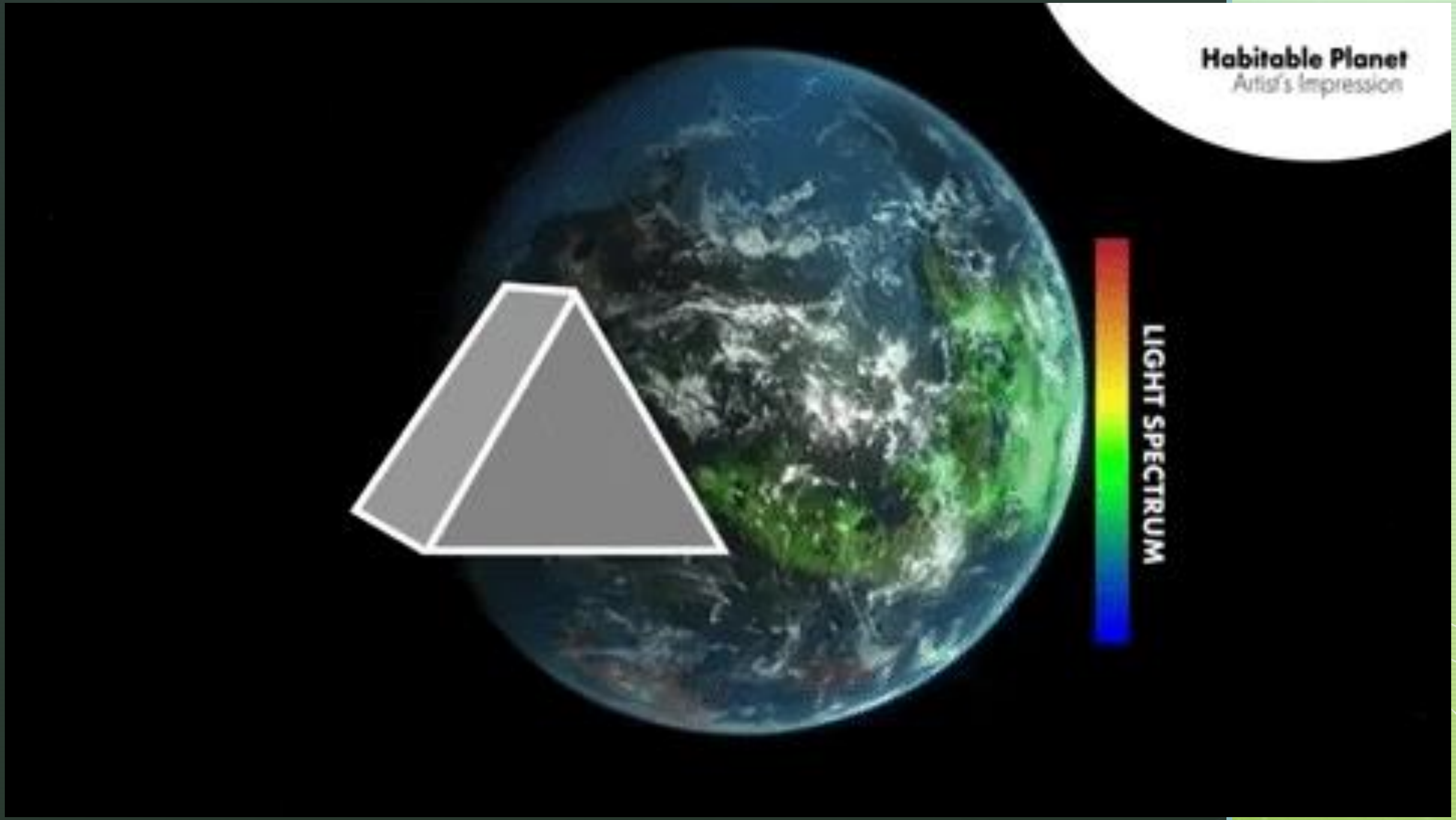
U. V. VISIBLE SPECTROSCOPY



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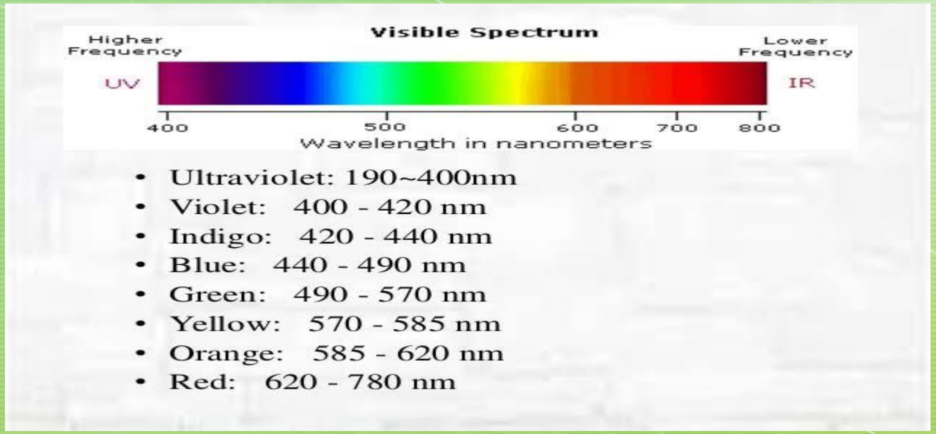
INTRODUCTION

In biochemistry, ultraviolet (UV)–visible spectrophotometry is commonly used for determining species and analysing biochemical processes. Because most biological chemicals absorb in the UV–visible region or can be transformed into an absorbing derivative, this approach allows for the determination of micromolar quantities of substances and has a wide range of applications in this field.

UV–visible (ultraviolet–visible) The absorption of near-UV (180–390 nm) or visible (390–780 nm) radiation by chemical species in solution is the subject of spectrophotometry, which is essentially a quantitative analytical technique.



Theory Of U.V Visible Spectroscopy

When a beam of monochromatic light is passed through a solution of an observing substance, it obeys the Beers Lambert law, which states that when a beam of monochromatic light is passed through a solution of an observing substance, it obeys the Beers Lambert law.

TYPES OF TRANSITIONS:

In U.V spectroscopy molecule undergo electronic transition involving σ, π and n electrons.

- Four types of electronic transition are possible.

i. $\sigma \rightarrow \sigma^*$ transition
ii. $n \rightarrow \sigma^*$ transition
iii. $n \rightarrow \pi^*$ transition
iv. $\pi \rightarrow \pi^*$ transition

BbbbLaw Of U.V Visible Spectroscopy

Lambert' s Law

When monochromatic radiation passes through a solution, the reduction in intensity with solution thickness is proportional to the intensity of the input light.

$$\ln I/I_o = -KL$$

$$2.303 \log I/I_o = -KL$$

Beers Law

When monochromatic radiation passes through a solution, the reduction in intensity of radiation with solution thickness is proportional to the intensity of input light as well as the solution concentration.

$$T = I/Io$$

$$- \log T = \log I/Io$$

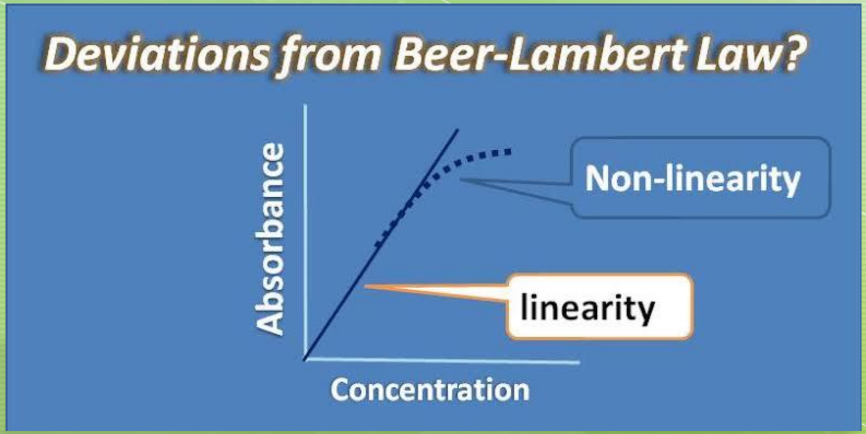
DEVIATION FROM BEER'S LAW

According to Beers law, if we plot obsorbance A against concentration, we get a straight line passing through the origin. The apparent failure of Beers law is based on a linear relationship between concentration and absorption.

Types Of Deviation

Positive Deviation – Small change in concentration produced a greater change in absorbance.

Negative Deviation - Large Change in concentration produces a smaller change in absorbance.



Terms Used In U.V Visible Spectroscopy

Chromophore - Any isolated covalently bound group with a distinctive absorption in the ultra violet or visible range (200-800nm) and the ability to give colour.

Auxochromes - Auxochrome is a term that refers to any group that does not operate as a chromophore but causes a shift in the absorption link between the longer wavelengths of the spectrum.

Intensity Shifs In U.V Spectroscopy

Bathochromic Shift (Red Shift) – Para Nitro Phenol

Hypochromic Shift (Blue Shift) - Aniline

Hyperchromic effect – Pyridine

Hypochromic effect - Naphthalene

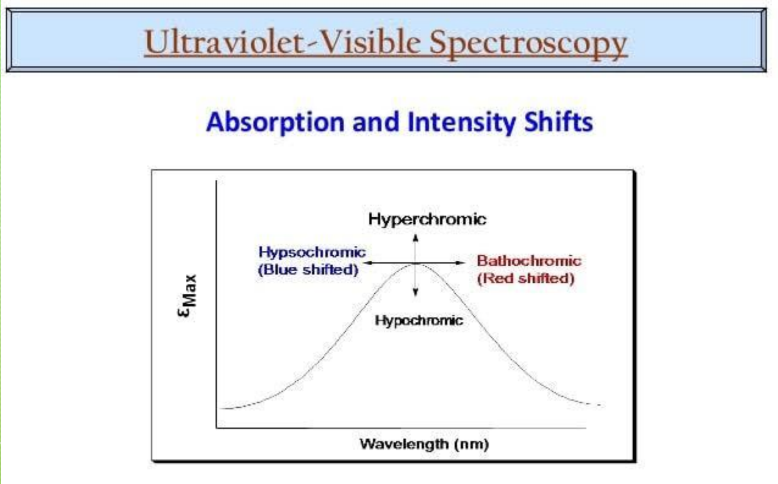


Figure: Absorption and intensity shifts

Choice Of Solvent

It should not absorb any radiation in the area being investiga. It should be less polar so that it interacts with the solute molecule as little as possible. Most frequently: Ethanol 95 percent It is inexpensive, has a high dissolving power, and does not absorb radiation beyond 210nm.

Wavelenght Cyclohexaneth of Solvent (nm) Methanol 210 Ethanol 210 Ether 210 Chloroform 245 Carbon tetra chloride 265 Cyclohexane 210 Water 205 Methanol 210 Ethanol 210 Ether 210 Chloroform 245 Carbon tetra chloride 265 Cyclohexane 210.

Solvents for electronic spectroscopy

SOLVENT	LOWER WAVELENGTH LIMIT/nm
Water	205nm
Ethanol	210nm
Hexane	210nm
Cyclohexane	210nm
Chloroform	245nm
Carbon tetrachloride	265nm
Benzene	280nm

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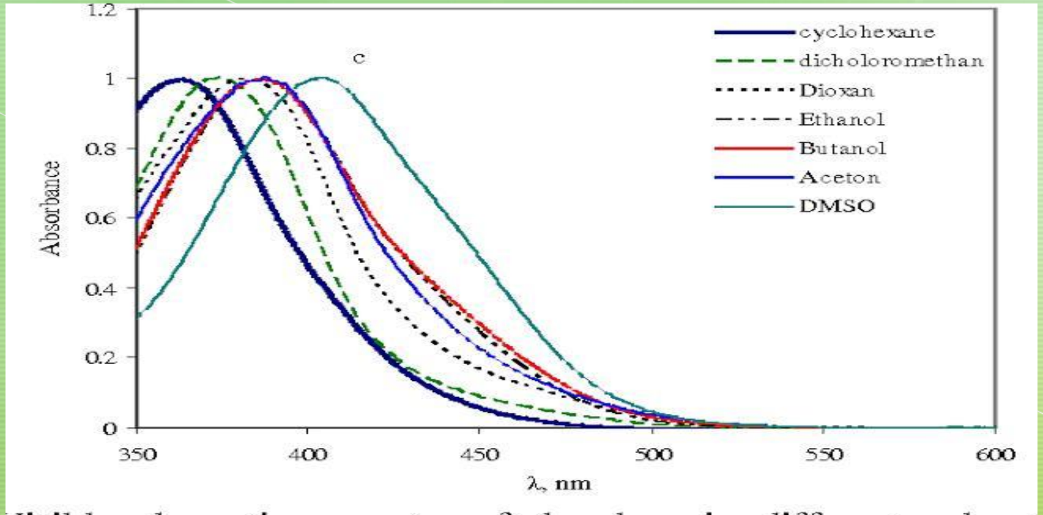
Effect Of Solvent

The solvent has a significant impact on the spectrum's quality and form. The absorption spectrum of a pharmaceutical drug is largely determined by the solvent used to solubilize the molecule. In one solvent, a medication may absorb a maximum amount of radiation energy at a specific wavelength, but only partially at the same wavelength in another solvent.

For example, acetone in n-hexane has a maximum absorption wavelength of 279nm. Acetone in water has a maximum wavelength of 264.5nm.



THANK YOU