②

UV-Visible Spectroscopy

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SPECTROSCOPY
(spectrochemical analysis)

An analytical method based on the interaction of the electromagnetic radiation and the matter (analyte).

The study of structure and properties of atoms and molecule by means of the spectral information obtained from the interaction of *electromagnetic radiant energy* with matter.

1

• Electromagnetic radiation (e.m.r.)

- Electromagnetic radiation is a form of energy
- Wave-particle duality of electromagnetic radiation

• Wave nature - expressed in term of frequency, wave-length and velocity.

• Particle nature - expressed in terms of individual photon, discrete packet of energy.

when expressing energy carried by a photon, we need to know the its frequency.

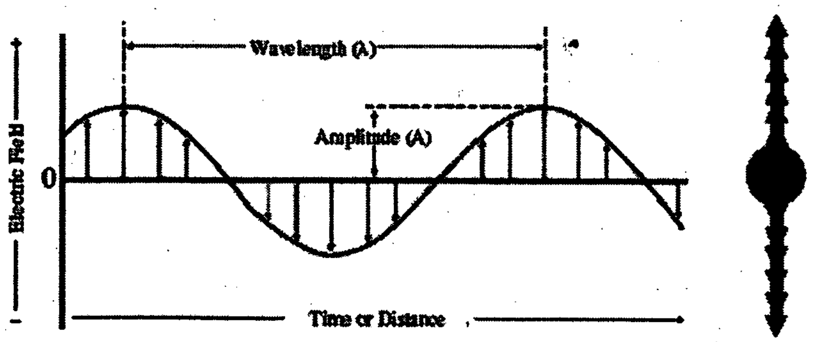
2

• Electromagnetic radiation

Characteristics of wave

- Frequency, ν - number of oscillations per unit time, unit: hertz (Hz) - cycle per second
- velocity, c - the speed of propagation, for e.m.r c=2.9979 x 108 m s^{-1} (in vacuum).
- wave-length, λ - the distance between adjacent crests of the wave.
- wave number, ν', - the number of waves per unit distance $\nu' = \lambda^{-1}$

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



3

Electromagnetic Radiation

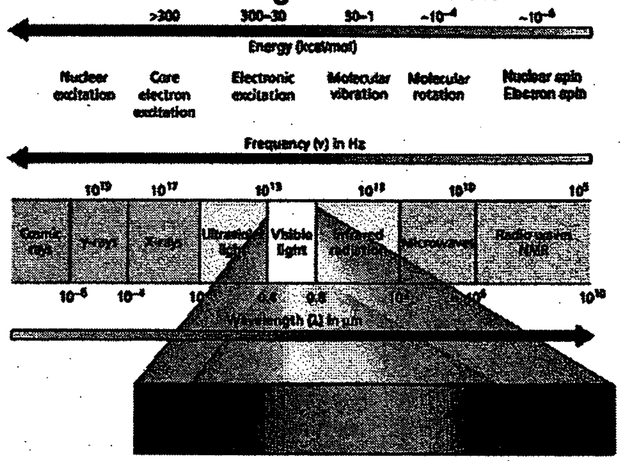
- The energy carried by an e.m.r. or a photon is directly proportional to the frequency, i.e.

$$E = h\nu = \frac{hc}{\lambda} = h\nu' c$$

where h is Planck's constant h=6.626x10^{-34} J s

4

Electromagnetic Radiation



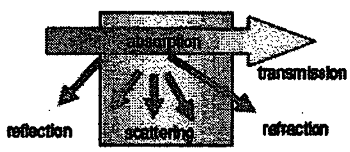
5

Interaction of electromagnetic radiation with Matter

– electromagnetic radiation interacts with materials because electrons and molecules in materials are polarizable.

– Types of interactions

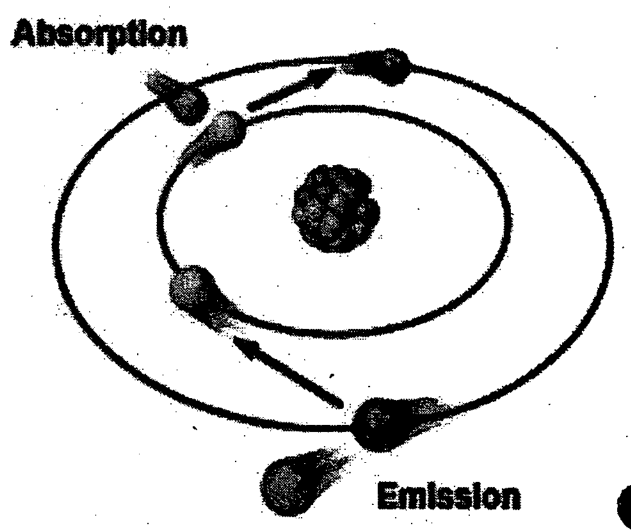
- **Absorption**
- **Reflection**
- **Transmission**
- **Scattering**
- **Refraction**



- Each interaction can disclose certain properties of the matter.
- When applying e.m.r. of different frequency (thus the nergy e.m.r. carried) different type information can be obtained.

6

Absorption and Emission of Photons



7

Atomic Absorption

•Passage of radiation through a medium that consists of monoatomic particles results in absorption of a few frequencies.

•Simplicity is due to small number of possible energy states for the absorbing particles.

9

Molecular Absorption

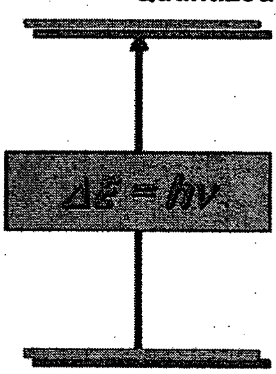
•More complex because the number of energy states is large compared to isolated atoms.

•The energy, E, associated with the molecular bands:

$$E = E_{electronic} + E_{vibrational} + E_{rotational}$$

8

Principles of Molecular Spectroscopy: Quantized Energy States



Electromagnetic radiation is absorbed when the energy of photon corresponds to difference in energy between two states.

9

Electromagnetic Radiation and Its Absorption by Molecules

Energy kJ/mol	*Wavelength (λ)* cm	*Region*	*Molecular effects*	*Type of spectroscopy*
10^7	10^{-9}	gamma rays		Gamma ray emission
10^5	10^{-7}	X rays	ionization	X-ray absorption, emission
10^3		vacuum UV		
	10^{-5}	near UV	electronic transitions	UV-Vis absorption, emission fluorescence
10^2	10^{-4}	visible		
10 1	10^{-3}	infrared (IR)	molecular vibrations	IR absorption, Raman
	10^{-1}	microwave	rotational motion	Microwave absorption
10^{-2} 10^{-4} 10^{-6}	10^2 10^4	radio	nuclear spin transitions	Nuclear magnetic Resonance (NMR)

10

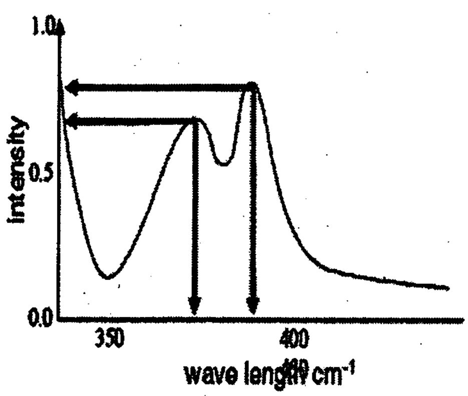
Spectrum

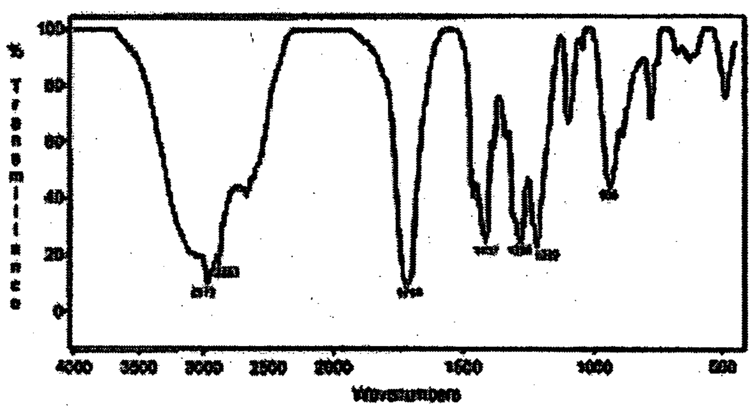
• Spectrum is the display of the energy level of e.m.r. as a function of wave number of electromagnetic radiation energy.

The energy level of e.m.r. is usually expressed in one of these terms

- absorbance (e.m.r. . being absorbed)
- transmission (e.m.r. passed through)
- Intensity

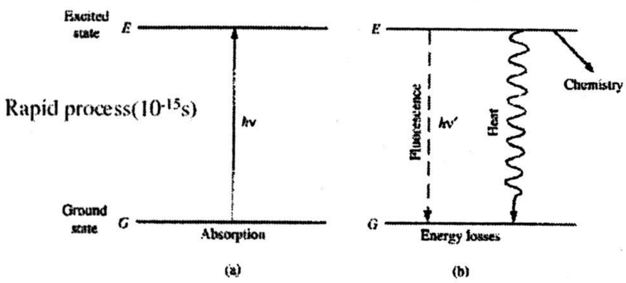
The term 'intensity' has the meaning of the radiant power that carried by an e.m.r





11

Absorption & Emission



12

Molecular spectroscopic methods

•**Absorption**: (light absorption)

spectrophotometric methods:
- -ultraviolet-visible (UV-VIS),
- -infrared (IR).

•**Emission** (light emission)
spectrophotometric methods:
- Fluorimetry (Fl).

•**Light scattering**: turbidimetry, nephelometry.

13

UV-Visible Spectroscopy

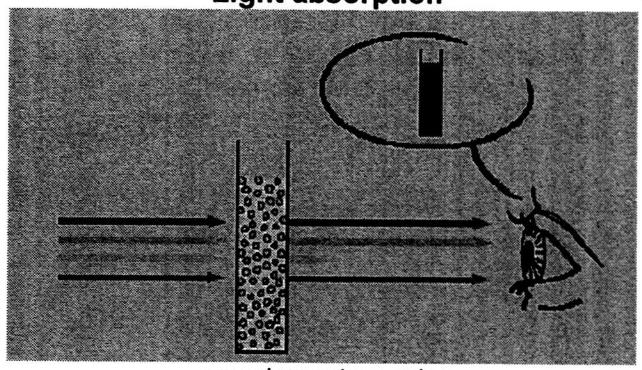
• Ultraviolet-visible spectroscopy involves the absorption of ultraviolet/visible light by a molecule causing the promotion of an electron from a ground electronic state to an excited electronic state.

• **Ultraviolet/Visible light:**

wavelengths (λ) between 190 and 800 nm

14

Light absorption



complementery color

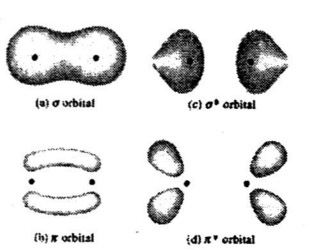
15

Absorbing Species

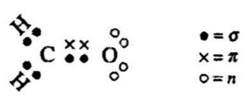
Absorbing species containing s,, p,, and *n* electrons

(organic compounds)

Bonding **Antibonding**

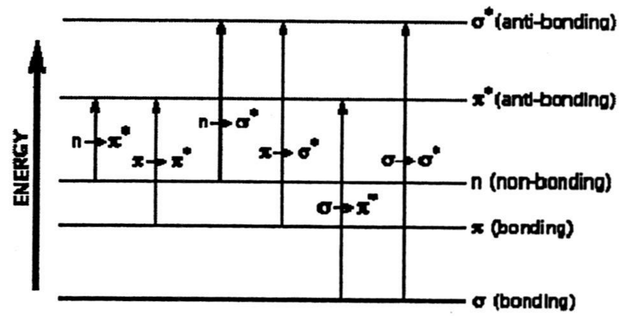


Formaldehyde



16

Possible Electronic Transitions

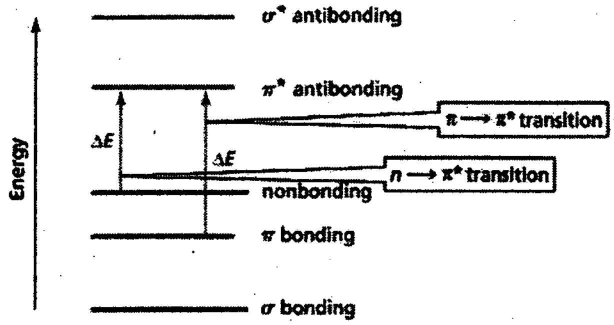


Electrons on outmost MO promoted: UV and visible radiation

UV-range: 200-400 nm
Visible range: 400-800 nm
Near IR range: from 800 nm

17

UV and Vis light cause only two kinds of electronic transition



- **Only organic compounds with π electrons can UV/Vis spectra.**
- **A visible spectrum is obtained if visible light is absorbed.**
- **A UV spectrum is obtained if UV light is absorbed.**

18

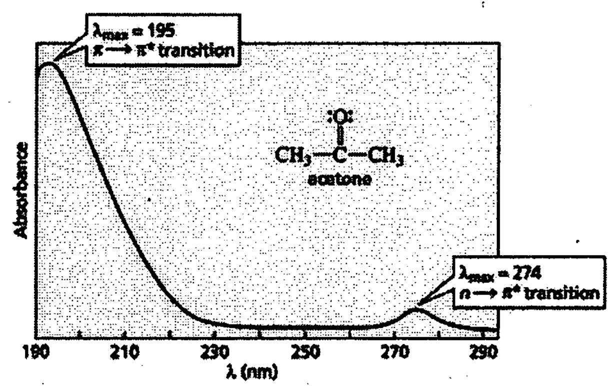
UV-Visible Spectroscopy

The method used to measure UV & visible light absorption is called spectrophotometry (colorimetry refers to the measurement of absorption of light in visible region only)

19

Absorbing Species

A chromophore is the part of a molecule which absorbs UV or visible light



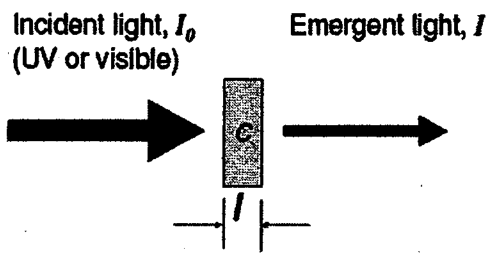
20

Chromophores

Chromophore	Example	Solvent	λ_{max}(nm)	ϵ_{max}	Type of Transition
Alkene	$C_6H_{13}CH{=}CH_2$	*n*-Heptane	177	13,000	$\pi \rightarrow \pi^*$
Alkyne	$C_5H_{11}C{\equiv}C{-}CH_3$	*n*-Heptane	178	10,000	$\pi \rightarrow \pi^*$
			196	2,000	—
			225	160	—
Carbonyl	$CH_3C(=O)CH_3$	*n*-Hexane	186	1,000	$n \rightarrow \sigma^*$
			280	16	$n \rightarrow \pi^*$
	$CH_3C(=O)H$	*n*-Hexane	180	large	$n \rightarrow \sigma^*$
			293	12	$n \rightarrow \pi^*$
Carboxyl	$CH_3C(=O)OH$	Ethanol	204	41	$n \rightarrow \pi^*$
Amido	$CH_3C(=O)NH_2$	Water	214	60	$n \rightarrow \pi^*$
Azo	$CH_3N{=}NCH_3$	Ethanol	339	5	$n \rightarrow \pi^*$
Nitro	CH_3NO_2	Isooctane	280	22	$n \rightarrow \pi^*$
Nitroso	C_4H_9NO	Ethyl ether	300	100	—

21

Absorption of UV-Vis light



22

BeeBeer-Lambert Law

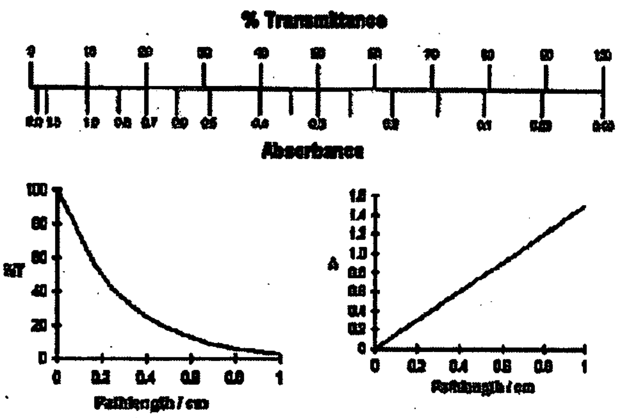
$$A = \log\frac{I_0}{I_t} = \varepsilon c l = -\log T$$

- **A Absorbance or optical density (OD)**
- **ε absorptivity; M^{-1} cm^{-1}**
- **c concentration; M**
- **T transmittance**

The molar absorptivity of a compound is a constant that is characteristic of the compound at a particular wavelength.

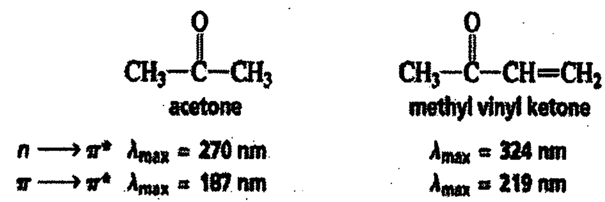
23

Transmittance, Absorbance, and Cell Pathlength



24

Effect of Conjugation on λ_{max}



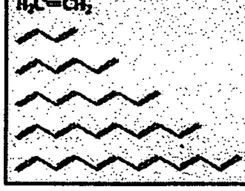
- Shifts λ_{max} to longer λ and ε is greater than expected due to the additive effect off conjugated double bonds.

- Conjugation causes delocalization off π electrons stabilizing π^*, therefore shifting absorbance to longer wavelength (lower energy).

25

Both the λ_{max} and ε increase as the number of conjugated double bonds increases

Table 9.3 Values of λ_{max} and ε for Ethylene and Conjugated Dienes

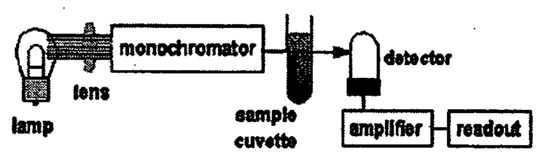


Compound	λ_{max} (nm)	ε
$H_2C{=}CH_2$	165	15,000
	217	21,000
	256	50,000
	290	85,000
	334	125,000
	364	138,000

26

UV & Visible Spectrophotometry

- **Instrumentation**



	UV	visible
Light source	Hydrogen discharge lamp	Tungsten-halogen lamp
Cuvette	QUARTZ	glass
Detectors	photomultiplier	photomultiplier

27

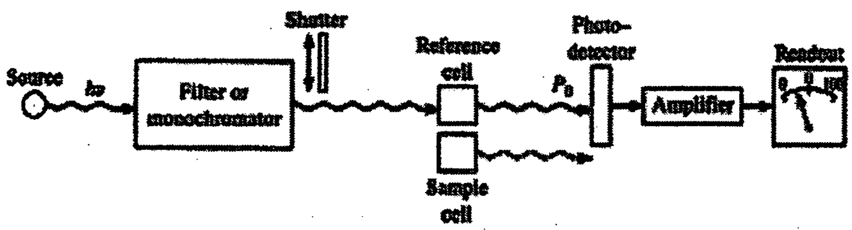
II. Instrumentation

A.. Four Basic Types of UV-Vis Instruments

- Single-beam
- Double-beam in space
- Double-beam in time
- Multi-channel

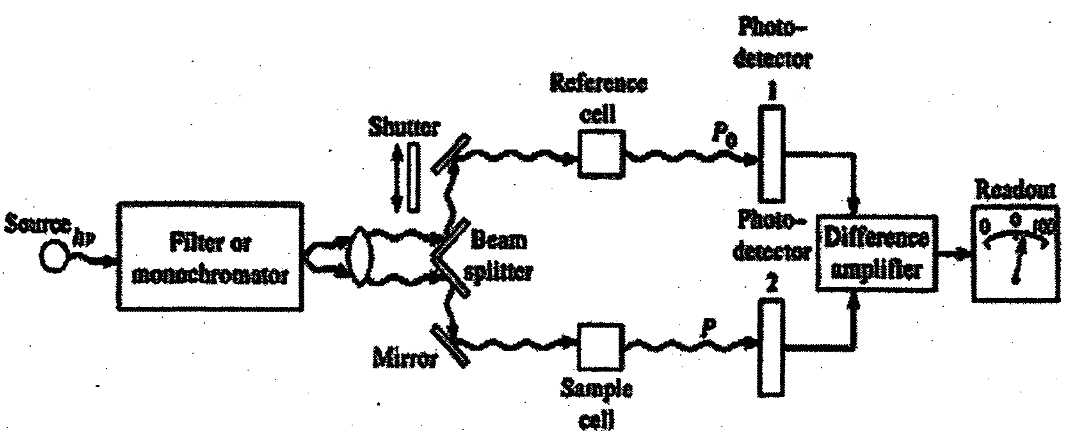
1. Single-beam Instrument

- These can be either single channel or scanning instruments.



- 0% T is set with shutter in the beam path.
- 100% T is set with a reference in the beam path.
- Measurement is then made with the sample in the beam path.

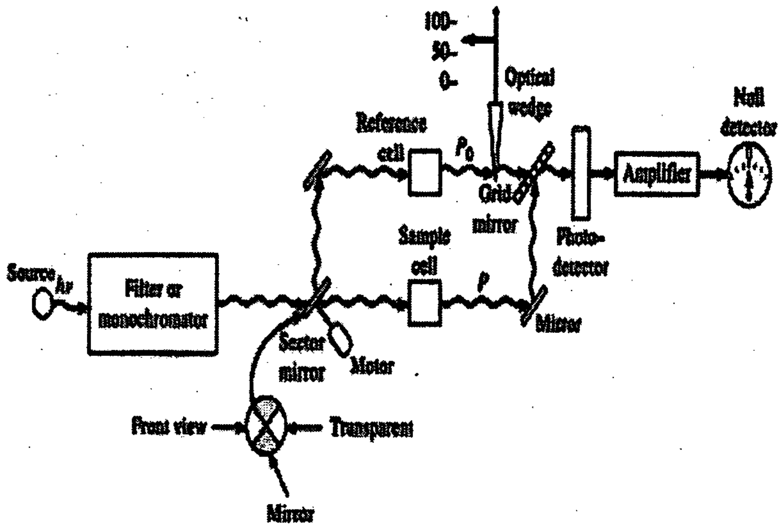
2. Double-beam in space instrument



- Sample and reference are measured simultaneously and the signal from the Reference is subtracted from the sample signal.

- A major drawback of this type of instrument is the requirement of two detectors, which makes the instrument more expensive.

3. Double-beam in time instrument



- Most common type of double-beam instrument commercially available.
- Advantages of a double-beam over a single-beam instrument:

a) Compensates for variations in the source intensity..
b) Compensates for drift in the detector and amplifier (DB in time only).
c) Compensates for variation in intensity as a function of wavelength.

30

UV & Visible Spectrophotometry

•Applications

* Analysis of unknowns using Beer's Law calibration curve.

* Absorbance vs. time graphs for kinetics.

* Single-point calibration for an equilibrium constant determination.

* Spectrophotometric titrations – a way to follow a reaction if at least one substance is colored – sudden or sharp change in absorbance at equivalence point

31

Some of the uses of UV/Vis Spectroscopy

- Measure the rates of a reaction.
- Determine the p*K*a of a compound.
- Estimate the nucleotide composition of DNA

32

References

1.Uv-Vis Spectroscopy and Its Applications, Author: Heinz-Helmut Perkampus, H. Charlotte Grinter, T.L. Threlfall (Translator), Publisher: Springer Verlag.

2. Molecular Spectroscopy, by John M. Brown - Paperback (Oxford Univ Pr on Demand; Nov 12, 1998).

3. Introduction to Spectroscopy, Donald L. Pavia, Gary M. Lampman, George S. Kriz, Brooks/Cole Pub Co, 2000

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