''बेटी बचाओ, बेटी पढ़ाओ''

JAYOTI VIDYAPEETH WOMEN'S UNIVERSITY, JAIPUR

Faculty of Pharmaceutical Science

Faculty Name - JV'n Dr. Parveen Parihar

Course - B. Pharm (7^{th} sem)

Session - Instrumental method of Analysis – (UV Spectroscopy)

Academic Day starts with –

- Greeting with saying **'Namaste'** by joining Hands together following by 2-3 Minutes Happy session, Celebrating birthday of any student of respective class and **National Anthem**

UV Spectroscopy

UV-Visible spectroscopy is a form of Absorption spectroscopy. Absorption spectroscopy in the UV-Visible region is to be one of the oldest and most frequently employed technique in pharmaceutical analysis for qualitative, quantitative and structural analysis of a substance in solution.

- The substance is analyzed by studying the spectrum produced by it due to absorption of certain wavelengths of UV-Visible light.
- Spectro scopically, visible light behaves in a similar way as UV light. Hence, the techniques of UV spectroscopy and Visible spectroscopy are studied together.

- The UV-Visible spectroscopy is concerned with the UV & Visible regions of the EMR which ranges between 200-800nm. UV wavelength range is 200-400 nm. Visible region wavelength range is 400-800 nm.

Principle

The principle involved in UV-Visible spectroscopy is absorption spectroscopy. The principle of UV-Visible spectroscopy is based on the fundamental law of absorption called Beer-Lambert's law. This law governs the absorption of radiation by an absorbing medium (dilute solution).

Beer's Law

According to Beer's law, when a beam of monochromatic radiation passes through an absorbing medium, the intensity radiation decreases exponentially with an increase in the concentration of the absorbing medium.

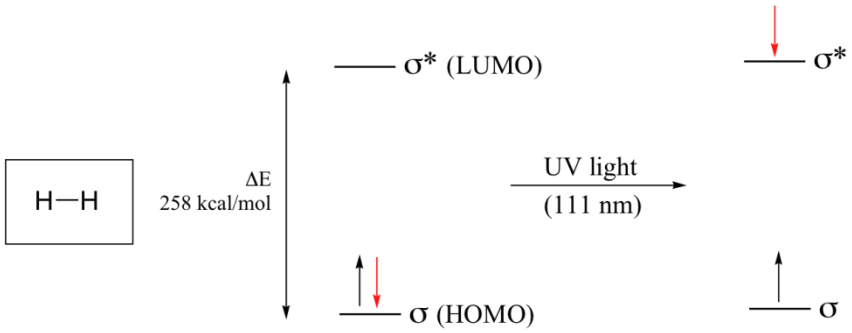
In other words, absorbance is directly proportional to the concentration of the absorbing substance.

Lambert's law:

According to Lambert's law, the rate of decrease in the intensity of the radiation (I) with the thickness of the medium (t) is directly proportional to the intensity of the incident light.

Electronic Transition

While interaction with infrared light causes molecules to undergo vibrational transitions, the shorter wavelength, higher energy radiation in the UV (200-400 nm) and visible (400-700 nm) range of the electromagnetic spectrum causes many organic molecules to undergo **electronic transitions**. What this means is that when the energy from UV or visible light is absorbed by a molecule, one of its electrons jumps from a lower energy to a higher energy molecular orbital.



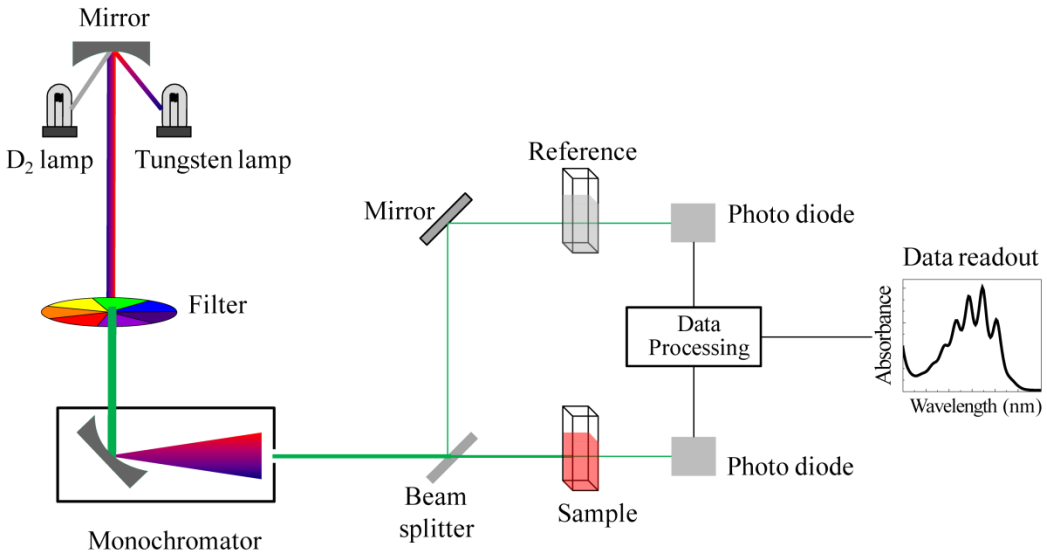
If the molecule is exposed to light of a wavelength with energy equal to **Δ**E, the HOMO-LUMO energy gap, this wavelength will be absorbed and the energy used to bump one of the electrons from the HOMO to the LUMO .

For some molecules, these electron transitions occur in the UV-visible region of the electromagnetic spectrum. Molecules or parts of molecules that absorb light strongly in the UV-vis region are called **chromophores**. These electronic transitions Where UV-vis spectroscopy becomes useful to most organic and biological chemists is in the study of molecules with conjugated π systems.

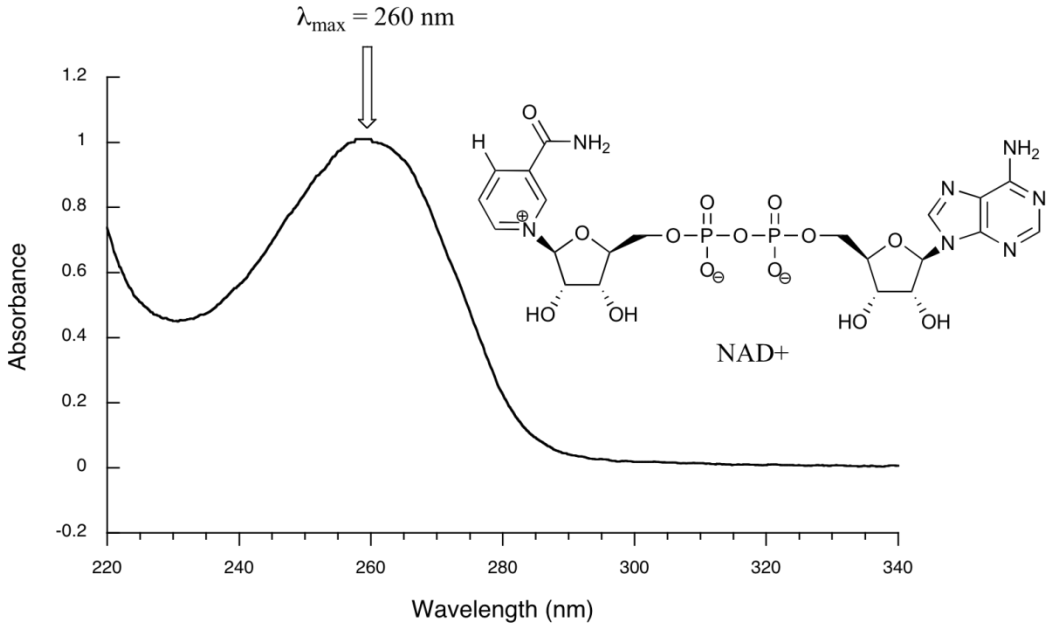
In molecules with extended pi systems, the HOMO-LUMO energy gap becomes so small that absorption occurs in the visible rather then the UV region of the electromagnetic spectrum. Beta-carotene, with its system of 11 conjugated double bonds, absorbs light with wavelengths in the blue region of the visible spectrum while allowing other visible wavelengths – mainly those in the red-yellow region - to be transmitted. This is why carrots are orange.

UV Visible Spectra

We have been talking in general terms about how molecules absorb UV and visible light – now let's look at some actual examples of data from a UV-vis absorbance spectrophotometer. The basic setup is the same as for IR spectroscopy: radiation with a range of wavelengths is directed through a sample of interest, and a detector records which wavelengths were absorbed and to what extent the absorption occurred.

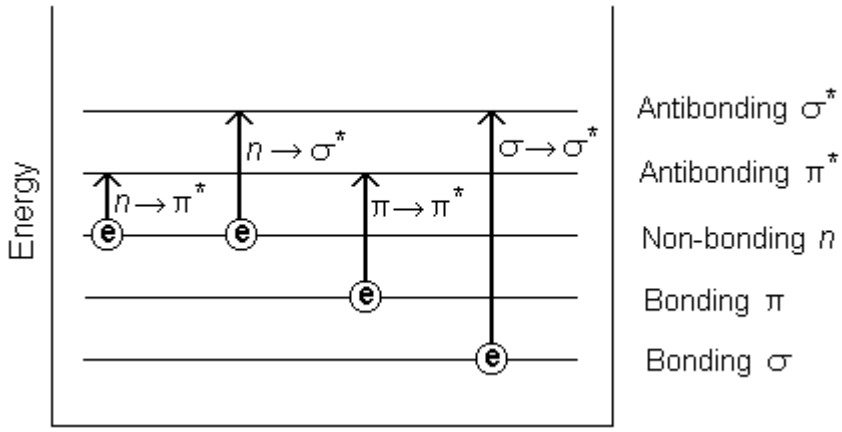


Below is the absorbance spectrum of an important biological molecule called nicotinamide adenine dinucleotide, abbreviated NAD^+. This compound absorbs light in the UV range due to the presence of conjugated pi-bonding systems.



Types of Electronic Transitions:

When we speak of a molecule as being raised to a higher electronic level we mean that an electron has been changed from one orbital to another orbital of higher energy. This electron can be of any kinds we have encountered –a σelectron, a π electron or an n electron. In Ultraviolet region we are confined only to the excitation of the comparatively loosely held n and πelectrons.



Out of the above mentioned transitions only $\mathbf{n \rightarrow \pi^*}$ and $\boldsymbol{\pi \rightarrow \pi^*}$ are of use to the analytical chemist working on the ultraviolet spectrophotometer.

- When light – either visible or ultraviolet – is absorbed by valence (outer) electrons. These Electrons are promoted from their normal (ground) states to higher energy (excited) states .
- The energies of the orbitals involved in electronic transitions have fixed values. Because energy is quantised, It seems safe to assume that absorption peaks in a UV/visible spectrum will be sharp peaks.
- However, this is rarely, if ever, observed. Instead the spectrum has broad peaks .This is because there are also vibrational and rotational energy levels available to absorbing materials.

- Ultraviolet- visible spectroscopyor ultraviolet - visible spectrophotometery (UV-Vis or UV/Vis) involves the spectroscopy of photons in the UV-visible region.
- There is an interaction between UV visible light and sample which is in solution form.
- As a result of this interaction some photons (photons of UV-Vis EMR) are absorbed and this absorption of UV visible is measured by an instrument named UV visible spectrophotometer.
- UV visible is low energy EMR hence generally no ionization is take place but electronic transition of lone pair and π electron take place (200-800 nm).

- **Next Topic-**

- Instrumental method of Analysis– (Instrumentation)

- **Academic Day ends with-**

National song 'Vande Mataram'