Spectroscopic Techniques for Pharmaceutical & Biopharmaceutical Industries
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Introduction to Spectroscopy - VI
Lecture 06

Hello students in the last two lectures we have looked at the basics of spectroscopy, in this lecture we will look at some specific features of spectroscopy, which is quite common to the different kind of spectroscopy, there are two things which we are going to discuss, one is resolution and second is intensity.

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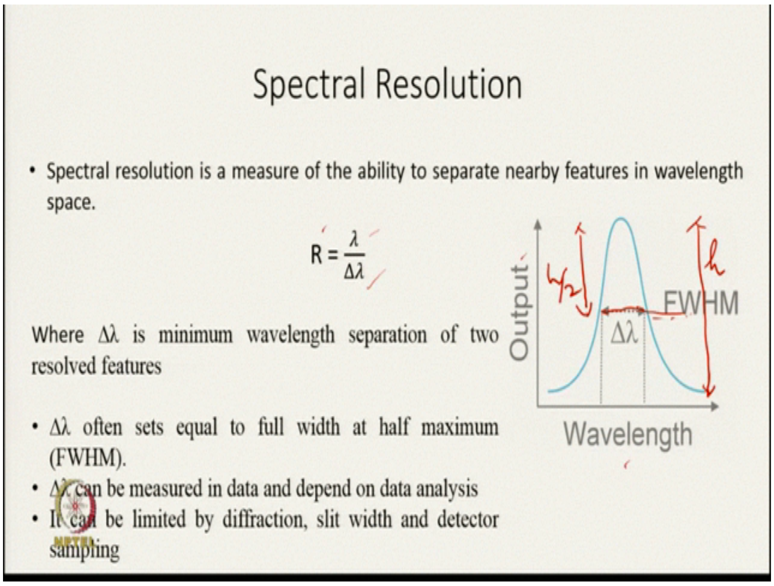

Spectral Resolution

- Spectral resolution is a measure of the ability to separate nearby features in wavelength space.

$$R = \frac{\lambda}{\Delta\lambda}$$

Where $\Delta\lambda$ is minimum wavelength separation of two resolved features

- $\Delta\lambda$ often sets equal to full width at half maximum (FWHM).
- $\Delta\lambda$ can be measured in data and depend on data analysis
- It can be limited by diffraction, slit width and detector sampling

So first one is spectral resolution, spectral resolution is a major of the ability to separate nearby features in wavelength space, and generally spectral resolution is given by lambda divided by delta lambda, where delta lambda is minimum wavelength separation of two resolved features.

So suppose there are two peak and if we can separate these two peaks or if we are able to separate these two peaks, which is quite close to each other, so that is basically what we mean by spectral resolution.

So for example here you see this is the output versus wavelength, your delta lambda is generally given by your full width at half maximum, which is known as FWHM, so full width mains this width, we are talking about this width at half maximum, what is that mean is, this is your height and half of this is this height, half of this is this height.

So if suppose this is g and this tends is your h by 2, then your width, spectral width at half height is called delta lambda, delta lambda can be measured in data and depends on that analysis, it can be limited by diffraction, slit width and detector sample, so if spectral resolution is high, what is that mean is, you will be able to separate two very close peaks, so a good resolution is important criteria in a spectroscopic.

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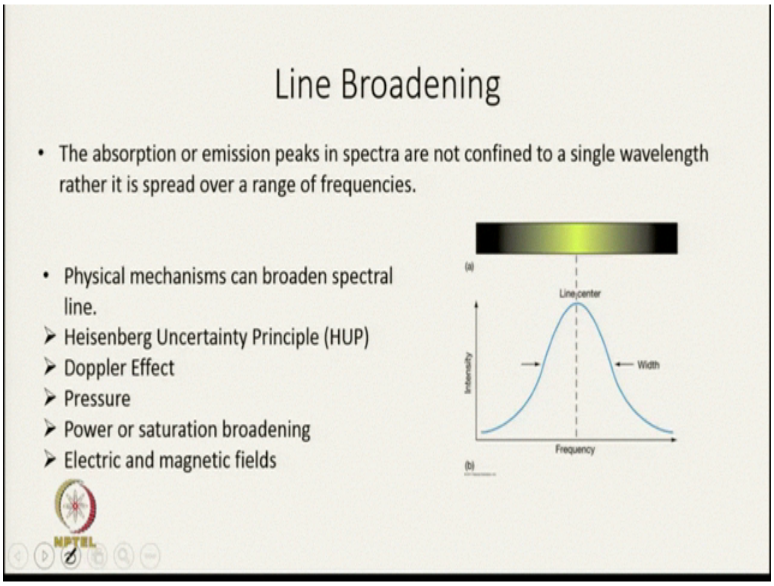

Line Broadening

- The absorption or emission peaks in spectra are not confined to a single wavelength rather it is spread over a range of frequencies.

- Physical mechanisms can broaden spectral line.
  - ➢ Heisenberg Uncertainty Principle (HUP)
  - ➢ Doppler Effect
  - ➢ Pressure
  - ➢ Power or saturation broadening
  - ➢ Electric and magnetic fields

The spectral resolution will depend on line broadening and line broadening can be due to several factors, so if line broadening is high, it means resolution is low, so broad peak has very low resolution, whereas a sharp peak has very high resolution, so what you are looking in the spectroscopy is that you should get very sharp peaks and so you will be able to distinguish between two nearby peaks.

The factors which can affect line broadening is your Heisenberg Uncertainty Principle, line can broaden due to Doppler effect, line can broaden due to pressure or power or saturation broadening and it can also broaden in electrical or magnetic fields, will go one by one and see how does this five factor affect the broadening of signals.

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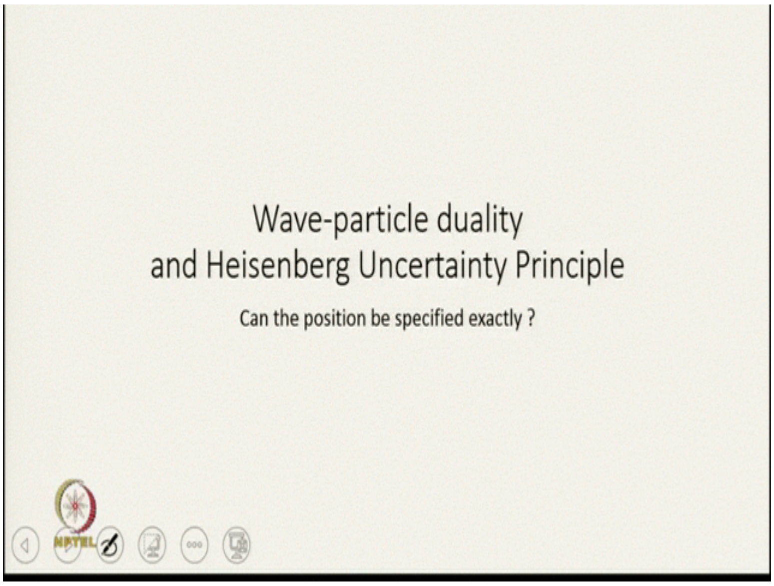

Wave-particle duality
and Heisenberg Uncertainty Principle

Can the position be specified exactly ?

So first we will discuss about Heisenberg uncertainty principle or this uncertainty is basically due to wave nature of the particle, so basically here what we will look at that whether position can be specified exactly.

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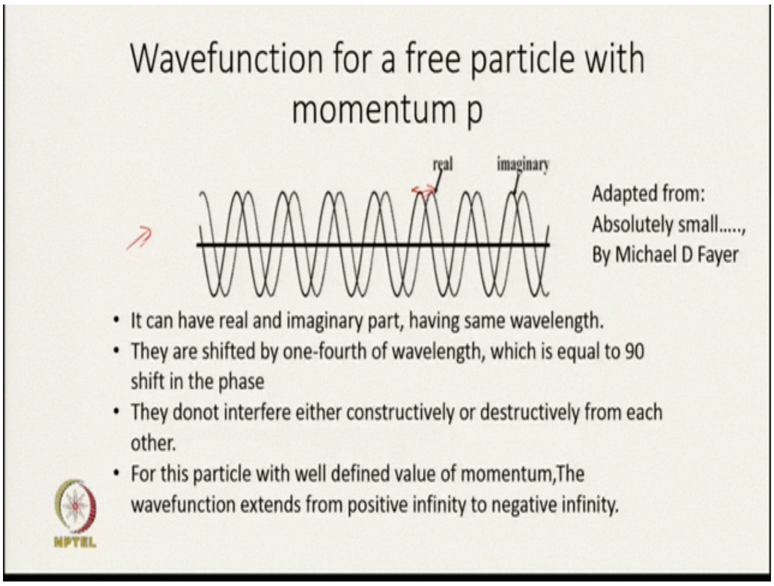

Wavefunction for a free particle with momentum p

Adapted from:
Absolutely small.....,
By Michael D Fayer

- It can have real and imaginary part, having same wavelength.
- They are shifted by one-fourth of wavelength, which is equal to 90 shift in the phase
- They donot interfere either constructively or destructively from each other.
- For this particle with well defined value of momentum,The wavefunction extends from positive infinity to negative infinity.

So here we saw wavefunction for a free particle of momentum p, so we are looking at wavefunction of a single a wavelength, which affects momentum, a wave can have two parts, one is real and imaginary part having same wavelength, they are shifted by the both real and imaginary part, are shifted by one-fourth of wavelength, so this is one-fourth of the wavelength.

Which is equal to 90° shift in the phase, these two waves do not interfere either constructively destructively from each other, for this particle with well-defined value of momentum, so here, since there is one wave of one wavelength, so it has well-defined value of momentum, here your wave function extends from positive infinity to negative infinity.

What does that mean is your since probability of finding a particle is proportional to a wavefunction square, so you can find particle everywhere, you can find particle between positive infinity to negative infinity.

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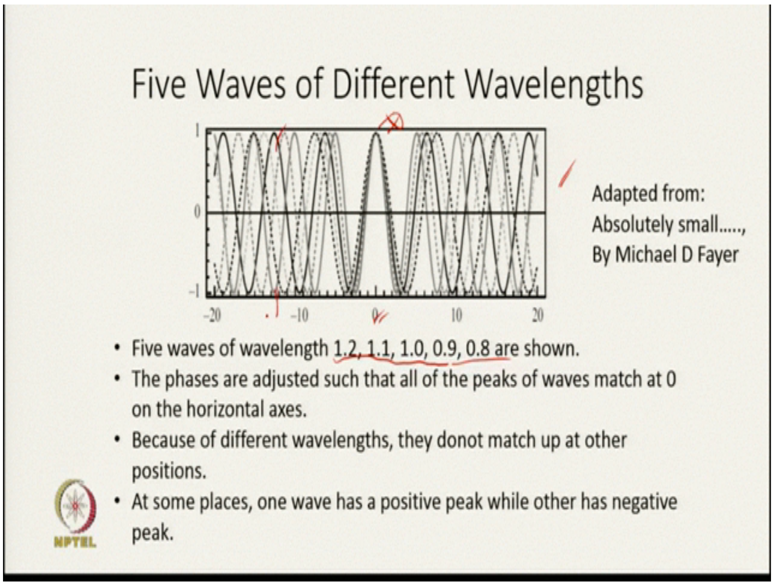

Five Waves of Different Wavelengths

Adapted from:
Absolutely small.....,
By Michael D Fayer

- Five waves of wavelength 1.2, 1.1, 1.0, 0.9, 0.8 are shown.
- The phases are adjusted such that all of the peaks of waves match at 0 on the horizontal axes.
- Because of different wavelengths, they donot match up at other positions.
- At some places, one wave has a positive peak while other has negative peak.

Now suppose we take five waves of different wavelength, which is shown here, so what we have done is we have taken five wavelength of 1.2, 1.1, 1.0, 0.9, and 0.8 and phases are adjusted such that all of the peaks of waves match add 0 on the horizontal axis.

So we can see here, you can see here that waves a matching at x is equal to 0, but if you go away from x is equal to 0 because of different wavelength, they are not going to matchup at other positions, at some places one wave has a positive peak, while other has negative peak, so you can see here for example here you see this has the black one has your positive peak and this one has negative peak. So at some positions one wave has of a positive peak, while other has negative peak.

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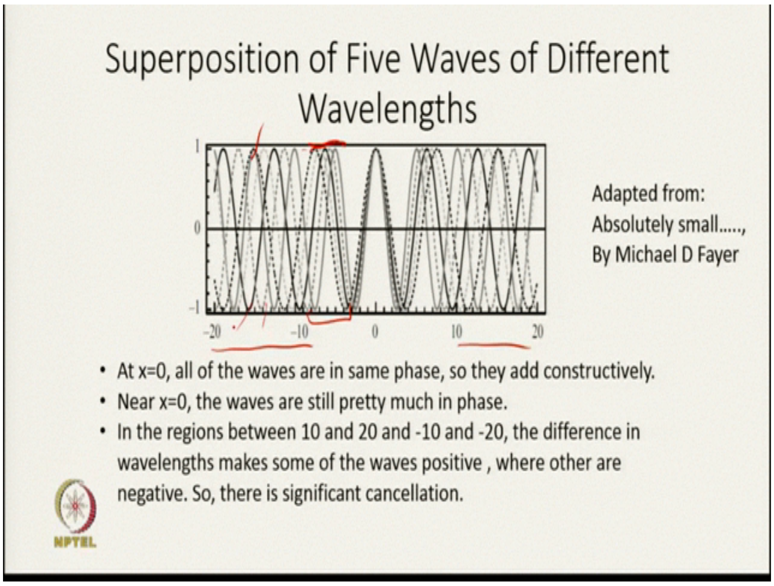

Superposition of Five Waves of Different Wavelengths

Adapted from:
Absolutely small.....,
By Michael D Fayer

- At x=0, all of the waves are in same phase, so they add constructively.
- Near x=0, the waves are still pretty much in phase.
- In the regions between 10 and 20 and -10 and -20, the difference in wavelengths makes some of the waves positive , where other are negative. So, there is significant cancellation.

What does that mean that at x is equal to 0, since all of the wave are in the same phase, so they add constructively, whereas near x is equal to 0, your talk this region, this region the waves are still pretty much in the phase, whereas in the regions between 10 and 20 and minus 10 and minus 20, the difference in wavelengths makes some of the wave is positive, the some of the waves are positive, where some of them are negative, some of them are negative, so there is a significant cancellation.

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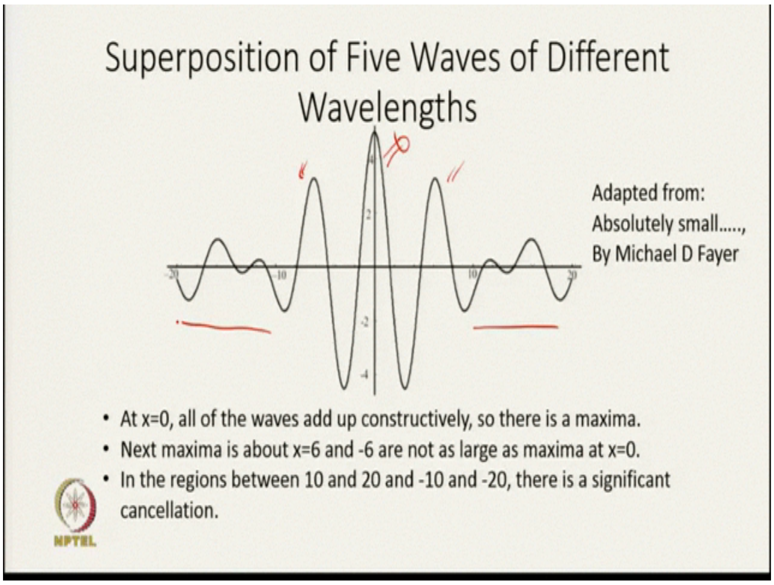

Superposition of Five Waves of Different Wavelengths

Adapted from:
Absolutely small.....,
By Michael D Fayer

- At x=0, all of the waves add up constructively, so there is a maxima.
- Next maxima is about x=6 and -6 are not as large as maxima at x=0.
- In the regions between 10 and 20 and -10 and -20, there is a significant cancellation.

So if we take combine wave it will look like this, so at x is equal to 0, since there is constructive interference all waves add up and so there is a maxima, so you can see there is a maxima, near 6 and minus 6 also 6 and minus 6, so this is 6 and this is minus 6, you can get some maxima but that will not be as large ar maxima at x is equal to 0, in the region between 10 and 20 and minus 10 and minus 20, so 10 and 20 and minus 10 and 20, there is a significant cancellation, so your value amplitude will not be that much its or amplitude will start decreasing.

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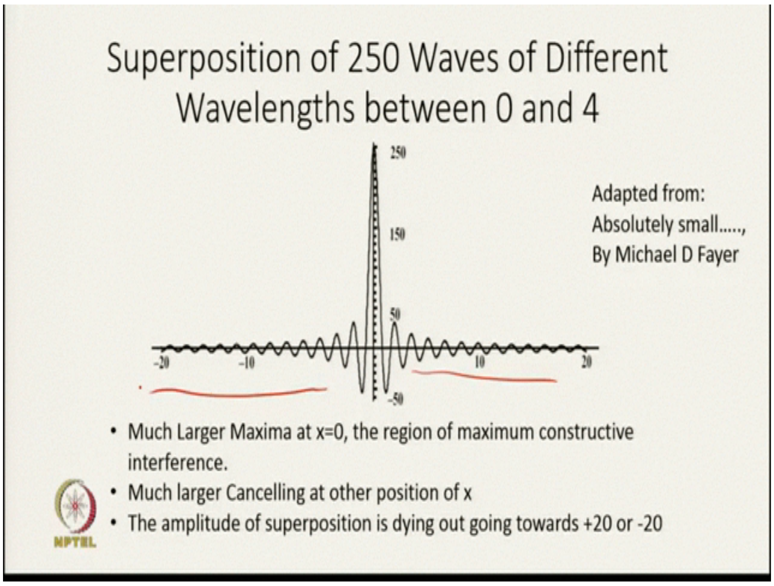

Superposition of 250 Waves of Different Wavelengths between 0 and 4

Adapted from:
Absolutely small.....,
By Michael D Fayer

- Much Larger Maxima at x=0, the region of maximum constructive interference.
- Much larger Cancelling at other position of x
- The amplitude of superposition is dying out going towards +20 or -20

Now think of if there are 250 waves what will happen, so this is the example where 250 waves are taken of different wavelengths between 0 and 4 and phases adjusted so that at x is equal to 0, there is a region of maximum constructive interference, in this case if you take very large number of waves what will happen is, there is much larger cancelling at other position of x and you can see almost 0 at other position of x, so the amplitude of superposition is dying out going towards plus 20 or minus 20.

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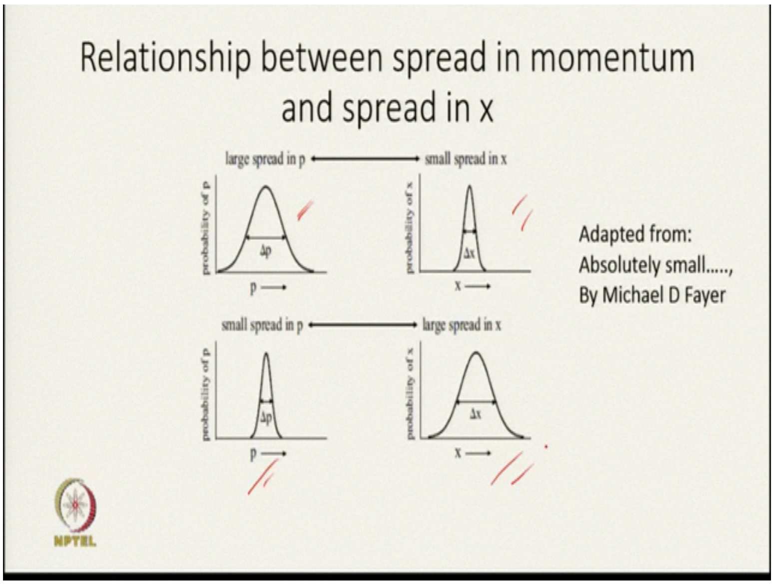

Relationship between spread in momentum and spread in x

Adapted from:
Absolutely small.....,
By Michael D Fayer

And so what is this tells you that when we have a large number of waves constituting your electromagnetic radiation, what is that mean is, your delta p will be large, delta p will be large in that case you will be able to specify the position of the particle, see specify the position of the particle, that is what is shown in this two diagrams, which tells you about relationship between spread in momentum and spread in x.

So when you are taking large number of waves it means your spread in momentum is high because each wavelength corresponds to single momentum and since there are large number of wavelength and so there is a spread in momentum, in that case, the spread in x is will low, spread in x is low and when we have only one, only one wave it means only one lambda which corresponds to one momentum, so you are talking about one space peak momentum, in that case, you cannot locate your particle because it can go from minus infinity to plus in infinity.

Spread in p and spread in x is related, so if there is high spread in p what is that mean there will be a small spread in x and if there is a small spread in momentum it means there is a large spread in x and this is the basis of Heisenberg uncertainty principle.

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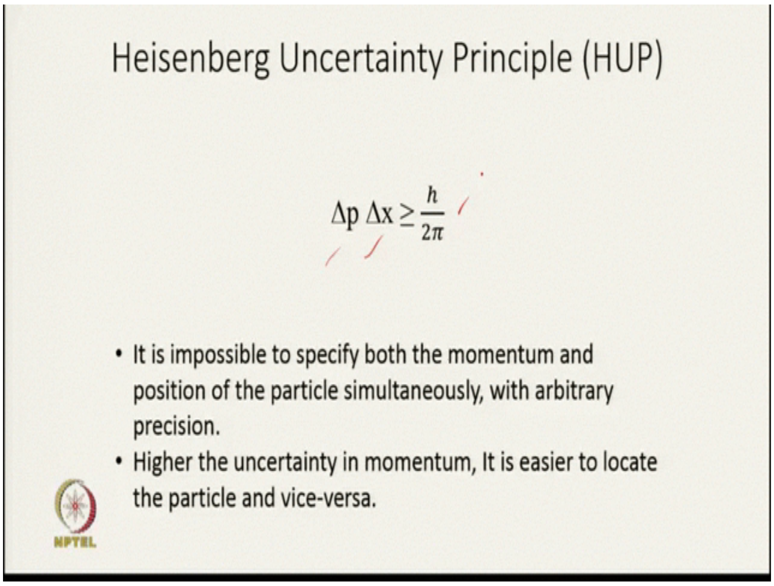

Heisenberg Uncertainty Principle (HUP)

$$\Delta p\, \Delta x \geq \frac{h}{2\pi}$$

- It is impossible to specify both the momentum and position of the particle simultaneously, with arbitrary precision.
- Higher the uncertainty in momentum, It is easier to locate the particle and vice-versa.

So Heisenberg uncertainty principle tells you that delta p which is your spread in p multiplied by delta x which is spread in position is greater than equal to h by 2 pie, what is that mean is that it is impossible to specify both the momentum and position of particles simultaneously, with arbitrary precision.

So if you can, if spread in momentum is high, then only you can be able to tell what is the position of particle, if there is a spread in position, then you will be able to tell what is the momentum but both p and x cannot be specified simultaneously, so higher the uncertainty in momentum it is easier to locate the particle and vice versa.

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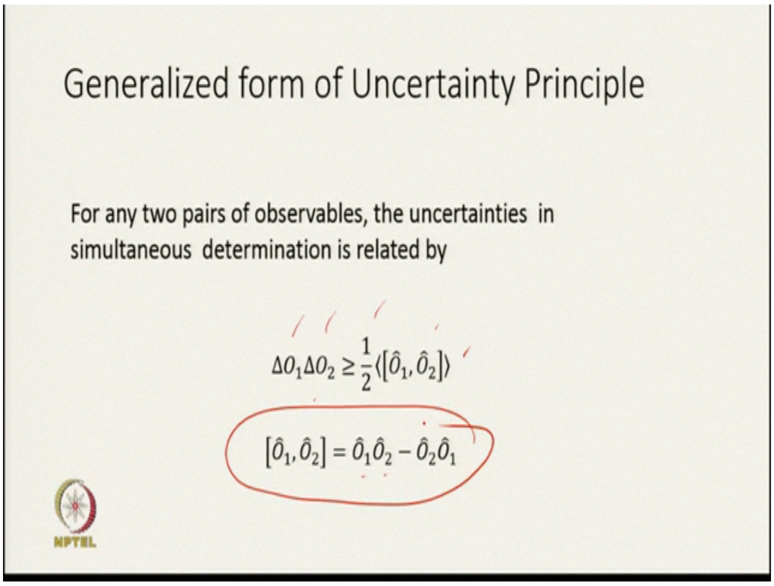

Generalized form of Uncertainty Principle

For any two pairs of observables, the uncertainties in simultaneous determination is related by

$$\Delta O_1 \Delta O_2 \geq \frac{1}{2}\langle[\hat{O}_1, \hat{O}_2]\rangle$$

$$[\hat{O}_1, \hat{O}_2] = \hat{O}_1\hat{O}_2 - \hat{O}_2\hat{O}_1$$

So this uncertainty principle has also a generalized form, so what this tells you that for any to pairs of observables, the uncertainties in simultaneous determination is related by this equation, this is equation which is generalize equation of uncertainty principle, that uncertainty in observables O1 multiplied by uncertainty in observable O2 is greater than equal to half and then this, commuting operator O1 and O2, where O1 and O2 is given by O1 into O2 minus O2 into O1.

So this is your what is known as commutator, so if this is equal to 0, then what will happen that this will be 0 and then you can precisely determine to two observable at the same time but if this is not equal to 0 and you cannot precisely determine observable O1 and O2 simultaneously.

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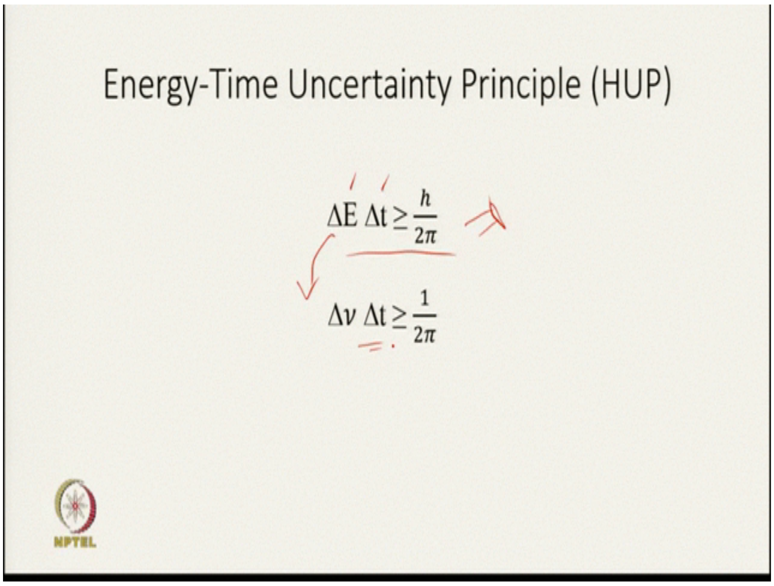

Energy-Time Uncertainty Principle (HUP)

$$\Delta E\,\Delta t \geq \frac{h}{2\pi}$$

$$\Delta \nu\,\Delta t \geq \frac{1}{2\pi}$$

So the second example of this Heisenberg uncertainty principle is between two other observables energy and time and again delta E into delta t will be greater than equal to h by 2 pie because they do not compute with each other and so delta E into delta t is not equal to 0 and this is related to a spectroscopy because what we generally look at is in a spectroscopy is broadening of signal, which are related to delta E or delta new.

So if delta E high it means or uncertainty in E is high it means peak is broad, you can convert delta E to delta nu and delta nu is again related to delta t, delta t is your what is known as life time, so if life time is higher, it means delta nu or delta E will be higher and it means peak will be more broaden.

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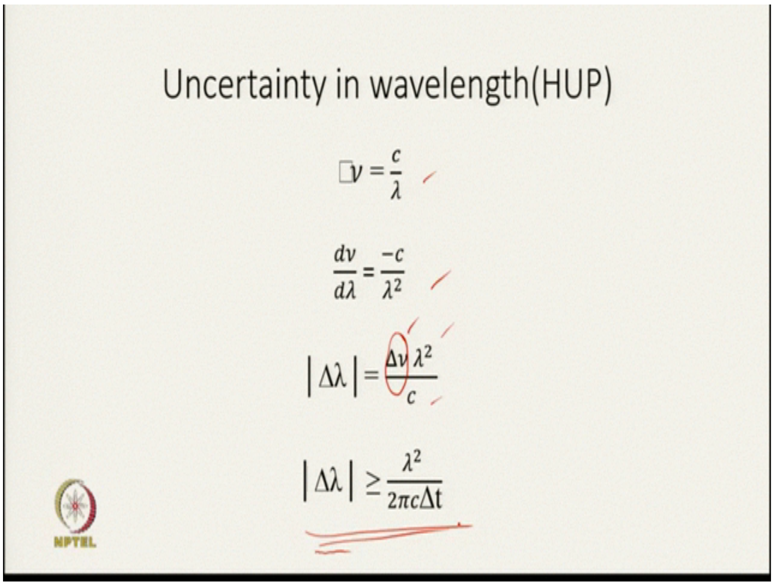

Uncertainty in wavelength(HUP)

$$\Box\nu = \frac{c}{\lambda}$$

$$\frac{d\nu}{d\lambda} = \frac{-c}{\lambda^2}$$

$$|\Delta\lambda| = \frac{\Delta\nu\,\lambda^2}{c}$$

$$\underline{|\Delta\lambda| \geq \frac{\lambda^2}{2\pi c\Delta t}}$$

Similarly you can relate this equation with uncertainty in wavelength, since you frequency is c by lambda, so dell nu by dell lambda is minus c by lambda square, so we can simply right delta lambda is equal to delta nu into lambda square by c and delta lambda and then if you put the this from your Heisenberg uncertainty principle you can get delta lambda, so this is your uncertainty in wavelength and that is related to delta t, which is life time.

So delta lambda is, you can see is inversely proportional to delta t, it means there is higher uncertainty in lambda than delta t must be low or if life time is a smaller than uncertainty in lambda is higher, whereas if life time is your, if life time is delta t is your high, then your delta lambda will be small.

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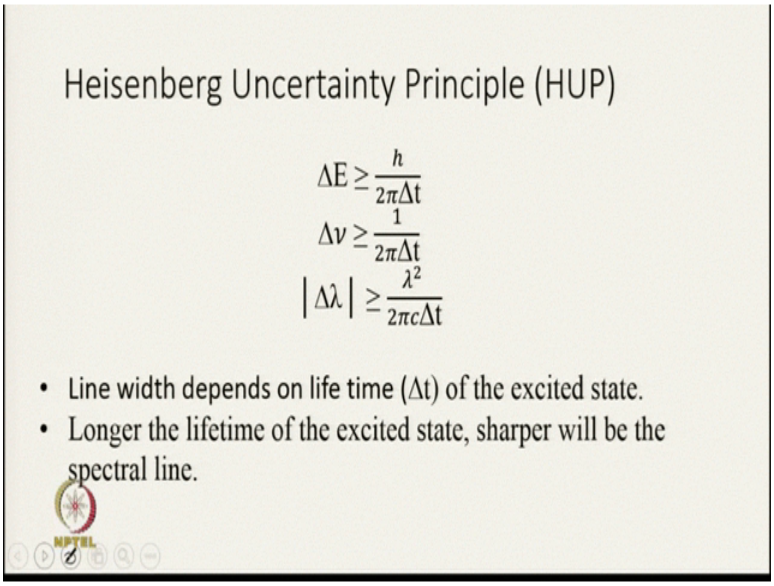

Heisenberg Uncertainty Principle (HUP)

$$\Delta E \geq \frac{h}{2\pi\Delta t}$$

$$\Delta \nu \geq \frac{1}{2\pi\Delta t}$$

$$\left|\Delta\lambda\right| \geq \frac{\lambda^2}{2\pi c\Delta t}$$

- Line width depends on life time (Δt) of the excited state.
- Longer the lifetime of the excited state, sharper will be the spectral line.

Line width depends on life time of the excited state, longer the lifetime of the excited state, sharper will be the spectral line, so if delta t is highly, it means delta lambda is small and so peak will be sharper, peak will be sharper.

So Heisenberg uncertainty principle tells you about your broadening of the peaks, so if you go from electronic transition to suppose NMR, what happens that in an electronic transition your lifetime of the excited state is very small, lifetime of the excited state is very small.

Since there is a larger difference between ground state and excited state and so there will be, the peaks obtained in electronic transition is quite broad, particularly in UV visible spectroscopy and if you go to NMR, since the energy difference between two levels, ground state and excited state is smaller and so lifetime of excited state is high, lifetime of the excited state is longer and so sharper lines are obtained and so NMR is high-resolution spectroscopy, whereas UV visible is low-resolution spectroscopy.

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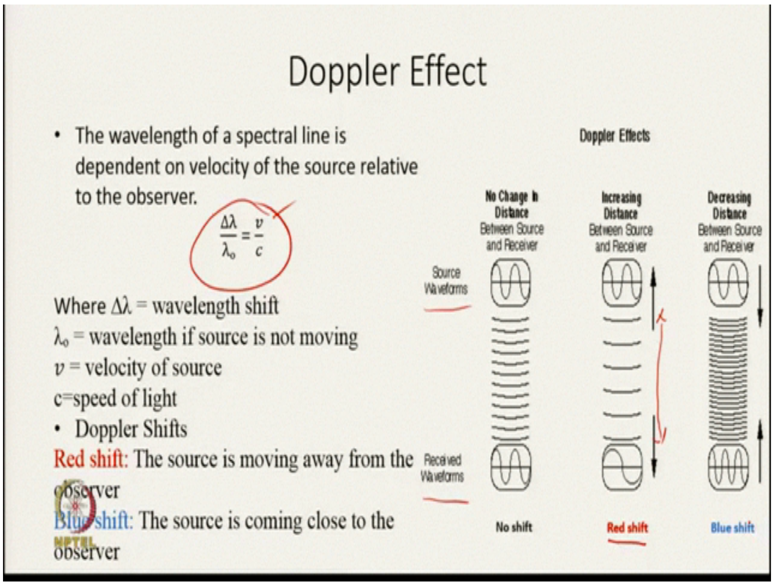

Doppler Effect

- The wavelength of a spectral line is dependent on velocity of the source relative to the observer.

$$\frac{\Delta\lambda}{\lambda_o} = \frac{v}{c}$$

Where $\Delta\lambda$ = wavelength shift
λ_o = wavelength if source is not moving
v = velocity of source
c=speed of light

- Doppler Shifts

Red shift: The source is moving away from the observer
Blue shift: The source is coming close to the observer

Apart from Heisenberg uncertainty principle, Doppler effect can also affect the broadening of spectra, so the wavelength of a spectral line is dependent on the velocity of source related to the observer and the relationship is given here that is delta lambda by lambda not is equal to v by c, where delta lambda is wavelength shift or you can say that line broadening and lambda 0 is wavelength of the source, when it is not moving, v is the velocity of your source, velocity of the source relative to observer and c is your speed of light.

So what you can see is there will be a red shift, if source is moving away from the observer, source is moving away from the observer and there will be a blue shift when sources coming close to the observer, so you can see here that in this case, this is your source waveform and this is your receiver waveform and here you are with increasing distance this lambda increases and there is a red shift, whereas if there moving towards each other than there is a blue shift.

So this is not only a applicable to light waves it is also applicable to other kind of wave, for example soundwaves, so if source is moving away from the observer, there will be red shift and if source is coming closer to the observer there will be blue shift and this is well-known Doppler effect, this is well-known Doppler effect.

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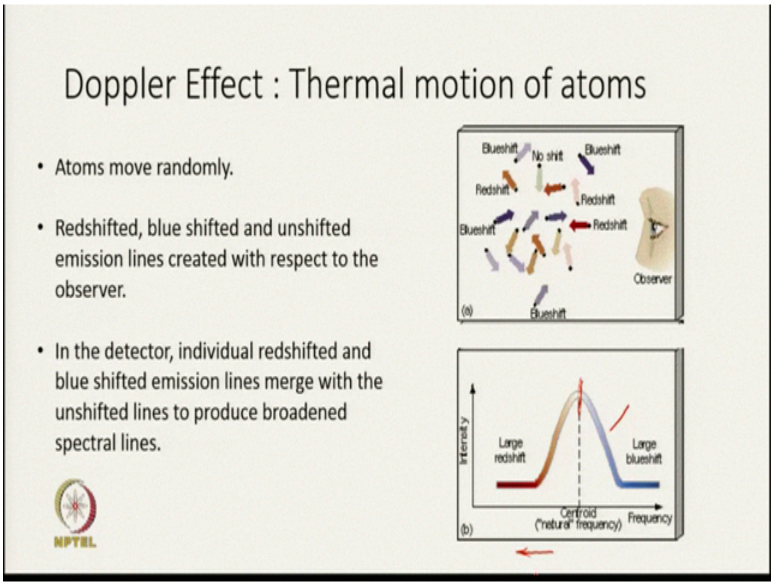

Doppler Effect : Thermal motion of atoms

- Atoms move randomly.
- Redshifted, blue shifted and unshifted emission lines created with respect to the observer.
- In the detector, individual redshifted and blue shifted emission lines merge with the unshifted lines to produce broadened spectral lines.

So now think of that if you are trying to look at spectra of some sample and suppose we are a, you are a observer, so what will happen is that some of the atoms of the sample will come towards you and some is going away from you, this is because of your Brownian motion, this is because of Brownian motion, something which is coming to words you is basically your blue shifted, the one which is going away from you is red shifted.

So what will happen that this is when there is no shift, if you are looking at the spectra of those molecule, so what will happen, some of the molecules are just at the position, at just random position and then what will happens some of them are going away from the detector and some are coming towards the detector, so those which are going towards the detector will have a blue shift and those which are going away from the detector will have red shift and so line will be broaden.

So this is the position the one which is moving away from you, well shift to, you know longer wavelengths and those who are doing towards you will shift to smaller wavelength and so there will be red shifted blue shift and so whole spectra will get broaden, so in the detector, individual red shifted and blue shifted emission lines merge with the unshifted lines to produce broaden spectral lines.

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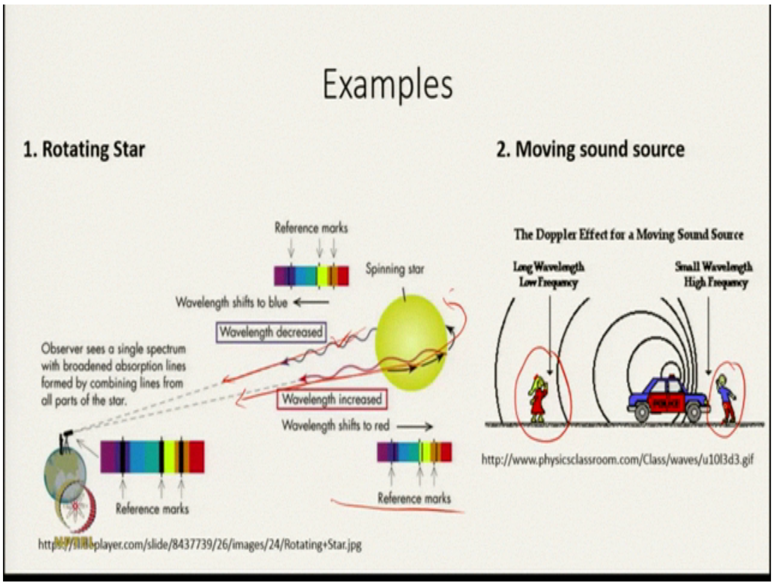

Examples

1. Rotating Star

2. Moving sound source

http://slideplayer.com/slide/8437739/26/images/24/Rotating+Star.jpg

http://www.physicsclassroom.com/Class/waves/u10l3d3.gif

Other things which shows the effect of Doppler or Doppler effect on the your spectra is when you are looking at the spectrum of rotating star, so what will happen is that your light is coming to you from the side and from the side okay, from two sides light is coming to you, and that is how you are observing your a rotating a star.

So what will happen, suppose there is a rotation from this side to this side, so basically light which is coming on this line, what is happening? It is going away from the observer and then it is going away from the observer what does that mean? It is red shifted, what about this ray, this light.

So this is basically coming towards the observer and so this will be your blue shifted, so the one which is going away from detector is red shifted, those which are coming from towards you will be blue shifted and this whole range of spectra will be obtained and that is why you see a continuous spectrum, so this is about the light, effect of Doppler effect on the light, what about sound source.

So now you see here if it is moving towards you, so car is moving towards you and there is a your siren and so this will appear to be of smaller wavelength and high-frequency to this person, to this person, whereas it will appear to be of longer wavelengths and low frequency to this observer, this is just because for this observer your sources is moving away, whereas for these of observer it is moving towards, the car is towards the observer.

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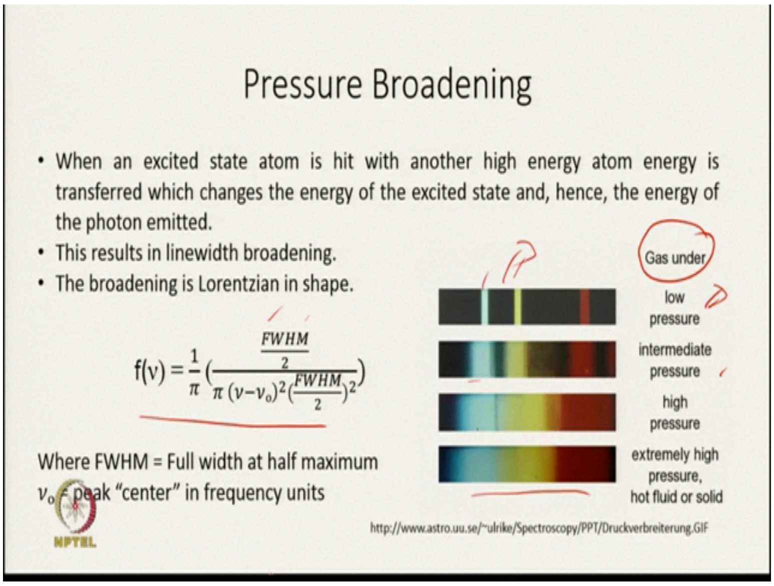

Pressure Broadening

- When an excited state atom is hit with another high energy atom energy is transferred which changes the energy of the excited state and, hence, the energy of the photon emitted.
- This results in linewidth broadening.
- The broadening is Lorentzian in shape.

$$f(\nu) = \frac{1}{\pi}\left(\frac{\frac{FWHM}{2}}{\pi\,(\nu-\nu_o)^2(\frac{FWHM}{2})^2}\right)$$

Where FWHM = Full width at half maximum

ν_o = peak "center" in frequency units

http://www.astro.uu.se/~ulrike/Spectroscopy/PPT/Druckverbreiterung.GIF

So apart from Heisenberg uncertainty principle and Doppler effect, you have pressure broadening, the spectra can broaden due to pressure, so when an excited state atom is hit with another high energy atom energy is transferred which changes the energy of excited state and hence the energy of the photon emitted and this results in linewidth broadening and you can see here that the gas under low pressure.

So this kind of spectra where is a intermediate pressure, you can see that there is broadening happening and this line looks like this under intermediate pressure, at high pressure you see again, this much broaden and add extremely high pressure you can see almost continuous kind of spectra is obtain, because almost continuous kind of spectra is obtained, this broadening is Lorentzian in shape and that will be given by this formula FWHM is full width at half maximum and nu not is your pick centre in frequency units.

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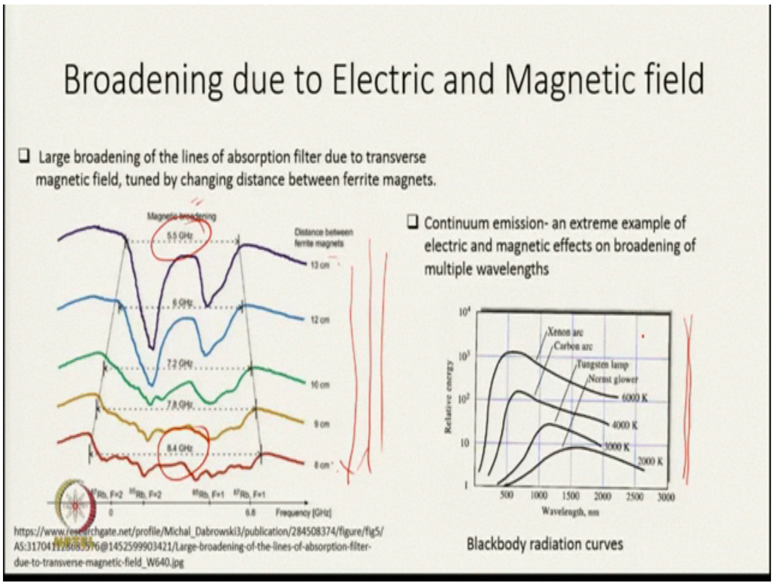

Broadening due to Electric and Magnetic field

- ☐ Large broadening of the lines of absorption filter due to transverse magnetic field, tuned by changing distance between ferrite magnets.
- ☐ Continuum emission- an extreme example of electric and magnetic effects on broadening of multiple wavelengths

https://www.researchgate.net/profile/Michal_Dabrowski3/publication/284508374/figure/fig5/AS:317041126083576@1452599903421/Large-broadening-of-the-lines-of-absorption-filter-due-to-transverse-magnetic-field_W640.jpg

Blackbody radiation curves

Peak may be broaden due to electric and magnetic field. So large broadening of lines of absorption due to transverse magnetic field tuned by changing distance between ferrite magnets.

So this is shown here and you can see that if you go from, so this is distance between ferrite magnets and this is magnetic broadening and you see you are going from distance, you are going from 13 cm to 8 cm, as you decrease the distance between ferrite magnets the line is getting broaden, line is getting broaden, you are basically increasing the magnetic field from 5.5 GHz to 8.4 GHz and there is a broadening of peaks okay.

Continuous emissions an extreme example of electric and magnetic field on broadening of multiple wavelengths, broadening of multiple wavelength and these are the your blackbody radiation curve fraud different kind of lamp at different temperature, so these are the few of the factors which affect the resolution of the peaks or the broaden the peaks and so it is important to understand what are the factors which can affect the broadening.

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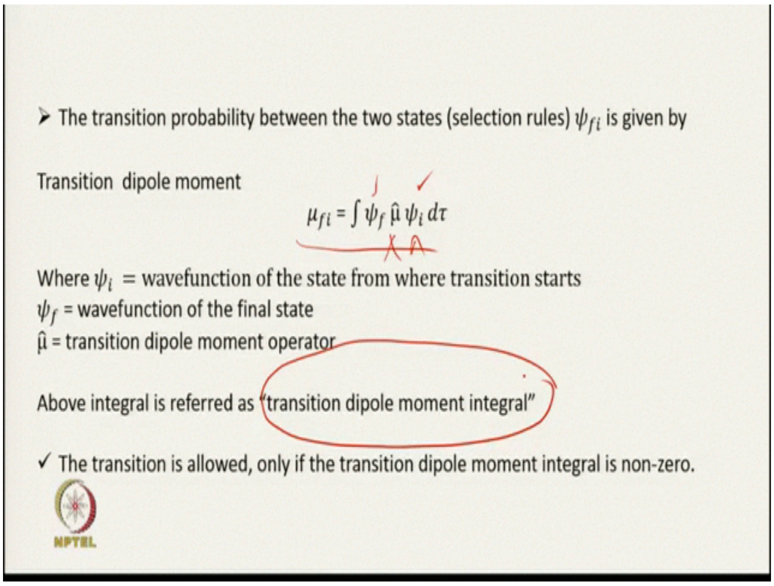

- The transition probability between the two states (selection rules) ψ_{fi} is given by

Transition dipole moment

$$\mu_{fi} = \int \psi_f \, \hat{\mu} \, \psi_i \, d\tau$$

Where ψ_i = wavefunction of the state from where transition starts
ψ_f = wavefunction of the final state
$\hat{\mu}$ = transition dipole moment operator

Above integral is referred as "transition dipole moment integral"

✓ The transition is allowed, only if the transition dipole moment integral is non-zero.

Now the second thing which is important in almost every kind of spectroscopy is intensity or signal to noise ratio, the intensity of spectral lines depends on transition probability between the two states, population of states and finally your concentration and path length of the sample which is given by Beer-Lambert law, so will go to one by one and see how does they affect the intensity of your spectral lines.

First thing is your transition dipole moment, the transition probability between two states, for example, you have to take wave function i or state i to state f is given by your this formula where mu fi is transition dipole moment and this is your psy f tells you about wave function of the final state, this operator, mu operator is your transition dipole moment operator and this is your wave function of ith state and this integral is known as transition dipole moment integral, this integral is referred as transition dipole moment integral.

The transition is allowed only if the transition dipole moment integral is non-zero, so if this integral is non-zero, it means transition is allowed if it is integral is 0, then transition is not allowed, so whenever we go and discuss are various types of spectroscopy will go, for example rotational spectroscopy will take wave function of rotational levels and then we see under what conditions this dipole moment integral is going to be non-zero and under those conditions your the spectra or transition that your rotational transition is going to be allowed, if you are transitions are allowed then you expect that signal intensity should be high.

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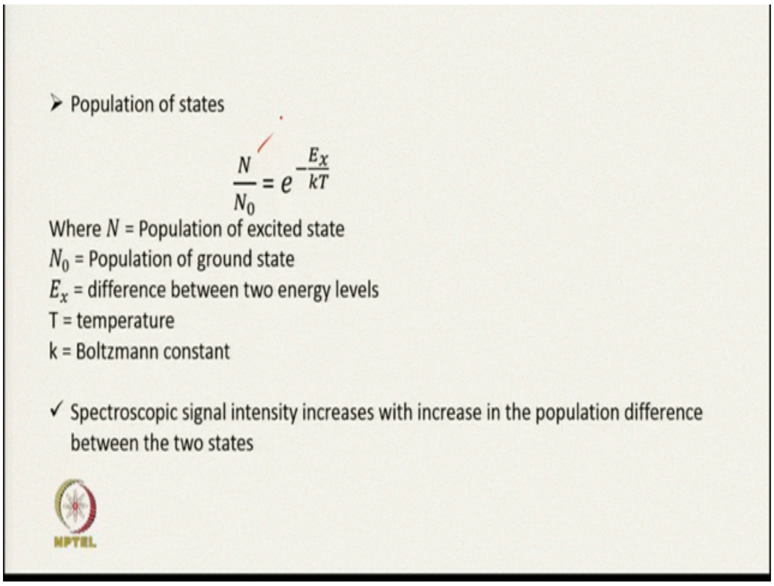

- Population of states

$$\frac{N}{N_0} = e^{-\frac{E_x}{kT}}$$

Where N = Population of excited state
N_0 = Population of ground state
E_x = difference between two energy levels
T = temperature
k = Boltzmann constant

✓ Spectroscopic signal intensity increases with increase in the population difference between the two states

The second factor which can affect intensity is population of a states, so if there is a difference of population between two states, ith state and f state or ground state and excited state, your intensity will be higher.

So higher the population difference higher will be intensity, lower the population difference lower will be intensity. Now the distribution of particles in different states is given by your Boltzmann distribution law, which tells you that N by N not is equal to exponential minus Ex by kT, where Ex is difference between two energy levels.

So the energy levels which your considering or where transition is happening, so the Ex is difference between an energy level of the state from where transition is taking place and the state where transition is happening. So Ex is difference between two energy levels, N0 is population of the ground state, T is temperature and k is Boltzmann constant.

So you can see that population in excited state, which is your N is going to be affected by a difference between two energy levels and temperature is, so if difference energy level is high, it means your population is going to be, population in the excited state is going to be lower and if temperature is high it means your population is going to be higher in the excited state compared to the population in the excited state at lower temperature.

So there are two factors which is going to affect the population of different energy levels, so spectroscopy signal intensity increases with increasing the population difference between the two states.

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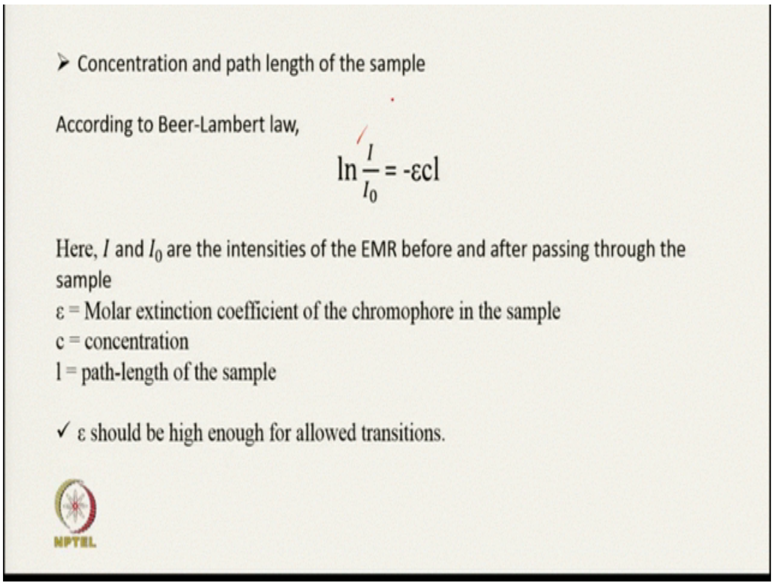

- Concentration and path length of the sample

According to Beer-Lambert law,

$$\ln\frac{I}{I_0} = -\varepsilon c l$$

Here, I and I_0 are the intensities of the EMR before and after passing through the sample

ε = Molar extinction coefficient of the chromophore in the sample
c = concentration
l = path-length of the sample

✓ ε should be high enough for allowed transitions.

And the last factor is concentration and path length of the sample and this is given by Beer-Lambert law which tells you ln I by I not is minus epsilons cl, where I the intensity is of the light after absorption and what we can say is after passing through the sample, where I not is the intensity of light before passing through the sample.

Epsilon smaller extension coefficient, c is concentration, l is path length, so epsilon would be high for transition which are allow transition and c is concentration, l is path lengths, so it concentration is high and again absorption is going to be high depending on delta E values, signal of some of the spectroscopy or spectrum will be high, some of them will be low, a question is how to improve intensity or signal to noise ratio.

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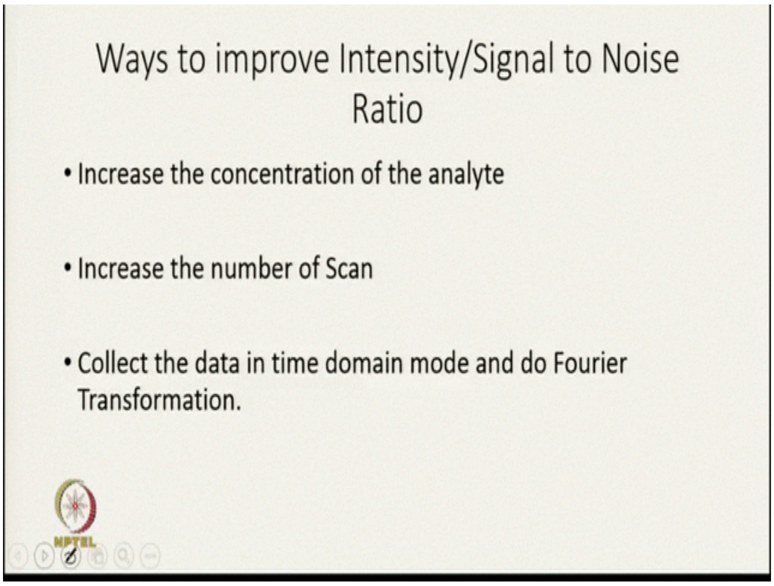

Ways to improve Intensity/Signal to Noise Ratio

- Increase the concentration of the analyte
- Increase the number of Scan
- Collect the data in time domain mode and do Fourier Transformation.

As we discussed in the last slide that concentration can affect the intensity, so one of the easiest way is increase the concentration of the analyte and that should increase the signal, but sometimes it is not possible to increase the concentration and so what can be done, the another way is to increase the number of scans.

So take spectra once, take another time, third time, four times, so increase the number of scans and add all the signals and third is collect the data in time domain mode and do Fourier transformation, we will go and tell you about advantage of the second and third factor on improving intensity signal to noise ratio.

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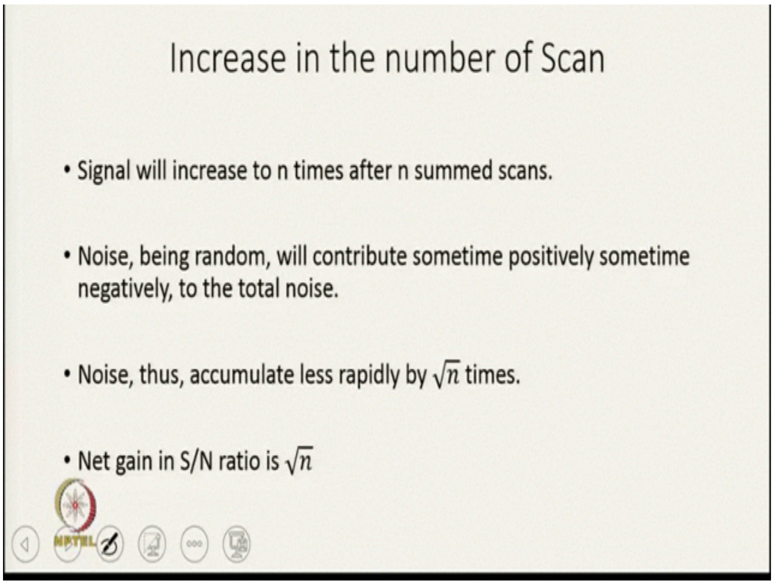

Increase in the number of Scan

- Signal will increase to n times after n summed scans.
- Noise, being random, will contribute sometime positively sometime negatively, to the total noise.
- Noise, thus, accumulate less rapidly by $\sqrt{n}$ times.
- Net gain in S/N ratio is $\sqrt{n}$

So now let us look at increase in the number of scans, signal will increase to n times after n summed scans, so if you have taken signal once, twice, thrice, four times and then you add the signal then it will your total signal will be four time a signal taken in one can, whereas noise being random will contribute sometime positively and sometime negatively to total noise copper.

So what happens since your noise is random, so it will not be true that every time noise or every at every point noise will be positive, sometimes it can be positive, sometimes it can be negative and so the total noise is not going to be like n times the noise.

So basically what happens that noise accumulate less rapidly and it goes up by root n times, root n times, if number of scans is n, so net gain in signal to noise ratio is root n times, so basically by increasing the number of scans you can increase the intensity or signal to noise ratio of the spectra.

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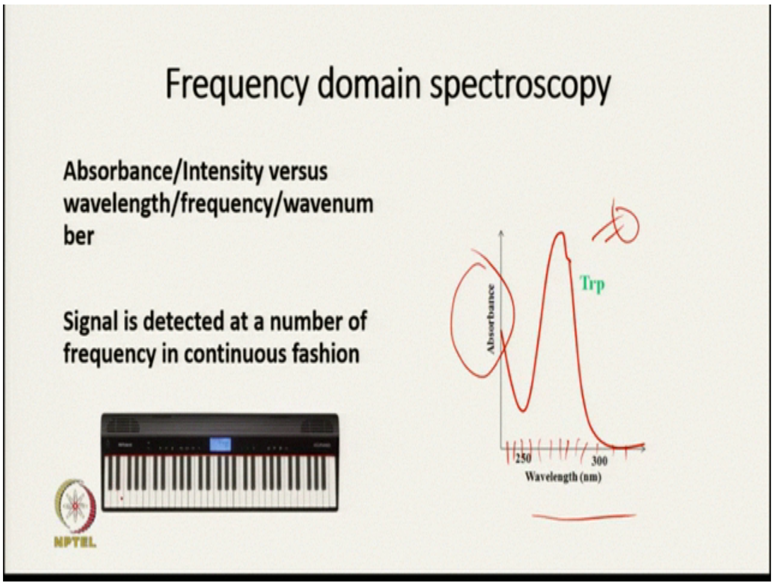

Frequency domain spectroscopy

Absorbance/Intensity versus wavelength/frequency/wavenumber

Signal is detected at a number of frequency in continuous fashion

Now let us go to the third factor which is basically, based on mode of collection of signal and based on this spectroscopy is also divided into two, one is your time domain spectroscope be an the another is your frequency domain spectroscopy, so first we will discuss frequency domain spectroscopy, frequency domain spectroscopy is often used when signal-to-noise ratio is high and basically what you are doing here, your plotting and absorbance or intensity versus wavelength.

So what you are doing here is you started getting the signal at every lambda or wave number or frequency, so it is like playing a piano where you are going and pressing one piano at each time, this key, then this key, followed by this key, this key, followed by this key, so you are going one at a time from one part to another part and looking at the signal.

So signal is detected at number of frequency in continuous fashion, so what you will see is spectra like this, so here your intensity is plotted against each wavelength or each frequency in a continuous fashion.

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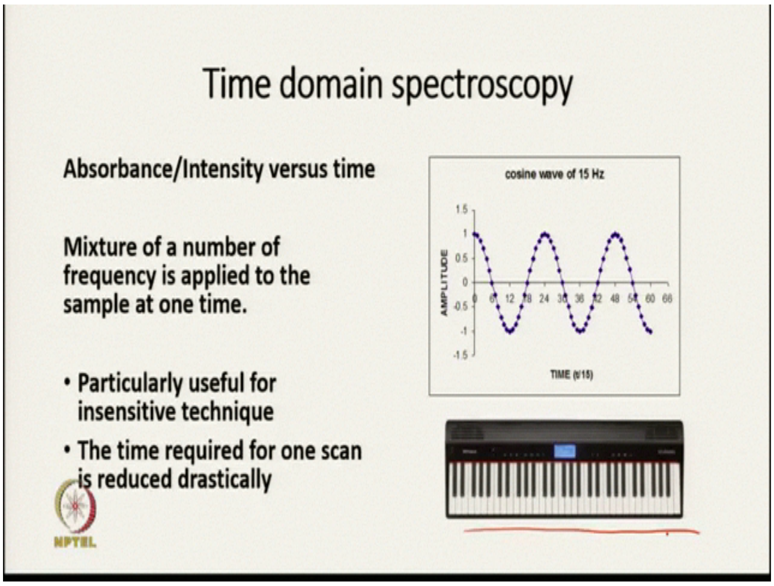

Time domain spectroscopy

Absorbance/Intensity versus time

Mixture of a number of frequency is applied to the sample at one time.

- **Particularly useful for insensitive technique**
- **The time required for one scan is reduced drastically**

Now let us go to time domain spectroscopy, this is the spectroscopy where what you are looking at is absorbance intensity versus time, so you can see here, this is generally use for low sensitive spectroscopy, what you do that in contrast to your frequency domain spectroscopy, you press all the keys at a same time, all the keys at the same time and so basically you are supplying a mixture of number of frequencies or you are applying the some of different frequencies to the sample at one time and this is particularly useful for insensitive technique because time required for one scan is reduce drastically.

So suppose there are sixty keys and pressing one key takes one second time, in frequency domain spectroscopy, if you are going from 1 to, first key to sixtieth key one by one, then it a sixty second time, while in the time domain spectroscopy, since all of the key is pressed, at one time it means that it is going to taken, take only one second.

So basically you are reducing the scan time by sixty, so it is time is basically the time taken in frequency domain spectroscopy divided by sixty and what is advantage, you can again go back, do more scans and then you can increase intensity by root sixty times.

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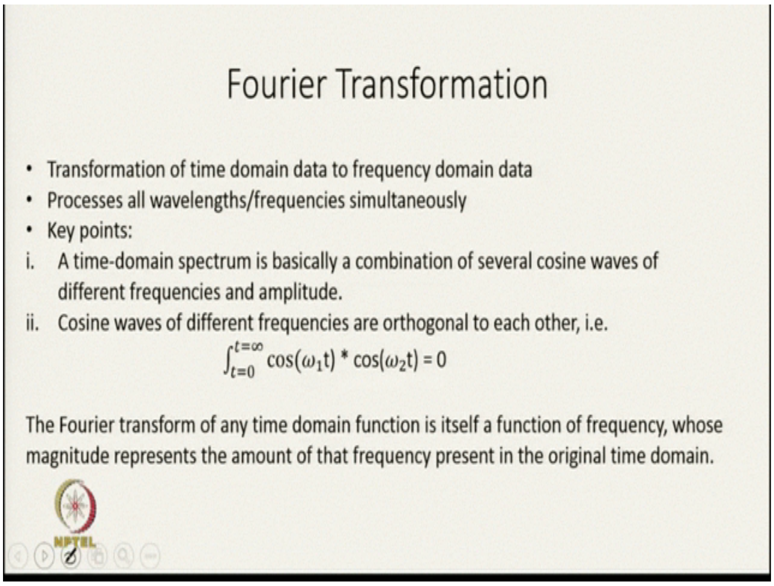

Fourier Transformation

- Transformation of time domain data to frequency domain data
- Processes all wavelengths/frequencies simultaneously
- Key points:

i. A time-domain spectrum is basically a combination of several cosine waves of different frequencies and amplitude.

ii. Cosine waves of different frequencies are orthogonal to each other, i.e.

$$\int_{t=0}^{t=\infty} \cos(\omega_1 t) * \cos(\omega_2 t) = 0$$

The Fourier transform of any time domain function is itself a function of frequency, whose magnitude represents the amount of that frequency present in the original time domain.

Now question is how to transform time domain data to frequency domain data, so once you obtain time domain data, you need to convert it into frequency domain data to get information out of it, so you need to convert time domain data to frequency domain data, so basically you need to process the data to get the frequency domain data, there are two key points in Fourier transformation, one is a time domain spectrum is basically a combination of several cosine waves of different frequencies and amplitude.

Suppose there are two chromo force, they absorb at two different lambda value, so your time domain spectrum is sum of cosine waves of two different lambda values, so you can think of suppose you are taking NMR, so if you have a five protons and they absorbs at different wavelengths, so your signal will be, time domain signal will be combination of five different cosine waves, five cosine waves of different frequencies.

So once you understand that now you will utilize one of the property to convert time domain spectrum to frequency domain spectrum and that property is, that cosine waves of different frequencies are orthogonal to each other, what I mean is that if you take cosine wave of frequency W1 and multiplied that by cosine wave of wavelength of frequency W2 and then if you add up between t is equal to 0 to t is equal to infinity, then you should get a value of 0, if omega 1 and omega 2 is different W1 and W2 is different.

Then if you multiply cosine wave of frequency omega 1 and cosine wave of frequency omega 2 and then sum up from t is equal to 0 and t is equal to infinity then you should get 0 and this property is used to convert your time domain spectrum to frequency domain spectrum.

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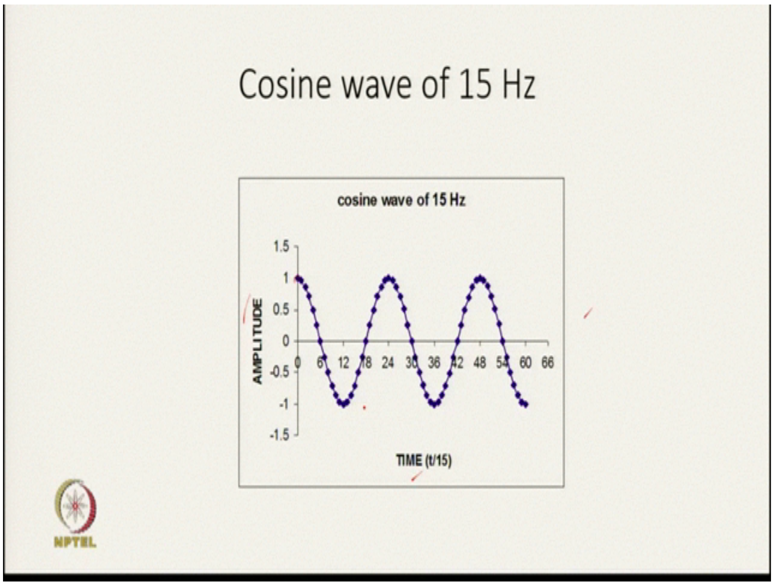

Cosine wave of 15 Hz

So let me show by example, so this is your cosine wave of 15 Hz, so this is the way your cosine wave of 15 Hz looks like and here your amplitude and there is a time and sometime it is at higher amplitude, then it will go to 0, then negative amplitude and then this is the way it is amplitude changes with time.

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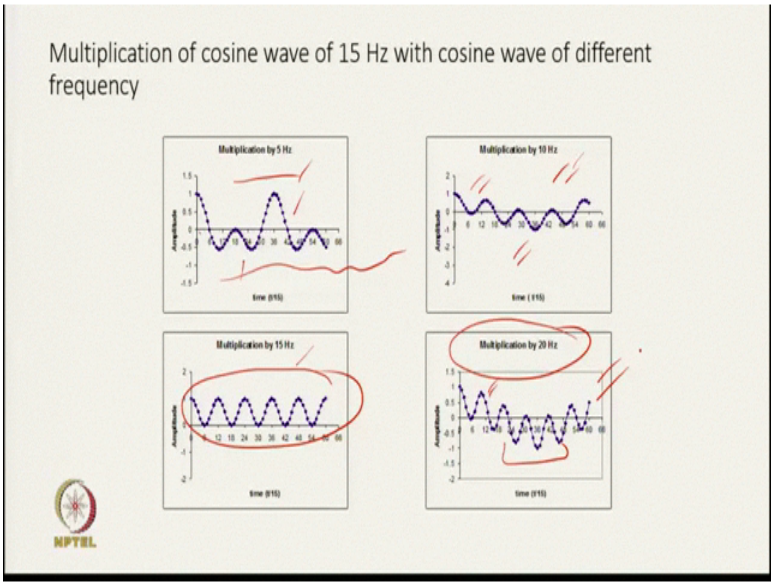

Multiplication of cosine wave of 15 Hz with cosine wave of different frequency

Now think of if I multiply this by this cosine wave of 15 Hz by cosine waves of different frequency, then how should is this look like, so first we have multiplied that 5 Hz and you see it looks like this, so some part has positive amplitudes, some part has negative amplitudes, whereas when we multiply by 10 Hz, again you can see there are some part which has positive amplitude and other part has negative amplitude, but when we multiply by 15 Hz then everything has positive amplitudes.

Whereas again if we multiply by 20 Hz then this part has positive amplitude, this part has negative amplitude and now if we sum up from t is equal to 0 to t is equal to infinity, what amplitude you are going to get is 0, some of the amplitude will be 0, if we multiply cosine wave of 15 Hz with cosine wave of 5 Hz, this will be 0 in this case also and this some of amplitude going to be 0 when we multiply by cosine wave of 20 Hz, in only one case it is not going to be 0, when you multiply cosine wave of 15 Hz with cosine wave of 15 Hz.

You multiply by any cosine wave, a cosine wave of any other frequency then 15 Hz, total amplitude will be 0, total amplitude is going to be 0, so that is what we mean by the cosine waves of two different frequencies are orthogonal to each other.

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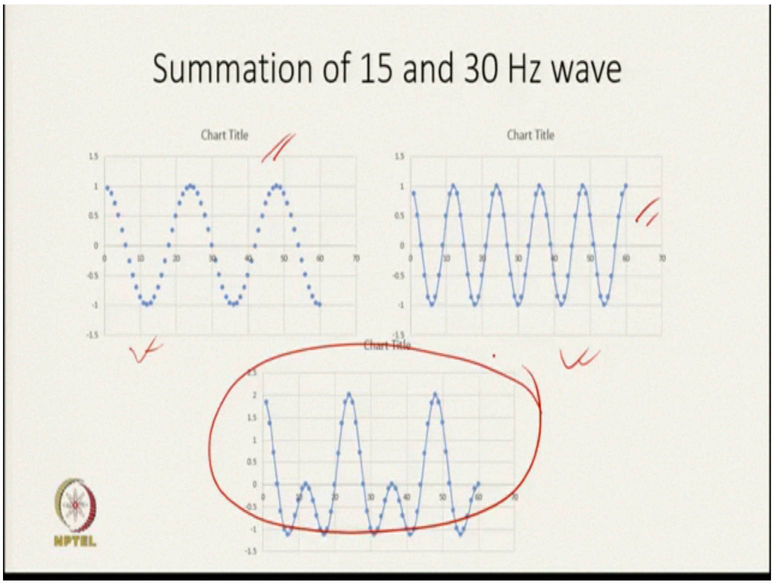

Summation of 15 and 30 Hz wave

Now let us think of that if our time domain data is function of two different wavelength okay, so suppose I take 15 Hz cosine wave or 30 Hz cosine waves, so this is your wave for 15 Hz and this is your amplitude versus time for your 30 Hz cosine wave, if we add up we are going to get this signal, so your time domain data is going to be something like that, it is going to be some of different, some of cosine waves of different wavelength okay.

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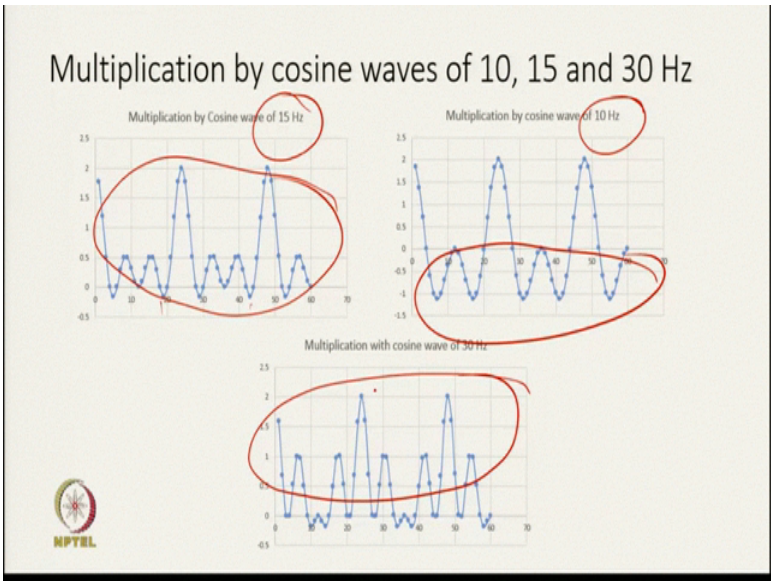

Multiplication by cosine waves of 10, 15 and 30 Hz

So let us think of, if I multiply the some of the wave consisting of 15 Hz and 30 Hz cosine wave by your 15 Hz cosine wave, then what you get you see, most of the time it is towards positive side, at very small place it is going to be negative, but if you look at, if I multiply by 10 Hz cosine wave, there are lot of time it has a negative amplitude, when I multiply by 30 Hz cosine wave, then again, it has positive value most of the time, so what you expect is that if your cosine wave or if you are time domain data is some of cosine waves of 15 Hz and 30 Hz and then you multiply by cosine waves of suppose 1 to 60 Hz.

Apart from two places, apart from two cosine waves, one with frequency 15 Hz and one with frequency 30 Hz it’s amplitude is going to be 0 if we integrate between t is equal to 0 to t is equal to infinity

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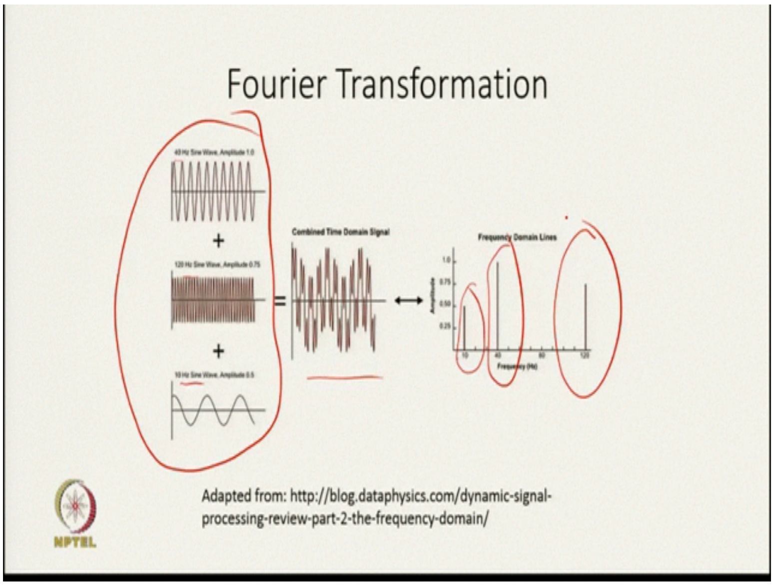

Fourier Transformation

Adapted from: http://blog.dataphysics.com/dynamic-signal-processing-review-part-2-the-frequency-domain/

And that gives us a very good tool to convert your time domain data to frequency domain data, so look at this picture and here there are three waves, one with 10 Hz, 120 Hz and 40 Hz, so these are three waves of different frequency, 10 Hz, 120 Hz and 40 Hz and this is your combine signal, if we do Fourier transformation.

So again we are going to different value of frequency and we are multiplying that by this combine wave function and then integrating amplitude between t is equal to 0 to t is equal to infinity, basically we are adding up what you are get a 0 at other values except at 10 Hz, 40 Hz and 120 Hz.

So if you do Fourier transformation of time domain data you will get a signal only at 10 Hz, 40 Hz, 120 Hz, if you are time domain data is mixture of three waves of frequency 10 Hz, 40 Hz and 120 Hz, so this is the basis of your for your transformation.

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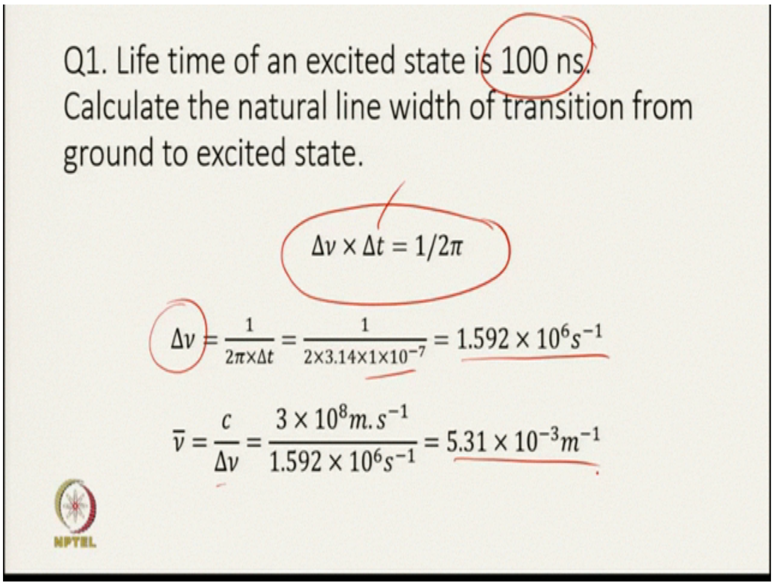

Q1. Life time of an excited state is 100 ns.
Calculate the natural line width of transition from ground to excited state.

$$\Delta\nu \times \Delta t = 1/2\pi$$

$$\Delta\nu = \frac{1}{2\pi\times\Delta t} = \frac{1}{2\times3.14\times1\times10^{-7}} = 1.592\times10^{6}s^{-1}$$

$$\bar{\nu} = \frac{c}{\Delta\nu} = \frac{3\times10^{8}m.s^{-1}}{1.592\times10^{6}s^{-1}} = 5.31\times10^{-3}m^{-1}$$

So we have looked at some special features of spectroscopy which is quite common to different kind of spectroscopy you will come across and now we are going to look at is some problems related to this, so first problem is, so suppose lifetime of an excited state is given, you need to calculate what is the natural line width of the transition from ground to excited state.

Just take this Heisenberg uncertainty principle, this equation from Heisenberg uncertainty principle that uncertainty in frequency multiplied by your delta t multiply by delta t, which is basically lifetime is equal to 1 by 2 pie.

Here lifetime is given that is 100 nanosecond and so it is quite easy to calculate what will be the uncertainty in frequency value and that is going to be 1 by 2 pie into delta t, delta t is 100 nanosecond and so 1 into 10 power minus 7 second and so you will get this much delta new, so this is your natural line width in frequency unit and you can calculate natural line width in wave number unit by simply dividing this c by delta new and this comes out to be 5.31 into 10 to the power minus 3 metre inverse.

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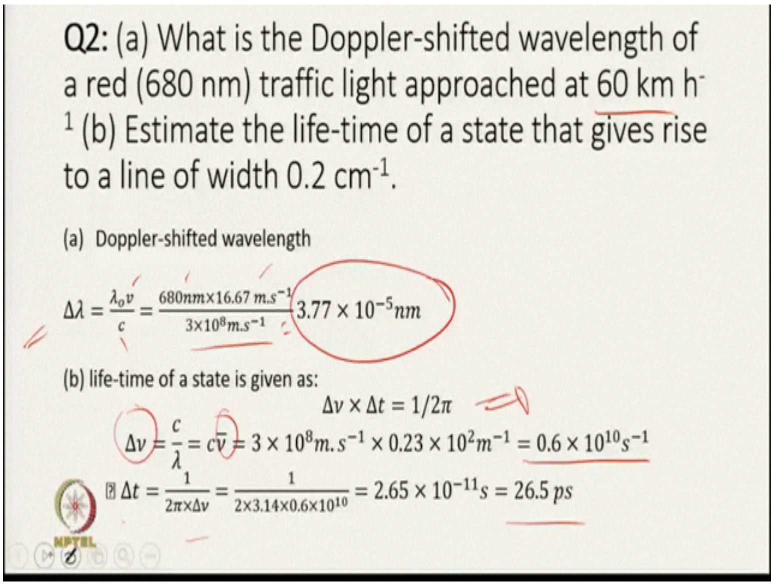

Q2: (a) What is the Doppler-shifted wavelength of a red (680 nm) traffic light approached at 60 km h^{-1} (b) Estimate the life-time of a state that gives rise to a line of width 0.2 cm^{-1}.

(a) Doppler-shifted wavelength

$$\Delta\lambda = \frac{\lambda_o v}{c} = \frac{680nm \times 16.67\ m.s^{-1}}{3\times10^{8} m.s^{-1}} = 3.77 \times 10^{-5} nm$$

(b) life-time of a state is given as:

$$\Delta\nu \times \Delta t = 1/2\pi$$

$$\Delta\nu = \frac{c}{\lambda} = c\bar{\nu} = 3 \times 10^{8} m.s^{-1} \times 0.23 \times 10^{2} m^{-1} = 0.6 \times 10^{10} s^{-1}$$

$$⍰\ \Delta t = \frac{1}{2\pi\times\Delta\nu} = \frac{1}{2\times3.14\times0.6\times10^{10}} = 2.65 \times 10^{-11} s = 26.5\ ps$$

Okay, next question is broadening based on Doppler shift, broadening off-peak based on Doppler shift, so what is the Doppler shifted wavelength of a red traffic light, which is basically wavelength 680 nm approached at 60 km/h and the second part is estimate the lifetime of a state that gives rise to a line width of 0.2 cm inverse, so your Doppler shifted wavelength can be calculated from the Doppler effect delta lambda is equal to lambda 0 into v by c, v is the velocity of source and c is velocity of light.

Velocity of sources given 60 km per hour and that is you can change in metre per second and that is comes out to be 16.67 meters per second multiplied by this wavelength and this is wavelength of your source when it is not moving, when your detector is not moving and this is your c velocity of light in metre per second and what you get is this much, so this is equal to this much nanometer, so this is your Doppler shift.

The second question is estimate the lifetime of state that gives rise to a line of width 0.2 cm inverse and it can be calculated similarly what we done question number one, so we use Heisenberg uncertainty principle, so delta new can be calculated and from that delta new bar can be calculated and that comes out to be this per second. Okay, so your delta t will be given by 1 x 2 pie into delta nu and then you can get the value of lifetime.

So line width this given in wave number unit, you need to calculate your line width in hertz or second inverse and then you put this into this equation Heisenberg uncertainty equation, that delta new into delta t is 1 by 2 pie, then you can get delta t which in this case is 26.5 picosecond.

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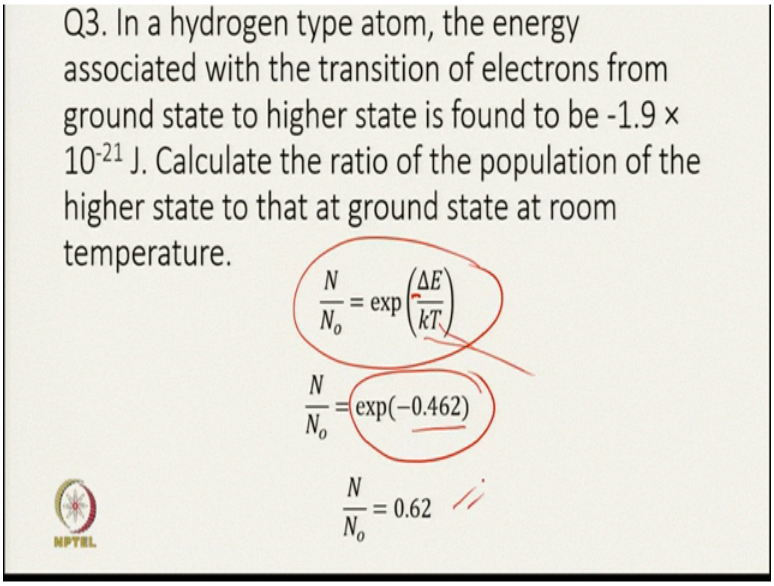

Q3. In a hydrogen type atom, the energy associated with the transition of electrons from ground state to higher state is found to be -1.9 × 10^{-21} J. Calculate the ratio of the population of the higher state to that at ground state at room temperature.

$$\frac{N}{N_0} = \exp\left(\frac{-\Delta E}{kT}\right)$$

$$\frac{N}{N_0} = \exp(-0.462)$$

$$\frac{N}{N_0} = 0.62 \; //$$

Now second question Delta E is given and then you need to calculate what will be the ratio of population at room temperature, ratio of population between higher state to that at ground state at room temperature and that can be calculated using your Boltzmann distribution law, which is given by this equation N by N not is equal to exponential minus delta E.

So just make this correction minus delta E by kT, where delta E is your difference between two energy levels, so basically you are looking at the difference between ground energy state and the higher excited state.

So this is your delta E and this is your T is temperature, which is in this case room temperature, so let us take 298K, K is Boltzmann constant here and what you are going to get is exponential minus 0.462, so you can calculate N by N2 which comes out to be 0.62.

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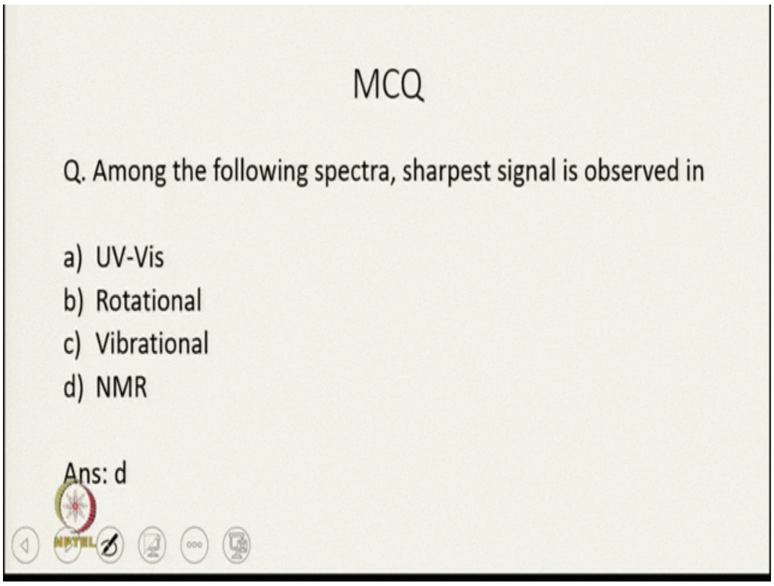

MCQ

Q. Among the following spectra, sharpest signal is observed in

a) UV-Vis
b) Rotational
c) Vibrational
d) NMR

Ans: d

So few multiple-choice questions among the following spectra, sharpest signal is observed in UV-Vis, rotational, vibrational and NMR, so again, you can go back and look at Heisenberg uncertainty principle, which tells you that if lifetime is high, then you can get sharpest signal and lifetime depends on the energy difference between two energy levels.

So if energy difference is high, then lifetime is going to be small because your molecule in excited state wants to come back to ground state very rapidly but when energy difference between two levels are small.

Then there is less tendency to come back to ground state, so lifetime is going to be high and so lifetime is going to depend on delta E value and delta E value is smallest for NMR out of all this four and so lifetime of excited state is going to be largest in place of NMR and so sharpest signal is obtained in NMR and thus NMR is known as high-resolution technique.

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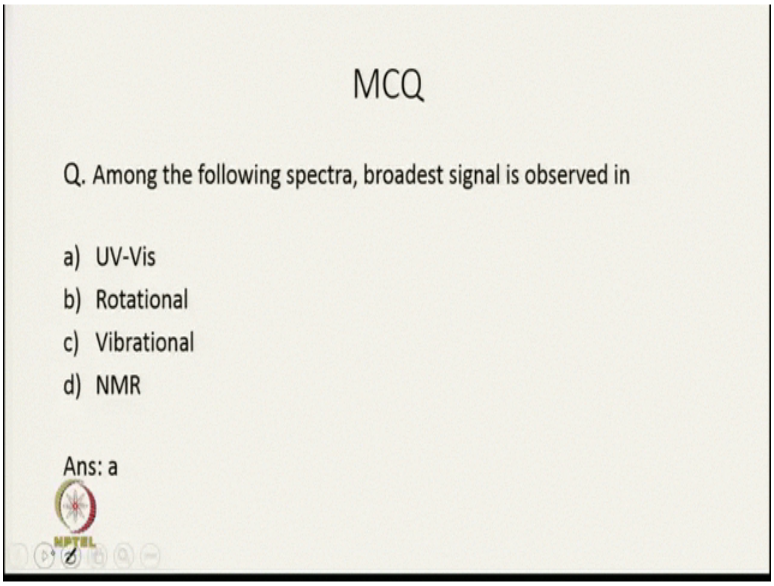

MCQ

Q. Among the following spectra, broadest signal is observed in

a) UV-Vis
b) Rotational
c) Vibrational
d) NMR

Ans: a

Among the following spectra, broadest signal is observed in again you have to look at the energy difference between different average levels corresponding to a particular spectra, so in UV-Vis that is a transition between electronic energy levels, where delta is very high, if delta is very high, then lifetime is going to be small and if lifetime is going to be small you are going to get a broad signal and so the broadest signal is obtained in UV-Vis spectra.

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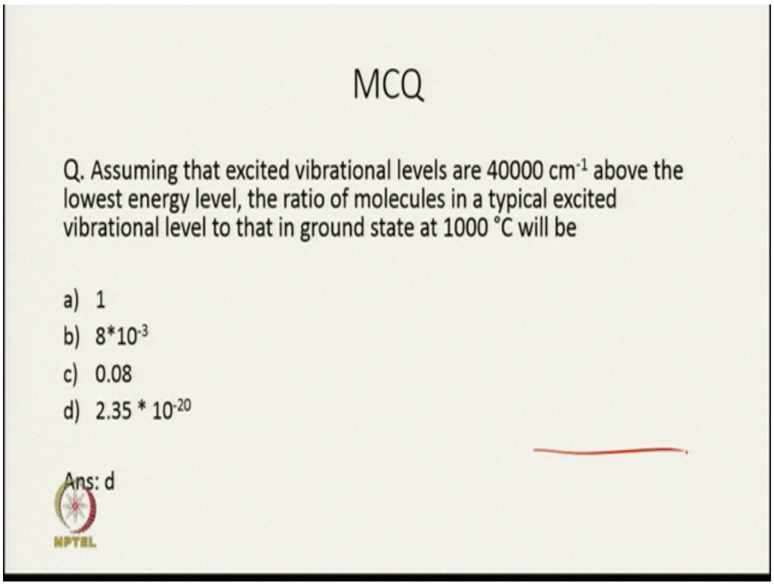

MCQ

Q. Assuming that excited vibrational levels are 40000 cm^{-1} above the lowest energy level, the ratio of molecules in a typical excited vibrational level to that in ground state at 1000 °C will be

a) 1
b) $8*10^{-3}$
c) 0.08
d) $2.35 * 10^{-20}$

Ans: d

Okay, next question is that, assume that excited, vibrational levels are 100 cm inverse above the lowest energy level, there ratio of molecules in typical excited vibrational level to that in ground state and 298K will be, so again we will apply your Boltzmann distribution law N by N not is equal to your exponential minus delta E by kT.

So delta E can be calculated, since we know that the wave number, since we know the wave number, so we can calculate delta E and delta E will be given by hc new bar and now here you must keep in mind that delta E should be expressed per molecule not per mole, if you are going to use Boltzmann constant.

If you are going to use R value, gas constant R value in place of Boltzmanns constant, then you must take delta E in joule per mole, otherwise you have to express delta in joule per molecule and if you do that you can get the value and the answer in this case is b which is 8 to 10 to the power minus 3.

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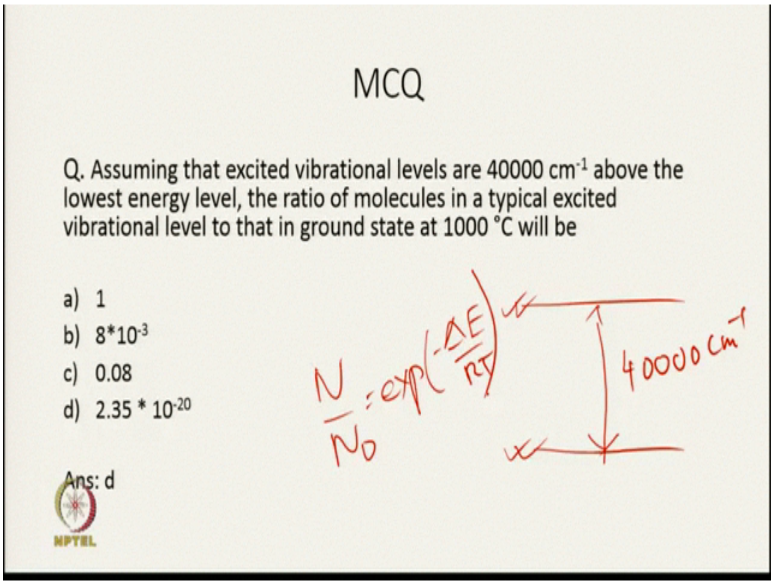

MCQ

Q. Assuming that excited vibrational levels are 40000 cm^{-1} above the lowest energy level, the ratio of molecules in a typical excited vibrational level to that in ground state at 1000 °C will be

a) 1
b) $8*10^{-3}$
c) 0.08
d) $2.35 * 10^{-20}$

Ans: d

$\frac{N}{N_0} = exp\left(-\frac{\Delta E}{RT}\right)$

In the next question one energy level is given, second energy level and this value is given N wave number, so this is 40,000 cm inverse and now again you have to find out ratio of molecules in typical excited vibrational level to that in the ground state.

So ratio of molecule in this excited level divided by the molecule in this ground level, so again we will calculate by same formula, your Boltzmann distribution law N by N not is equal to exponential minus delta E by kT, your temperature is 1000 degree Celsius, you must be to convert this in Calvin and when you do that you will get the answer is 2.35 into 10 to the power minus 20.

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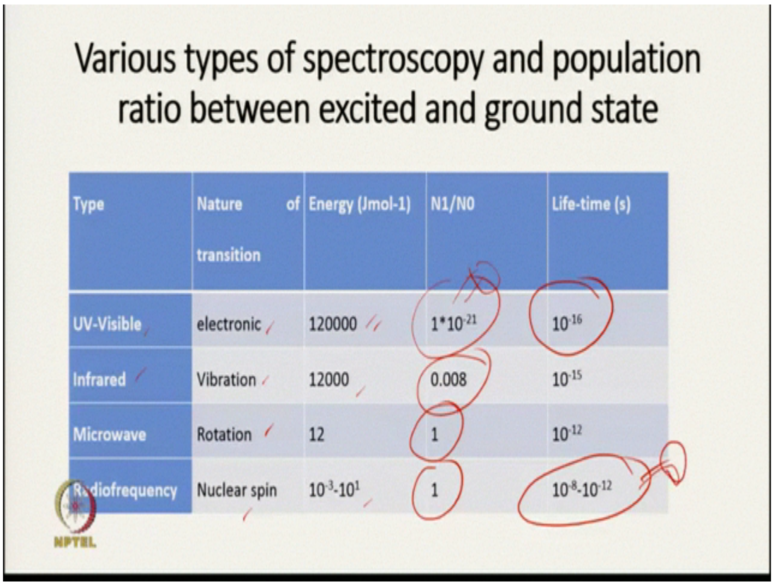

Various types of spectroscopy and population ratio between excited and ground state

Type	Nature of transition	Energy (Jmol-1)	N1/N0	Life-time (s)
UV-Visible	electronic	120000	$1*10^{-21}$	10^{-16}
Infrared	Vibration	12000	0.008	10^{-15}
Microwave	Rotation	12	1	10^{-12}
Radiofrequency	Nuclear spin	10^{-3}-10^{1}	1	10^{-8}-10^{-12}

So just to summarize various types of spectroscopy and population ratio between excited and ground state is given and lifetime is also given, lifetime will tell you about your broadening and N by N0 or ratio between excited and ground state will tell you about intensity.

So these are the various spectroscopy UV-Vis, IR, microwave, a radio-frequency, the nature of transition is electronic in UV-Vis, vibration in infrared, rotation in microwave, nuclear spin in radio-frequency and the energy of transition is this 120,000, 12,000 in infrared, 12 in microwave and 10 to the power minus 3 to 10 to the power 1 and if you try to calculate the ratio between excited and ground state, population ratio between excited and ground state at room temperature this is going to be this value.

So now can see in NMR almost your excited and ground state are poorly populated, almost there are equally populated, lifetime is lowest in the UV-Vis and highest in the NMR and so intensity is going to be highest in your UV-Vis spectroscopy, whereas the solution is going to be highest in NMR.

So UV-Vis spectroscopy is low resolution technique, whereas NMR is high-resolution technique but NMR is low sensitive technique, it means signal to noise ratio in NMR is going to be low and to get higher signal to noise ratio in NMR, you need to take more scans and generally data is collected in time domain model and then Fourier transformation is done, so thank you for listening and see you.