



INSTRUMENTAL ANALYTICAL METHODS



INTRODUCTION TO SPECTROSCOPY

- It is the branch of science that deals with the study of interaction of matter with light.

OR

- It is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.



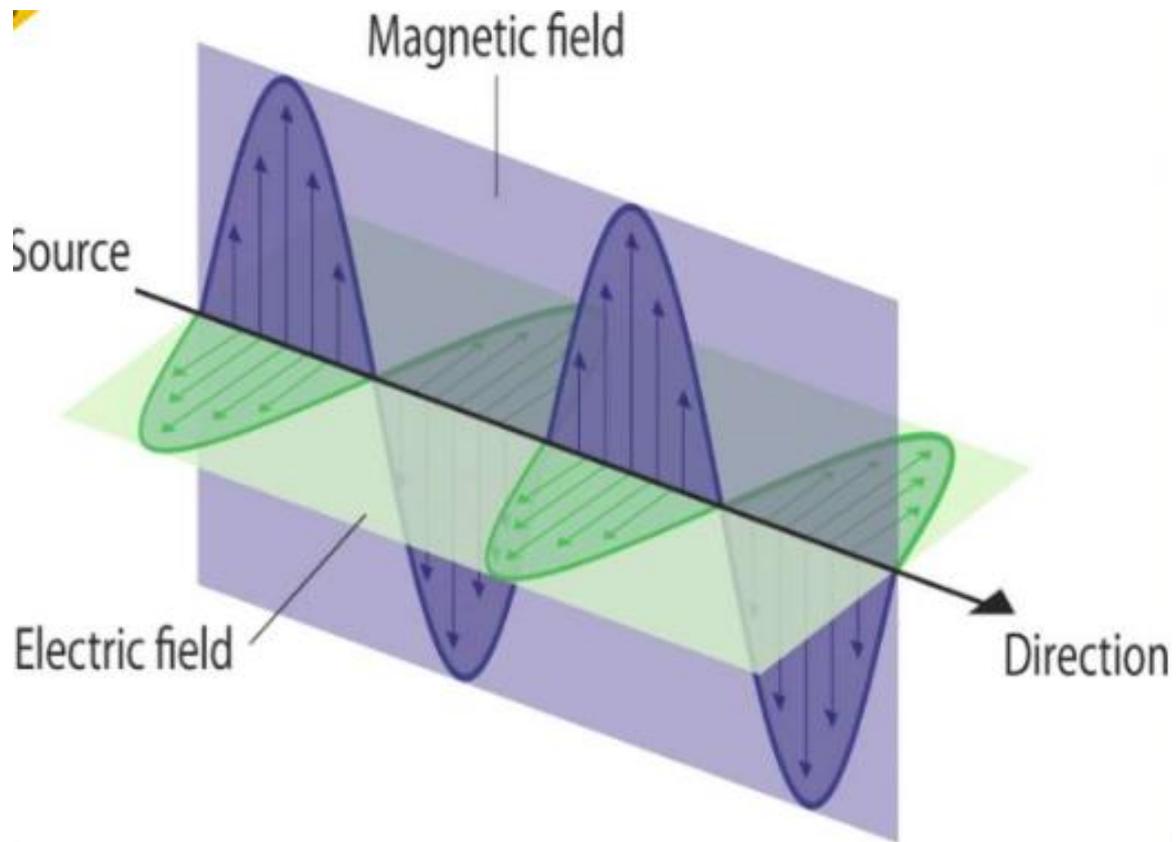
ELECTROMAGNETIC RADIATION



ELECTROMAGNETIC RADIATION

- **Electromagnetic (EM) radiation** is a form of energy that is all around us and takes many forms, such as radio waves, microwaves, X-rays and gamma rays
- Electromagnetic radiation consist of discrete packages of energy which are called as photons.
- A photon consists of an oscillating electric field (E) & an oscillating magnetic field (M) which are perpendicular to each other





MEASURING ELECTROMAGNETIC RADIATION

Electromagnetic radiation can be expressed in terms of energy, frequency or wavelength.

- Energy is measured in electron volts. Each of these three quantities for describing EM radiation are related to each other in a precise mathematical way.



ELECTROMAGNETIC RADIATION

- Frequency (ν):

- It is defined as the number of times electrical field radiation oscillates in one second.

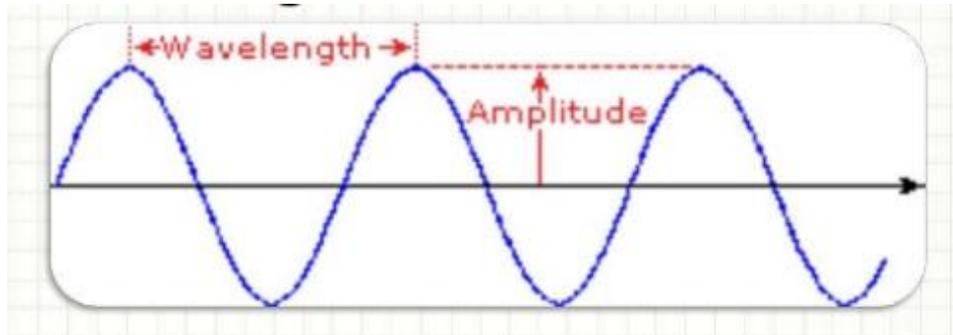
- Frequency is measured in cycles per second, or Hertz.

- The unit for frequency is Hertz (Hz).

 - 1 Hz = 1 cycle per second



- Wavelength (λ):
 - It is the distance between two nearest parts of the wave in the same phase.
- Wavelength is measured in meters.



Electromagnetic Radiation

- The relationship between wavelength & frequency can be written as: $c = v\lambda$
- As photon is subjected to energy, so $E = hv = hc/\lambda$

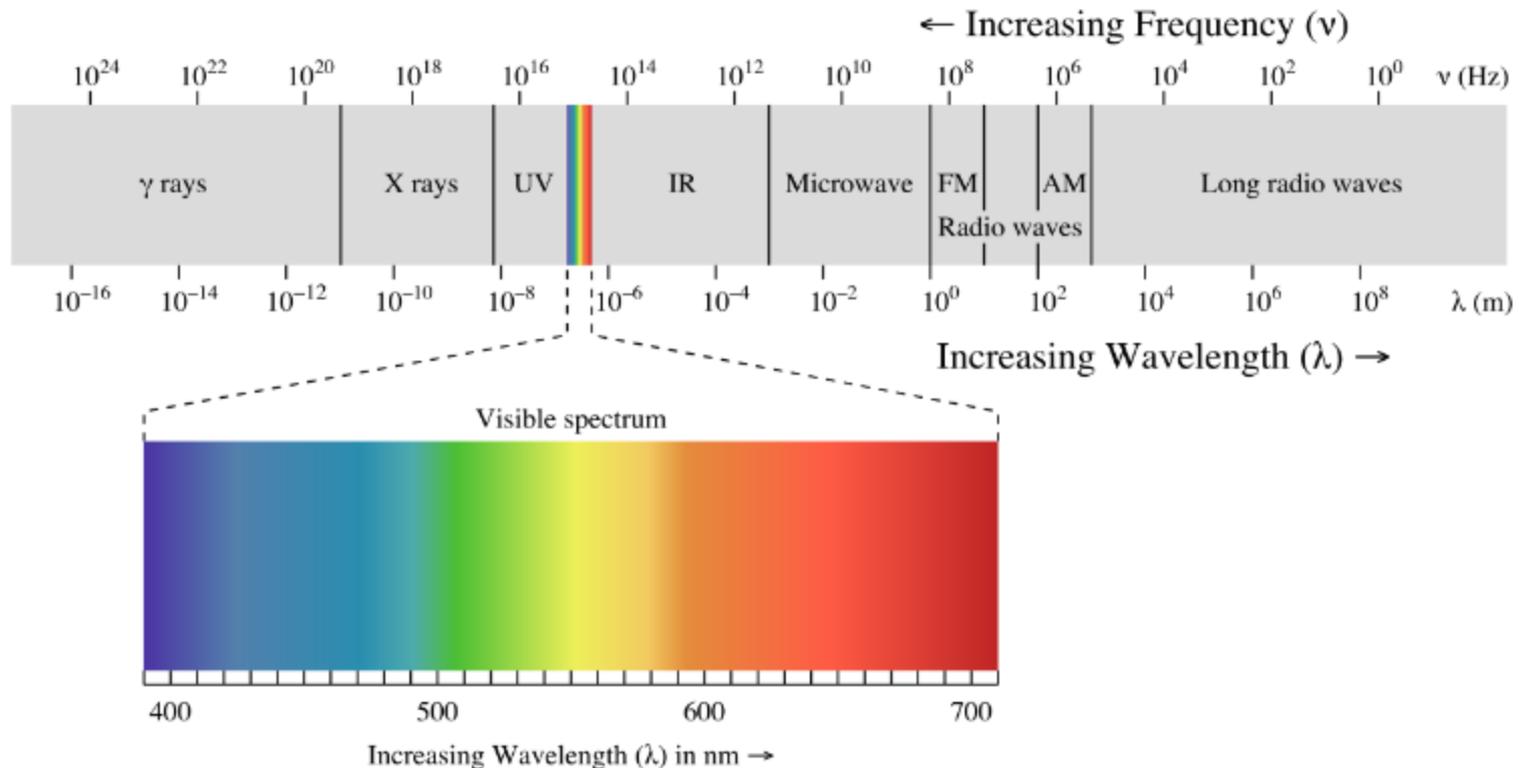


measure	symbol	unit
wavelength	λ (lambda)	m
frequency	ν (nu)	/s
speed of light	c	m/s



REGIONS OF THE SPECTRUM

The types of electromagnetic radiation are broadly classified into the following classes:



GAMMA RAYS

- These are the most energetic photons, having no defined lower limit to their wavelength.
- In astronomy they are valuable for studying high-energy objects or regions, however as with X-rays this can only be done with telescopes outside the Earth's atmosphere.
- They are used for irradiation of foods and seeds for sterilization, and in medicine they are used in radiation cancer therapy.



X-RAYS

- One notable use is diagnostic X-ray imaging in medicine (a process known as radiography). X-rays are useful as probes in high-energy physics. In astronomy, the accretion disks around neutron stars and black holes emit X-rays, enabling studies of these phenomena.
- However, X-ray telescopes must be placed outside the Earth's atmosphere to see astronomical X-rays



ULTRAVIOLET RADIATION

- The amount of penetration of UV relative to altitude in Earth's ozone
- The wavelength of UV rays is shorter than the violet end of the visible spectrum but longer than the X-ray.
- UV in the very shortest wavelength range is capable of ionizing atoms, greatly changing their physical behavior.
- At the middle range of UV, UV rays cannot ionize but can break chemical bonds, making molecules unusually reactive. Sunburn, for example, is caused by the disruptive effects of middle range UV radiation on skin cells, which is the main cause of skin cancer.



ULTRAVIOLET RADIATION

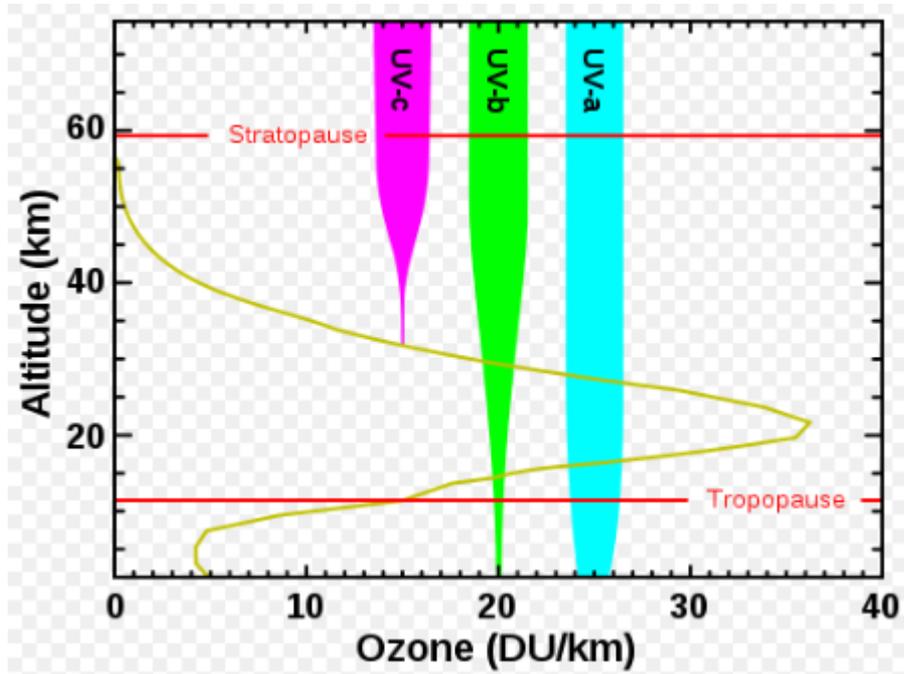
- Most of the UV in the mid-range of energy is blocked by the ozone layer, which absorbs strongly in the important 200-315 nm range.
- The very lowest energy range of UV between 315 nm and visible light (called UV-A) is not blocked well by the atmosphere, but does not cause sunburn and does less biological damage.



UVA, OR NEAR UV (315–400 nm)

UVB, OR MIDDLE UV (280–315 nm)

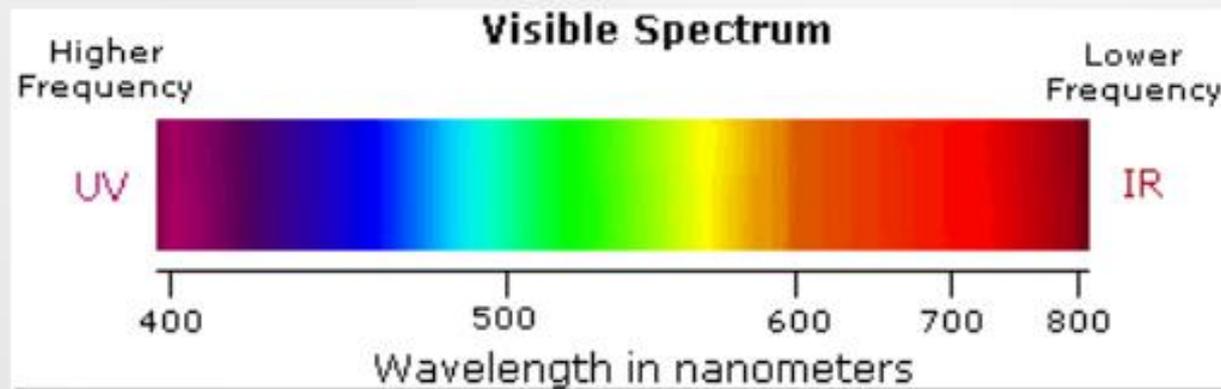
UVC, OR FAR UV (180–280 nm)



VISIBLE RADIATION (LIGHT)

- By definition, visible light is the part of the EM spectrum the human eye is the most sensitive to. Visible light is typically absorbed and emitted by electrons in molecules and atoms that move from one energy level to another.
- A rainbow shows the optical (visible) part of the electromagnetic spectrum; infrared would be located just beyond the red side of the rainbow with ultraviolet appearing just beyond the violet end.
- Electromagnetic radiation with a wavelength between 400 nm and 780 nm is detected by the human eye and perceived as visible light.
- White light is a combination of lights of different wavelengths in the visible spectrum. Passing white light through a prism splits it up into the several colors of light observed in the visible spectrum between 400 nm and 780 nm.





- Ultraviolet : 190 ~400 nm
- Violet : 400 - 420 nm
- Indigo : 420 - 440 nm
- Blue : 440 - 490 nm
- Green : 490 - 570 nm
- Yellow : 570 - 585 nm
- Orange : 585 - 620 nm
- Red : 620 - 780 nm



INFRARED RADIATION

- The infrared part of the electromagnetic spectrum covers the range from roughly 1 mm (10^6 nm) to 750 nm. It can be divided into three parts:
- **Far-infrared**, from 300 GHz to 30 THz (1 mm - 10 μ m). The lower part of this range may also be called microwaves or terahertz waves.



INFRARED RADIATION

- **Mid-infrared**, from 30 to 120 THz (10 - 2.5 μm).
- This radiation is absorbed by molecular vibrations, where the different atoms in a molecule vibrate around their equilibrium positions. This range is sometimes called the *fingerprint region*, since the mid-infrared absorption spectrum of a compound is very specific for that compound.
- **Near-infrared**, from 120 to 400 THz (2.500 - 750 nm). Physical processes that are relevant for this range are similar to those for visible light.



MICRO WAVES

- Microwaves are absorbed by molecules that have a dipole moment in liquids. In a microwave oven, this effect is used to heat food.
- Volumetric heating, as used by microwave ovens, transfers energy through the material electromagnetically, not as a thermal heat flux.
- The benefit of this is a more uniform heating and reduced heating time; microwaves can heat material in less than 1% of the time of conventional heating methods.

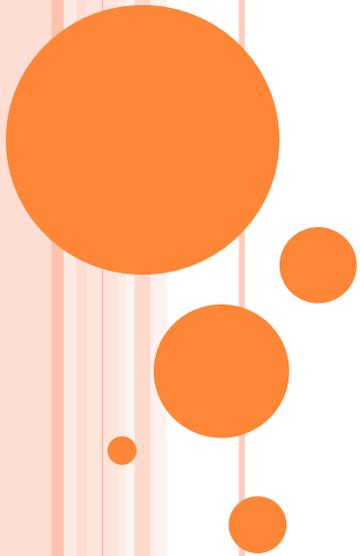


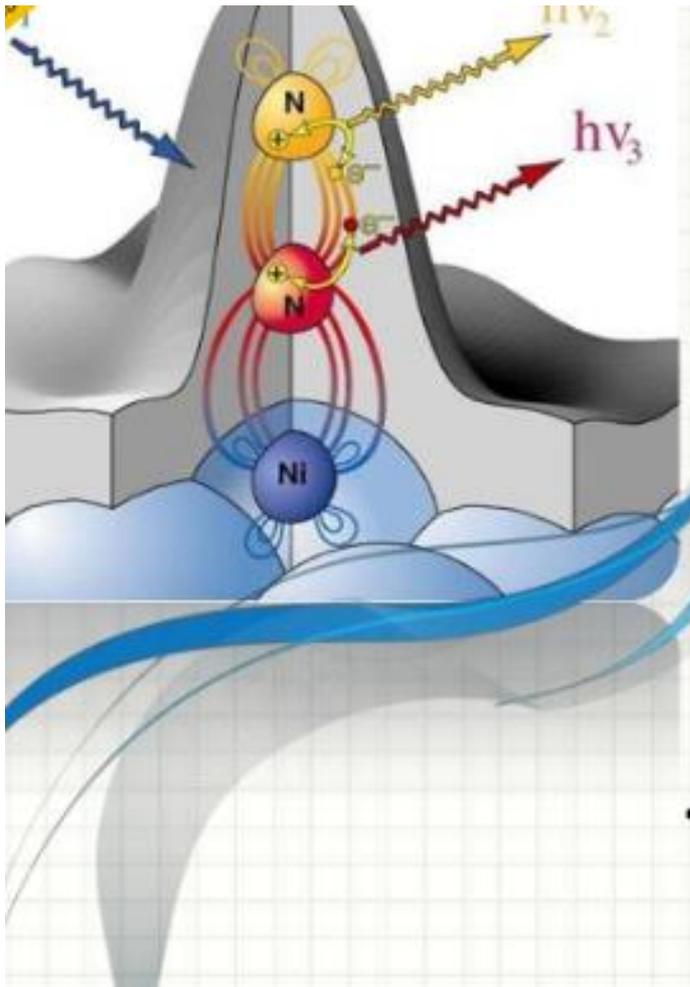
RADIO FREQUENCY

- Radio waves generally are utilized by antennas of appropriate size (according to the principle of resonance), with wavelengths ranging from hundreds of meters to about one millimeter.
- They are used for transmission of data, via modulation. Television, mobile phones, wireless networking use radio waves.
- Radio waves can be made to carry information by varying a combination of the amplitude, frequency, and phase of the wave within a frequency band.



UV-VISIBLE SPECTROSCOPY



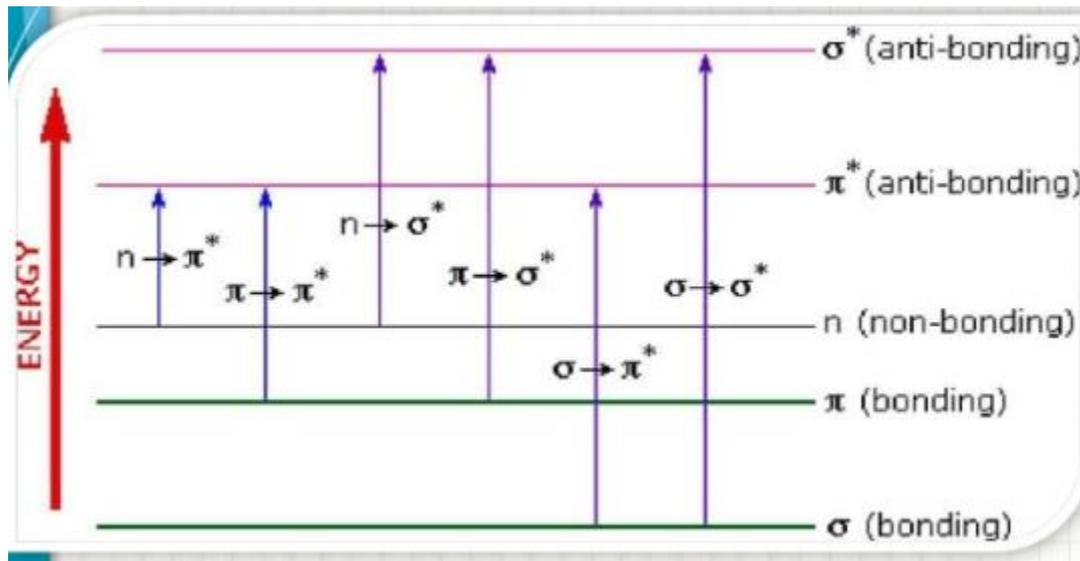


ELECTRONIC TRANSITIONS



PRINCIPLES OF UV - VISIBLE SPECTROSCOPY

The possible electronic transitions can graphically shown as:



The possible electronic transitions are

- 1 • $\sigma \rightarrow \sigma^*$ transition
- 2 • $\pi \rightarrow \pi^*$ transition
- 3 • $n \rightarrow \sigma^*$ transition
- 4 • $n \rightarrow \pi^*$ transition
- 5 • $\sigma \rightarrow \pi^*$ transition
- 6 • $\pi \rightarrow \sigma^*$ transition



1

• $\sigma \rightarrow \sigma^*$ transition

- σ electron from orbital is excited to corresponding anti-bonding orbital σ^* .
- The energy required is large for this transition.
- e.g. Methane (CH_4) has C-H bond only and can undergo $\sigma \rightarrow \sigma^*$ transition and shows absorbance maxima at 125 nm.



2

• $\pi \rightarrow \pi^*$ transition

- π electron in a bonding orbital is excited to corresponding anti-bonding orbital π^* .
- Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds... undergo $\pi \rightarrow \pi^*$ transitions.
- e.g. Alkenes generally absorb in the region 170 to 205 nm.



3

• $n \rightarrow \sigma^*$ transition

- Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of $n \rightarrow \sigma^*$ transition.
- These transitions usually requires less energy than $\sigma \rightarrow \sigma^*$ transitions.
- The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in UV region is small (150 - 250 nm).



4

• $n \rightarrow \pi^*$ transition

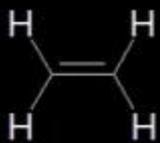
- An electron from non-bonding orbital is promoted to anti-bonding π^* orbital.
- Compounds containing double bond involving hetero atoms ($C=O$, $C\equiv N$, $N=O$) undergo such transitions.
- $n \rightarrow \pi^*$ transitions require minimum energy and show absorption at longer wavelength around 300 nm.



5	• $\sigma \rightarrow \pi^*$ transition	
&	• $\pi \rightarrow \sigma^*$ transition	6

- These electronic transitions are forbidden transitions & are only theoretically possible.
- Thus, $n \rightarrow \pi^*$ & $\pi \rightarrow \pi^*$ electronic transitions show absorption in region above 200 nm which is accessible to UV-visible spectrophotometer.
- The UV spectrum is of only a few broad of absorption.





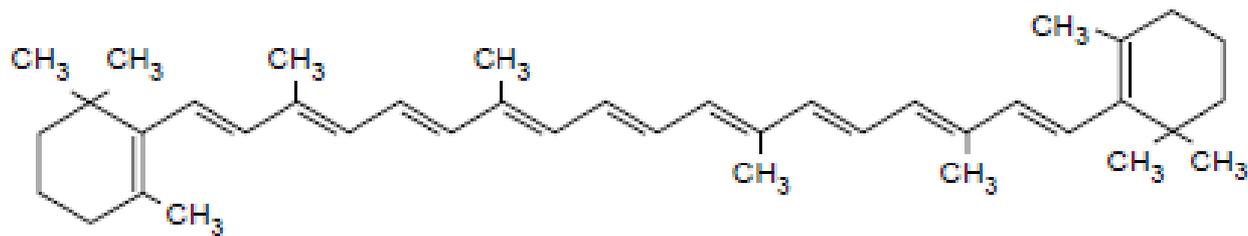
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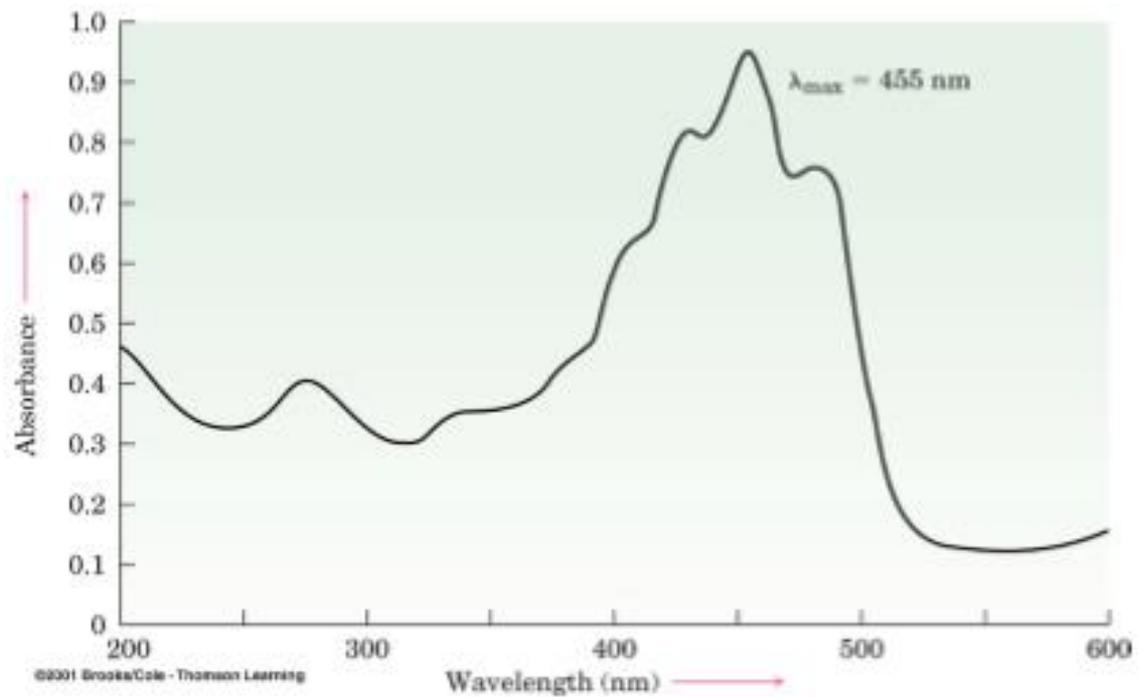
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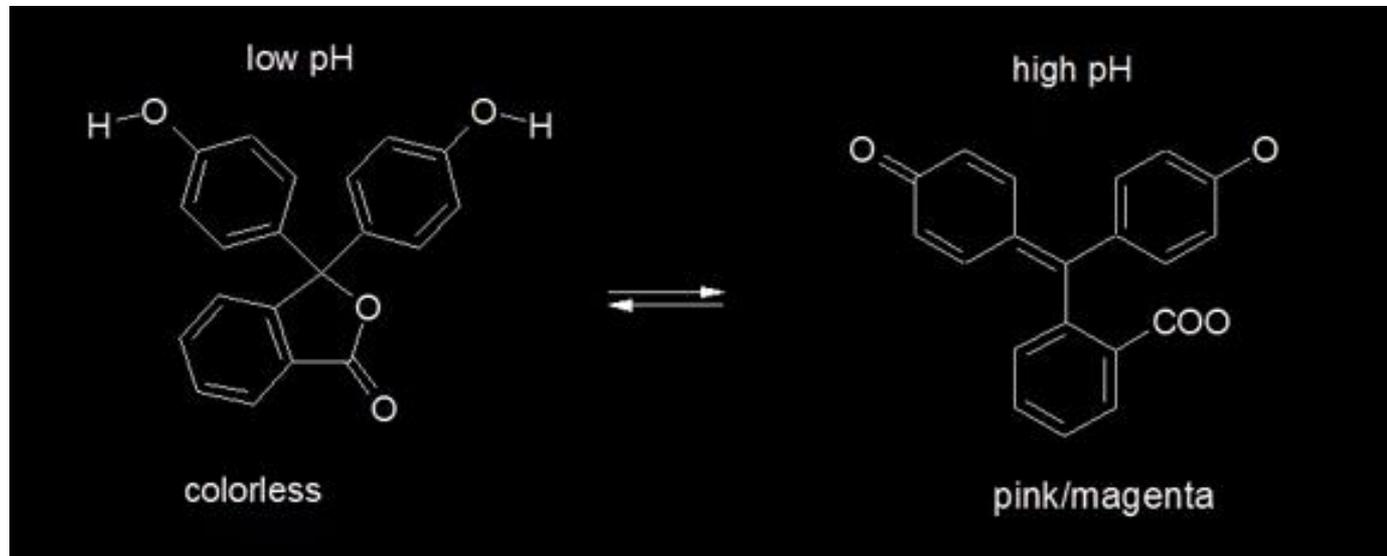
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B-carotene (orange)





ABSORPTION & INTENSITY SHIFTS



-
- 1 • Bathochromic Shift (Red Shift)
 - 2 • Hypsochromic Shift (Blue Shift)
 - 3 • Hyperchromic Effect
 - 4 • Hypochromic Effect



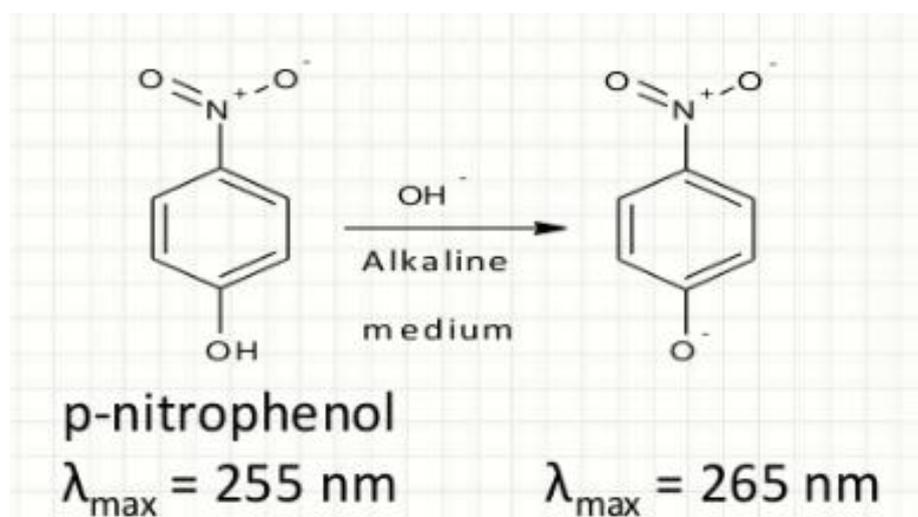
1. BATHOCHROMIC SHIFT (RED SHIFT)

- When absorption maxima (λ_{max}) of a compound shifts to longer wavelength, it is known as bathochromic shift or red shift.
- The effect is due to presence of conjugation or by the change of solvent.
- e.g. An auxochrome group like -OH, -OCH₃ causes absorption of compound at longer wavelength.



1. BATHOCHROMIC SHIFT (RED SHIFT)

- In alkaline medium, p-nitrophenol shows red shift. Because negatively charged oxygen delocalizes more effectively than the unshared pair of electron.



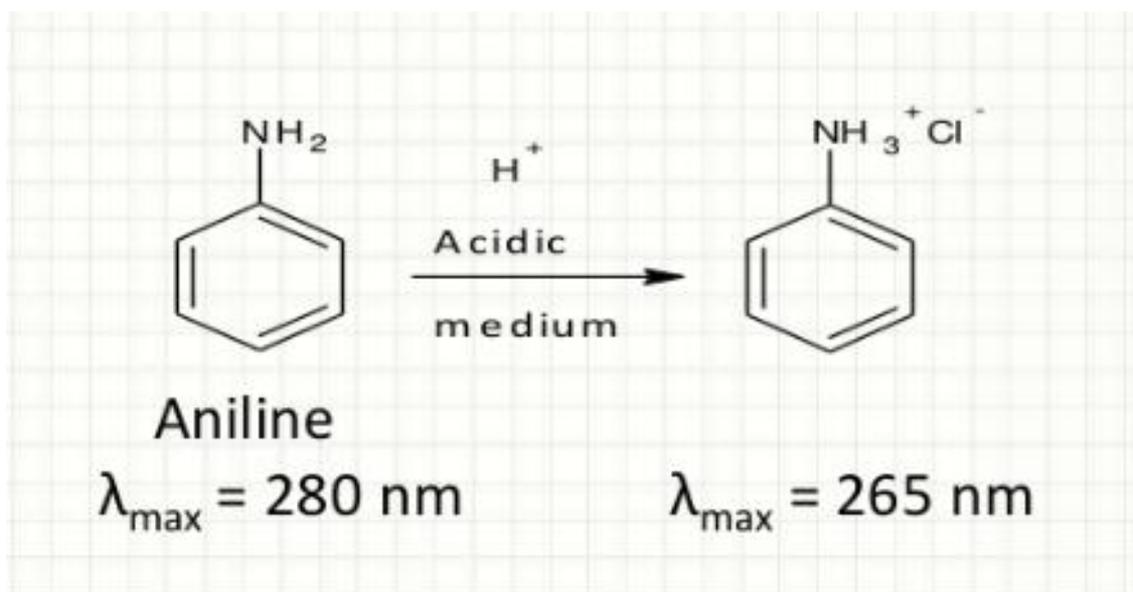
2. HYPSOCHROMIC SHIFT (BLUE SHIFT)

- When absorption maxima (λ_{max}) of a compound shifts to shorter wavelength, it is known as hypsochromic shift or blue shift.
- The effect is due to presence of an group causes removal of conjugation or by the change of solvent



2. HYPSOCHROMIC SHIFT (BLUE SHIFT)

- Aniline shows blue shift in acidic medium, it loses conjugation.



3 • HYPERCHROMIC EFFECT•

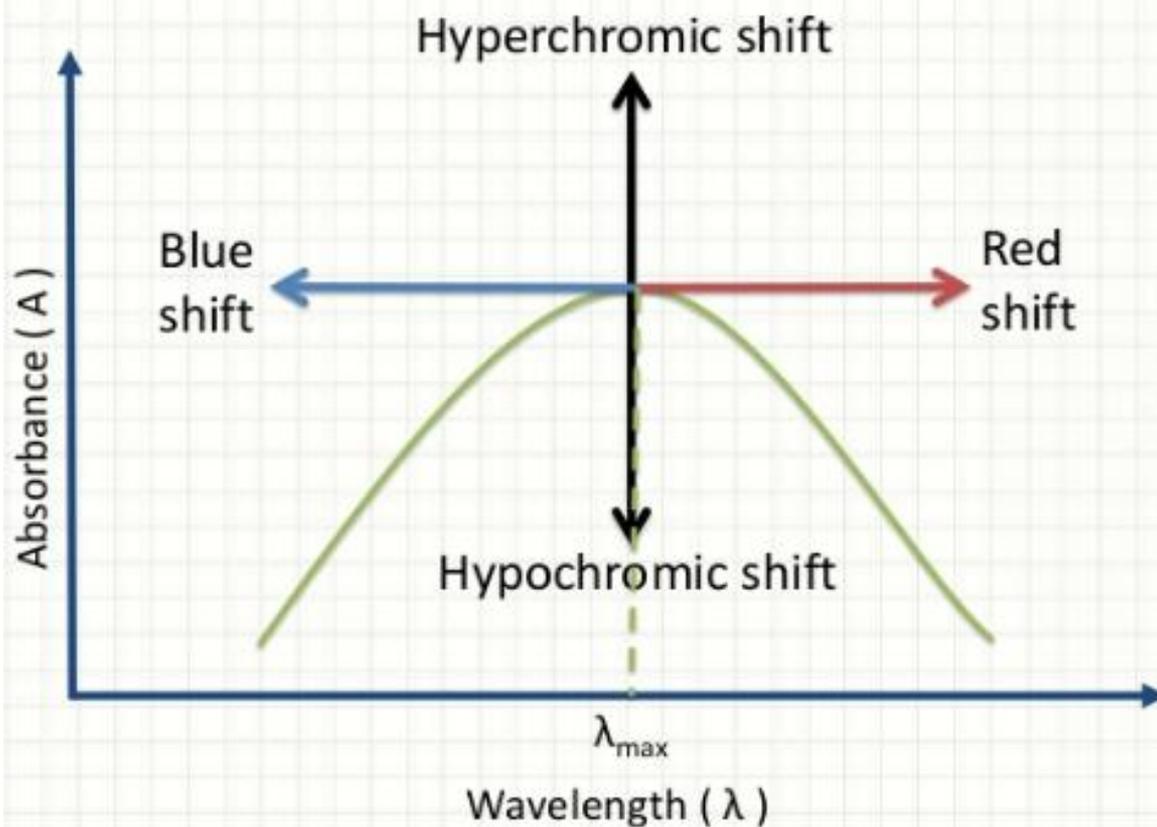
- When absorption intensity (ϵ) of a compound is increased, it is known as hyperchromic shift.
- If auxochrome introduces to the compound, the intensity of absorption increases.

4 • HYPOCHROMIC EFFECT

- When absorption intensity (ϵ) of a compound is decreased, it is known as hypochromic shift



Shifts and Effects



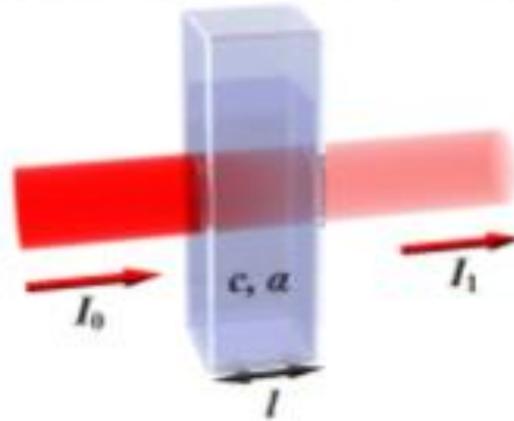
THE ABSORBANCE OF A SOLUTION

Transmittance and Absorbance

The *transmittance* is the ratio of the light that is detected when the sample is present to the ratio when the sample is not present.

$$T = \frac{I}{I_0}$$

Transmittance is measured in the following way:



THE ABSORBANCE OF A SOLUTION

Beer-Lambert Law

Transmittance is related to concentration in a non-linear way (exponential), so it is usually converted to the much more useful quantity 'absorbance', where:

$$\text{Absorbance} = A = \log \frac{I_0}{I}$$

Absorbance *is* related to concentration in a linear way according to the Beer-Lambert Law.

$$\text{Absorbance} = A = \epsilon lc$$

Where l is the *pathlength* in centimeters

And c is the *concentration* of the absorbing species in Molarity (mol/L)

And ϵ is the *molar absorptivity* or *molar extinction coefficient*



HOW DO WE CHARACTERIZE VARIOUS VALUES OF EPSILON?

- **Strong** ($\epsilon > 1000$)
- **Weak** ($\epsilon < 100$)
- **No absorption** ($\epsilon = 0$)



IF $E > 1000$, WHAT KIND OF PARTIAL STRUCTURE IS INDICATED?

- The compound **MUST CONTAIN** a **conjugated π system**.
- It might be **aromatic** (like benzene).
- It might be a **conjugated polyene** (like 1,3-butadiene).



IF $E < 100$, WHAT KIND OF PARTIAL STRUCTURE IS INDICATED?

- The compound must have an aldehyde or ketone carbonyl.
- There is no heteroatom next to the carbonyl carbon (only C or H).



APPLICATIONS OF UV / VISIBLE SPECTROSCOPY



APPLICATIONS

- Qualitative & Quantitative Analysis:
 - It is used for characterizing aromatic compounds and conjugated olefins. Absorbance above 210 gives indication of conjugated systems
 - It can be used to find out molar concentration of the solute under study.
- Detection of impurities:
 - It is one of the important method to detect impurities in organic solvents.Detection of isomers are possible.



UV-VIS INSTRUMENTATION



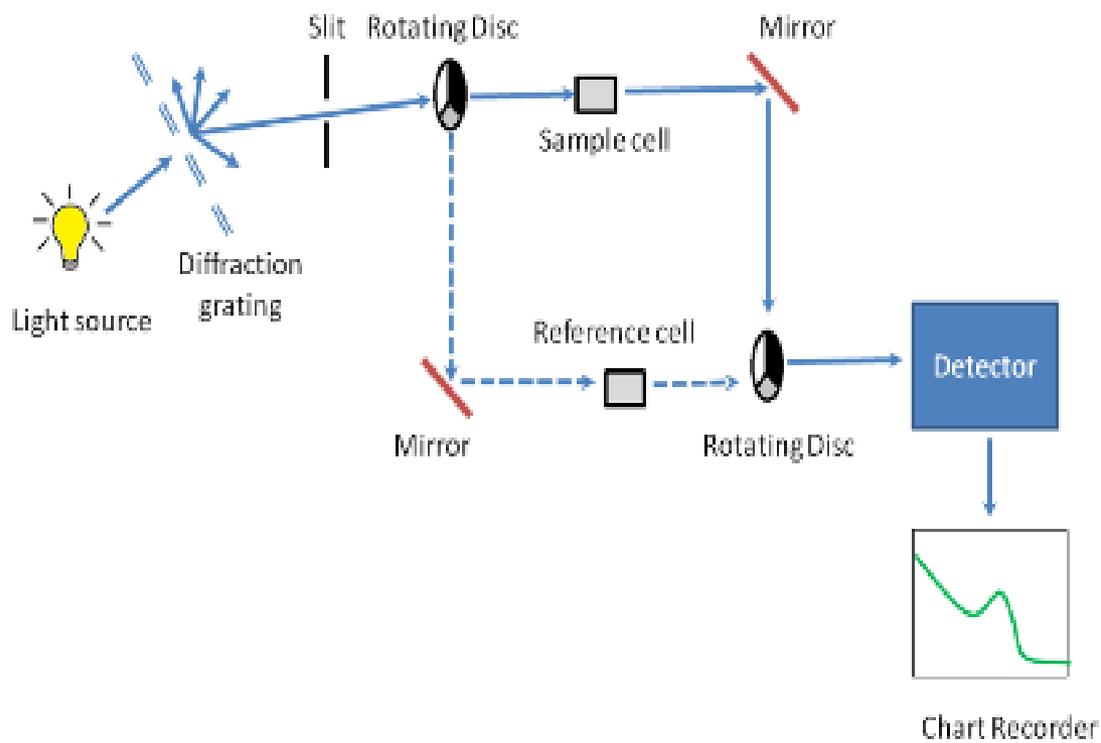


Figure 1. Schematic of UV-visible spectrophotometer.



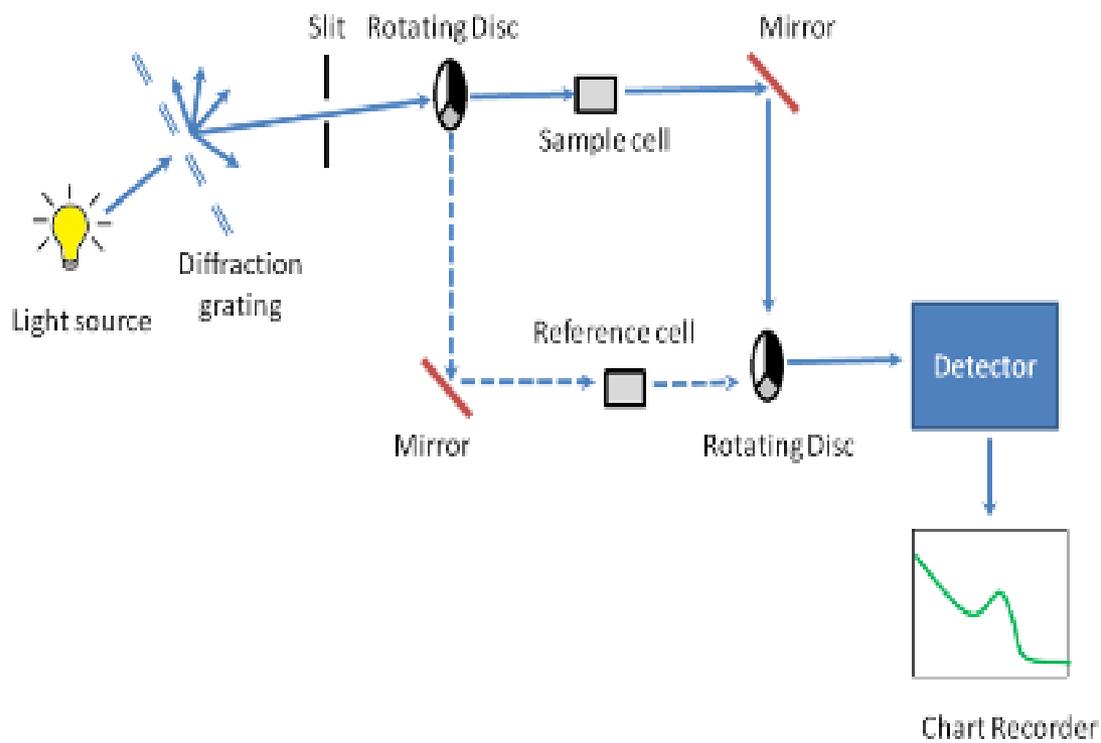


Figure 1. Schematic of UV-visible spectrophotometer.



The following cases are possible:

- Where $I < I_0$ and $A < 0$.
- Where $I = I_0$ and $A = 0$.
- When $A = 1$.
- When $A > 1$.



SAMPLE PREPARATION

- Selection of cuvettes

Cuvette	Wavelength (nm)
UV plastic	220 - 780
Quartz	200 - 900

- Selection of solvent: The solvents used in preparing solutions to be analyzed must be spectral grade.



SOURCES OF CONFUSION

Major sources of confusion arise from faulty sample preparation and from incorrect use of the UV/VIS spectrometer.

- Dirty cells.
- Impure solvents

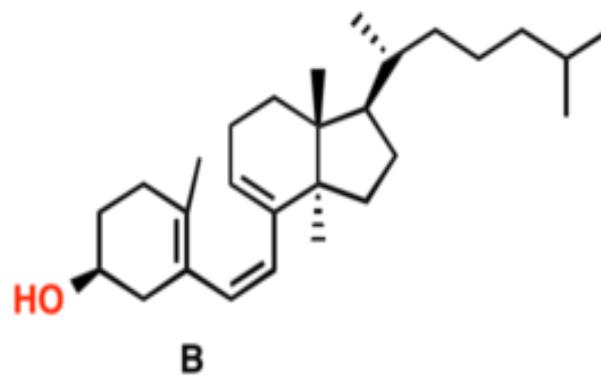
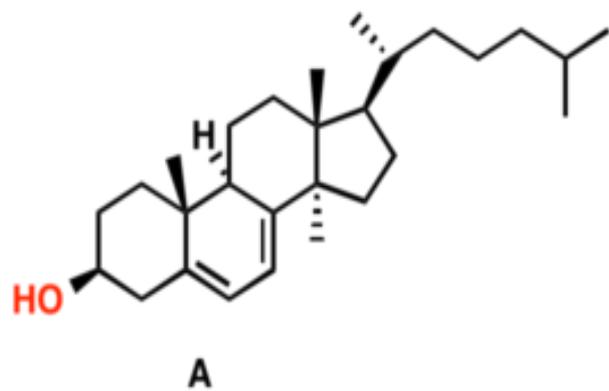


UV-VISIBLE SPECTROSCOPY -

EXAMPLES

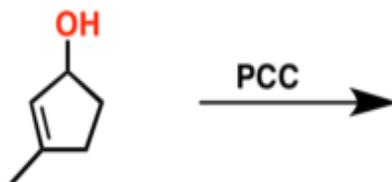
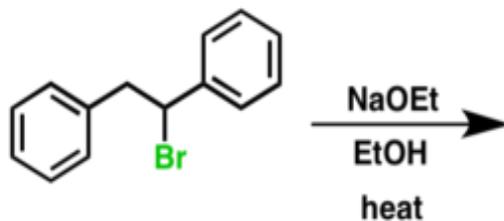
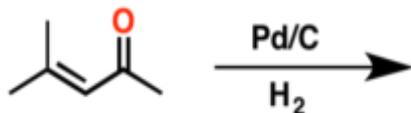


Which molecule absorbs at a longer wavelength, A or B ?

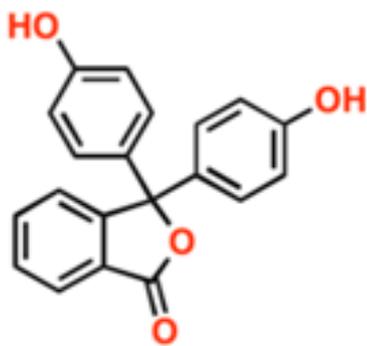


What are the products of these reactions?

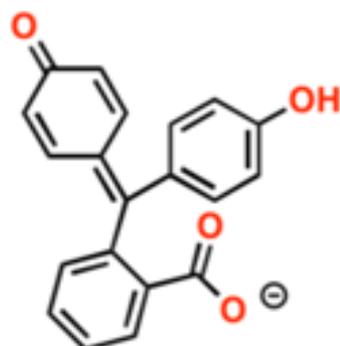
Would you expect them to have higher or lower λ_{max} than the starting material?



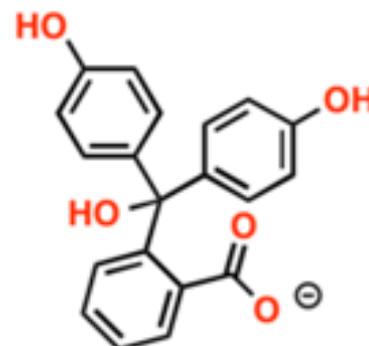
These are three forms of the pH indicator dye phenolphthalein.
One of them is responsible for its characteristic pink color
Which one?



A



B



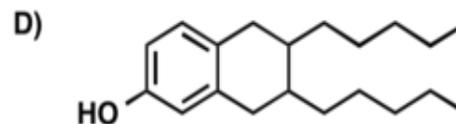
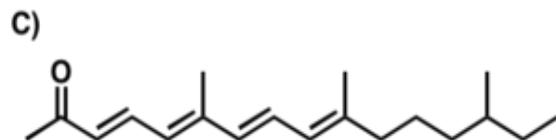
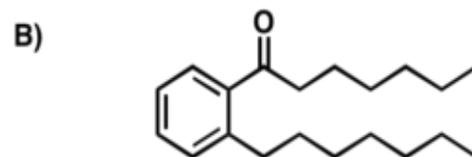
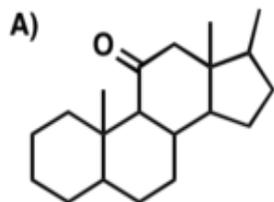
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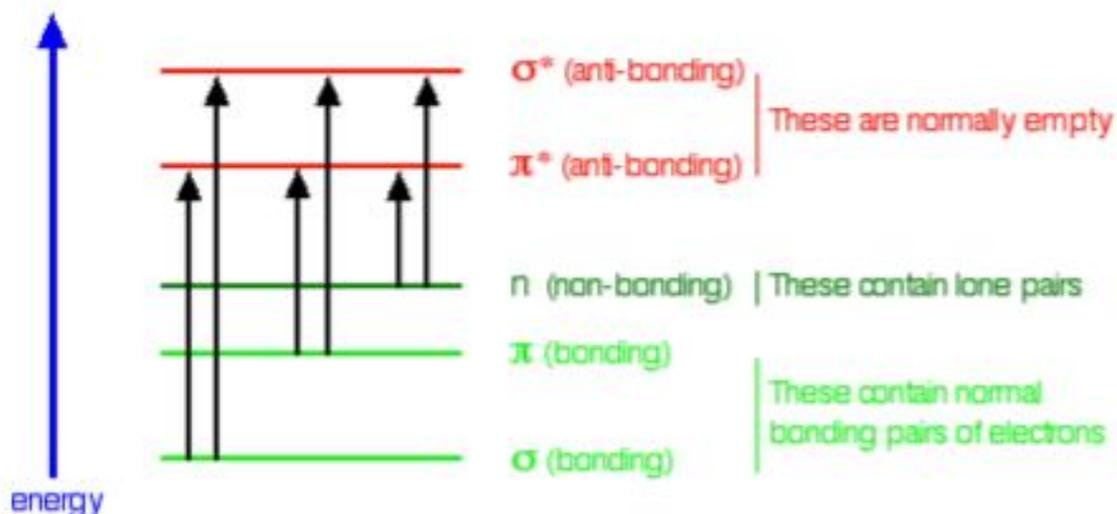
A compound has the formula $C_{20}H_{32}O$ and a UV spectrum with $\lambda_{max} = 275$ nm.

- Subjecting the compound to hydrogenation with Pd/C and H_2 led to no change in λ_{max}
- Treatment with $NaBH_4$ or $LiAlH_4$ led to no change in the λ_{max}

What is the most likely structure?



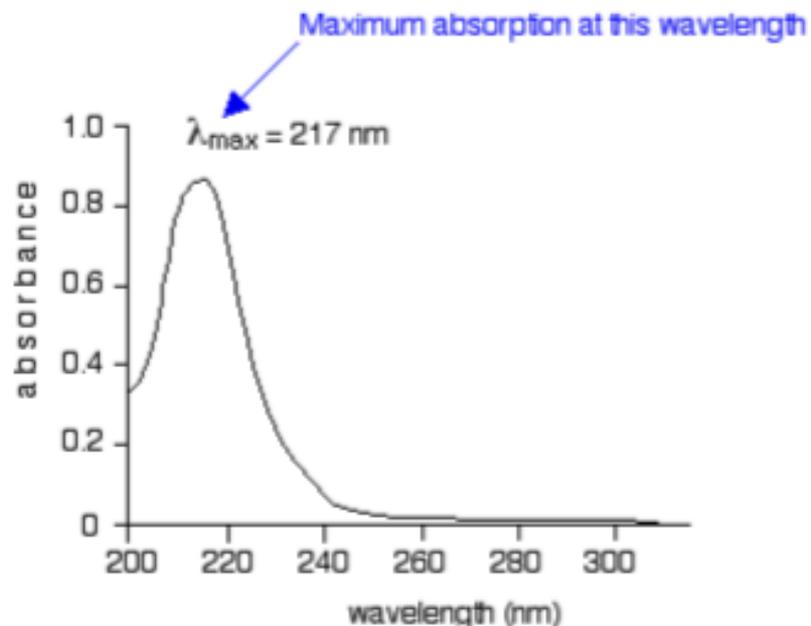
1. When light passes through a compound some wavelengths are absorbed because their energy is used to promote an electron into a higher energy orbital. The diagram shows the electron jumps which are possible.



- Which jump needs the highest energy?
- Which jump absorbs light of the highest frequency? Explain your answer.
- Which jump absorbs light of the highest wavelength? Explain your answer.
- The three longest jumps aren't useful in UV-visible spectrometry. Explain why.



e) The spectrum for buta-1,3-diene, $\text{CH}_2=\text{CH}-\text{CH}=\text{CH}_2$, looks like this:



What sort of jump is responsible for the peak? Explain your answer.

f) Ethanal, CH_3CHO , produces two peaks involving jumps from the pi electrons in the $\text{C}=\text{O}$ double bond and from a lone pair on the oxygen. The peaks are at 180 and 290 nm. Which jump produces which peak?

