

UCB008 - APPLIED CHEMISTRY



Molecular Spectroscopy Series Lecture - IX

UV-Visible Spectroscopy –Analysis and Applications

by

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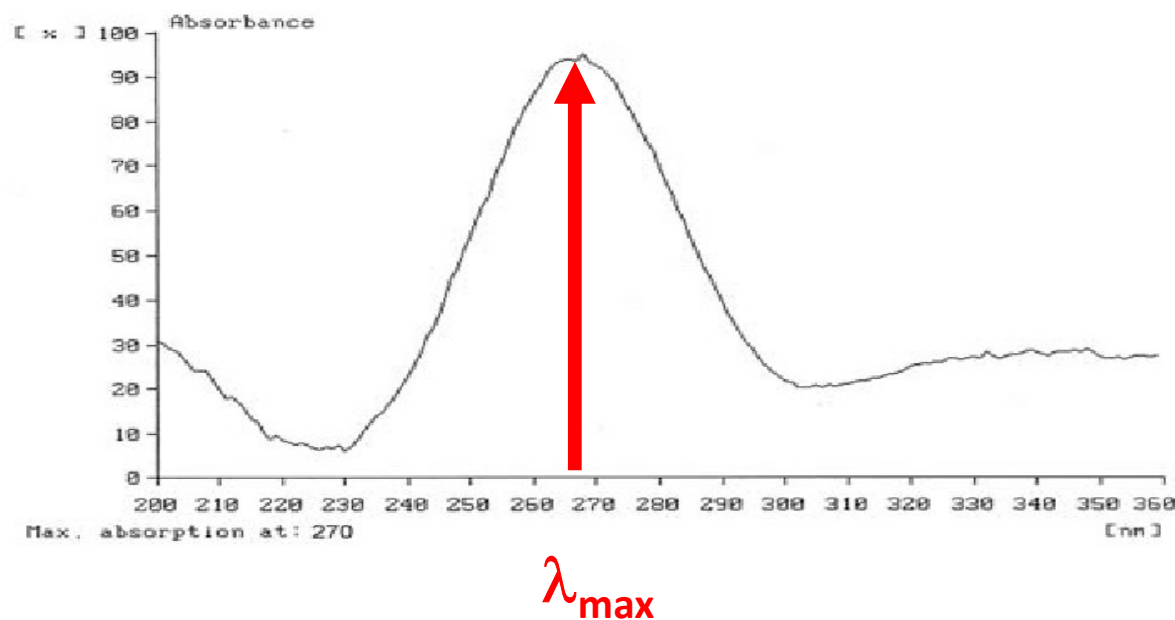
Learning Outcomes

At the end of this session participants should be able to:

- Understand the analytical procedure of qualitative and quantitative analysis using UV-visible spectrophotometer
- Gain knowledge on applications of UV-visible spectroscopy

Qualitative analysis using UV-visible spectrophotometer

- λ_{max} of organic compounds can be determined using UV-visible spectra
- This value can be used to detect the extent of conjugation
- ... and absence/presence of the chromophore in an organic compound.



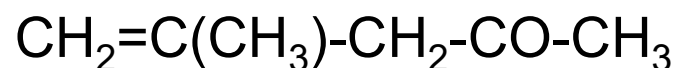
Applications

• Qualitative Analysis:

- Extent of conjugation in polyenes can be estimated.
- Shift in the absorption, due to addition of unsaturation in a compound, towards longer wavelength can be detected.
- Presence or absence of a chromophore can be detected.
- If UV-visible spectrum of a compound has no peaks above 200 nm, it shows the absence of conjugation, carbonyl group and aromatic ring.
- It is used for characterizing aromatic compounds and conjugated olefins.
- Distinction between conjugated and non-conjugated compounds



A

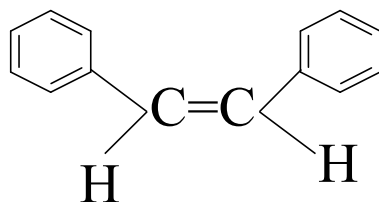


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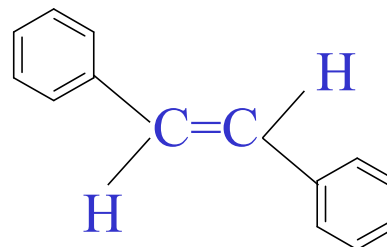
Applications

- **Qualitative Analysis:**

- Geometric isomers can be distinguished



cis-stilbene
 λ_{max} : 283 nm



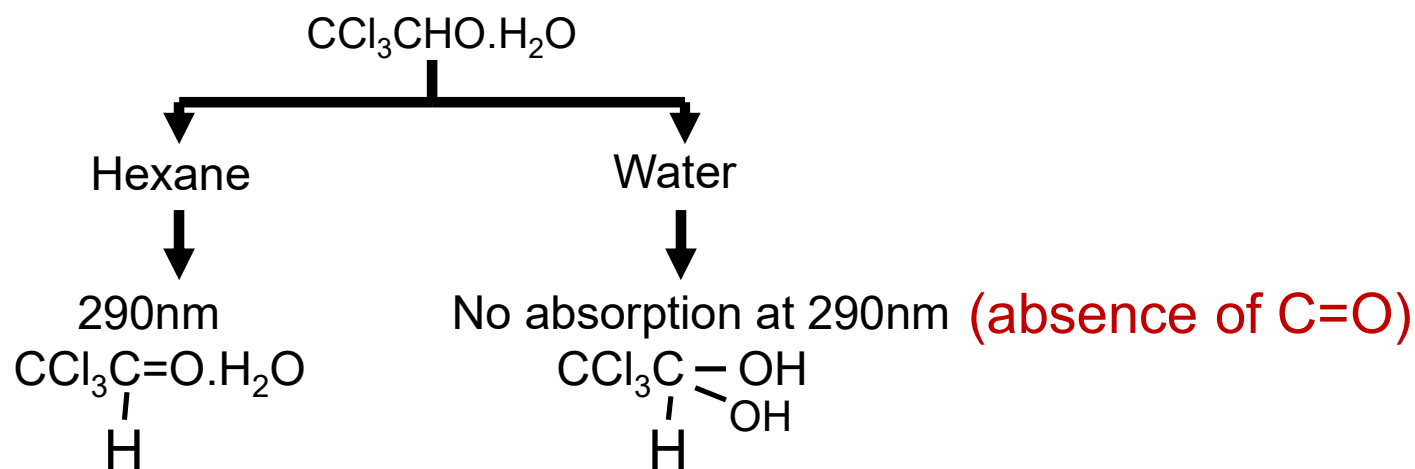
trans-stilbene
 λ_{max} : 295 nm

- Trans isomer always absorb at higher wavelength

Applications

• Qualitative Analysis:

- Identification of a compound in different solvents- If structure of a compound changes in different solvents, the change can be detected by uv-vis spectra.



Sharma YR, 2010, Elementary Organic Spectroscopy, S.Chand & Co.

Quantitative analysis of an unknown sample solution using UV-visible spectrophotometer

- Series of standard solutions having known concentration of analyte X are prepared.
- One of these solutions is analyzed by the spectrophotometer and its λ_{\max} is determined.
- Absorbance of all the standard solutions are analyzed at the determined λ_{\max} .
- **A calibration curve** (absorbance vs concentration) is prepared for the standard samples.
- Signal intensity of unknown sample of analyte X is taken.
- The concentration of the analyte X is then determined based on the absorbance value from the calibration curve.

Quantitative analysis of an unknown sample solution using UV-visible spectrophotometer

Step 1: Series of standard solutions having known concentration of analyte X are prepared.

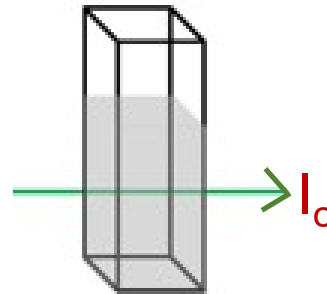


Quantitative analysis of an unknown sample solution using UV-visible spectrophotometer

Step 2: Solvent is taken as **blank solution** for which absorbance is measured and absorbance value is set to **Zero**.

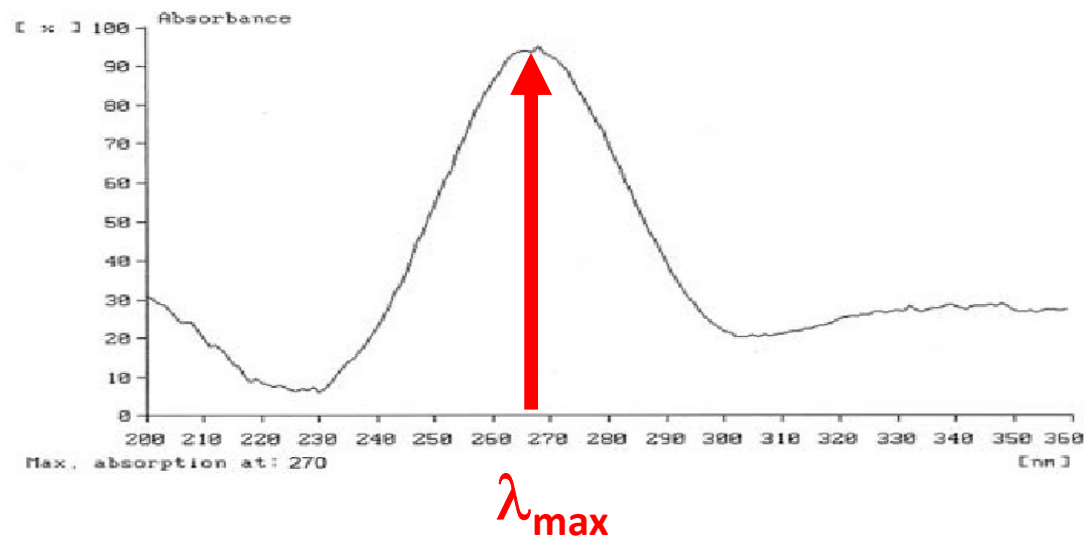


Blank Solution



Quantitative analysis of an unknown sample solution using UV-visible spectrophotometer

Step 3: One of the standard solutions is analyzed by the UV-visible spectrophotometer and its λ_{max} is determined.

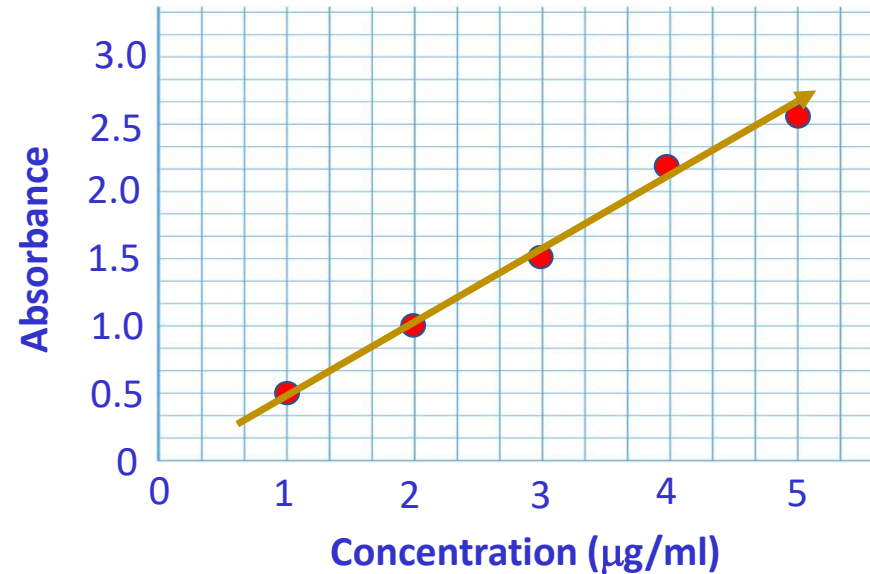


Instrument is calibrated with blank, periodically, when wavelength is changed, in case of single beam spectrophotometer.

Quantitative analysis of an unknown sample solution using UV-visible spectrophotometer

Step 4: Absorbance of all the standard solutions are analyzed at the determined λ_{max} .

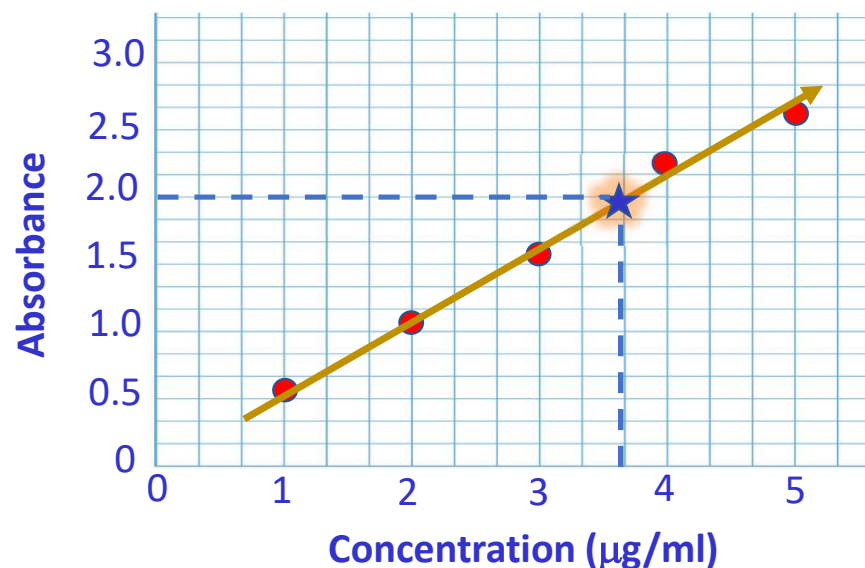
Step 5: **A calibration curve** (absorbance vs concentration) is prepared by analyzing the standard samples.



Quantitative analysis of an unknown sample solution using UV-visible spectrophotometer

Step 6: Absorbance value of the solution of analyte X (unknown) is determined at the same λ_{\max} .

Step 7: The concentration of the analyte X is then determined based on the absorbance value from the calibration curve by extrapolating towards concentration in X axis.

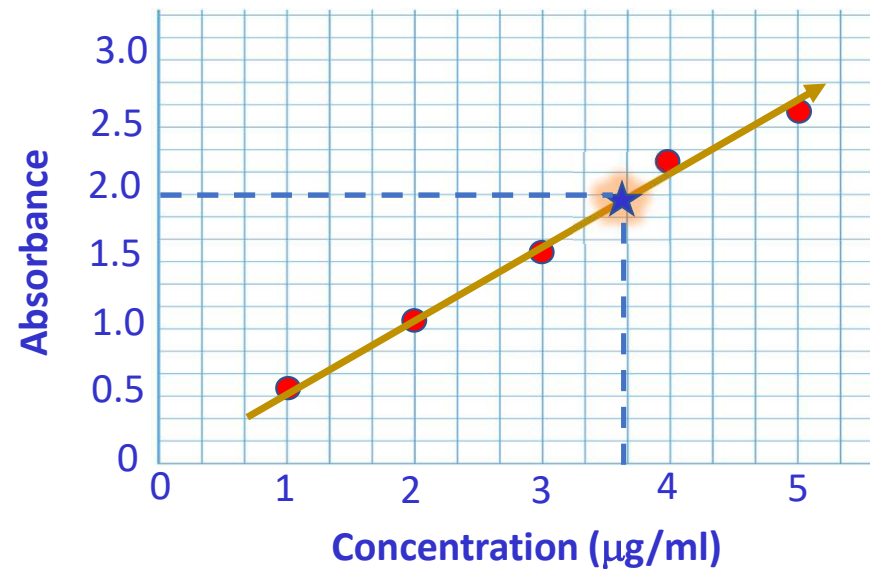


★ - concentration of analyte X
- e.g., ~3.66 µg/ml in this case

Applications

- **Quantitative Analysis:**

- It can be used to find out molar concentration of the solute under study.



With this session.....

- We complete the understanding of the fundamental aspects associated with UV-visible spectroscopy

From the next session.....

- We will be exploring the principle, instrumentation and applications of Infra-red spectroscopy