

GPAT Online Class for B.Pharm Students



Ananthapuramu Local Branch

Pharmaceutical Analysis – Part 2 (2nd July 2020)

By

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Pharmaceutical Analysis – Part 2 UV-Visible Spectroscopy : Woodward rules & IR Spectroscopy

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Woodward- Feiser Rules



Woodward rules

These are several sets of empirically derived rules which attempt to predict the wavelength of the absorption maximum (λ_{max}) in an ultraviolet–visible spectrum of a given compound.

Its applied to,

- **Conjugated dienes**
- **Alpha-beta unsaturated carbonyl compounds,**
- **Substituted benzoyl compounds**
- **Polyenes**



- Rules developed by Woodward in 1941 then expanded by Feiser in 1948.
- Woodward rules work well for conjugated dienes and polyenes with up to 4-double bonds or less.



■ In 1959 Fieser modified these rules with more experimental data, and the modified rule is known as **Woodward- Fieser** Rules.

■ The Fieser rule must be applied for certain plants pigments such as carotenoids with even more than 4 conjugated double bonds.

■ This may differ from observed values by 5-6.5 nm



According to Woodward's rules the λ_{\max} of the molecule can be calculated using a formula:

$$\lambda_{\max} = \text{Base value} + \Sigma \text{Substituent Contributions} + \Sigma \text{Other Contributions}$$

Here the base value depends upon whether the diene is a heteroannular or homoannular diene or cyclic or acyclic.



Class of Compounds that suitable for Woodward rules

Conditions: Structure should possess conjugation

1. 1,3 butadiene
2. α , β - unsaturated carbonyl compounds
3. Benzoyl compounds
4. Polyenes



λ max for 1,3-butadiene

Simplified formula for remembrance

Absorption maxima = $B + A + E + R + S$

B - Basic value

A - Additional conjugation

E - Exocyclic double bond

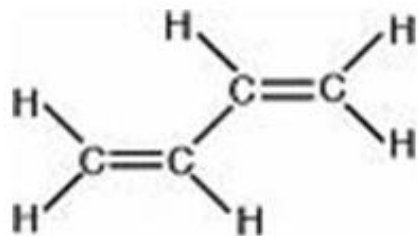
R - Ring residue

S - Substitution on double bonded carbon only



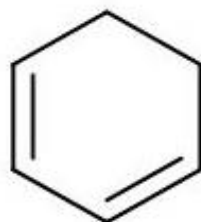
Acyclic dienes: 1,3-Butadiene with the structural formula

Basic Value

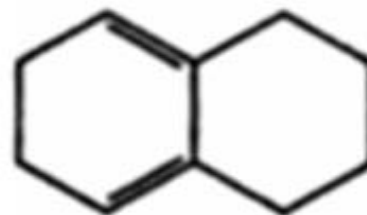


215 nm

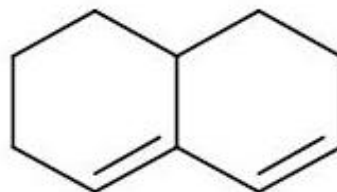
Homo-annular conjugated dienes: Both conjugated double bonds are in same ring



253 nm



Hetero-annular dienes: Conjugated double bonds are not present in same ring



217 nm

Values to be used in calculations

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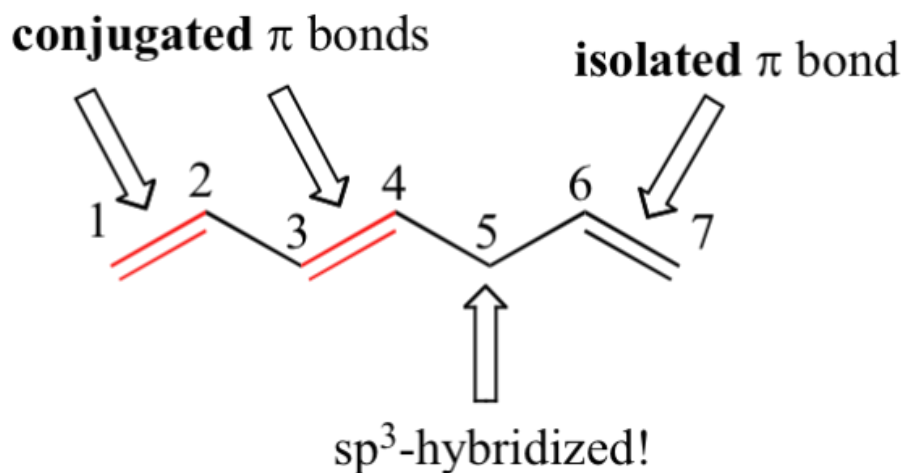
Descriptors	Value to be considered in calculation (in nm)
Homoannular (cisoid) diene	253 nm
Heteroannular (transoid) diene	217 nm
Acyclic diene	215 nm
Double bond extending conjugation	30 nm
Alkyl substituent or ring residue	5 nm
Exocyclic double bond	5 nm
-OC(O)CH ₃	0 nm
-OR	6 nm
-Cl, -Br	5 nm
-NR ₂	60 nm
-SR	30 nm
Phenyl ring	75 nm



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Additional Conjugation

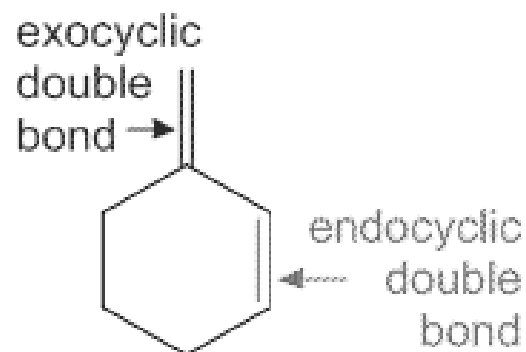
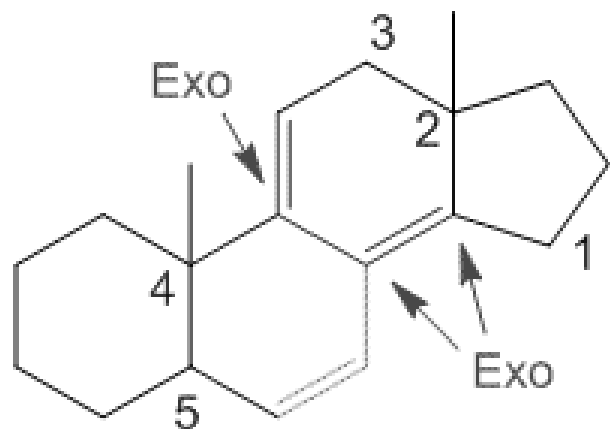
Bonds in alternative position are considered to be extending conjugation. In the below structure the bond between **C⁶** and **C⁷** is isolated from diene, so it cannot be considered as extending conjugation or addition conjugation. **Thus it has 0 (zero) value in calculation.**



In the below structure, there is a ONE extended or additional conjugation to diene. So (1 × 30 nm) 30 nm has to be added in the calculation.



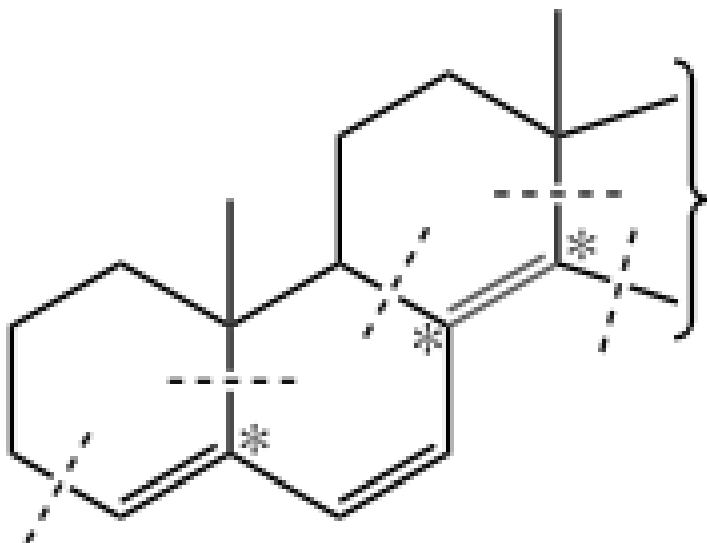
Exocyclic Double Bond



The above structure has both **Heteroannular** diene and **Homoannular** diene. But basic value has **to** be based on **Homoannular** diene.

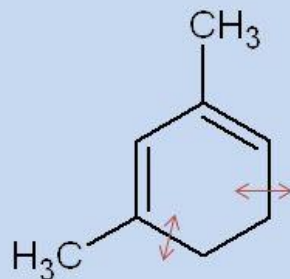
The exocyclic double bond has to be calculated based on the position, thus above structure has **THREE** exocyclic double bond
($3 \times 5 = 15$ nm)

Ring Residue

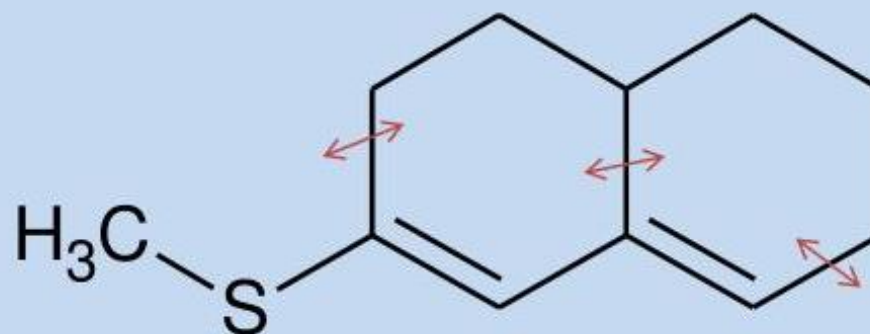


It is the number of bond cleavage to be done on the ring structure to isolate the entire conjugated chain, thus above structure has five ring residue ($5 \times 5 = 25$ nm) and 25 nm has to added in the calculation

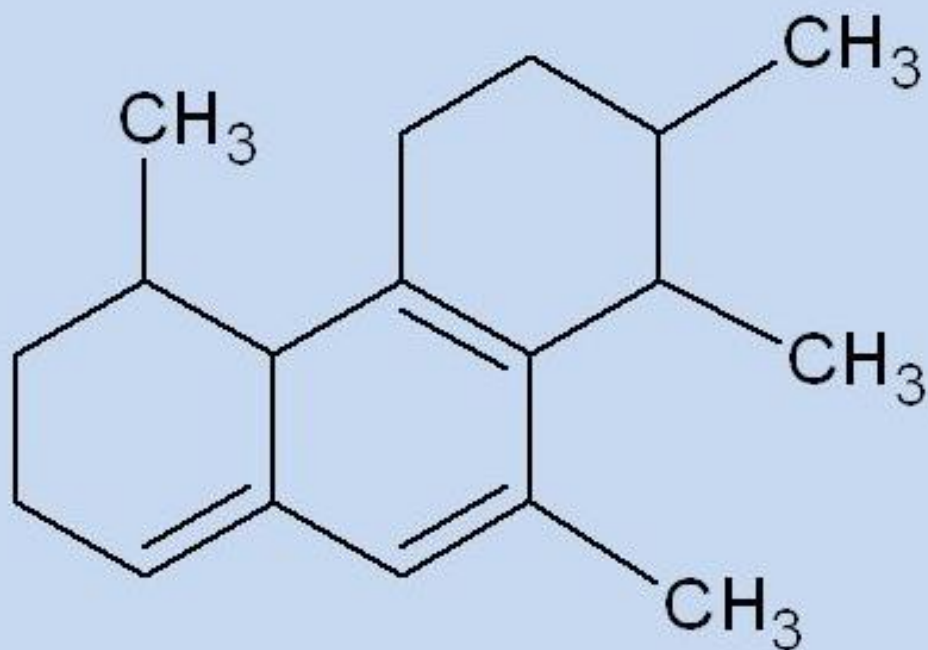
2. Calculate λ_{max} for 1,4- dimethylcyclohex-1,3-diene



- | | |
|---------------------------------------|---------------|
| • Parent value for homoannular ring : | = 253 nm |
| • Two alkyl substituents : | 2 x 5 = 10 nm |
| • Two ring residue : | 2 x 5 = 10 nm |
| <hr/> | |
| • calculated value : | = 273 nm |
| • observed value : | = 263 nm |

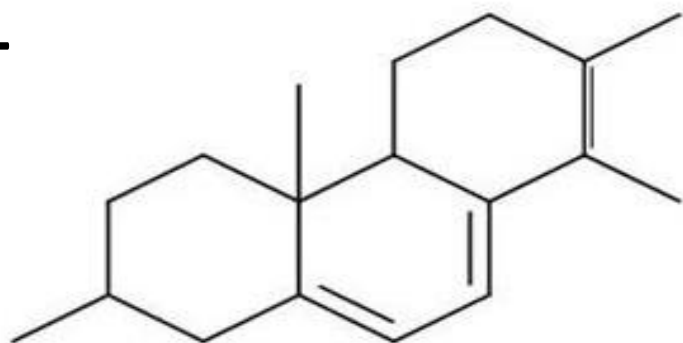


- Parent value for heteroannular diene : = 214 nm
- 3 ring residue : 3 X 5 = 15 nm
- Exocyclic double bond = 5 nm
- Thiomethyl substituent = 30 nm
- Calculated value : = 264 nm
- Observed value : = 268 nm



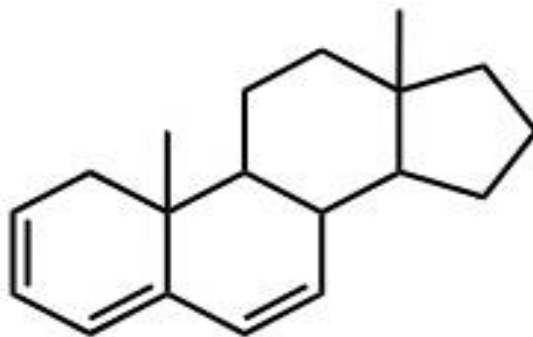
Parent value for homoannular (the highest value) :		= 253 nm
Extended conjugation	1 X30	= 30 nm
Exocyclic double bond		=5 nm
Alkyl substitution or ring residue	6x5	=30 nm
Calculated value :		= 318 nm

Example 1



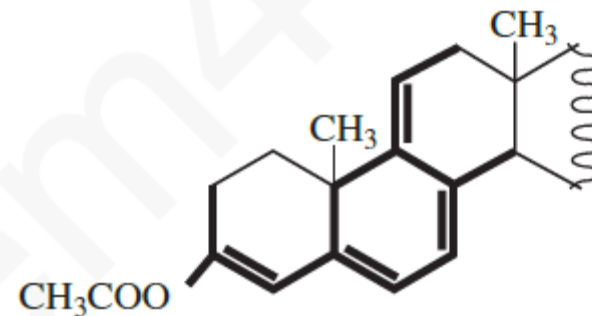
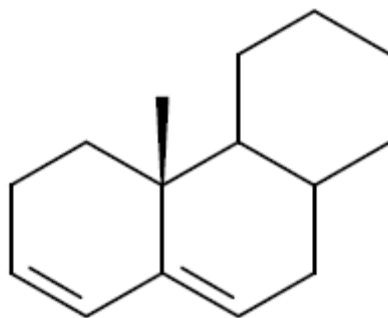
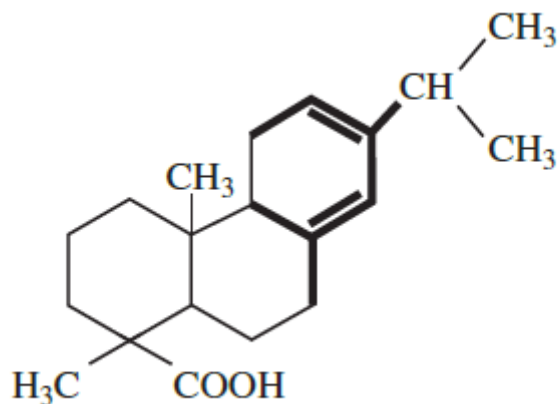
Formula	Value	Final value
B	253	253nm
A	0	0nm
E	2x5nm	10nm
R	4x5nm	20nm
S	0	0nm
Total		283nm

Example 2



Formula	Value	Final value
B	253	253nm
A	1x30nm	30nm
E	1x5nm	5nm
R	4x5nm	20nm
S	0	0nm
Total		303nm

Problems

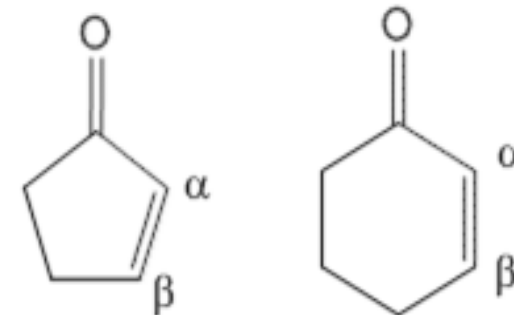


Calculate the λ_{\max} by using Woodward- Fieser rule for above structures

Unsaturated Carbonyl System

Basic value for Cyclic Unsaturated Carbonyl System

Base value:

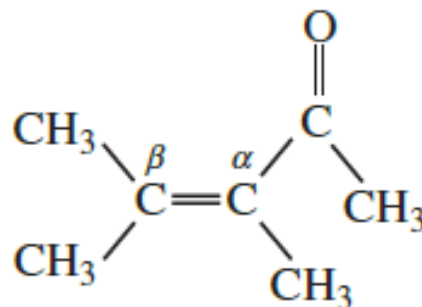


(a)	Acyclic α , β unsaturated ketones	=	214 nm
(b)	6 membered cyclic α , β unsaturated ketones	=	215 nm
(c)	5 membered cyclic α , β unsaturated ketones	=	202 nm
(d)	α , β unsaturated aldehydes	=	210 nm
(e)	α , β unsaturated carboxylic acids & esters	=	195 nm
(f)	Homoannular diene	=	39 nm

Auxochrome	Alpha	Beta	Gamma	delta
OH	35	30	--	50
OR (-OCH ₃)	35	30	17	31
Br	25	30	--	--
Cl	15	12	--	--
CH ₃ or Any alkyl group	10	12	18	18
Ring residue	10	12	18	18
-OCOR	6	6	6	6
NR ₂	--	95	--	--

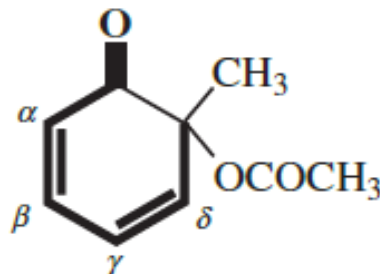


Example 1



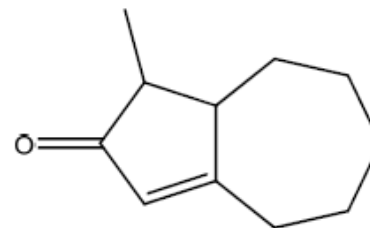
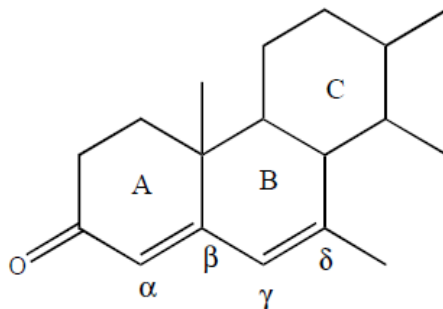
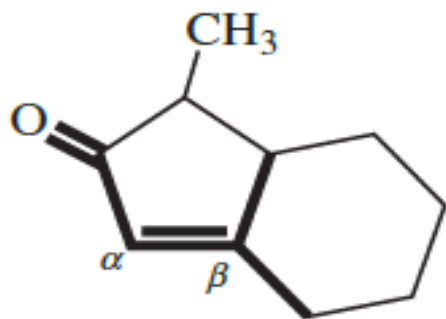
Formula	Value	Final value
B	214	214nm
A	0	0nm
E	0	0nm
R	0	0nm
S	$\alpha = 10\text{nm}$ $\beta = 2 \times 12 = 24\text{nm}$	34nm
Total		248nm

Example 2



Formula	Value	Final value
B	215	215nm
A	1x30nm	30nm
E	1x39nm	39nm
R	1x18nm	18nm
S	0	0nm
Total		302nm

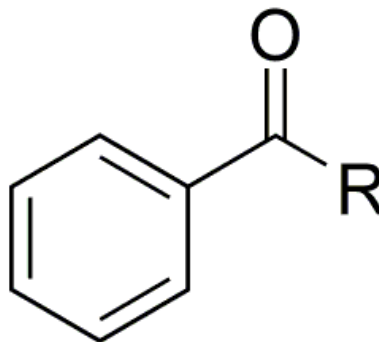
Problems



Calculate the λ_{\max} by using Woodward- Fieser rule for above structures

λ max Calculation for Aromatic (benzoyl system) compounds

$$\lambda_{\max} = B + S$$



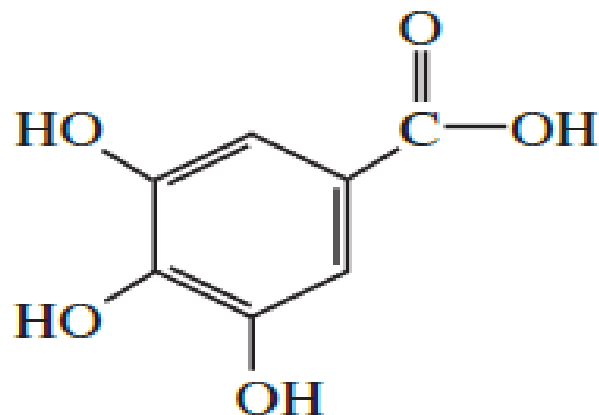
λ_{\max} = Basic value + Substitution effect (ortho or meta or para)
Basic values:

- | | | |
|--------------------------------------|---|--------|
| • (a) Ar -COR (ketone) | = | 246 nm |
| • (b) Ar -CHO (Aldehyde) | = | 250 nm |
| • (c) Ar -CO ₂ H (Acid) | = | 230 nm |
| • (d) Ar -CO ₂ R (Ester) | = | 230 nm |

Auxochrome	ortho	meta	para
-NR ₂ (amine)	20	20	85
-NH ₂	13	13	58
-NHCH ₃	-	-	73
-NHCOCH ₃	20	20	45
-OH , -OR	7	7	25
Aliphatic (- R)	3	3	10
-Br	2	2	15
-Cl	0	0	10
-O ⁺ (oxonium)	11	20	78

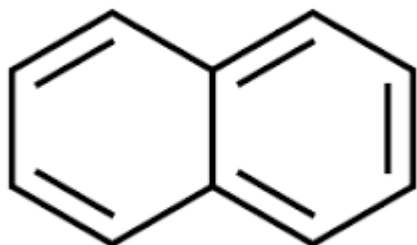


Example 1



Formula	Value	Final value
B	230	230nm
S	m- 2x7= 14nm p- 1x25nm	39nm
Total		269nm

Problems



Calculate the λ_{max} by using Woodward- Fieser rule for above structures ?

Infra-Red Spectroscopy

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- **Infrared spectroscopy** (IR spectroscopy) is a type of absorption spectroscopy based on absorption of **Middle** infrared region (**MID**-IR radiation) of the electromagnetic spectrum.
- MID-IR is absorbed by the vibrating bonds in the molecule (dipole moment should not be Zero).
- **It is used to identify** the chemical **compound** based on functional groups, **bonds**, isomerism (except optical isomerism) and chemicals **derivatives**.

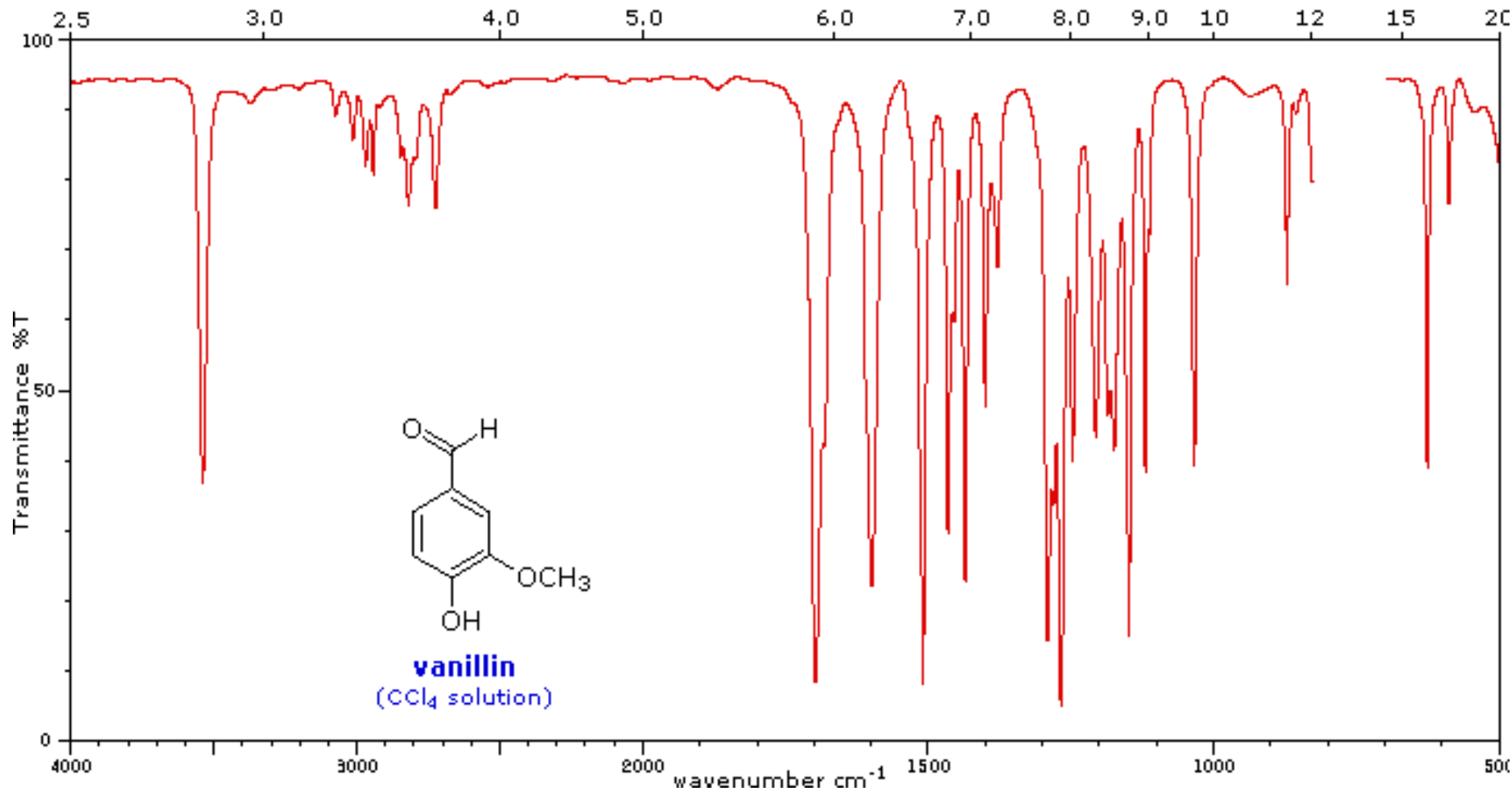


- A basic IR spectrum is based on infrared light absorbance (or % transmittance) on the vertical Y-axis Vs frequency (Hz) or wavelength (micron) or wave number (Cm^{-1}) on the horizontal X-axis.
- More commonly, wave number (reciprocal centimeters of wavelength) is used in interpretation of spectra.
- A common laboratory instrument in present day is a Fourier transform infrared (FTIR) spectrometer.



IR Spectrum

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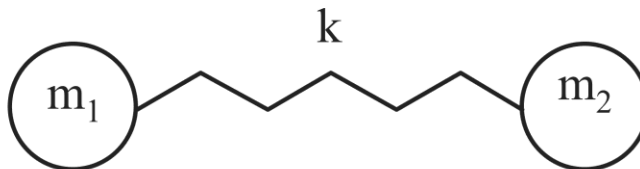
Different units and regions of IR radiation and their effect on organic molecules

Region	Frequency	Effect on molecule
Near IR	14000–4000 cm^{-1} (0.8–2.5 μm)	The higher-energy near-IR, approximately can excite overtone or harmonic vibrations
Middle IR	4000–400 cm^{-1} (2.5–25 μm)	The mid-infrared approximately may be used to study the fundamental vibrations and associated rotational-vibrational structure. (organic and pharmaceuticals)
Far IR	400–10 cm^{-1} (25–1000 μm),	The far-infrared, approximately lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy



Molecular Vibration and IR Absorption: Hooke's Law

Every bond in the molecule is under vibration, due to **variety of reasons**. If, m_1 and m_2 are mass of the atom and K is the force constant, the vibration of bond can be calculated using **Hooke's law**



Fundamental
frequency of the bond
(natural) =

$$\bar{\nu} = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}$$

$\bar{\nu}$ = frequency

$$\mu = \frac{m_1 \cdot m_2}{m_1 + m_2}$$

μ = reduced mass

NOTE: For a molecule to be "IR active (absorbs IR radiation), it must be associated with changes in the dipole, remember that permanent dipole is not necessary, but requires only a change in dipole moment.

- Simple diatomic molecules have only one bond and only one Vibrational band.
- If the molecule is symmetrical, e.g. N_2 , H_2 , Cl_2 , etc, the band is not observed in the IR spectrum but observed only in the Raman spectrum.
- Asymmetrical diatomic molecules, e.g. CO , absorb in the IR spectrum.



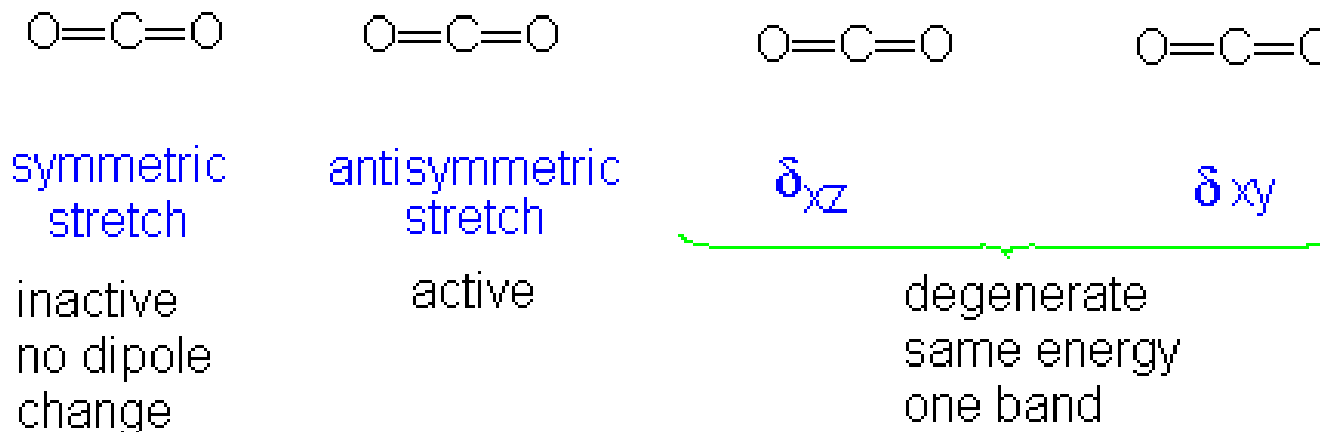
Number of vibrations in a molecule

A molecule can vibrate in several ways (called *vibrational mode*). If, N is the number of atoms in a molecule,

- Linear molecules will have $3N - 5$ degrees of Vibrational modes.
- Nonlinear molecules will have $3N - 6$ degrees of Vibrational modes

As an example H_2O , a non-linear molecule, will have $3 \times 3 - 6 = 3$ degrees of Vibrational freedom, or modes.



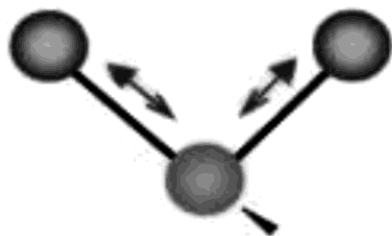


In carbon dioxide, bond under symmetrical stretching bond (dipole moment = 0) does not absorb IR frequency, but it scatter the IR frequency with Raman shift.

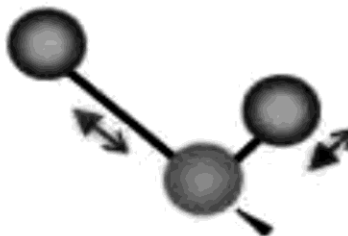
Thus Raman Spectroscopy is the complimentary technique to IR spectroscopy

Types of vibrations in an organic molecules.

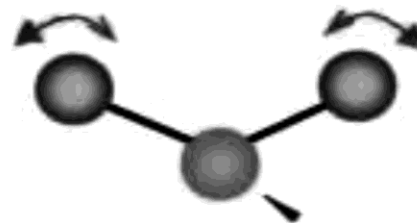
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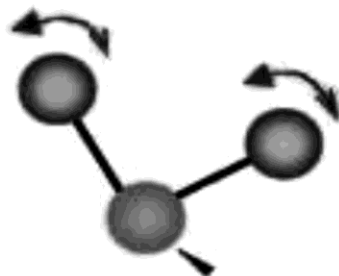
Symmetric stretching



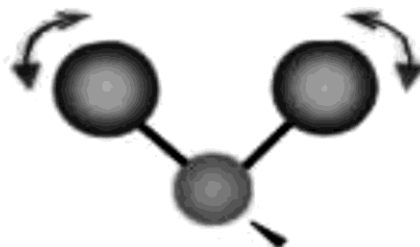
Antisymmetric stretching



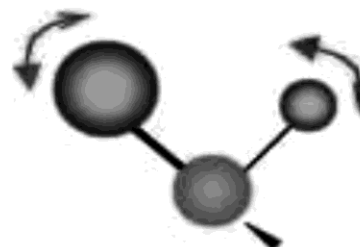
In-plane scissoring



In-plane rocking



Out-of-plane wagging



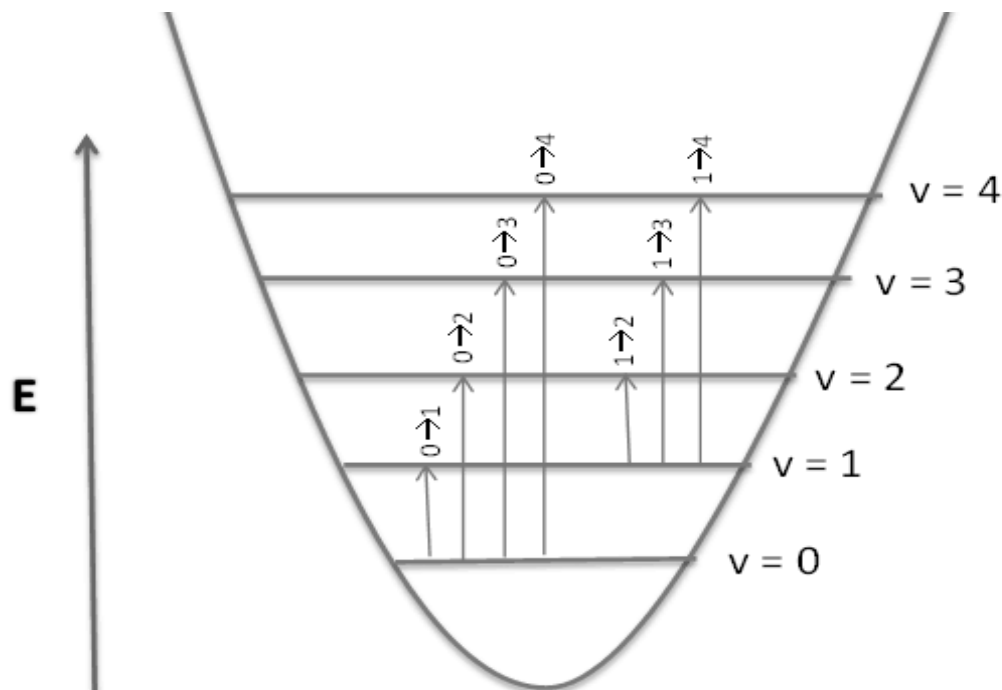
Out-of-plane twisting

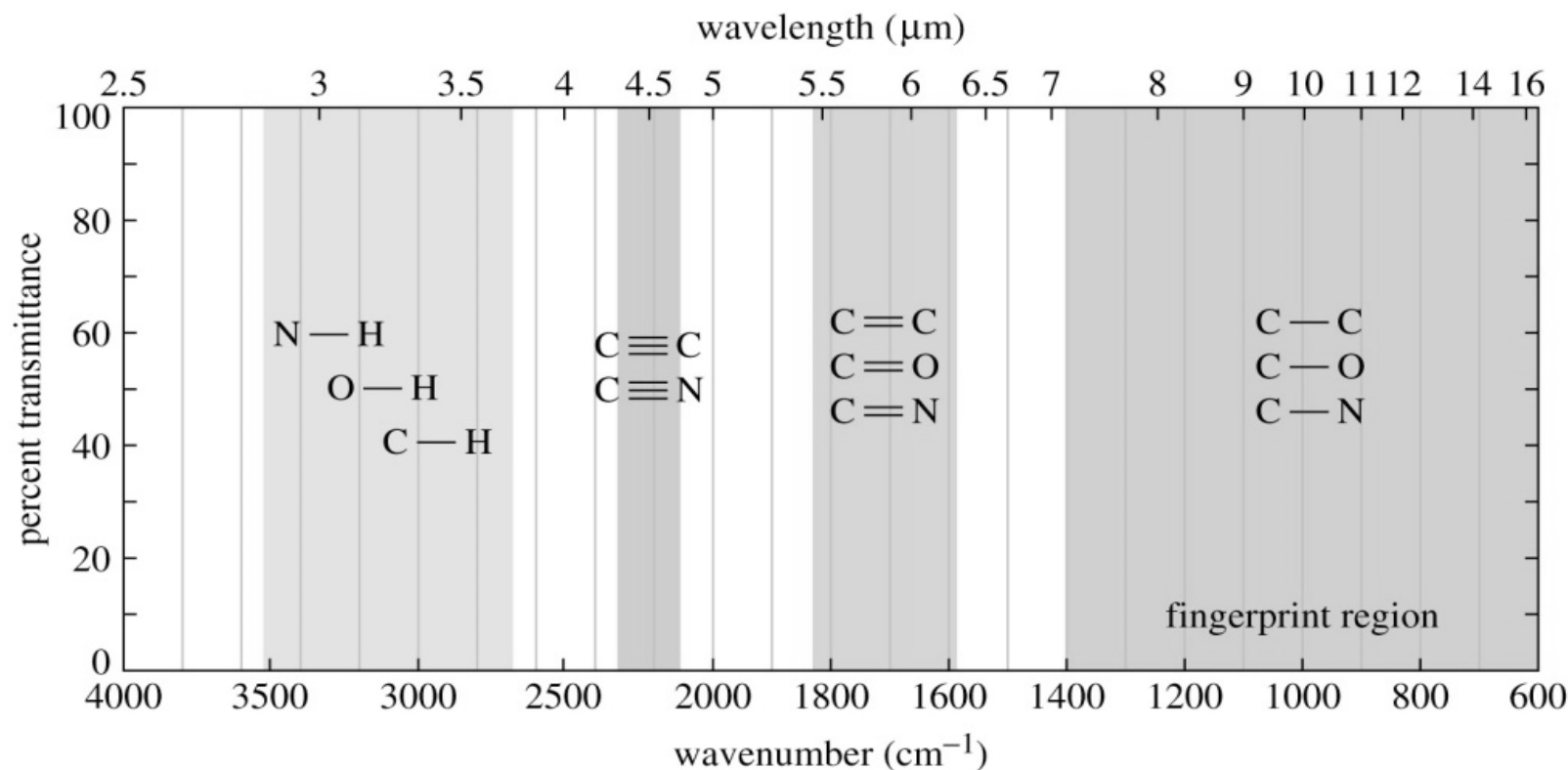
IR absorption by a vibrating bond

The irradiated IR radiation is absorbed by the bond when fundamental frequency is equal to the applied IR frequency, as a result the change vibration energy takes place (V_0 to V_1).

In Overtone is due to

V_0 to V_2 or V_3 .



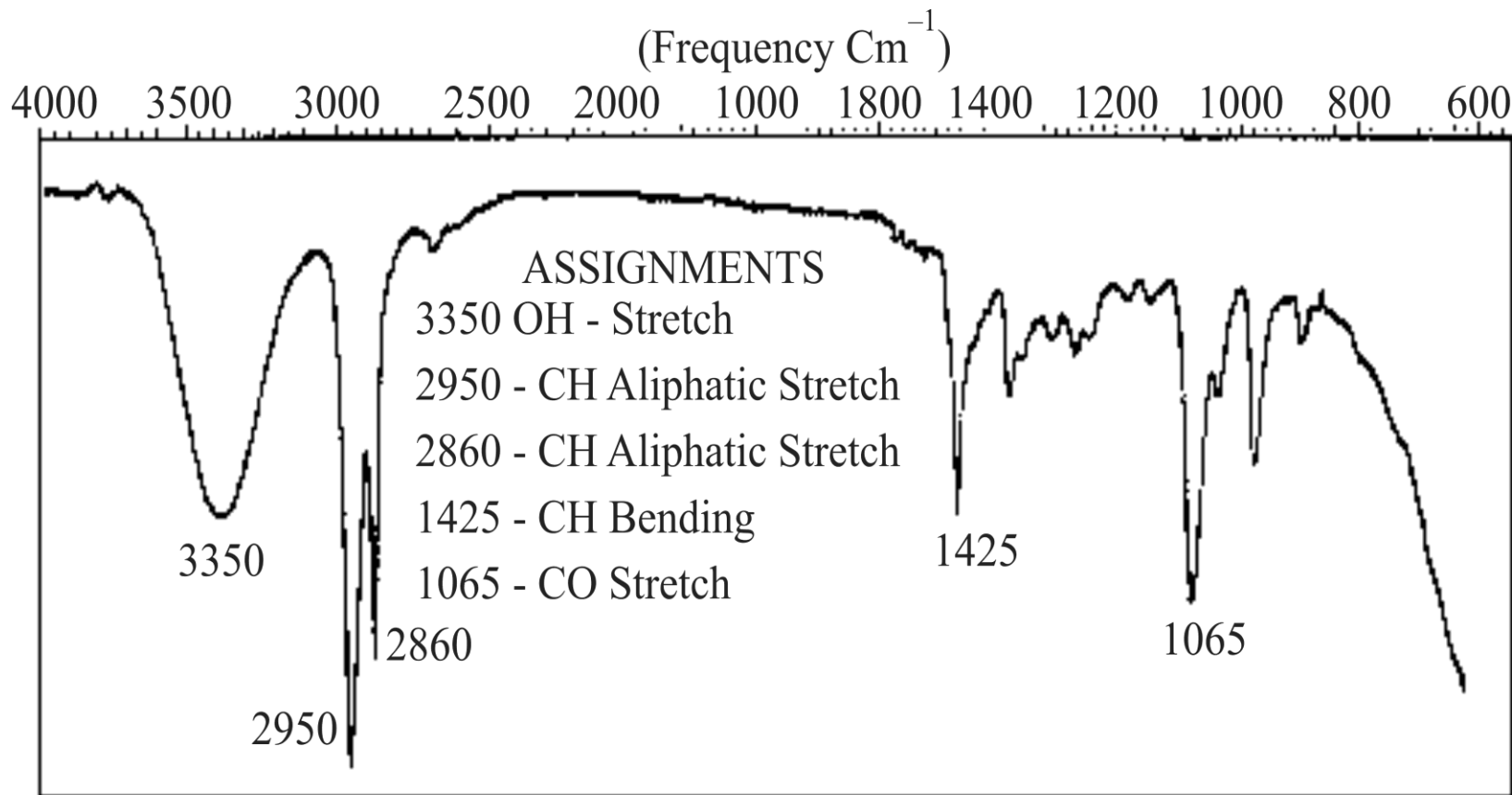


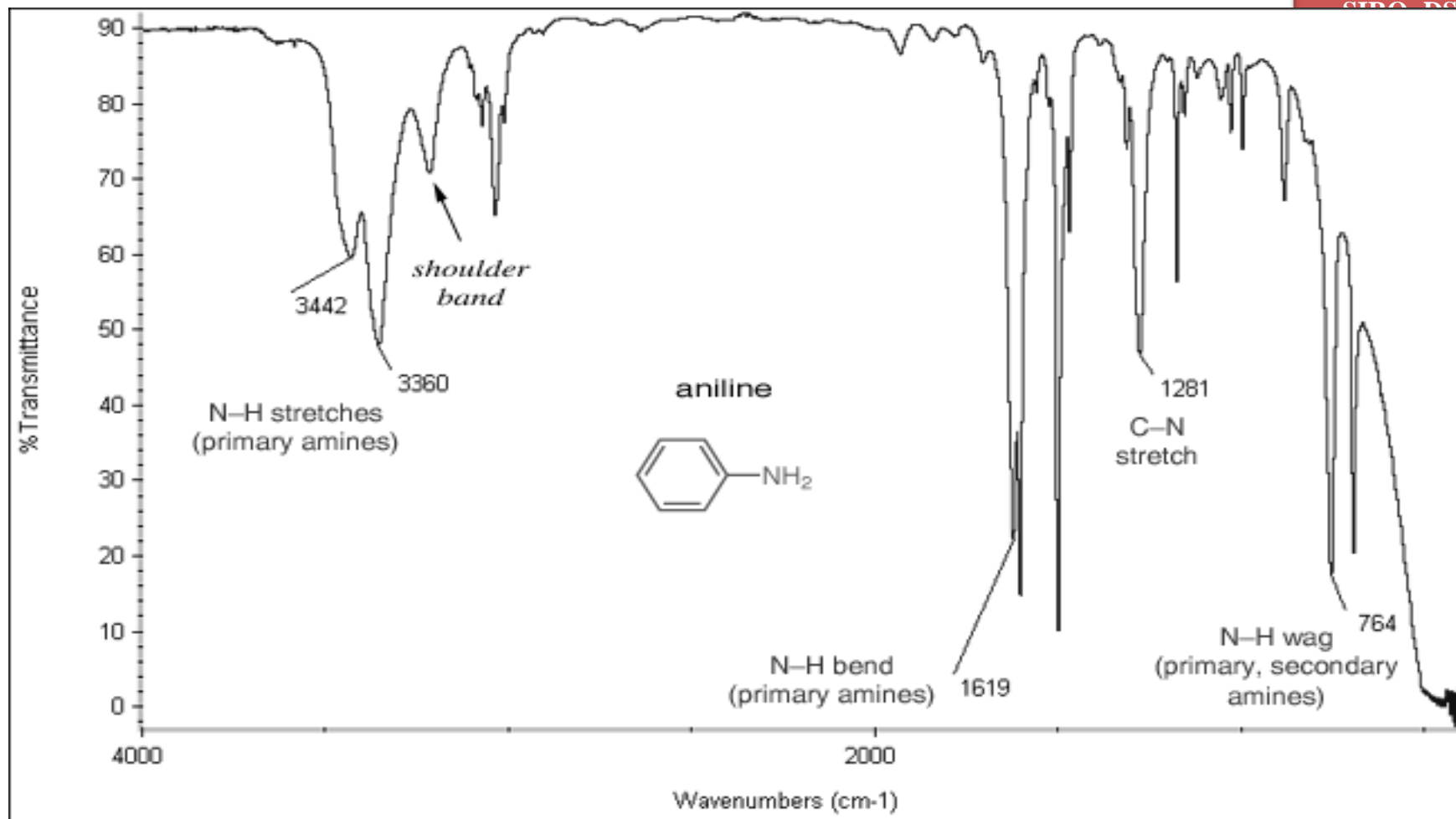
Group frequency region (4000-1100 cm⁻¹) and finger print region (1100-400 cm⁻¹).

Finger print region is very sensitive for small change in the structure, so it can be used for identification and authentication.

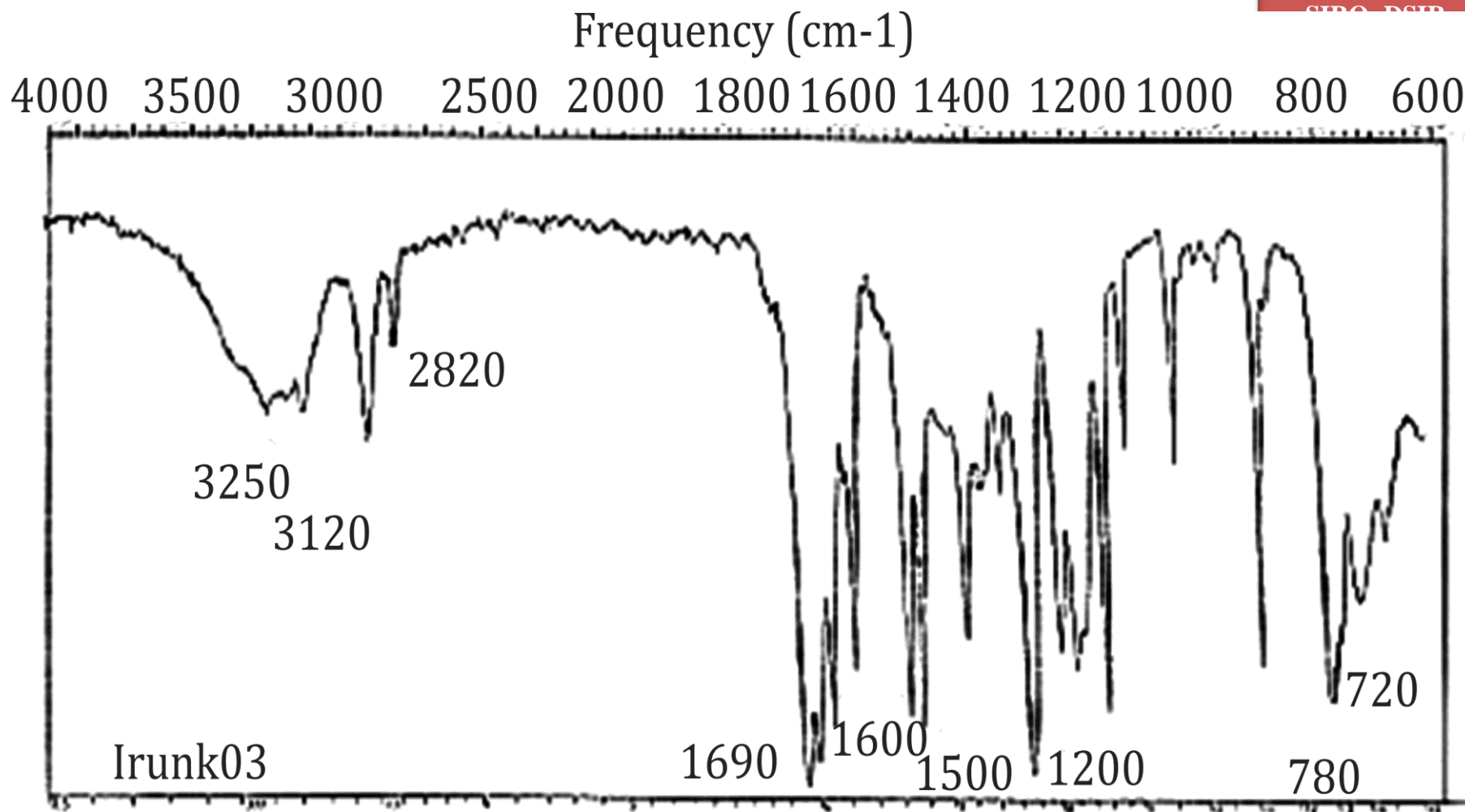
$$v = \frac{1}{2\pi} \sqrt{\frac{k}{m}}$$

Group	Cm-1 (str)	Cm-1 bend	Group	Cm-1 Str	Group	Cm-1
O-H	3600	1100	C=O	1700	C-O	1200
N-H	3300	1200	C=N	1600	C-N	1100
C-H (Ar)	3000	1400	C=C	1500	C-S	1000
C-H (Ali)	2900	1400	C=S	1500	C-X	800-1000
C-H(Ald)	2800	1400	N=O	1400	Phenyl	800-600
			S=O	1300		

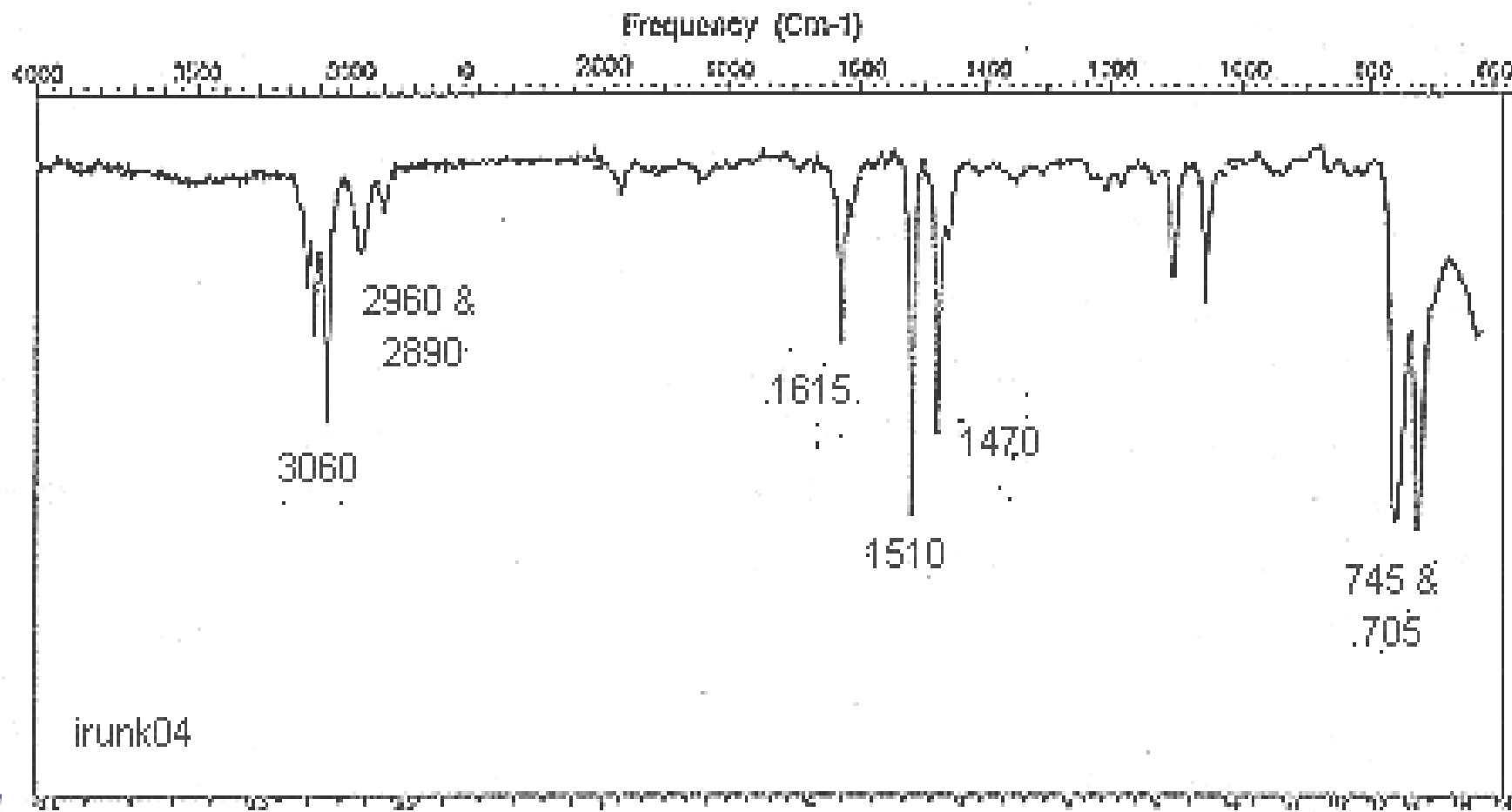




Identify the functional group in the given IR-Spectrum



Identify the functional group in the given IR-Spectrum



Factors affecting the Vibrational frequency of the bond

- Coupled vibration (AX₂ system)
- Electronic effect
- Overtone
- Fermi-Resonance
- Resonance – Electronic factors
- Hydrogen bonding

COUPLED VIBRATIONS

- An isolated C-H bond has only one stretching vibrational frequency where as methylene group shows two stretching vibrations, symmetrical and asymmetrical.
- Because of mechanical coupling or interaction between C-H stretching vibrations in the CH₂ group.
- Assymetric vibrations occur at higher frequencies or wave numbers than symmetric stretching vibrations.
- These are known as coupled vibrations because these vibrations occur at different frequencies than that required for an isolated C-H stretching.
- A strong vibrational coupling is present in carboxylic acid anhydrides in which symmetrical and asymmetrical stretching vibrations appear in the region 1720 – 1825 cm⁻¹.



Electronic effect

- Changes in the absorption frequencies for a particular group take place when the substituents in the neighbourhood of that particular group are changed.

It includes :

- Inductive effect
- Mesomeric effect
- Field effect



INDUCTIVE EFFECT

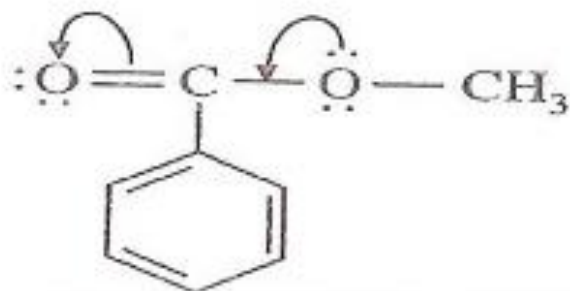
- The introduction of alkyl group causes +I effect which results in the lengthening or the weakening of the bond
- Hence the force constant is lowered and wave number of absorption decreases.
- Let us compare the wave numbers of ν (C=O) absorptions for the following compounds :

• Formaldehyde (HCHO)	1750 cm^{-1} .
• Acetaldehyde (CH ₃ CHO)	1745 cm^{-1} .
• Acetone (CH ₃ COCH ₃)	1715 cm^{-1} .
- Introduction of an electronegative atom or group causes -I effect which results in the bond order to increase.
- Hence the force constant increases and the wave number of absorption rises.

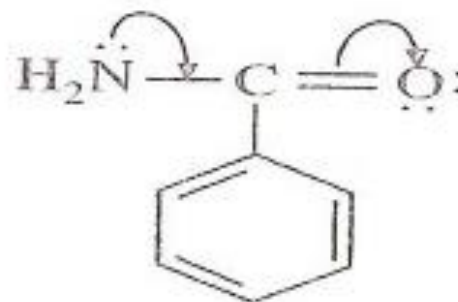


Mesomeric effect :

- It causes lengthening or the weakening of a bond leading in the lowering of absorption frequency.



Methyl benzoate
 $\nu \text{ C}=\text{O} \text{ } 1730 \text{ cm}^{-1}$

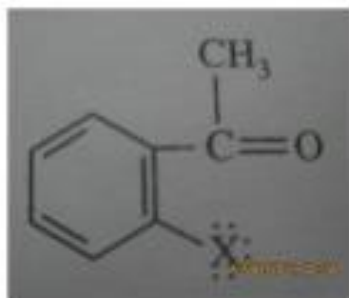


Benzamide
 $\nu \text{ C}=\text{O} \text{ } 1693 \text{ cm}^{-1}$

- As nitrogen atom is less electronegative than oxygen atom, the electron pair on nitrogen atom in amide is more labile and participates more in conjugation.
- Due to this greater degree of conjugation, the $\text{C}=\text{O}$ absorption frequency is much less in amides as compared to that in esters.

Field effect :

- In ortho substituted compounds, the lone pair of electrons on two atoms influence each other through space interactions and change the vibrational frequencies of both the groups.
- This effect is called field effect.



Ortho halo acetophenone

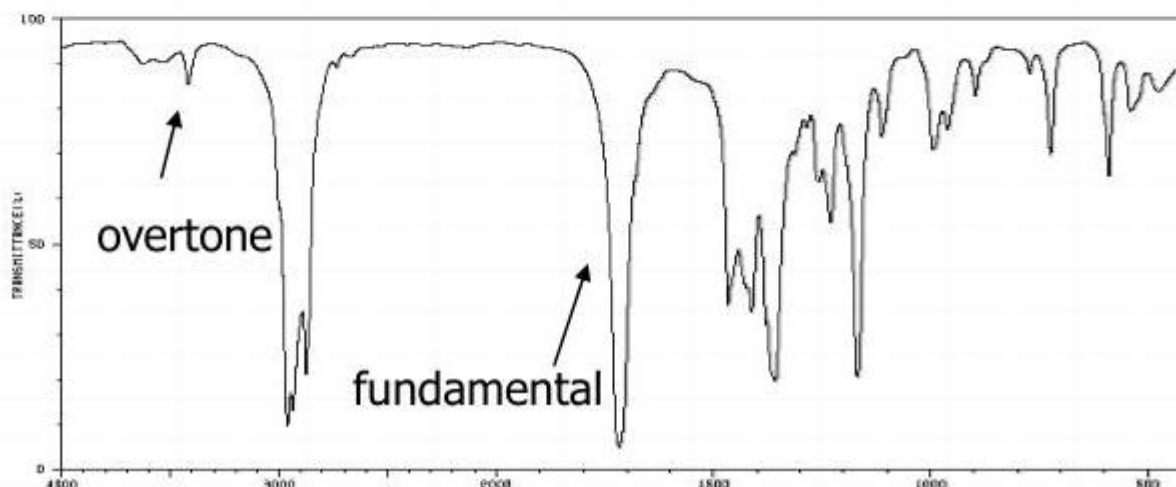
FERMI RESONANCE

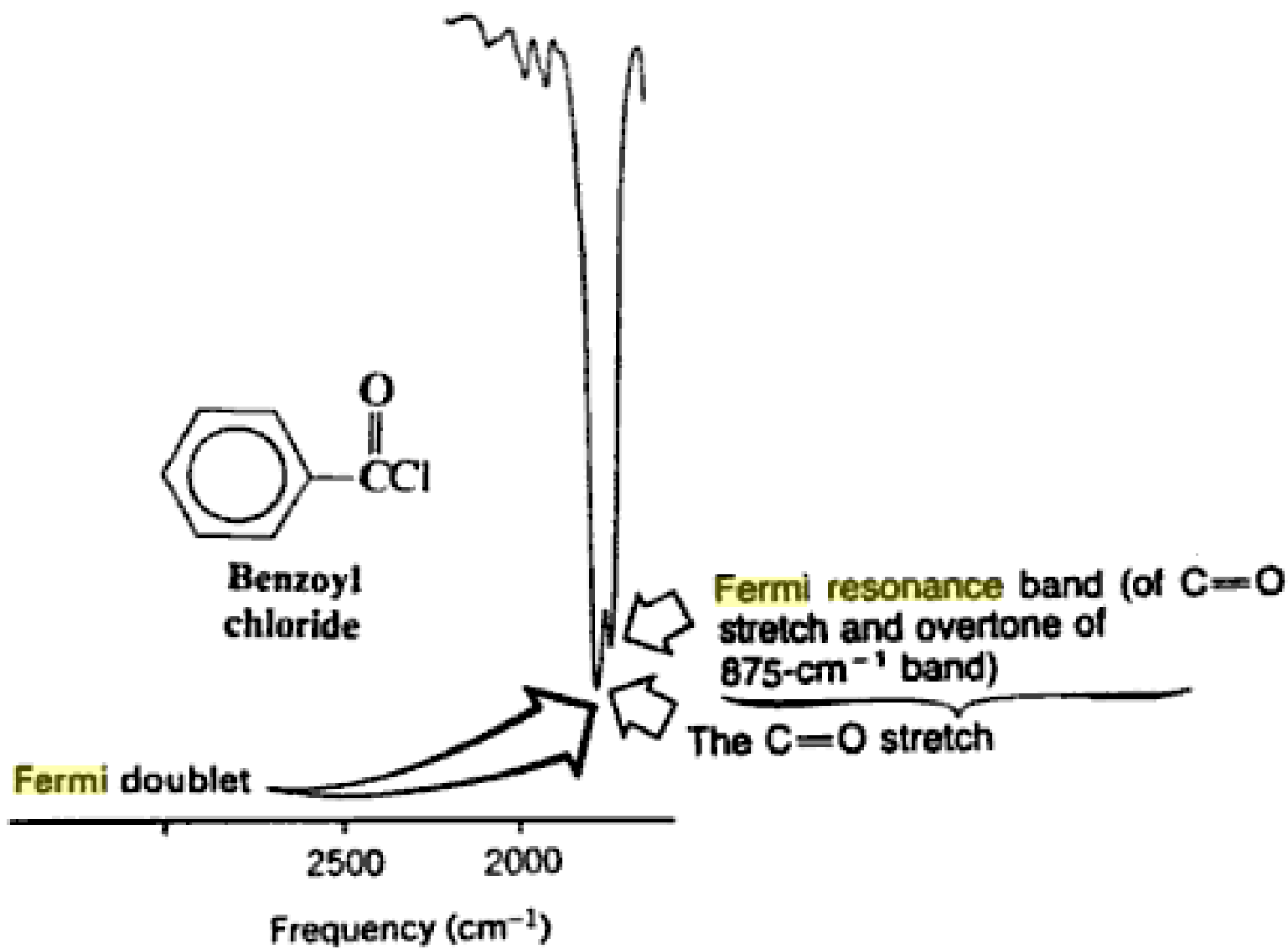
- Resonance
 - A vibration of large amplitude produced by a relatively small vibration.
- Coupling of two fundamental vibration modes produces two new modes of vibration ,with frequencies higher and lower than that observed in absence of interaction. Interaction can also take place between fundamental vibrations and overtones or combination tone vibrations and such interactions are known as **Fermi Resonance**.



Fermi Resonance

- A Fermi Resonance is a special case of mechanical coupling
- It is often called an “accidental degeneracy”
- In understanding this, for many IR bands, there are “overtone” of the fundamental (the ν 's you are taught) at twice the wavenumber
- In a good IR spectrum of a ketone (2-hexanone, here) you will see a C=O stretch at 1715 cm^{-1} and a small peak at 3430 cm^{-1} for the overtone

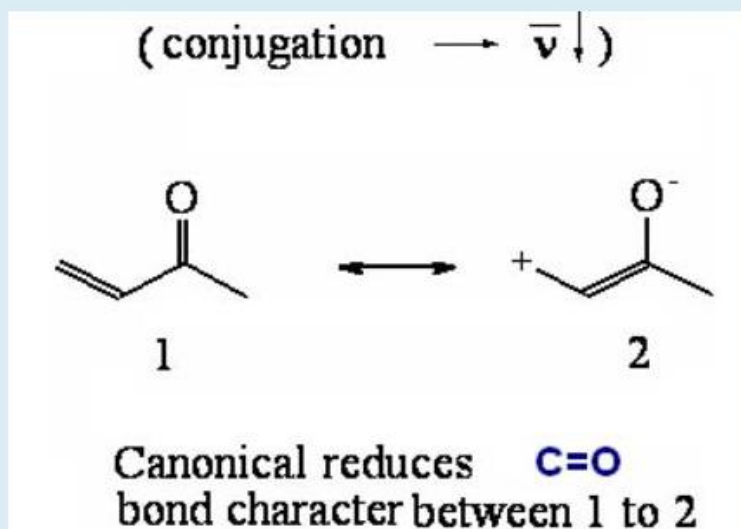




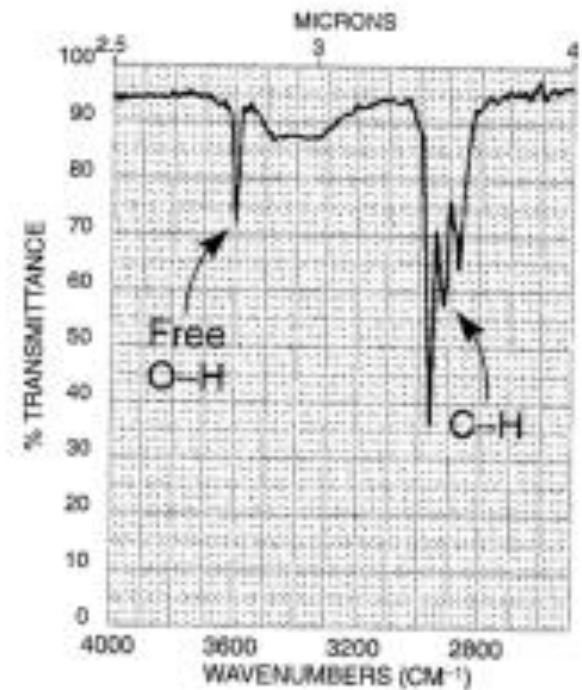
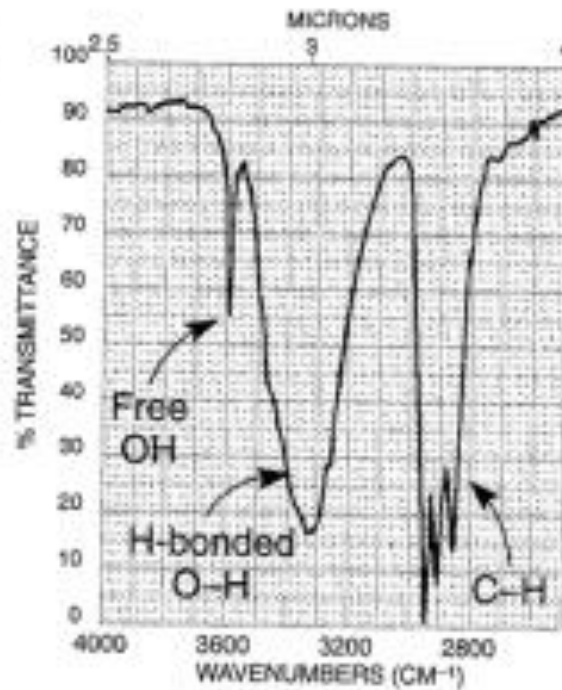
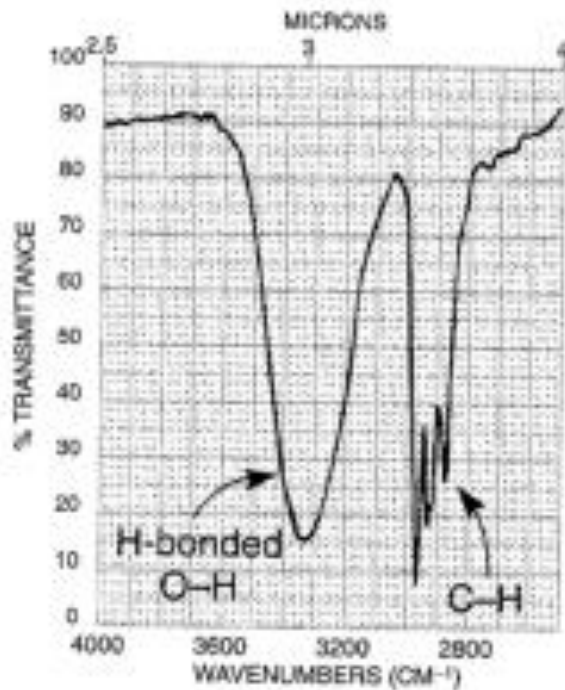
Factors Affecting Absorption

Frequency: Resonance

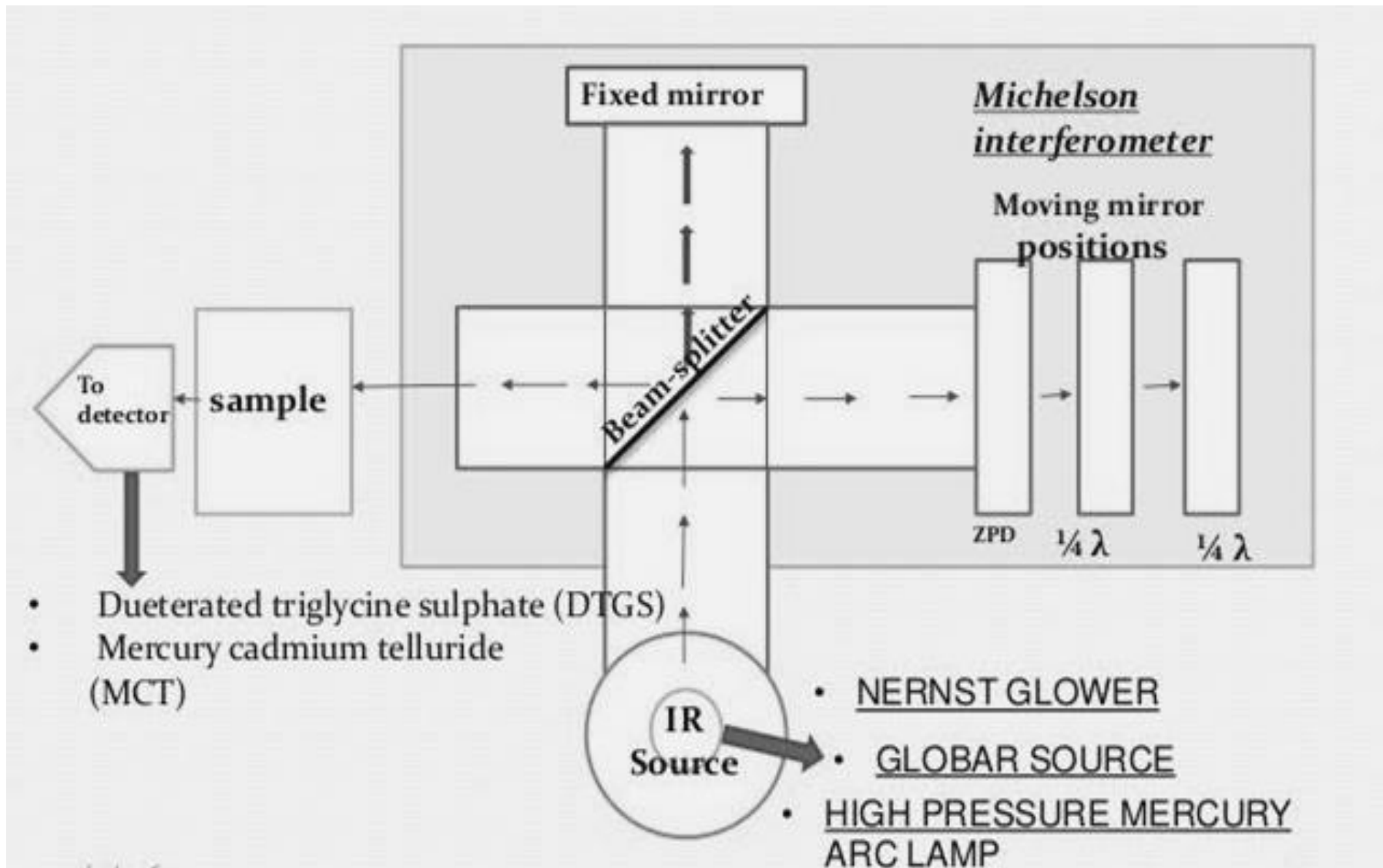
- Resonance: electronic factors
- Conjugation lowers the energy to vibrate bond
- isolated ketones: 1710 cm^{-1}
- α, β -unsaturated ketones: 1690 cm^{-1}
- $\alpha, \beta, \gamma, \delta$ -unsaturated ketones: 1675 cm^{-1}



H-Bonding in IR spectra (Broadening effect)



FT-IR Spectrophotometer



	Raman	IR
1	It is due to the scattering of light by the vibrating molecules.	It is the result of absorption of light by vibrating molecules.
2	The vibration is Raman active if it causes a change in polarisability .	The vibration is IR active if there is a change in dipole moment during the vibration.
3	The molecule need not possess a permanent dipole moment .	The vibration concerned should have a change in dipole moment due to that vibration.
4	Water can be used as a solvent.	Water cannot be used due to its intense absorption.
5	Sample preparation is not very elaborate sample can be almost in any state.	Sample preparation is elaborate Gaseous samples can rarely be used.
6	Gives an indication of covalent character in the molecule.	Gives an indication of ionic character in the molecule.
7	Cost of instrumentation is very high	Comparatively inexpensive .