SPECTROSCOPY

Presented By

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Synopsis

- > What is Spectroscopy?
- Interaction of EMR with Matter
- Classification of Spectroscopy
- > UV-Visible Spectroscopy
- ✓ Principle
- ✓ Types of Electronic Transitions
- ✓ Beer-Lamberts Law
- ✓ Instrumentation
- ✓ Applications
- ✓ Wood-Word Fieser Rules
- Question Bank

What is spectroscopy?

Spectrum + Scopies

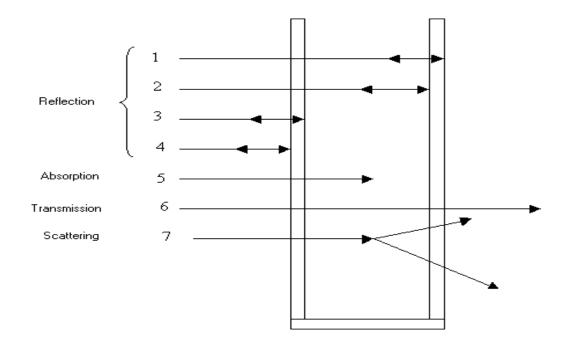
"When a beam of light is allowed to pass through a prism or grating, it will dispersed into seven colors from red to violet and the set of colors or band produced is called spectrum" + Examination

"Spectroscopy is the branch of the science dealt with the study of interaction of Electro Magnetic Radiation (EMR) with matter"

So the spectroscopy means examination of spectrum.

From the type of radiation, which is absorbed, we can get idea about the nature (type) of the compound and From the amount of the radiation which is absorbed, we can get idea about the concentration (amount) of the substance. So the spectroscopy is used for qualitative and quantitative analysis.

Interaction of EMR with matter



When a beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of the light may occur.

This is due to

- 1) Reflection at the inner and outer surfaces of the cell
- 2) Scattered by the particles present in the solution
- 3) Absorption of the light by the molecules in the solution

Classification of Spectroscopy:

1)Absorption Spectroscopy: the type and amount of the radiation, which is absorbed depend upon the structure of the molecules and the numbers of molecules interacting with the radiation. The study of these dependencies is called absorption spectroscopy. (UV, IR, NMR, X-Ray, ESR)

2)Emission spectroscopy: if sufficient energy gets impinged upon a sample, the outer electrons in the species will be raised from their stable ground state to higher energy level (unstable in nature). These excited species rapidly emits a photon and return to their ground stable energy level. The type and amount of radiation, which is emitted, is studied, this type of spectroscopy is called emission spectroscopy. (AES, MES, Fluorimetry) 3)Scattering spectroscopy: if the incoming radiation strikes with the solid particles suspended in the solution, the light transmitted at an angle other than 180° from the incident light. This spectroscopy is called scattering spectroscopy. (turbidimetry, nephelometry)

around

singlet state

excited

singlet state

excited

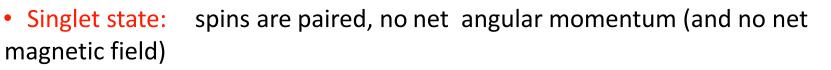
triplet state

More Complex Electronic Processes

• Fluorescence:

absorption of radiation to an excited state, followed by emission of radiation to a lower state of the same multiplicity

 Phosphorescence: absorption of radiation to an excited state, followed by emission of radiation to a lower state of different multiplicity



• Triplet state: spins are unpaired, net angular momentum (and net magnetic field)

What is EMR?

-EMR is a form of energy that is transmitted through space at an enormous velocity

- It can travel in space with the same speed as that of light. As the name implies an EMR is an alternating electrical and associated magnetic force field in space (It contains electrical and magnetic components)

-The two components oscillate in planes perpendicular to each other and perpendicular to the direction of propagation of the radiation.

- -EMR consist of a stream of discrete packets (particles) of pure energy, which is called photons or quanta.
- -The energy of photon is proportional to the frequency
- E = hυ where E= Energy of photons, h= plank's constant (6.624 x 10⁻²⁷ erg. Sec) and υ=frequency of radiation in cycles/second

-Wavelength (λ): it is the distance between two successive maxima on an electromagnetic wave. (m, cm, mm, μ m, nm, and A⁰)

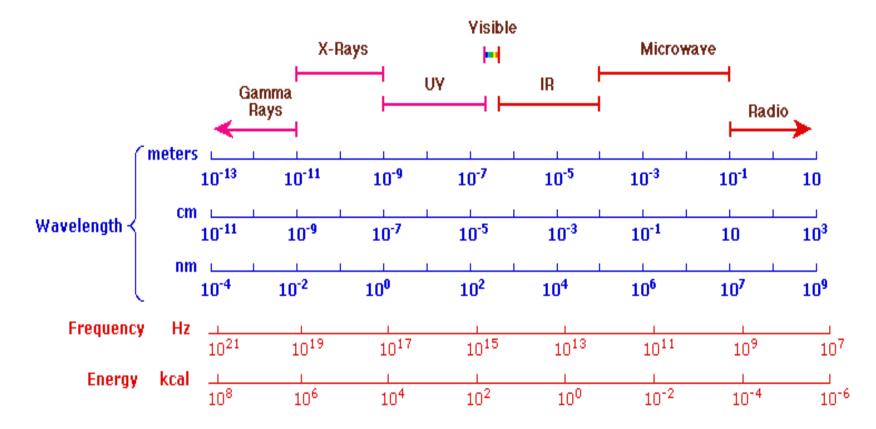
-Frequency (υ): is the numbers of waves passing through a given point in unit time. (T⁻¹, sec⁻¹, cycles/second, hertz, fresnel)

-Wave numbers ($\ddot{\upsilon}$): is the numbers of waves per centimeter in vacuum. (cm⁻¹)

-Velocity (V): is the product of wavelength and frequency ($\lambda X \upsilon = V$) (cm/sec, m/sec)

Classification of EMR: EMR is arbitrarily classified in to different regions according to wavelength.

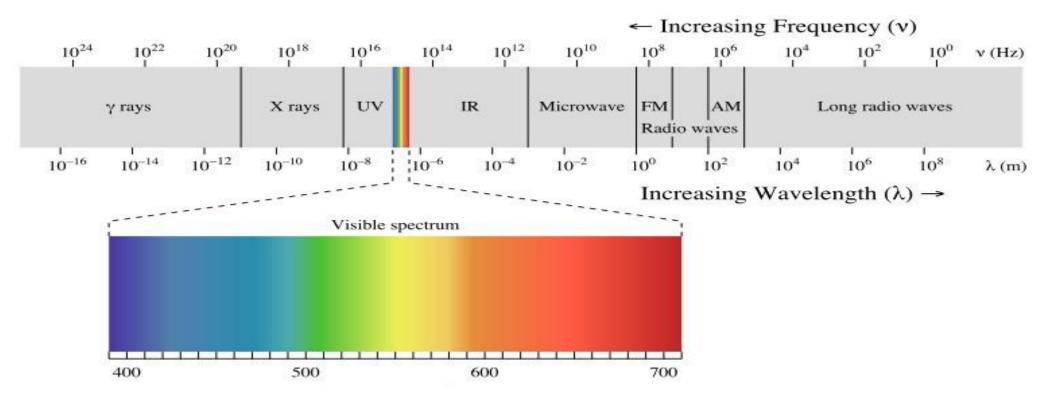
The Electromagnetic Spectrum

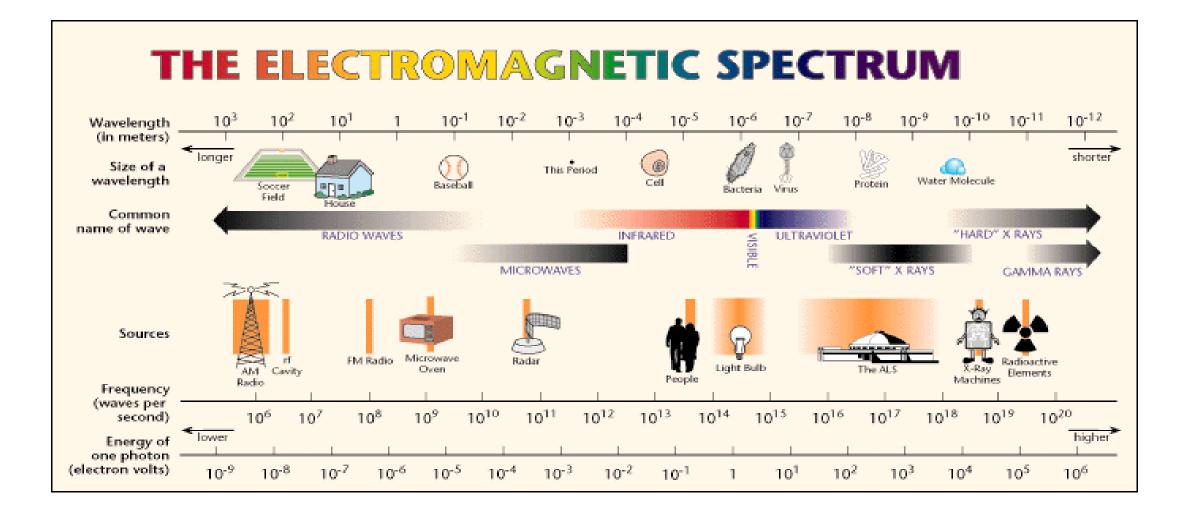


Spectroscopy: A technique of producing, detecting and analyzing spectra of electromagnetic radiation

Self-prpagating waves in vacuum or in matter whose spectrum will have a range of wavelength from 10⁻³m to 10³m

Can be classified into several types depending on the wavelength of the wave





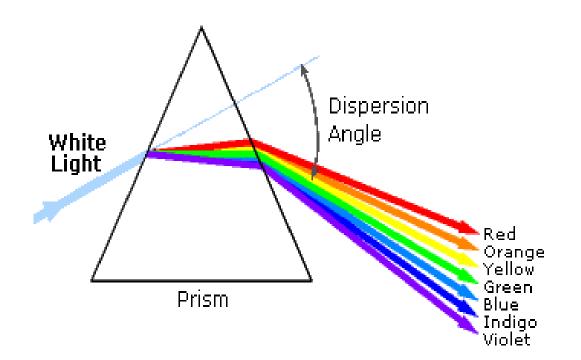
UV-Visible Spectroscopy

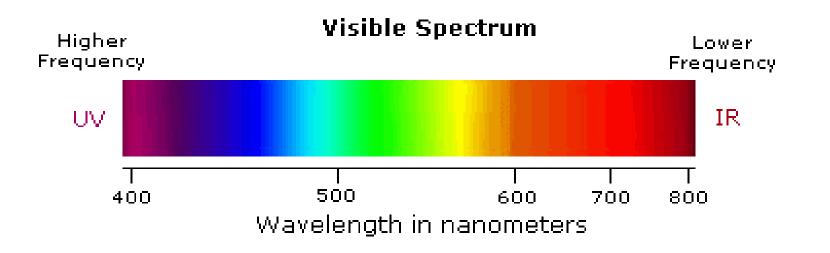
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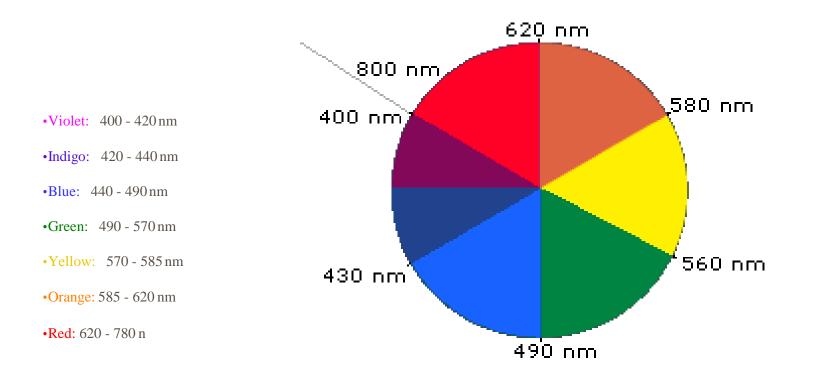
UV-Visible Spectroscopy

▲ The wavelength range of UV radiation is from 200nm – 400nm.

 The wavelength range of Visible radiation is from 400nm – 800nm







Energy associated with the molecules:

1)The molecule as a whole may move this is called translation and the energy associate with this movement is called transnational energy. (E_{trans})

2)The part of the molecules, that is atom or groups of atoms, may move with respect to each other. This motion is called vibration and the associated energy is called vibrational energy. (E_{vib})

3)The molecule may rotate about an axis. And such rotation is characterized by the rotational energy. (E_{rot})

4)Besides these modes of movements, the molecule possesses an electronic configuration and the energy associated with this configuration is called electronic energy. (E_{ele}) E total = E trans + E vib + E rot + E ele.

Theoretical principles

If a molecule is allowed to interact with the EMR of a proper frequency, the energy of the molecule is raised from one level to a higher one; we say that absorption of radiation takes place. In order for absorption to occur, the energy difference between the two energy level must be equal to the energy of the photon absorbed E2 - E1 = hv where E1 is energy of lower level and E2 is the energy of upper level

- This energy jump from one level to another is called transition

-The graph of the light absorption against the frequency is called absorption spectra.

-Visible and Ultraviolet light provides enough energy for electronic transition there for called electronic spectra.

The range is from 10⁻³m to 10³m This range will be associated with range of energy

 $E = hc / \lambda,$ $c = \sim 300,000,000 \text{ m s}^{-1}$ h = 6.626 X 10-34 J s (Planck's Constant)

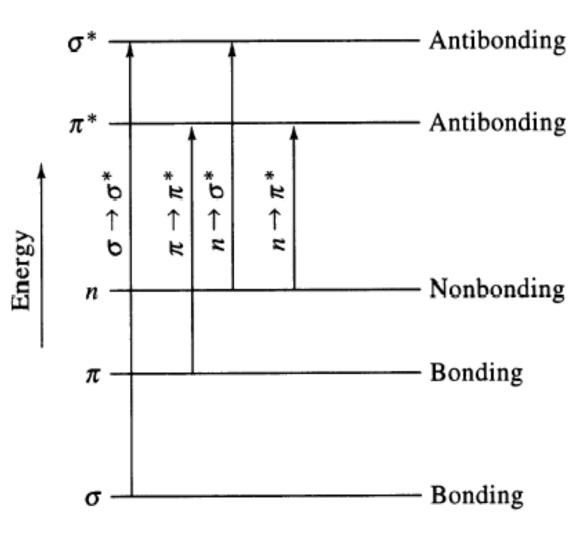
High Wave length Low wave length Short Energy High Energy

Types of transitions:

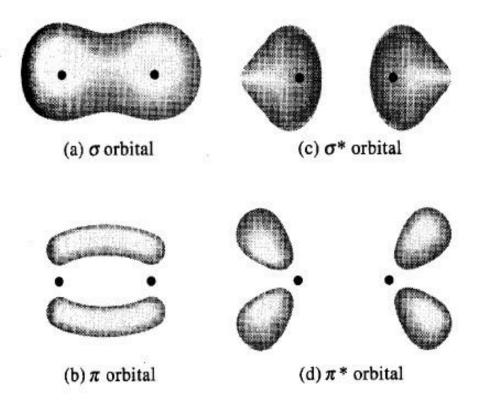
1)6 6*: A transitions of electrons from a bonding sigma orbital to the higher energy antibonding orbitals. (eg. Alkane). Sigma bonds are, in general, very strong, there fore they require high energy for the transitions and this transitions requires very short wavelength (near about 150 nm)

2)n 6*: This transition involves saturatedcompounds with one hetero atom with unshared pairof electrons (n electrons). Corresponding band appearsat 180-200 nm.

3) π π^* : This transition is available in compounds with un- saturation (eg. Alkene). Corresponding band appears at 170-190 nm. 4)n π^* : This type of transitions are shown by the unsaturated molecules containing one or more hetero atoms. (O, N, S)



Sigma and Pi orbitals



| Chromophore | Example | Excitation | λ_{max} , nm | 3 | Solvent |
|-----------------|---------------------------------------|---|----------------------|--------------|------------------|
| C=C | Ethene | π -> π^* | 171 | 15,000 | hexane |
| C≡C | 1-Hexyne | $\pi \rightarrow \pi^*$ | 180 | 10,000 | hexane |
| C=O | Ethanal | $\begin{array}{ccc} n & \longrightarrow & \pi^* \\ \pi & \longrightarrow & \pi^* \end{array}$ | 290 180 | 15 10,000 | hexane hexane |
| N=O | | $\begin{array}{cccc} n & \longrightarrow & \pi^* \ \pi & \longrightarrow & \pi^* \end{array}$ | | | |
| C-X X=Br X=I | Methyl bromide Methyl Iodide | n _> σ* n _> σ* | 205 255 | 200 360 | hexane hexane |

Solvents for UV (showing high energy cutoffs)

| Water | 205 | THF | 220 | |
|-----------------|-----|---------------------------|-----|--|
| $CH_3C\equiv N$ | 210 | CH_2Cl_2235 | | |
| $C_{6}H_{12}$ | 210 | CHCl ₃ | 245 | |
| Ether | 210 | CCl_4 | 265 | |
| EtOH | 210 | benzene | 280 | |
| Hexane | 210 | Acetone | 300 | |
| MeOH | 210 | Various buffers for | | |
| Dioxane | 220 | HPLC, check before using. | | |

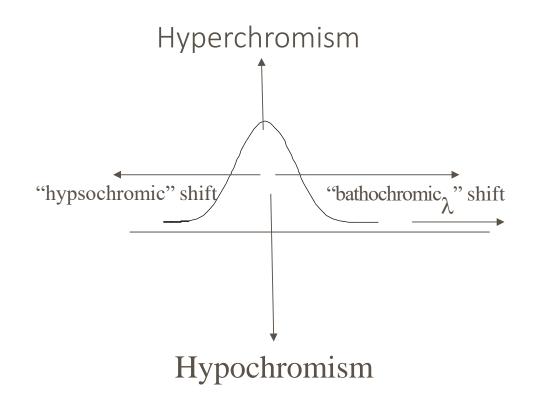
Some important terms:

1)Chromophore: It is a group of molecules, which is responsible for the absorption of light by molecules. It is conjugated dienes. It is minimum structural requirements for the absorption of radiation in UV range. E.g., -NO2, -N=N, -C=C-C=C-

2)Auxochrome: It is a saturated group containing unshared electrons which when attached to a Chromophore changes both intensity as well as the wavelength of the absorption maxima. e.g. OH, NH2, Cl etc.

3) λ -max: It is a wavelength at which there is a maximum absorption or absorption intensity. It is a physical constant and characteristic of structure and so useful for identification of compounds. It is independent of concentration.

4)Bathochromic shift: The shifting of absorption to a longer wavelength due to substitution or solvent is called as bathochromic shift. It is also called as Red shift. e.g., λ_{max} of Ascorbic acid=243nm, λ_{max} of Ascorbic acid in alkali medium=299nm.



5)Hypsochromic shift (Blue shift): Shifting of λ_{max} to lower value or left hand side due to substitution, solvent, pH etc is called as Hypsochromic shift. e.g. λ_{max} of Phenol in basic

media=297nm, λ_{max} of Phenol in acidic media=277nm. 6)Hyperchromism: Increase in absorption intensity (ϵ) due to solvent, pH or some other factors called hyperchromic effect.

7)Hypochromism : Decrease in absorption intensity due to substituent, solvent, pH etc. called hypochromic effect.

8) (A one percent one centimeter): Is the

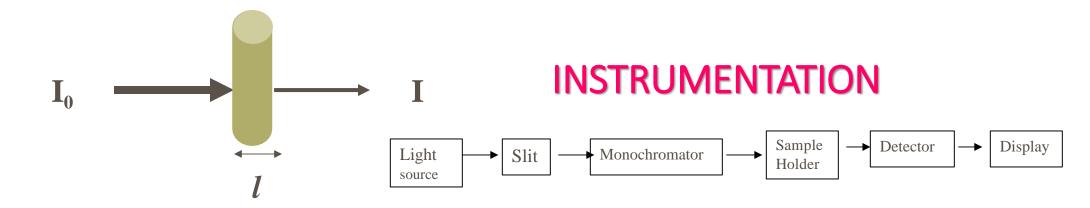
absorbance of the solution having concentration 1 gm per 100 ml of the solution and 1cm path length.

9) Molar absorptivity (ϵ): Is the absorbance of the solution having concentration gm.mol.weight/1000 ml of the solution. [$\epsilon = (A^{1\%}_{1cm} X \text{ Mol. Wt.})/10$].

BEER's – LAMBERTS Law

1)Thickness of the medium: Lambert's law: "when a beam of monochromatic light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of incident radiation". It gives relationship between absorbance and the thickness of the medium. 2)Concentration of absorbing solute: Beer's law: "when a beam of monochromatic light is allowed to pass through a transparent medium, the rate of decrease of intensity with the concentration of absorbing solute is directly proportional to the intensity of incident radiation". It gives relationship between absorbance and the concentration of the medium.

A = a b c (Fundamental equations of spectroscopy)



1)

Transmittance,
$$T = I / I_0$$

Absorbance, $A = -\log_{10}I/I_0$

- = $-\log_{10} T$ = $\log_{10} I_0 / I$

Light Source: (source of electromagnetic radiation): The tungsten filament lamp is a satisfactory light source for the region 350 to 2000 nm. It consists of a tungsten filament contained in a glass envelope. The most convenient light source for UV radiation is discharge lamp. Generally deuterium discharge lamp is used. It is consisting of deuterium-filled silica envelope. It gives radiation from 185 to 380 nm.

2) Slit: (Radiation intensity controlling device):

Enough light must pass through the sample to elicit a measurable response from the detector.

3) Monochromator: (wavelength selecting device).

It converts polychromatic light in monochromatic light (light having one wavelength).

a) Filters:

Glass filters are pieces of colored glass, which transmit limited wavelength ranges of the spectrum. The color is produced by incorporating oxides of such metals.

b) Prisms:

When a beam of light passes through a prism, it is bent or refracted. The amount of deviation is dependent on the wavelength.

The prism is made up of quartz for use in the UV light, since glass absorbs wavelengths shorter than about 330 nm.

Glass prism are preferable for the visible region of the spectrum, as the dispersion is much greater than that obtained with quartz.

c) Grating:

Most modern UV spectrophotometer uses diffraction grating as a Monochromator. It consisting of a very large number of equispaced lines (200-2000 per mm) ruled on a glass plate.

They can be used either as transmission grating or when aluminized, as reflection grating.

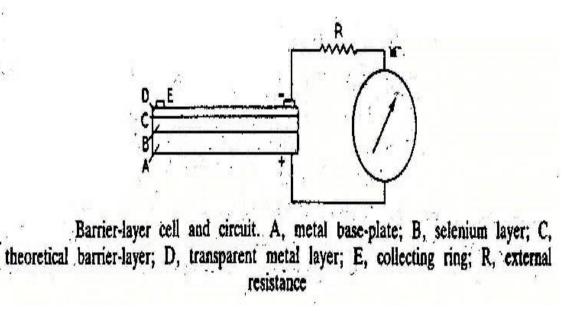
4) Sample Holder:

The sample holder is known as cuvettes. Cuvettes must be transparent to the light, so the glass cells are used in the visible region and quartz or silica cells are used in the UV region. The cells used in the UV spectrophotometers are usually 1 cm in path length but cells are available from 0.1 cm to 10 cm or more.

5) Detectors (Radiation measuring device):

It is also known as photocell. They convert radiation energy in electrical energy. For the determination of substances by spectrophotometric techniques, precise determinations of the light intensities are necessary. Photoelectric detectors are most frequently used for this purpose. They must be employed in such a way that they give a response linearly proportional to the light input and they must not suffer from drift or fatigue.

a) Barrier-layer photoce":



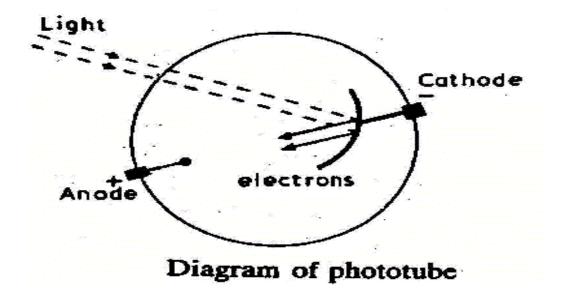
It one of the simplest detectors, which has the advantage that it requires no power supply but gives a current, which is directly proportional to the light intensity. It is consists of a metallic plate, usually copper or iron, upon which is deposited a layer of selenium.

An extremely thin transparent layer of a good conducting metal, e.g. silver, platinum or copper, is formed over the selenium to act as one electrode, the metallic plate acting as the other. Light passes through the semitransparent silver layer causes release of an electron, which migrates, to the collector.

The electron accumulating on the collector resulting in a potential difference between the base and collector, which can be measured by a low resistance galvanometer circuit.

The useful working range of selenium photocell is 380-780 nm. Their lack of sensitivity compared to phototube and photo multiplier tube, restricts their use to the cheapest colorimeters and flame photometers.

b) Photo emissive tube:



It consists of an anode and a cathode sealed in an evacuated glass tube, which may have a quartz or silica window for UV measurement.

The cathode is coated with a layer of light sensitive material that emits electrons upon absorption of photons.

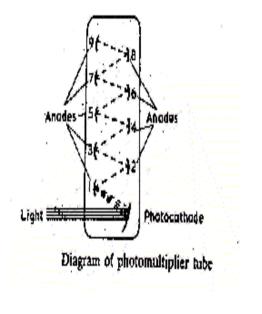
A power supply maintains the anode positive with respect to the cathode so that the photoelectrons are collected at the anode.

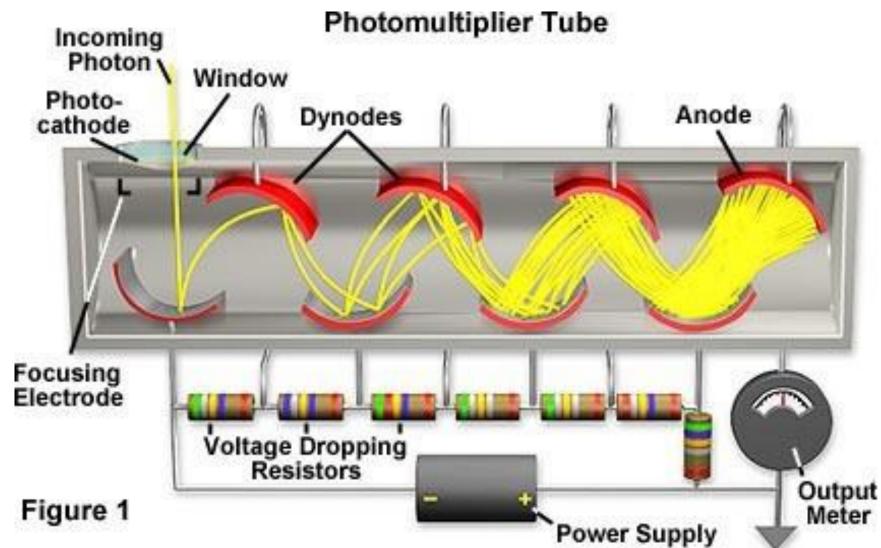
This current is directly proportional to the light intensity. Phototubes are available for use over the entire UV/visible region of the spectrum, but no single tube covers the entire range satisfactorily.

Therefore many instruments with phototube detectors employ interchangeable blue and red sensitive phototube in order to provide sufficient sensitivity over the entire spectrum.

c) Photo multiplier tube:

It is very sensitive detectors with very short response times. It contains a photo cathode and a series of dynodes, which are also photosensitive.



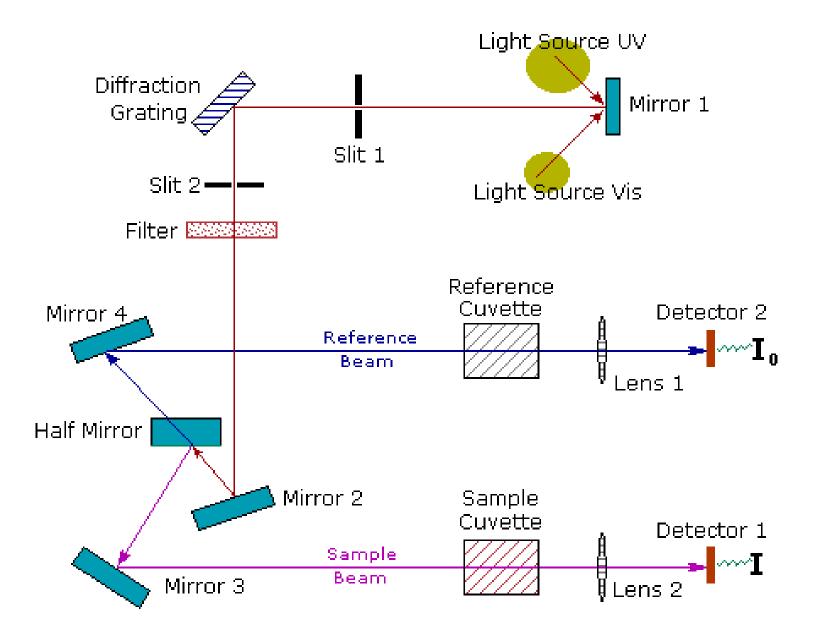


A higher successive potential is maintained between each dynodes.

A photoelectrons released from the photo cathode is accelerated toward the first dynode by their voltage difference, where it strikes to release several electrons.

The secondary electrons are then accelerated toward the second dynode where the process repeats. In this way multiplication of the electrons can be achieved.

The current from phototubes and photo multiplier tubes never falls to zero. A small residual current called dark current is produced, due to long exposure of the light.



APPLICATIONS:

1. Qualitative Analysis:

The UV spectra of most compounds are of limited value for qualitative analysis as compared to IR and Mass spectra. Qualitative analytical use of UV spectra has largely involved λ -max and absorptivities, occasionally includes absorption minima. In pharmacopoeias, absorption ratios have found use in identity tests, and are referred to as Q-values in USP.

2. Quantitative Analysis:

UV spectroscopy is perhaps the most widely used spectroscopic techniques for the quantitative analysis of chemical substances as pure materials and as components of dosage forms.

Why should we learn this stuff?

After all, nobody solves structures with UV any longer!

Many organic molecules have chromophores that absorb UV

UV absorbance is about 1000 x easier to detect per mole than NMR

Still used in following reactions where the chromophore changes. Useful because timescale is so fast, and sensitivity so high. Kinetics, esp. in biochemistry, enzymology.

Most quantitative Analytical chemistry in organic chemistry is conducted using HPLC with UV detectors One wavelength may not be the best for all compound in a mixture. Affects quantitative interpretation of HPLC peak heights 1) Structural Elucidation Detection of conjugation

It helps to show the relationships between different groups, particularly with respect to conjugation.

- The conjugation may be
 - Between two or more C-C double/triple bonds.
 - Between C=C or C=O double bonds. Between double

bond and aromatic ring.

From the spectra the presence of an aromatic ring and its number can be revealed.

Locations of substituents attached to the C atom in the conjugation system can also be revealed.

2) Detection of Geometrical Isomers

• In case of geometrically isomeric compounds, the *trans* isomers exhibit λ_{ma} at slightly longer wavelength than *cis*

isomers.

Also, trans isomers will possess larger extinction coefficients than cis isomers.

♦ For example, Of the two Stilbenes (C₆H₅-CH=CH-C₆H₅), λ_{max} ε
Trans isomer 294 nm 24000
Cis isomer 278 nm 9350

3) Detection of Impurities

- This is one of the best methods for detecting impurities. The reasons for this are:
- 1. The band due to impurities are very intense.

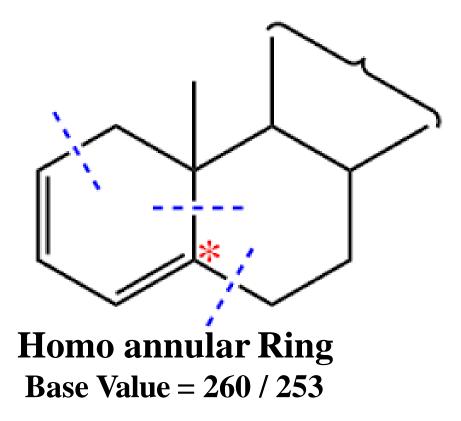
e.g. An impurities in the amount of 0.05% has an ϵ value of 2000. Therefore such impurities can be detected in a transparent major component.

- 2. The organic compounds can be classified into:
 - Saturated compounds having little absorption.
 - Unsaturated compounds having strong absorption bands.

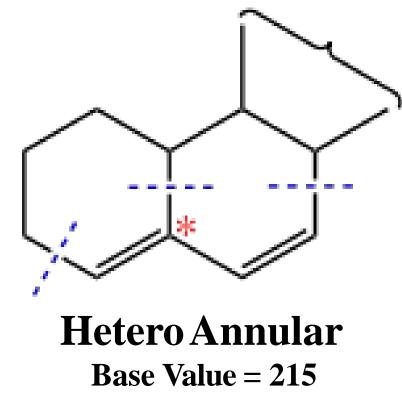
Woodward - Fieser Rules

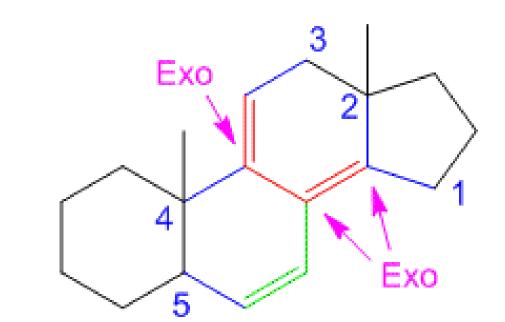
Woodward-Fieser Rules for Calculating the λ_{max} of Conjugated Dienes and Polyenes

Empirical Rules for Absorption Wavelengths of Conjugated Systems



Double bonds present in the same ring and * Represents that the carbon has Exocyclic double bond





Double bond extending from ring to another

Exocyclic double bonds

Two exocyclic double bonds

Each exocyclic double bond adds 5 nm.

*When a homoannular (same ring) a base value of 260 nm should be choosen.

*When a hetero annular (different ring) a base value of 215 nm should be choosen.

 λ_{max} (calculated) = Base (215 or 260) + SubstituentContributions

Substituent and Influence

 R- (Alkyl Group) +5 nm

 RO- (Alkoxy Group) ... +6

 X- (Cl- or Br-) +10

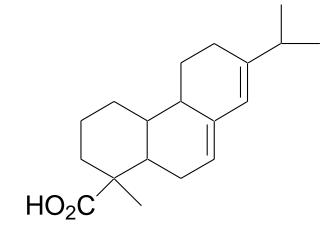
 RCO₂- (Acyl Group) 0

 RS- (Sulfide Group) ... +30

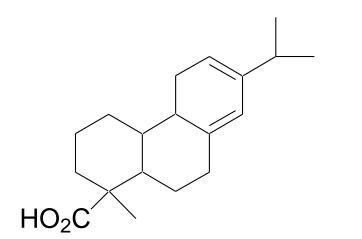
 R₂N- (Amino Group) ... +60

Further π -ConjugationC=C (Double Bond) ... +30C_6H_5 (Phenyl Group) ... +60

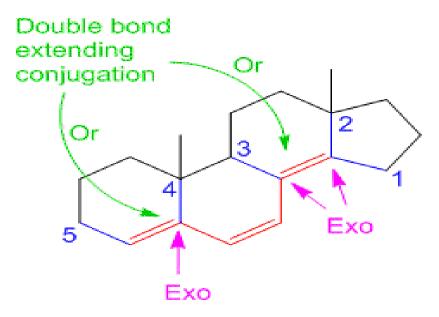
Distinguishing Isomers



| Base value | 215 |
|------------------|-----|
| 4 X alkyl subst. | 20 |
| exo DB | 5 |
| Total | 240 |
| Obs | 238 |



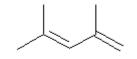
| Base value 2Xalkyl subst | 260 10 |
|-----------------------------|-----------|
| Total | 270 |
| Obs | 273 |



 $\lambda max = ? nm$

| Woodward-Fieser Rules for Dienes | | | |
|-----------------------------------|-------------------------|-----------------------------|--|
| | Homoannular (cisoid) | Heteroannular (transoid) | |
| Parent | λ =253 nm | λ=214 nm =217 (acyclic) | |
| Increments for: | | | |
| Double bond extending conjugation | 30 | 30 | |
| Alkyl substituent or ring residue | 5 | 5 | |
| Exocyclic double bond | 5 | 5 | |
| Polar groupings: | | | |
| | | | |
| -OC(O)CH ₃ | 0 | 0 | |
| -OR | 6 | 6 | |
| -CI, -Br | 5 | 5 | |
| -NR ₂ | 60 | 60 | |
| -SR | 30 | 30 | |

Example 1:

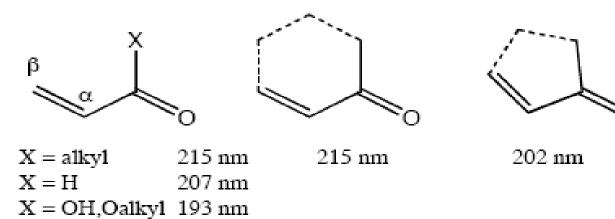


| Transoid: | | 217 nm |
|--------------------------------|---------|--------------|
| Alkyl groups or ring residues: | 3 x 5 = | <u>15 nm</u> |
| Calculated: | | 232 nm |
| Observed: | | 234 nm |
| | | |

| Hetero annualr: | | 214 nm |
|--------------------------------|---------|-------------|
| Alkyl groups or ring residues: | 3 x 5 = | 15 nm |
| Exocyclic double bond: | | <u>5 nm</u> |
| Calculated: | | 234 nm |
| Observed: | | 235 nm |

UV/VIS SPECTROSCOPY: CARBONYL COMPOUNDS

Woodward rules

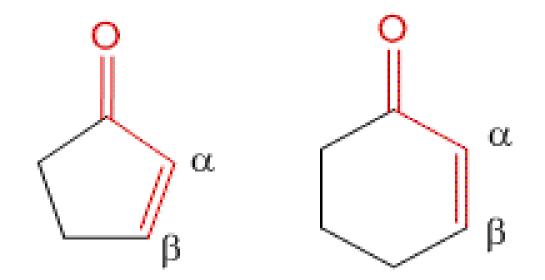


additional conjugated double bond +30 nm exocyclic double bond homoannular C=C-C=C

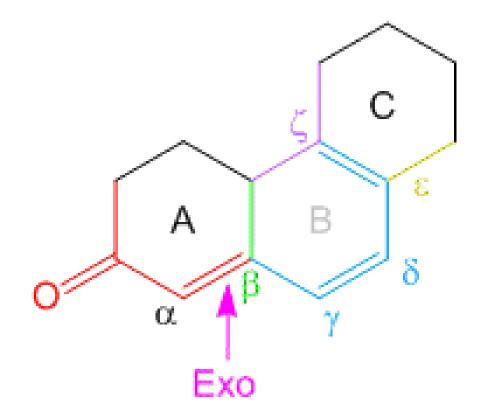
+5 nm +39 nm

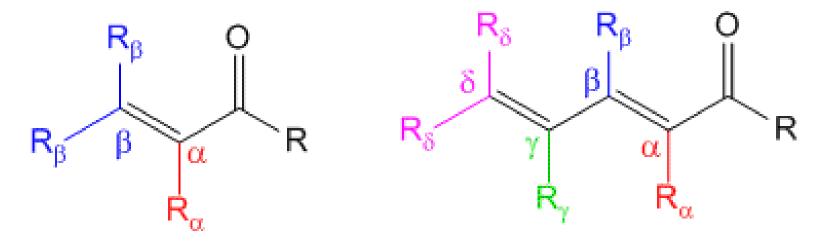
δ

| | | - | 1 | |
|--------------|-------------------|----|----|----|
| substituent: | Substituent | α | β | γ |
| | R | 10 | 12 | 18 |
| | OH | 35 | 30 | |
| | O ₂ CR | 6 | 6 | 6 |
| | Oalky1 | 35 | 30 | 17 |
| | C1 | 15 | 21 | |
| | Br | 25 | 30 | |
| | N(alkyl)2 | | 95 | |

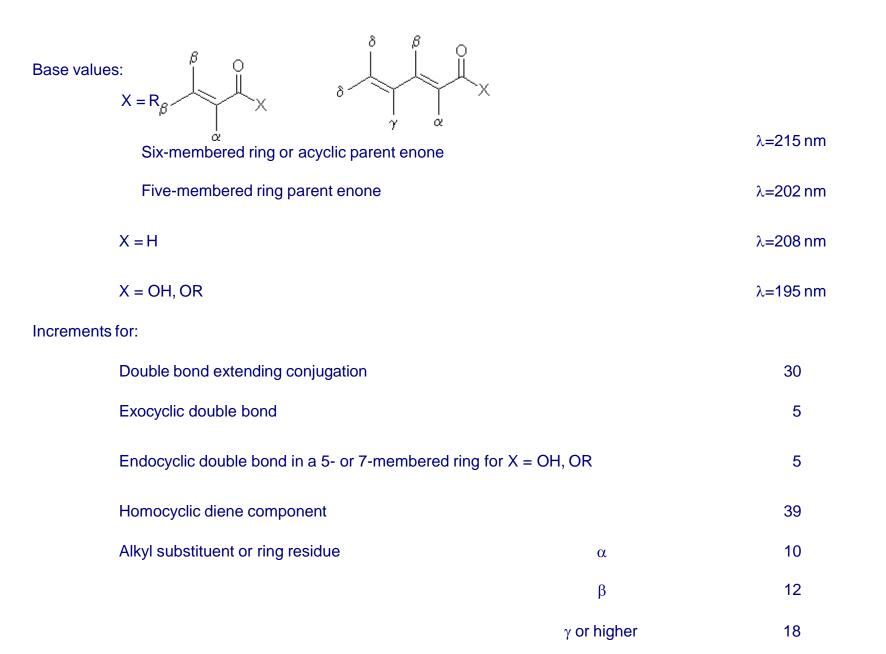


Cyclopentenone Cyclohexenone





Woodward's Rules for Conjugated Carbonyl Compounds



Polar groupings:

-OH α β δ $-OC(O)CH_3$ α,β,γ,δ $-OCH_3$ α β γ δ -Cl α β,γ,δ -Br β α,γ,δ

*Solvent shifts for various solvents:

| Solvent | $\lambda_{max} shift$ (|
|-------------|-------------------------|
| water | + 8 |
| chloroform | - 1 |
| ether | - 7 |
| cyclohexane | - 11 |
| dioxane | - 5 |
| hexane | - 11 |
| | |

-NR₂

| λ | max | shift | (nm) | |
|---|-----|-------|------|--|
|---|-----|-------|------|--|

β

35

30

50

6

35

30

17

31

15

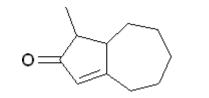
12

30

25

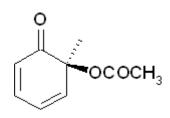
95

| Acyclic enone: | | 215 nm |
|--|----------|--------------|
| α -Alkyl groups or ring residues: | | 10 nm |
| β -Alkyl groups or ring residues: | 2 x 12 = | <u>24 nm</u> |
| Calculated: | | 249 nm |
| Observed: | | 249 nm |



, o

| Five-membered ring parent enone: | | 202 nm |
|----------------------------------|----------|-------------|
| β-Alkyl groups or ring residues: | 2 x 12 = | 24 nm |
| Exocyclic double bond: | | <u>5 nm</u> |
| Calculated: | | 231 nm |
| Observed: | | 226 nm |



| Six-membered ring or alicyclic parent enone: | 215 nm |
|--|--------------|
| Extended conjugation: | 30 nm |
| Homocyclic diene component: | 39 nm |
| δ-Alkyl groups or ring residues: | <u>18 nm</u> |
| Calculated: | 302 nm |
| Observed: | 300 nm |

o Br

| Five-membered ring parent enone: | | 202 nm |
|----------------------------------|----------|-------------|
| α-Br: | | 25 nm |
| β-Alkyl groups or ring residues: | 2 x 12 = | 24 nm |
| Exocyclic double bond: | | <u>5 nm</u> |
| Calculated: | | 256 nm |
| Observed: | | 251 nm |

Limitation: Woodward's rule is applicable upto 2-4 conjugation.

Fieser-Kuhn rule:

• Another empirical rule to calculate the absorption band maxima and *extinction coefficients* of *conjugated molecules* specially polyenes.

Long chains (Fieser-Kuhn rules)

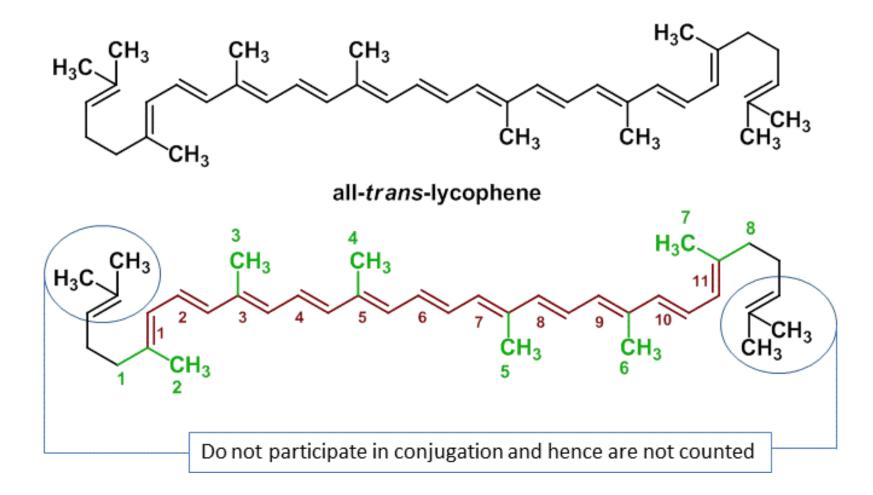
$$\lambda_{max} = 114 + 5M + n(48.0 - 1.7n) - 16.5 R_{endo} - 10 R_{exo}$$

and

 $E_{max} = (1.74 \text{ x } 10^4) \text{n}$

n = nos. of conjugated double bonds M = nos. of alkyl substituents on the conjugated system R_{endo} = no. of rings with endocyclic double bonds in the

conjugated system R_{exo} = no. of rings with exocyclic double bonds in the conjugated system



For Lycopene :

For this compound with eleven conjugated double bonds

 $\lambda \max(\text{cal}) = 114 + 5(8) + 11 [48-1.7 (11)] - 0 - 0 = 476 \text{ nm}$

 $\lambda max = 476 \text{ nm}$

Question Bank

> Explain interaction of electromagnetic radiation with molecules

Explain Lambert's law and Lambert's – Beers law?

> What are Electronic Transitions? Explain various types of Electronic transitions

> Explain a) Chromophore b) Auxochrome.

