PHARMACEUTICAL ANALYSIS

Ultraviolet and Visible Spectrophotometry

Dr. Hinna Hamid
Lecturer
Dept. of Chemistry
Faculty of Science
Jamia Hamdard
Hamdard Nagar
New Delhi- 110062

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Keywords
Spectrometry, Absorption, Emission, Spectrum, absorbance, Transmittance
**Introduction**

Among the most dynamic challenges in the analytical laboratory is the quest to determine the chemical structure of an unknown compound. The process of elucidation can serve a variety of purposes, such as the identification and classification of drug metabolites or products being investigated as drug candidates, quantify compounds, process impurities, etc. Elucidation of chemical compounds can be done by many different methods.

Chemical methods:
- Functional group testing
- Chemical transformations

Physical measurements:
- Physical properties
- Spectroscopy

Electromagnetic radiations can interact with matter in various ways. Each interaction can disclose certain properties of that matter and use of electromagnetic radiations of different energies can give different information about the matter under study.

**Electromagnetic Spectrum**

Different regions of electromagnetic spectrum have different kinds of spectroscopic techniques associated with them.

- Radiowaves: ESR and NMR spectroscopy
- Microwaves: Rotational spectroscopy
- Infrared waves: IR spectroscopy
- UV/ VIS waves: UV/VIS spectroscopy

**Principle**

Molecules absorb energy and this energy can bring about translational, rotational or vibrational motion or ionization of the molecules depending upon the frequency of the electromagnetic radiation they receive. Excited molecules are unstable and quickly drop down to ground state again giving off the energy they have received as electromagnetic radiation.
The wavelength and intensity of the electromagnetic radiation absorbed or emitted can be recorded to get a spectrum. Spectral analysis yields qualitative and quantitative information about the matter under study.

**Spectrum**

Spectrum is the display of the energy level of an e.m.r. as a function of wave number of electromagnetic radiation energy. The energy level of an e.m.r. may be expressed in any of the following terms:

- Absorbance (e.m.r. absorbed)
- Transmittance (e.m.r. passed through)
- Intensity (radiant power of e.m.r)

**Ultraviolet and Visible Spectroscopy**

Ultraviolet-Visible (UV-VIS) spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of compounds and technologically important materials, such as pigments, coatings etc. The UV-VIS spectra have broad features that are of some use for sample identification but are very useful for quantitative measurements.
Qualitative Analysis

**UV-VIS spectroscopy** studies the electronic transitions of molecules as they absorb light in the UV and visible regions of the electromagnetic spectrum. The data is used to produce absorbance spectra.

The visible region of the spectrum comprises photon energies of 36 to 72 Kcal/mole, and the near ultraviolet region, out to 200 nm, extends this energy range to 143 kcal/mole. This energy is enough to promote the outer electrons to higher energy levels. As a rule, the energetically favored electron promotion will be from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The resulting species is said to be in an excited state. An optical spectrometer records the wavelengths at which absorption occurs, together with the degree of absorption at each wavelength to produce a spectrum.

For example for isoprene, wavelength of maximum absorption is caused by the $\pi-\pi^*$ electronic transition within the conjugated system present in the molecule.

UV absorptions of molecules are generally broad because vibrational and rotational levels are "superimposed" on top of the electronic levels. In addition to undergoing electronic transitions the atoms can rotate and vibrate with respect to each other. These vibrations and rotations also have discrete energy levels. This large number of available levels produces multiple absorptions and appearance of broad bands in an UV/VIS spectrum, rather than narrow peaks. For this reason, the wavelength of maximum absorption ($\lambda_{\text{max}}$) is usually reported.
**Electronic Transitions**
The absorption of UV or visible radiation corresponds to the three types of electronic transition:

1. Transitions involving $\pi$, $\sigma$, and $n$ electrons
2. Transitions involving charge-transfer electrons
3. Transitions involving $d$ and $f$ electrons

Possible *electronic* transitions of $\pi$, $\sigma$, and $n$ electrons are;

**$\sigma \rightarrow \sigma^*$ Transitions:**
The energy required for an electron in a bonding $\sigma$ orbital to get excited to the corresponding antibonding orbital is large. For example, methane (which has only C-H bonds, and can only undergo $\sigma \rightarrow \sigma^*$ transitions) shows an absorbance maximum at 125 nm and are thus not seen in typical UV-VIS spectra (200 - 780 nm).

**$n \rightarrow \sigma^*$ Transitions:**
Saturated compounds containing atoms with lone pairs (non-bonding electrons) like saturated alcohols, amines, halides, ethers etc are capable of showing $n \rightarrow \sigma^*$ transitions. Energy required for these transitions is usually less than $\sigma \rightarrow \sigma^*$ transitions. Such compounds absorb light having wavelength in the range 150 - 250 nm, e.g., absorption maxima for water, methyl chloride and methyl iodide are 167 nm, 169 nm and 258 nm respectively.

**$\pi \rightarrow \pi^*$ Transitions:**
These transitions need an unsaturated group in the molecule to provide the $\pi$ electrons, e.g., in alkenes, alkynes, aromatics, acyl compounds or nitriles. Most absorption spectroscopy of organic compounds is based on transitions of $n$ or $\pi$ electrons to the $\pi^*$ excited state and the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 780 nm). $\pi \rightarrow \pi^*$ transitions normally give molar absorptivities between 1000 and 10,000 L mol$^{-1}$ cm$^{-1}$. Unconjugated alkenes absorb at 170-190 nm.
$n \rightarrow \pi^*$ Transitions:

Unsaturated compounds containing atoms with lone pairs (non-bonding electrons) show this transition. For $n \rightarrow \pi^*$ transitions the molar absorptivities are relatively low (10 to 100 L mol$^{-1}$ cm$^{-1}$), e.g., saturated aliphatic Ketones absorb at 280 nm.

Most UV/vis spectrometric studies are carried out between 180-780 nm. Region below 180 nm is referred to as the vacuum ultraviolet region. Atmospheric gases and most glasses and quartz absorb below 180 nm making measurements difficult without taking special measures. The only molecular moieties that absorb light in the 200 to 800 nm region are pi-electron functions and hetero atoms having non-bonding valence-shell electron pairs. Chemical functionalities that are responsible for absorption are referred to as chromophores. Thus any species, which is colored, absorbs visible light and any moiety with an extended system of alternating double and single bonds will absorb UV light. This makes UV-vis spectroscopy applicable to a wide range of samples.

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Transition</th>
<th>$\lambda_{\text{max}}$</th>
<th>log(e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>alkene</td>
<td>$n \rightarrow \pi^*$</td>
<td>160</td>
<td>1.0</td>
</tr>
<tr>
<td>alkyne</td>
<td>$\pi \rightarrow \pi^*$</td>
<td>170</td>
<td>3.0</td>
</tr>
<tr>
<td>alkene</td>
<td>$\pi \rightarrow \pi^*$</td>
<td>175</td>
<td>3.0</td>
</tr>
<tr>
<td>alcohol</td>
<td>$n \rightarrow \sigma^*$</td>
<td>180</td>
<td>2.5</td>
</tr>
<tr>
<td>ether</td>
<td>$n \rightarrow \sigma^*$</td>
<td>180</td>
<td>3.5</td>
</tr>
<tr>
<td>ketone</td>
<td>$\pi \rightarrow \pi^*$</td>
<td>189</td>
<td>3.0</td>
</tr>
<tr>
<td>aldehyde</td>
<td>$\pi \rightarrow \sigma^*$</td>
<td>260</td>
<td>1.5</td>
</tr>
<tr>
<td>amine</td>
<td>$n \rightarrow \sigma^*$</td>
<td>180</td>
<td>3.5</td>
</tr>
<tr>
<td>acid</td>
<td>$n \rightarrow \sigma^*$</td>
<td>265</td>
<td>1.5</td>
</tr>
<tr>
<td>ester</td>
<td>$n \rightarrow \sigma^*$</td>
<td>269</td>
<td>1.5</td>
</tr>
<tr>
<td>amide</td>
<td>$n \rightarrow \sigma^*$</td>
<td>210</td>
<td>1.5</td>
</tr>
<tr>
<td>thiol</td>
<td>$n \rightarrow \sigma^*$</td>
<td>210</td>
<td>3.0</td>
</tr>
<tr>
<td>sulfonate</td>
<td>$\pi \rightarrow \pi^*$</td>
<td>271</td>
<td>1.0</td>
</tr>
<tr>
<td>azo</td>
<td>$n \rightarrow \pi^*$</td>
<td>340</td>
<td>1.0</td>
</tr>
</tbody>
</table>

List of some simple chromophores and their light absorption characteristics
Table 1: Absorption by molecules with heteroatoms

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound</th>
<th>$\lambda_{\text{max}}$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH$_3$OH</td>
<td>167</td>
</tr>
<tr>
<td>2</td>
<td>(CH$_3$)$_2$O</td>
<td>184</td>
</tr>
<tr>
<td>3</td>
<td>CH$_3$Cl</td>
<td>173</td>
</tr>
<tr>
<td>4</td>
<td>CH$_3$I</td>
<td>258</td>
</tr>
<tr>
<td>5</td>
<td>(CH$_3$)$_2$S</td>
<td>229</td>
</tr>
<tr>
<td>6</td>
<td>CH$_3$NH$_2$</td>
<td>215</td>
</tr>
<tr>
<td>7</td>
<td>(CH$_3$)$_3$N</td>
<td>227</td>
</tr>
</tbody>
</table>

The presence of an absorbance band at a particular wavelength often is a good indicator of the presence of a chromophore. However, the position of the absorbance maximum is not fixed but depends partially on the molecular environment of the chromophore and on the solvent in which the sample may be dissolved. Other parameters, such as pH and temperature, also may cause changes in both the intensity and the wavelength of the absorbance maxima.

In addition to such organic compounds early transition metals also absorb in UV region depending on the nature of the ligand.

Many inorganic species called as charge-transfer complexes show charge-transfer absorption. In such a complex one of its components must have electron-donating properties and another component must be able to accept electrons. The transfer of an electron from the donor to an orbital associated with the acceptor leads to absorption. Charge transfer agents have high molar absorptivity.
Molar absorptivities of compounds vary significantly
The magnitude of \( \varepsilon \) reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed when it strikes the chromophore. It may be very large for strongly absorbing chromophores (>10,000) and very small if absorption is weak (10 to 100).

The transitions between the electronic states are governed by Selection Rules. Some transitions are “allowed”, and others may be “forbidden”. Allowedness is associated with the geometries of HOMO and LUMO and the symmetry of the molecule as a whole.
For example

The two common transitions of an isolated carbonyl group are
\[ n \rightarrow \pi^* \] transition which is lower in energy (\( \lambda_{\text{max}} = 290 \text{ nm} \))
\[ \pi \rightarrow \pi^* \] transition which is higher in energy (\( \lambda_{\text{max}} = 180 \text{ nm} \))
but the former is a forbidden transition and later an allowed transition. This can be explained on the basis of the spatial distribution of these orbitals.

The \( n \)-orbitals do not overlap at all well with the \( \pi^* \) orbital, so the probability of this excitation is small. The \( \pi \rightarrow \pi^* \) transition, on the other hand, involves orbitals that have significant overlap, and the probability is near 1.0. Allowed transitions have higher \( \varepsilon_{\text{max}} \) values than the forbidden transitions.
Although UV-visible spectra do not enable absolute identification of an unknown compound, UV spectra and Visible spectra can be used to identify a compound by comparative analysis. One can compare the UV or Visible spectra of the unknown with the spectra of known suspects.

Besides detecting the presence of distinctive chromophores in a molecule, UV/VIS spectroscopy can also be used to detect the extent of conjugation in a molecule, more the conjugation the longer the wavelength of absorption. Each additional double bond in the conjugated pi-electron system shifts the absorption maximum about 30 nm towards longer wavelength. This is due to decreasing energy gap between HOMO and LUMO as the conjugation increases.

A common feature of all these colored compounds is a system of extensively conjugated pi-electrons.
UV/vis spectroscopy can also be used to study geometric isomerism of molecules. The trans isomer absorbs at longer wavelength with a larger molar extinction constant than cis isomer. This can be explained by the steric strain introduced in the cis isomer resulting in lesser $\pi$ orbital overlap.

![UV absorption by Stilbene](image)

UV/Vis spectroscopy can also be used to study the degree of strain; the degree of conjugation may suffer in strained molecules (e.g., 2-substituted biphenyls) by correlating changes in spectrum with angular distortion. It can also be used to distinguish the most stable tautomeric forms. The UV spectrum of the solution will be found to be complimentary to more abundant tautomer.

![π MO's of cis-Stilbene and trans-Stilbene](image)

For example, for 2-hydroxy pyridine

![Diagram of 2-hydroxy pyridine and pyridine-2-one](image)

The equilibrium has been shown to lie far to the right, i.e., the uv spectrum of the solution resembles that of the pyrid-2-one rather than 2-hydroxy pyridine.

**Effect of solvent on absorption:** The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. Peaks resulting from $n \rightarrow \pi^*$ transitions are shifted to shorter wavelengths (blue shift) with increasing solvent polarity because of increased solvation of the lone pair in the ground state, which lowers the energy of the $n$ orbital. Often the reverse (i.e., red shift) is seen for $\pi \rightarrow \pi^*$ transitions. This is caused by attractive polarisation forces.
between the solvent and the absorbing molecule, which lower the energy levels of both the excited and unexcited states. The effect being greater for the excited state, the energy difference between the excited and unexcited states is slightly reduced - resulting in a small red shift. This effect can also influence $\pi \rightarrow \pi^*$ transitions but cannot be observed due to the dominant blue shift resulting from solvation of lone pairs.
For example, for Mesityl oxide, following shifts are observed for the two electronic transitions on moving from low polarity solvent hexane to water, which has higher polarity.

\[
\text{Hexane} \quad 230 \ (12,600) \quad 327 \ (98) \\
\text{Ethanol} \quad 237 \ (12,600) \quad 315 \ (78) \\
\text{Water} \quad 245 \ (10,000) \quad 305 \ (60)
\]

Spectroscopists use the following terms when describing shifts in absorption.

<table>
<thead>
<tr>
<th>Nature of Shift</th>
<th>Term used</th>
</tr>
</thead>
<tbody>
<tr>
<td>To longer wavelength</td>
<td>Bathochromic shift/ red shift</td>
</tr>
<tr>
<td>To shorter wavelength</td>
<td>Hypsochromic shift/ blue shift</td>
</tr>
<tr>
<td>To greater absorbance</td>
<td>Hyperchromic shift</td>
</tr>
<tr>
<td>To lesser absorbance</td>
<td>Hypochromic shift</td>
</tr>
</tbody>
</table>

**Instrumentation**

Spectrophotometer is an instrument for measuring the transmittance or absorbance of a sample as a function of the wavelength of electromagnetic radiation. The key components of a spectrophotometer are:

- a source that generates a broad band of electromagnetic radiation
- a dispersion device that selects from the broadband radiation of the source a particular wavelength (or, more correctly, a waveband)
- a sample area
- one or more detectors to measure the intensity of radiation
- other optical components, such as lenses or mirrors, relay light through the instrument.

A schematic representation of a UV/VIS spectrophotometer is shown below. Normal working range for a spectrometer is 190 – 900 nm, working beyond 180 nm requires special arrangements.
The Light Source:
- A deuterium discharge lamp for UV region (160-375 nm)
- A tungsten filament lamp or tungsten-halogen lamp for Visible and NIR regions (350 - 2500 nm)
- The instruments automatically swap lamps when scanning between the UV and VIS-NIR regions

The Monochromator: All monochromators contain the following component parts:
- An entrance slit
- A collimating lens
- A dispersing device
- A focusing lens
- An exit slit

Ideally, the output from a monochromator is monochromatic light. In practice, however, the output is always a band, optimally symmetrical in shape.

Dispersion devices: Dispersion devices cause different wavelengths of light to be dispersed at different angles. When combined with an appropriate exit slit, these devices can be used to select a particular wavelength (or, more precisely, a narrow waveband) of light from a continuous source. Two types of dispersion devices, prisms and holographic gratings, are commonly used in UV-VIS spectrophotometers.

![Dispersion devices](image)

Light falling on the grating is reflected at different angles, depending on the wavelength. Holographic gratings yield a linear angular dispersion with wavelength and are temperature insensitive. However, they reflect light in different orders, which may overlap. As a result, filters must be used to ensure that only the light from the desired reflection order reaches the detector.

Detectors: A detector converts a light signal into an electrical signal. Ideally, it should give a linear response over a wide range with low noise and high sensitivity. Spectrophotometers normally contain either a photomultiplier tube detector or a photodiode detector.

The photomultiplier tube combines signal conversion with several stages of amplification within the body of the tube. It consists of a photoemissive cathode, a number of dynodes (which emit several electrons for each electron striking them) and an anode.

Photodiodes are increasingly being used as detectors in modern spectrophotometers. Photodiode detectors have a wider dynamic range and are more robust than photomultiplier tube detectors. In a photodiode, light falling on the semiconductor material allows electrons to flow through it, thereby depleting the charge in a capacitor connected across the material. The amount of charge
needed to recharge the capacitor at regular intervals is proportional to the intensity of the light. The limits of detection are approximately 170–1100 nm for silicon-based detectors.

Some modern spectrophotometers contain an array of photodiode detectors instead of a single detector. A diode array consists of a series of photodiode detectors positioned side by side on a silicon crystal. Each diode has a dedicated capacitor and is connected by a solid-state switch to a common output line. Initially, the capacitors are charged to a specific level. When photons penetrate the silicon, free electrical charge carriers are generated that discharge the capacitors. The capacitors are recharged at regular intervals. The amount of charge needed to recharge the capacitors is proportional to the number of photons detected by each diode, which in turn is proportional to the light intensity.

The absorption spectrum is obtained by measuring the variation in light intensity over the entire wavelength range.

**Configuration**
Various configurations of spectrophotometers are commercially available. Each has its advantages and disadvantages.

- Single-beam design
- Dual-beam design
- Split-beam design
- Dual-wavelength design
Cells
These are containers for the sample and reference solutions. They must be transparent to the radiation passing through. For UV region: Quartz or fused silica cuvettes are usually used. VIS/NIR regions: Silicate glass or plastic cuvettes (350-2000 nm) can also be used.

Choice of solvent
UV/VIS spectroscopy is usually applied to molecules or inorganic complexes in solution. Ideally the solvent used should be transparent at all wavelengths.

Properties of some common solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Polarity*</th>
<th>Cut-off wavelength (nm)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>78.5</td>
<td>&lt; 195</td>
</tr>
<tr>
<td>Hexane</td>
<td>1.9</td>
<td>199</td>
</tr>
<tr>
<td>Ethanol (absolute)</td>
<td>24.3</td>
<td>207</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.6</td>
<td>210</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>2.0</td>
<td>211</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4.8</td>
<td>246</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>none</td>
<td>270</td>
</tr>
<tr>
<td>Acetone</td>
<td>20.7</td>
<td>331</td>
</tr>
</tbody>
</table>

* Dielectric constant at ambient temperature
** Wavelength at which transmittance of 10-mm path length is < 25%

Key Chromophores for UV/VIS Spectroscopy
Isolated Double bond
Due to $\pi \rightarrow \pi^*$ electronic transition unconjugated alkenes absorb below 200 nm, e.g., ethene shows an absorption at 171 nm. Alkyl substituents or the ring residues attached to the olefinic carbon shift the absorption band towards longer wavelength as can be seen with absorption pattern of compounds like 1-Octene and cyclohexene which absorb at 177 nm and 182 nm, respectively.

Conjugated dienes: With the increase in conjugation the absorption moves towards longer wavelengths, thus compared with ethene, which absorbs at 171 nm, 1,3-butadiene absorbs at 217 nm. Sufficient conjugation shifts the absorption to wavelengths that reach the visible region of
the spectrum. An acyclic diene largely exists in the preferred S-trans conformation, as is so in the case of butadiene as rotation about the central single bond is possible. In case of a cyclic diene, it is forced into a cisoid configuration resulting in shifting the absorption to longer wavelengths but the intensity is lowered.

\[
\text{Cisoid} \quad \rightarrow \quad \text{Transoid}
\]

**Forced (Cisoid) Conformation**

Each alkyl substituent or ring substituent when attached to the conjugated diene chromophore displaces absorption by about 5nm towards longer wavelengths and each exocyclic location of the double bond causes a red shift of \( \lambda_{\text{max}} \) by 5nm.

Woodward and Fieser formulated a set of empirical rules, which could be used to predict absorption maxima of conjugated systems like dienes, enones, aromatic systems (bezene and its derivatives), benzoyl compounds etc.

**Woodward-Fieser Rules for Dienes**

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Influence on ( \lambda_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>R- (Alkyl Group)</td>
<td>+5 nm</td>
</tr>
<tr>
<td>RO- (Alkoxy Group)</td>
<td>+6 nm</td>
</tr>
<tr>
<td>X- (Cl- or Br-)</td>
<td>+10 nm</td>
</tr>
<tr>
<td>RCO\textsubscript{2} (Acyl Group)</td>
<td>0</td>
</tr>
<tr>
<td>RS- (Sulfide Group)</td>
<td>+30 nm</td>
</tr>
<tr>
<td>R\textsubscript{2}N- (Amino Group)</td>
<td>+60 nm</td>
</tr>
</tbody>
</table>
Double bond extending conjugation  
+30 nm
Exocyclic double bond  
+5 nm

Examples

214 (base value) + 5 (one alkyl group) = 219 nm

214 (base value) + 30 (one conjugated DB) = 244 nm

214 (base value) + 5x2 (two alkyl groups) + 5 (one exocyclic DB) = 229 nm

214 (base value) + 5x3 (three alkyl groups) + 5 (one exocyclic DB) = 234 nm

214 (base value) + 5x2 (two alkyl groups) = 224 nm

214 (base value) + 5x3 (three alkyl groups) + 5 (one exocyclic DB) = 234 nm

214 (base value) + 5x3 (three alkyl groups) + 5 (one exocyclic DB) + 6 (one OR group) = 241 nm

For X = EtO
215 (base value) + 5x3 (three alkyl groups) + 5 (one exocyclic DB) + 6 (one OR group) = 241 nm

For X = MeS
215 (base value) + 5x3 (three alkyl groups) + 5 (one exocyclic DB) + 30 (one SR group) = 265 nm

For X = Br
215 (base value) + 5x3 (three alkyl groups) + 5 (one exocyclic DB) + 5 (one Br group) = 240 nm
Polyenes
The Woodward’s rules work well only for conjugated polyenes having four double bonds or less. For conjugated polyenes with more than four double bonds the Fieser-Kuhn rules are used.

\[
\lambda_{\text{max}} = 114 + 5M + n (48 - 1.7n) – 16.5 \text{ Rendo} - 10 \text{ Rexo}
\]

where
\( n \) = no. of conjugated double bonds
\( M \) = no. of alkyl groups
\( \text{Rendo} \) = no. of endocyclic double bonds
\( \text{Rexo} \) = no. of exocyclic double bonds

For example

For this compound with eleven conjugated double bonds

\[
\lambda_{\text{max}}(\text{cal}) = 114 + 5(8) + 11 [48-1.7 (11)] – 0 – 0 = 476 \text{ nm}
\]

Aldehydes and ketones
Saturated ketones and aldehydes show three absorption bands, two of which are observed in far UV region

\[\pi \rightarrow \pi^* \text{ transition (150 nm)} \quad n \rightarrow \sigma^* \text{ transition (190 nm)} \quad n \rightarrow \pi^* \text{ transition (270-300 nm)}\]

Introduction of polar substituents: Halogen on the alpha carbon atom has no effect on \( n \) to \( \pi^* \) transition in case of aliphatic compounds but in case of cyclic ketones the presence of such substituents raises \( \lambda_{\text{max}} \) by 10-30 nm (when substituent is axial) or lowers it by 4-10 nm (when substituent is equatorial). This data is useful to provide information to know the preferred conformation in a non-rigid system.

When auxophoric groups like a hydroxyl, an ester or an amino group are attached to a carbonyl group, the \( \pi^* \) orbital is raised in energy but \( n \) level is left unaltered. So \( n \) to \( \pi^* \) transition shows a blue shift.

Acetaldehyde (293 nm)  Ethyl acetate (207 nm)
Acetic acid (204 nm)  Acetamide (220 nm)
Alpha- Beta unsaturated aldehydes and ketones (enones)
Bathochromic shift in both $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ bands

\[
\begin{align*}
\text{Methyl ethyl ketone} & \quad \text{Methyl vinyl ketone} \\
\text{n to } \pi^* & \quad 277 \text{ nm} \quad 324 \text{ nm (24)} \\
\text{\pi to } \pi^* & \quad 185 \text{ nm} \quad 219 \text{ nm (3600)}
\end{align*}
\]

A set of empirical rules can be used to calculate the position of intense $\pi \rightarrow \pi^*$ transition band (K band) for these compounds.

**Woodward-Fieser Rules for Calculating the $\pi \rightarrow \pi^*$ $\lambda_{\text{max}}$ of Conjugated Carbonyl Compounds (enones)**

<table>
<thead>
<tr>
<th>Core Chromophore</th>
<th>Substituent and Influence</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Core Chromophore" /></td>
<td><img src="image" alt="Substituent Influence" /></td>
</tr>
</tbody>
</table>

- **$\alpha$-Substituent**
  - R- (Alkyl Group) $+10$ nm
  - Cl- (Chloro Group) $+15$ nm
  - Br- (Chloro Group) $+25$ nm
  - HO- (Hydroxyl Group) $+35$ nm
  - RO- (Alkoxyl Group) $+35$ nm
  - RCO$_2$- (Acyl Group) $+6$ nm

- **$\beta$-Substituent**
  - R- (Alkyl Group) $+12$ nm
  - Cl- (Chloro Group) $+12$ nm
  - Br- (Chloro Group) $+30$ nm
  - HO- (Hydroxyl Group) $+30$ nm
  - RO- (Alkoxyl Group) $+30$ nm
  - RCO$_2$- (Acyl Group) $+6$ nm
  - RS- (Sulfide Group) $+85$ nm
  - R$_2$N- (Amino Group) $+95$ nm

- **$\gamma$ & $\delta$-Substituents**
  - R- (Alkyl Group) $+18$ nm (both $\gamma$ & $\delta$)
  - HO- (Hydroxyl Group) $+50$ nm ($\gamma$)
  - RO- (Alkoxyl Group) $+30$ nm ($\gamma$)

**Further $\pi$-Conjugation**
- C=C (Double Bond) $+30$ nm
- C$_6$H$_5$ (Phenyl Group) $+60$ nm

$\lambda_{\text{max}}$ (calculated) = Base + Substituent Contributions and Corrections

(i) Each exocyclic double bond adds $5$ nm.
(ii) Homoannular cyclohexadiene component adds $+35$ nm (ring atoms must be counted separately as substituents)
(iii) Solvent Correction: water = $-8$; methanol/ethanol = $0$; ether = $+7$; hexane/cyclohexane = $+11$
For example

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>215 nm</td>
</tr>
<tr>
<td>Conj.</td>
<td>60</td>
</tr>
<tr>
<td>Subs.</td>
<td>66</td>
</tr>
<tr>
<td>Exo</td>
<td>5</td>
</tr>
<tr>
<td>Homoann. Ring</td>
<td>35</td>
</tr>
</tbody>
</table>

381 nm

When cross conjugation is present in a compound (Alpha- Beta unsaturation on both sides of the carbonyl group) the value of absorption is calculated by considering the most highly substituted conjugated system.

Non conjugated Interacting Chromophores

<table>
<thead>
<tr>
<th>X</th>
<th>( \lambda_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMe₂</td>
<td>238 nm, 308.5 nm</td>
</tr>
<tr>
<td>CH₂</td>
<td>229 nm, 318.5 nm</td>
</tr>
</tbody>
</table>
The unsaturated ketones in some cases sometimes display their \( n \text{ to } \pi^* \) and \( \pi \text{ to } \pi^* \) transitions shifted in opposite directions when \( X \) becomes electronegative.

**Alpha- and beta diketones- exist in enolic forms.**
The absorption depends on the concentration of the different forms present

\[
\begin{array}{c}
\text{O} \\
\text{O} \\
\text{OH}
\end{array}
\]  
\[\lambda_{\text{max}} \text{Obs. 270 nm}\]

\[
\begin{array}{c}
\lambda_{\text{max Cal}}: \\
215 \text{nm (base)} \\
24 \text{ (2-beta subst.)} \\
35 \text{ (alpha hydroxy)} \\
274 \text{ nm}
\end{array}
\]

**Benzene Chromophore**

Benzene is the classic example of a molecule with an extended \( \Pi \) system, as can be seen from its circular arrangement of alternating double and single bonds.

Electronic transitions in benzene could be complex due to closely spaced \( \pi \) orbitals. Three absorptions are observed for \( \pi-\pi^* \) transitions

- \( 184 \text{ nm; } \varepsilon \sim 60,000 \) (second primary band)
- \( 204 \text{ nm; } \varepsilon \sim 8,000 \) (primary band or K band)
- \( 256 \text{ nm; } \varepsilon \sim 2,00 \) (secondary band or B band)

K and B bands are forbidden transitions.
B band often displays a fine structure in non-polar solvents.

**Substituted Benzenes**

- Any substituent shifts K band to longer \( \lambda \)
- EWG has little effect on B band
- EDG shifts B band to longer \( \lambda \)
- When benzene is substituted by an alkyl group the absorption is shifted to longer wavelengths. This may be attributed to the hyperconjugation between the alkyl group and the \( \pi \) system of the ring. Para isomer exerting the maximum effect.
Disubstituted Benzenes

For ortho and meta substitution, substituent effects are additive for $\lambda_{\text{max}}$

In case of para substitution, when both groups are EWG or EDG, only the group with the larger effect is used to estimate $\lambda_{\text{max}}$.

When one group is EWG and one EDG, the observed $\lambda_{\text{max}}$ is much higher than the calculated $\lambda_{\text{max}}$ due to extension of the chromophore.

### Absorption for some di-substituted Benzene derivatives

<table>
<thead>
<tr>
<th>R</th>
<th>R’</th>
<th>Orientation</th>
<th>K</th>
<th>$\lambda_{\text{max}}$</th>
<th>$\varepsilon_{\text{max}}$</th>
<th>$\lambda_{\text{max}}$</th>
<th>$\varepsilon_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-OH</td>
<td>-OH</td>
<td>ortho</td>
<td>214</td>
<td>6000</td>
<td>278</td>
<td>2630</td>
<td></td>
</tr>
<tr>
<td>-OR</td>
<td>-CHO</td>
<td>ortho</td>
<td>253</td>
<td>11000</td>
<td>319</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>-NH$_2$</td>
<td>-NO$_2$</td>
<td>Ortho</td>
<td>229</td>
<td>16000</td>
<td>275</td>
<td>5000</td>
<td></td>
</tr>
<tr>
<td>-OH</td>
<td>-OH</td>
<td>Meta</td>
<td>277</td>
<td>8300</td>
<td>314</td>
<td>2800</td>
<td></td>
</tr>
<tr>
<td>-OR</td>
<td>-CHO</td>
<td>Meta</td>
<td>235</td>
<td>16000</td>
<td>373</td>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>-NH$_2$</td>
<td>-NO$_2$</td>
<td>Meta</td>
<td>225</td>
<td>5100</td>
<td>293</td>
<td>2700</td>
<td></td>
</tr>
<tr>
<td>-OH</td>
<td>-OH</td>
<td>Para</td>
<td>277</td>
<td>14800</td>
<td>375</td>
<td>16000</td>
<td></td>
</tr>
<tr>
<td>-OR</td>
<td>-CHO</td>
<td>Para</td>
<td>229</td>
<td>5000</td>
<td>280</td>
<td>25000</td>
<td></td>
</tr>
<tr>
<td>-NH$_2$</td>
<td>-NO$_2$</td>
<td>Meta</td>
<td>251</td>
<td>44000</td>
<td>375</td>
<td>16000</td>
<td></td>
</tr>
<tr>
<td>-Ph</td>
<td>-Ph</td>
<td>Meta</td>
<td>280</td>
<td>25000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Woodward-Fieser Rules for predicting absorptions of the principal band of substituted benzene.

**X-C6H4-CO-Z**

**Base:**

where Z = H, 250; or X-C6H4-COH (benzaldehydes)
where Z = aliphatic, 246; or X-C6H4-COR (acetophenones, tetralones, etc.)
where Z = O-H / O-R, 230; or X-C6H4-COOH / X-C6H4-COOR (acids / esters)

**Auxochromes:**

<table>
<thead>
<tr>
<th>R</th>
<th>o-, m-</th>
<th>p-</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR₂</td>
<td>20</td>
<td>85</td>
</tr>
<tr>
<td>O-H, O-R</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>aliphatic</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Br</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Cl</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>NH₂</td>
<td>13</td>
<td>58</td>
</tr>
</tbody>
</table>

**Examples**

![Chemical structures](image)

Parent value 246 nm  
Ortho alkyl 3 nm  
Para methoxy 25 nm  
Calc. $\lambda_{\text{max}}$ (EtOH) 274 nm  
Obs. $\lambda_{\text{max}}$ (EtOH) 276 nm

The spectra of heteroatomic compounds are similar to their corresponding hydrocarbons.

**Quantitative UV/vis Analysis**

Absorption spectroscopy is one of the most useful tools available to the chemist for quantitative analysis and its applications are not only numerous but also touch upon every field in which chemical quantitative analysis is required.

Quantitative UV/vis is used to determine the concentration of an analyte, which can absorb in this region, in a solution. Beer's Law, which gives a linear relationship between absorbance and concentration for dilute solutions, is used. A calibration plot is formed by measuring the absorbance of a series of analyte solutions with different known concentrations and the concentration of the target analyte in the sample under study is determined from the plot.
Spectrophotometry

Beer – Lambert Law is central to spectrophotometry and for many current applications a spectrometer is increasingly becoming the measurement device of choice. This law forms the basis of nearly all the chemometric methods for spectroscopic data.

Simply stated, the law claims that when a sample is placed in the beam of a spectrometer, there is a direct and linear relationship between the amount (concentration) of its constituent(s) and the amount of energy it absorbs.

In mathematical terms:

Absorption \( (A) = -\log(P/P_0) = \varepsilon bc \)

Where \( A \) is the sample’s Absorbance value at specific wavelength (or frequency), \( P_0 \) is the intensity of incident light, \( P \) is the intensity of transmitted light, \( \varepsilon \) is the absorptivity coefficient of the material (constituent) at that wavelength, \( b \) is the path length through the sample and \( c \) is the concentration.

The absorptivity coefficient for every material is different, but for a given compound at a selected wavelength, this value is a constant. \( \varepsilon \) is also known as molar extinction coefficient.

The amount of radiation absorbed may be measured in a number of ways:

Absorbance,

\[ A = \log_{10} \frac{P_0}{P} \]

\[ A = \log_{10} \frac{l}{T} \]
\[ A = \log_{10} \frac{100}{\%T} \]
\[ A = 2 - \log_{10} \%T \]

**Transmittance**, \( T = \frac{P}{P_0} \)

\% **Transmittance**, \( \%T = 100 \times \frac{T}{1} \)

The magnitude of \( \varepsilon \) reflects both the size of the chromophore and the probability that light of a given wavelength will be absorbed when it strikes the chromophore.

\[ \varepsilon = 0.87 \times 10^{20} \times P \times a \]

where

- \( P \) is the transition probability (0 to 1)
- \( a \) is the chromophore area in cm\(^2\)

The factors that influence transition probabilities are "Selection Rules" and the peaks with low \( \varepsilon \) values arise from transitions, which are formally ‘forbidden’.

**Beer’s Law and Mixtures**

The total absorbance of a solution at a given wavelength is equal to the sum of the absorbances of the individual components present. This makes it possible quantitatively estimate the individual components present.

- Each analyte present in the solution absorbs light.
- The magnitude of the absorption depends on its \( \varepsilon \)
- \( A_{\text{total}} = A_1 + A_2 + \ldots + A_n \)
- \( A_{\text{total}} = \varepsilon_1 b c_1 + \varepsilon_2 b c_2 + \ldots + \varepsilon_n b c_n \)
- If \( \varepsilon_1 = \varepsilon_2 = \ldots = \varepsilon_n \) then simultaneous determination is impossible

**Limitations to Beer’s Law**

Deviations from the direct proportionality between the measured absorbance and concentration when path length is constant may be encountered.

Some of the deviations are inherent to the law whereas others occur as a consequence of the manner in which the absorbance measurements (instrumental deviations) are made or as a result of chemical changes associated with concentration changes (chemical deviations).

Assumptions of the absorption law:

1. The incident beam is monochromatic
2. The absorbers absorb independently of each other.
3. Incident radiation consists of parallel rays perpendicular to the surface of the absorbing medium.
4. Path length traversed is uniform over the cross section of the beam
5. Absorbing medium is homogenous and does not scatter the radiation.
6. The incident flux is not large enough to cause saturation effects.
To compensate for these effects, the power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an identical cell containing only solvent.

Beer’s law is successful in describing the absorption behaviour of media containing relatively low analyte concentrations. At high concentrations usually $> 0.01$M - the average distance between the absorbing molecules is diminished, each molecule affecting the charge distribution of its neighbors, resulting in alteration in the ability of the molecules to absorb.

Similar effect is observed at low absorber concentration but high electrolyte concentration (ions).

Deviations are also due to the fact that $\varepsilon$ is dependent on refractive index of the medium. Thus changes in concentration of the solutions cause significant change in refractive index leading to deviations from Beer’s law.

Deviations from Beer’s law also arise when an analyte associates, dissociates or reacts with a solvent to produce a product having a different absorption spectrum from the analyte.

Consider the equilibrium

$$A + C \rightleftharpoons AC$$

If $\varepsilon$ is different for $A$ and $AC$ then the absorbance depends on the equilibrium $[A]$ and $[AC]$ depend on $[A]_{total}$.

And a plot of absorbance vs. $[A]_{total}$ will not be linear.
How to Approach UV-VIS Data

Range of Electronic spectroscopy, 200-400 nm UV; 400-800 nm, visible.

- UV-VIS data alone gives little structural information
- UV-VIS data more useful when a general idea of structural features is known; then empirical rules can be applied.

1. Single band of low intensity with $(\varepsilon_{100 \text{ to } 10,000})$ and $\lambda_{\text{max}} < 220 \text{ nm}$: Usually $n \rightarrow \sigma^*$ transition possible and the groups present may be alcohols, ethers, amines, thiols, etc.

2. Single band of low intensity $(\varepsilon_{10 \text{ to } 100}) 250 \text{ nm} < \lambda_{\text{max}} < 360 \text{ nm}$ usually $n \rightarrow \pi^*$ transition, without absorption at lower wavelength implies that a simple chromophore is present.

3. Two bands of medium intensity with $(\varepsilon_{1,000 \text{ to } 10,000})$ and both $\lambda_{\text{max}} > 200 \text{ nm}$: Usually $\pi \rightarrow \pi^*$ of aromatic system: Look for fine structure in longer wavelength band.

4. Bands of high intensity with $(\varepsilon_{10,000 \text{ to } 20,000})$ and $\lambda_{\text{max}} > 220 \text{ nm}$: Usually conjugated $\pi$ system: Check dienes and $\alpha,\beta$-unsaturated carbonyls

5. Band of low intensity with $\lambda_{\text{max}} > 300 \text{ nm}$ $(n \rightarrow \pi^*)$ and band of high intensity with $\lambda_{\text{max}} < 250 \text{ nm}$ $(\pi \rightarrow \pi^*)$: Unconjugated ketones, esters, acids, etc.

Suggested Readings:
- Introduction to spectroscopy: Pavia; Lampman, Kriz, Books/cole.
- Spectrometric identification of organic compounds, R. M. Silverstein, John Wiley and Sons publication.
- Spectroscopic methods in organic chemistry; H. Williams; I. Fleming, Tata Mc Grawhills
- Organic spectroscopy, W. Kemp, Palgrave publications.