?

Ans : Two products, 1,1-dichloroethane and 1,2-dichloroethane. **Hence there are two types of H in chloroethane**

So, counting the number of different kinds of hydrogen, there are 3 methods that can be used to count the number of kinds of hydrogen each achieved the same result, you will probably find it best to master the first of this method, because it is more easy which you are going to discuss and over time you will find yourself moving towards the third method. The first method is called substitution method. As I told it is the simplest one.

The idea is that you replace each hydrogen in a molecule in turn with a dummy atom, for example, chlorine to see if you get a different product. For example, 1 that will require a different name, the different position, each new product indicate a different type of hydrogen. This idea is related to the radical chlorination of alkanes where some of each possible product is usually obtained.

For example, if you remove 1 hydrogen from chloroethane and substitute it for another chlorine atom, how many different molecules can you make? 2 products? 1, 1-dichloroethane and 1, 2-dichloroethan hence there are 2 type of hydrogen in chloroethane. So, in that way, you have to do the substitution. So, you get chloromethane and you substitute 1 hydrogen with chlorine. And you see how many compounds you are making.

(Refer Slide Time: 39:23)

Verbal description

The verbal method requires that you describe the position of each H within the molecule. If you need to use different words to describe two H atoms, then they represent different types of H.

For example:

an -OH is different to a -CH (based on what they are attached to)

a -CH₃ is different to a -CH₂- (because the number of H at each C are different)

an sp³ C-H is different to an sp C-H.

Other differences could be position on a chain, across a ring or double bond etc.

Symmetry (most difficult but fastest)

The symmetry method is the most sophisticated but the quickest method and requires that you look for mirror planes, rotation axes or inversion centers that interchange H atoms

H atoms that can be interchanged by symmetry are equivalent to each other



Verbal description. The verbal method requires that you describe the position of each hydrogen within the molecule. If you need to use different words to describe 2 hydrogen atoms, then they represent different type of hydrogen. For example, an OH is different to a CH Based on what they are attached to. A CH₃ is different to CH₂ because the number of hydrogen it is carbon a different. SP³ carbon hydrogen is different when SP² or SP carbon hydrogen.

Other differences could be position on a chain across a ring or double bond etcetera. So, you look at them you start naming if there is a different name, this is a different hydrogen. Symmetry, the symmetry method is the most sophisticated but the quickest method and requires that you look for the mirror planes rotation axis or inverse and centers that interchange hydrogen atoms because you are coming from crystallography.

So, we talk about all of this we talk about mirror planes, we talk about rotation axis we talk about inverse in the symmetry. So, the hydrogen atoms that can be interchanged by symmetry are equivalent to each other.

(Refer Slide Time: 40:47)

Terminology:

Homotopic	Replacement of the groups gives the same product	For example, it doesn't matter which of the H atoms in bromomethane is replaced with chlorine, we always get bromochloromethane. Hence these three H are said to be homotopic .	Homotopic H always give the same NMR signal.
Enantiotopic	Replacement of the groups gives enantiomers	Consider the H atoms in the methylene group in bromoethane. If we replace one of those H with a Cl, we create a chirality center. Therefore depending on which of the two H is replaced, we get one enantiomer or the other. Hence these two H are said to be enantiotopic .	Enantiotopic H give the same NMR signal in an achiral environment (normal NMR conditions)
Diastereotopic	Replacement of the groups gives diastereomers.	Consider the H atoms in the methylene group in 1-bromo-1-chloropropane. There is already a chirality center at C1. If we replace one of those H with a Cl, we create a new chirality center. Therefore depending on which of the two H is replaced, we get one diastereomer or the other. Hence these two H are said to be diastereotopic .	Diastereotopic H : are different and in principle they give rise to different signals and hence they can couple to each other (see later).



S



R S



S S



S



R

So, here is the terminology there are majorly 3 type Homotopic, Enantiotopic and Diastereotopic. Homotopic is replacement of the group give the same product for example, it does not matter which of the hydrogen atoms in bromomethane is replaced with chlorine, we always get bromochloromethane hence, these 3 hydrogens are said to be homotopic. Homotopic hydrogen always gives the same NMR signal.

Enantiotopic replacement of the groups gives enantiomers consider the hydrogen atom in a methylene group in bromoethane if we replace 1 of those hydrogen with a chlorine we create a chirality center because then there is a difference. Therefore, depending on which of the 2 hydrogen is replaced, we get 1 in enantiomer or the other. Hence, these 2 hydrogens are said to be an enantiotopic.

An enantiotopic hydrogen keep the same NMR signal in an achiral environment which is the normal NMR condition. Diastereotopic considered the hydrogen atom in a methylene group in 1-bromo-1-chloropropane. Now, there is already a chirality center at C 1 if we replace one of those hydrogen with a chlorine we create a new chirality center. Therefore, depending on which of the 2 hydrogen is replaced, we get 1 diastereomer or the other.

Hence, these 2 hydrogens are said to be diastereotopic. Diastereotopic hydrogens are different and in principle, they give rise to different signals and hence they can couple to each other. So, there are 3 types in terms of terminology: homotopic, enantiotopic and diastereotopic.

(Refer Slide Time: 42:57)

What do we mean by integration ?

Integration is a mathematical term that means the area under a curve

In NMR, the curve is the spectra, and the integration is a measure of the area of the peaks in the spectra

The key thing, is that the area of the peak is proportional to the number of atoms that it represents

So in an H NMR, the integration of a peak gives the area of the peak and this area gives us a measure of the number of H atoms it represents, *i.e.* the number of H of that type

In many modern NMR spectra, the peaks on the spectra often look to be "lines" rather than peaks, and this tends to lead to students thinking about peak height rather than area... but it is the area that is important.

Now, we are going to the spectra and we are looking at what we call integration. Integration is a mathematical term that means the area under a curve. In NMR, the curve is the spectra and the integration is a measure of the area of the peaks in the spectrum. The key thing here is that the area of the peak is proportional to the number of atoms that it represents. So, in an H NMR, the integration of a peak gives the area of the peak.

In this area gives us a measure of the number of hydrogen atoms it represents the number of hydrogen of that type. In many modern NMR spectra the peaks on the spectra often look to be lines rather than peaks. And this tends to lead to students thinking about peak height rather than area but it is actually the area which is important.

(Refer Slide Time: 44:09)

How do we measure the integration ?

There are two methods that may be encountered

First though, we should point out that the area is measured by the spectrometer itself and plotted on the spectra, we are talking about what we need to do to interpret that information:

Numbers on spectra method (modern)

The numbers are typically printed under the peak, often with a bar or two marks that represent the limits of the integration (i.e. the x axis start and end points for the area measurement). FYI : the limits are set by the person processing the spectrum. In the example to the right, the green bar shows the limits and the area = 3.00



So, how do we measure the integration? Here to measure the area of the integration there are 2 methods which are principally used. We should point out that the area is measured by the spectrometer itself and plotted on the spectra we are talking about what we need to do to interpret that information. So, numbers on spectra method. The numbers are typically printed under the peak, often with a bar or 2 marks that represent the limits of the integration.

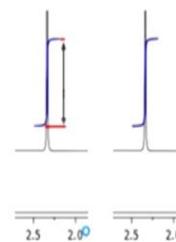
For example, the x axis, start and end points for the area measurement the limits are set by the person processing the spectrum. In the example, here, the green bar is shows the limit and the area is 3.

(Refer Slide Time: 45:11)

Steps on integration curve (traditional)

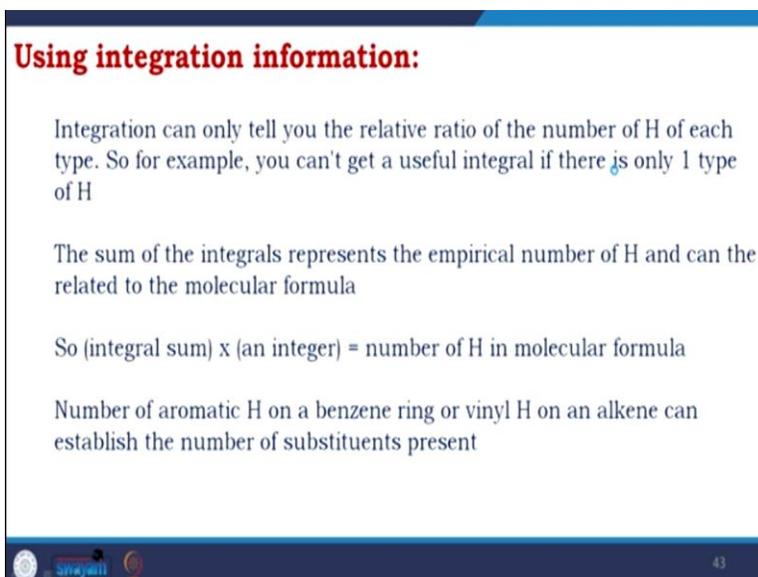
Older instrumentation used to plot a second curve (in the example to the right in blue) above the actual spectra where the integration was recorded as a series of steps.

The height (represented by the arrow in the furthest right image) of this step needs to be measured with a ruler and recorded (e.g. in mm) and then compared to other steps in the same spectrum to get the relative ratio.



Steps on integration curve which is traditional. Older instrumentation used to plot a second curve in the example to the right in blue, above the actual spectra, where the integration was recorded as a series of steps. The height which is represented by the arrow in the furthest right image here, of this step needs to be measured with a ruler and record and then compared to the other steps in the same spectrum to get the relative ratio. So, that is steps on integration curve.

(Refer Slide Time: 45:55)



Using integration information:

Integration can only tell you the relative ratio of the number of H of each type. So for example, you can't get a useful integral if there is only 1 type of H

The sum of the integrals represents the empirical number of H and can be related to the molecular formula

So (integral sum) x (an integer) = number of H in molecular formula

Number of aromatic H on a benzene ring or vinyl H on an alkene can establish the number of substituents present

43

So, what we do using integration information. Integration can only tell you the relative ratio of the number of hydrogen of each type. For example, you cannot get a useful integral if there is only 1 type of hydrogen. The sum of the integrals represent the empirical number of hydrogen and can it relate it to the molecular formula, so, the integral sum into an integer equal to the number of hydrogen in the molecular formula. The number of aromatic hydrogen on a benzene ring or vinyl hydrogen on an alkene can establish the number of substituents present.

(Refer Slide Time: 46:39)

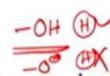
Limitations:

Under normal conditions, the accuracy is likely close to + or - 5%. This can be improved if required by modifying the experimental conditions

Overlapping peaks make the situation more difficult, but a total area for the overlapping peaks should be possible

Exchangeable H (e.g. -OH) often integrate low. This can be improved if required by modifying the experimental conditions

Integral accuracy is affected by sample quality and processing so it is important to pay attention to these factors.



What are the limitations of integration method, under normal condition, the accuracy is likely close to 5%. This can be improved if required by modifying the experimental condition. Overlapping peaks make the situation more difficult but a total area of the overlapping peaks should be possible to measure. Exchangeable hydrogen often integrate low this can be improved if required by modifying the experimental condition.

Exchangeable hydrogen means in hydroxide it could be in 2 state an o minus show here hydrogen is present, here hydrogen is absent. Integral accuracy is affected by sample quality and processing so, it is important to pay attention to this factor also.

(Refer Slide Time: 47:39)

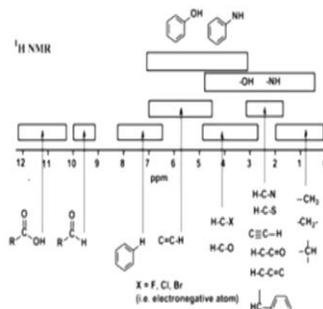
H-NMR Chemical shifts:

The chemical shift is the position on the δ scale (in ppm) where the peak occurs

Typical δ /ppm values for protons in different chemical environments are different

In the figure typical chemical shifts for protons are shown which is being influenced by a single group

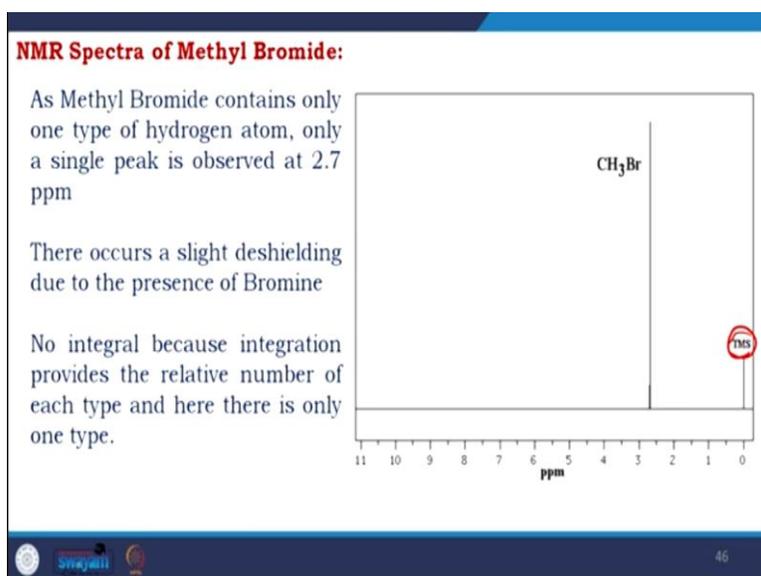
In cases where a proton is influenced by more than one group, the effects are essentially cumulative



H-NMR chemical shifts, the chemical shift is the position of the delta scale where peak occurs. Typical delta ppm values for protons in different chemical environments are different as we told. And if you look at here, the chemical shifts for protons are shown which is being influenced by a single group. So, if you see the methyl, methylene that all this carbon with single bond, they come around 0 to 1 more towards 1.

Then when there are nitrogen, sulfur that all, they come under 2 to 3, then with oxygen and other negative group, they come around 4, then with double bond, they come between 5 to 6, then aromatic hydrogen comes in 7, then with carbonyl it come between 9 and 10, then the OH group in a carboxylic group it comes around 11. In cases where a proton is influenced by more than 1 group, the effects are essentially cumulative.

(Refer Slide Time: 48:56)

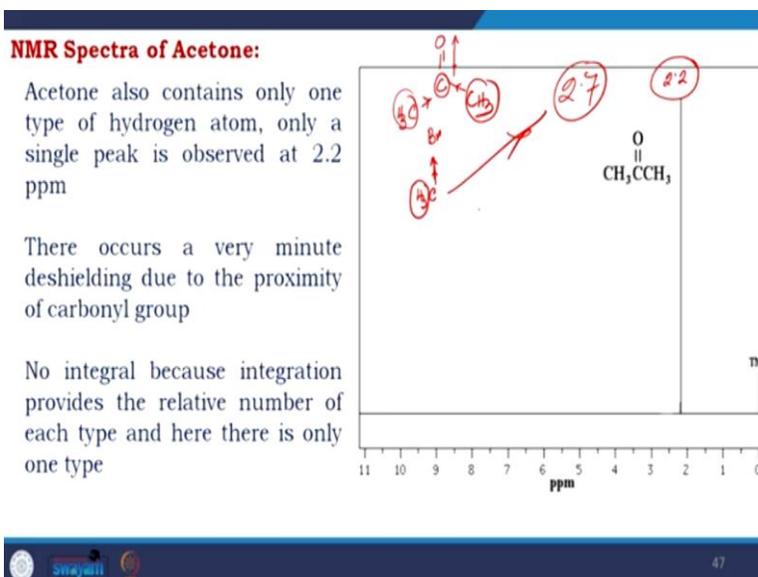


We talked about those compounds and their effects, so let us discuss them. So, let us take Methyl bromide. So, this is methyl bromide, this is bromine that this is carbon and this is 3 hydrogen making this methyl. So, there are 3 hydrogens that all of them are equivalent because they only have they are connected to carbon and the effect of bromine is same on them. So, as the methyl bromide contents only one type of hydrogen atoms, only a single peak is observed.

And that peak is at 2.7. So, this is the standard at 0 TMS working as a 0 standard like standard molecule. So, if you remember with carbon to hydrogen, they are between 1 to 2 because of the

presence of bromine which is having electron withdrawing effect negatively charged. So, it draws the electron have less shielding effect, we see the shift from here to higher values, there occurs a slight deshielding due to the presence of bromine as I told. No integral, because integration provide the relative number of each type and here there is only one type as again we discussed.

(Refer Slide Time: 50:32)



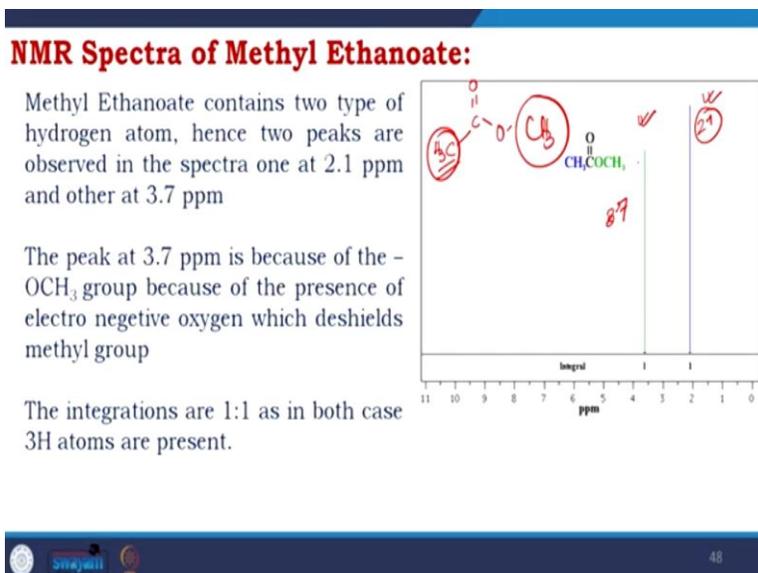
Coming to the spectra of acetone. Acetone is CH_3COCH_3 , 2CH_3 and a double bonded oxygen. So, this is acetone. Acetone also contains only 1 type of hydrogen atom only. So, if you see there are 6 hydrogens and all of the 6 hydrogens if you separate they are connected to carbon and all of them are connected to 1 oxygen. So, the value comes at 2.2. This value is 2.2 there occurs a very minute deshielding due to the proximity of carbonyl group.

So, if you think this is CH_3COCH_3 so, the carbon the oxygen is now taking the electron but this deshielding is divided between 6 of them. So, you get minor, if you remember when we are talking about bromine which is more electronegative in terms of its power it was working on. So, when I say bromine is more electronegative what I mean is it have a direct effect these oxygen does not have a direct effect.

This is affecting on this carbon and then that is why there is a deshielding but there is a direct deshielding and also there is only 3. So, if you go back you will see that this was having a value

of 2.7. So, in comparison to 2.2 there is a shifting 2.7 for this compound, again no integral because integration provides the relative number of each type and here there is only 1 type.

(Refer Slide Time: 52:59)



Now, we are talking about CH₃COOCH₃ which is Methyl Ethanoate. So, to make this I have to integrate an oxygen here. So, now, if you see this is Methyl Ethanoate, what you have is CH₃COOCH₃. So, here in Methyl Ethanoate you have an extra oxygen which is first of all differentiating between this CH₃ and this CH₃ because you have 2 different type of hydrogen now, you have 2 peaks.

So, Methyl Ethanoate contents 2 type of hydrogen atom hints 2 peaks are observed in the spectra, 1 at 2.1 mostly the same we got in the last one and other at 3.7. The peak at 3.7 ppm is because of the OCH₃ group. So, if you look at this is because of the OCH₃ group because here it is directly taking. So, the peak at 3.7 ppm is because of the presence of the OCH₃ group, because the presence of the electronegative oxygen which deshield the methyl group. The integration are 1:1 as in both case 3 hydrogens are present. So, you see mostly same size of peak.

(Refer Slide Time: 55:11)

NMR Spectra of t-butyl Bromide:

As t-butyl bromide contains only one type of hydrogen atom, only a single peak is observed at 1.8 ppm

Since the Br atom is further away from the H-atoms than methyl bromide, deshielding is relatively less than methyl bromide

No integral because integration provides the relative number of each type and here there is only one type

49

Now, coming to tertiary butyl bromide, again there is a bromide, there is a bromine but all 3 methyl are there. So, there are 9 hydrogens and all of them are equal. As tertiary butyl bromide contents only one type of hydrogen atom only a single peak is observed at 1.8. So, if you remember when it was CH₃ Br here whatever it is taking, it is distributed the deshielding is distributed, here it was not distributed and that is why the peak shifted.

Since the bromine atom is farther away from the hydrogen atoms, then methyl bromide deshielding is relatively less than methyl bromide, no integral because integration provides the relative number of each type and here also we have only one.

(Refer Slide Time: 56:35)

Coupling in H-NMR:

1,1-dichloroethane

The compound contains two types of hydrogen atoms, so a doublet peak is observed at 2.1 ppm and a quartet at 5.9 ppm

So far the H-NMR spectra that we have looked at have all had different types of protons that are seen as **singlets** in the spectra

This is *not* the normal case, spectra usually have peaks that appear as groups of peaks due to **coupling** with neighboring protons.

$\delta = 2.1$ ppm, integration = 3H : agrees with -CH₃ unit

$\delta = 5.9$ ppm, integration = 1H deshielded : agrees with the -CHCl₂ unit

50

The last one is coupling where we have 1, 1-dichloroethane. So, it is methyl group carbon and 2 chlorine, CH_3CHCl_2 . So, here the compound you know 2 type of hydrogen, so, 2 peak. The compound content to type of hydrogen atom, so, a doublet peak is absorb one at 2.1 ppm for this and a quartet at 5.9. So, first of all, why this we know right? Methyl group 3 hydrogen more intense peak.

This quartet is because of this right? Why? So, so far the H-NMR spectra that you have looked at have all had different type of protons that are seen as singlets in the spectra, we see singlets but here we see a quartet and a doublet here, this is not the normal case, the spectra usually have peaks that appear as groups of peaks due to coupling with neighboring protons. So, what is happening here is the electron withdrawing plus the coupling effect.

If you look at here, we have the splitting, why we have the splitting? We have because if you see in this you have positive inductive effect, electron withdrawing effect up to chlorine that what we were thinking about on bond. This is because of the coupling with neighboring protons. So, we know about chemical shift up to now we are looking at their effect but we did not see what is coupling. Due to neighboring cup the presence of neighboring protons.

In the next class, we will talk about the effects we have observed here the coupling and many more and then we will see the effect there and up to now, whatever you are studying we are studying in one day spectra proton spectra. In the next class we will talk about different spectras together a proton spectra, a carbon spectra, in one graph that is called 2d spectra. A proton, carbon that nitrogen spectra and in 1 graph which is a 3d spectra.

So, these will be complicated but this will, show, how powerful NMR is in deducting the informations So, in this class, we talk about sample preparation, good and bad spectrum, chemical shift and different type of protons that the effect of chemical shift in the plotted spectrum. We are ending with a concept of coupling which we try to see but we will go in details in the next class. Thank you very much.