A very brief history of the study of light

1. Sir Isaac Newton 1672:
   Showed that the component colors of the visible portion of white light can be separated through a prism, which acts to bend the light (refraction) in differing degrees according to wavelength. Developed a “corpuscular” theory of light.

2. Christian Huygens 1692:
   Developed a wave theory of light

3. Hans Christian Oersted 1820
   Showed that there is a magnetic field associated with the flow of electric current

4. Michael Faraday 1831
   Showed the converse i.e. that there is an electric current associated with a change of magnetic field
5. James Clark Maxwell: 1865

Published his “Dynamical theory of the electromagnetic field” which combined the discoveries of Newton, Young, Foucault, Oersted and Faraday into a unified theory of electromagnetic radiation

Showed that light consists of electromagnetic transverse waves whose frequency of vibration ($\nu$) and wavelength ($\lambda$) are related by

$$\nu \lambda = c$$

Where $\nu$ is the speed of light in the medium of study (for a vacuum $\nu = c$, where $c = 3 \times 10^{10}$ cm/sec) so $\lambda \nu = c$

We need to concern ourselves with how molecules interact with electromagnetic waves.
Absorption spectroscopy of electronic states
### Energy levels of a small molecule

<table>
<thead>
<tr>
<th>Type of Transition</th>
<th>Energy Unit 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electronic transitions</td>
<td>160 Kcal/M</td>
</tr>
<tr>
<td></td>
<td>5 eV</td>
</tr>
<tr>
<td></td>
<td>200 nm</td>
</tr>
<tr>
<td></td>
<td>50000 cm⁻¹</td>
</tr>
<tr>
<td>Vibrational transitions</td>
<td>3 kcal/M</td>
</tr>
<tr>
<td></td>
<td>1000 cm⁻¹</td>
</tr>
<tr>
<td>Rotational transitions</td>
<td>0.2 Kcal/M</td>
</tr>
<tr>
<td></td>
<td>50 cm⁻¹</td>
</tr>
</tbody>
</table>

At room temperature only the ground electronic state and the lowest vibrational state is populated.
If light of correct frequency is absorbed, the molecule can be excited to one of the many rotational-vibrational levels of the electronic excited state S1. The probability of excitation depends on the magnitude of the transition dipole.

In gas phase, very narrow lines appear in the absorption spectrum.

In liquid phase each individual line is so broadened that only a rotation-vibration envelope can be recognized.

The result is that a very detailed and informative spectrum is lost.
The extinction coefficient

The extinction coefficient of lysozyme at 280 nm is 25,000.

What that means?

The spectroscopy experiment

A monochromatic light beam of intensity $I_0$ impinges on a sample (liquid) containing an absorbing molecule at concentration $C$ (in moles/liter). A fraction $I$ of the light is transmitted.

We define $I/I_0$ the transmission coefficient of the sample.

Consider a thin layer of the sample in a direction perpendicular to the light
The fraction of light absorbed in this layer is proportional to the number of molecules in the thin layer and to the probability to be absorbed by a molecule.

After the layer \( d \ell \) the intensity decreases

\[-d\ell = I_0 \varepsilon \, d\ell\]

\( \varepsilon \) is proportional to the probability of absorption and is called the molar extinction coefficient.

Integrating this equation over the length \( L \) of the sample.
Converting in base 10 and defining the absorbance

\[ A = \log_{10} \frac{I_0}{I} \]

A is sometimes called the **OPTICAL DENSITY**

The extinction coefficient of 25,000 for lysozyme means that a solution of concentration of 1 molar over a length of 1 cm will have an optical density of 25,000.

Extinction coefficients range from 1 to 100,000
Absorption: general principles

The Beer-Lambert Law

The absorption strength of a molecule can be determined by absorption measurements using the Beer-Lambert Law, which is expressed as:

\[
\text{Absorption (Optical Density)} = \log \frac{I_o}{I} = \varepsilon c l
\]

$I_o$ and $I$ are the intensities entering and leaving the sample respectively.

$\varepsilon$ is the molar extinction coefficient or molar.

$l$ is the pathlength of the sample (1 cm).
Dynamic range of absorption values

✓ An OD of 1.0 - for every 100 photons entering the sample, 10 leave without being absorbed
✓ An OD of 2.0 - for every 100 photons entering the sample, only 1 leaves without being absorbed
✓ OD =3? - measuring the difference between 999 and 1000 photons is difficult!
✓ The useful range of absorption is 0.01-2.0 OD units

Fluorescein

The extinction coefficient of fluorescein is ~72,000 M⁻¹cm⁻¹

An absorption of 0.022 would correspond to a concentration of ~3 x 10⁻⁷ M
An absorption of 2.16 would correspond to a concentration of 3 x 10⁻⁵ M
Two-state system

\[ h \nu \text{ is the energy difference between B and A} \]

The rate of transfer from A to B in the presence of light is

\[ \frac{dP_b}{dt} = \frac{1}{2\hbar} |\langle \Psi_b | \mu | \Psi_a \rangle \cdot \vec{E}_0|^2 \]

\( \mu \) is the dipole operator; \( E \) is the electric field of the incident light beam.
To calculate absorption intensity, we must know the rate at which energy is taken from the incident beam

$$\frac{dP_B}{dt} = B_{ab} I(\nu)$$

$B_{ab}$ is the transition rate per unit energy density. $I(\nu)$ is the energy density incident on the sample.

$$I(\nu) = |E_0|^2$$

In a solution of non-oriented molecules

$$\langle \Psi_b | \mu | \Psi_a \rangle \cdot \overrightarrow{E_0} \cdot \overrightarrow{E_0} = \frac{1}{3} \langle \Psi_b | \mu | \Psi_a \rangle |E_0|^2$$

$$B_{ab} = 2/3 \hbar \langle \Psi_b | \mu | \Psi_a \rangle^2$$

In general the rate at which energy is removed from the incident light beam depends on the $A \rightarrow B$ and from $B \rightarrow A$ transition.
\[-dI(\nu)/dt = h \nu (N_a B_{ab} - N_b B_{ba}) I(\nu)\]

where \(N_a\) and \(N_b\) are the number of molecules per \(\text{cm}^3\) in the a and b state, respectively.

\(B_{ab}\) and \(B_{ba}\) are the Einstein coefficients. For a two-state system \(B_{ab} = B_{ba}\).

**Classical - induced transition moment**
Transition dipole moment has an orientation but not a direction.

\[B_{ab} = \frac{1000 \varepsilon' c}{N_0 h \nu}\]

\[B_{ba} = \frac{1000 c}{N_0 h} \int \frac{\varepsilon'(\nu) d\nu}{\nu}\]

Since \(\varepsilon'\) depends on wavelength.
MOLECULAR ORBITALS

Formaldehyde molecule

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1s</td>
<td>1 electron</td>
</tr>
<tr>
<td>C</td>
<td>1s² (2sp²)³2p_z</td>
<td>6 electrons</td>
</tr>
<tr>
<td>O</td>
<td>1s² 2s² 2p_x 2p_y 2p_z</td>
<td>8 electrons</td>
</tr>
</tbody>
</table>

C--H bonds are formed between the s electron of H and the carbon hybrid 2sp². The third sp² orbital on carbon forms a σ orbital with the 2p_x orbital of oxygen. The remaining orbitals are a 2p_z for C and 2p_z and 2p_y for O.

A molecular orbital can be formed by overlap of the single occupied 2p_z of O with 2p_z of C.
The remaining 2 electrons of O must be in the 2p<sub>y</sub> atomic orbital (non-bonding n orbitals).

The two highest unoccupied states in formaldehyde are the binding π and the atomic n (2p<sub>y</sub>) orbital of oxygen.

Considering only the π<sup>*</sup> level, two transitions are possible from the n state and from the-π state. To estimate absorption intensities we need to evaluate the matrix element of the transition dipole moment.
Consider symmetry first.

<table>
<thead>
<tr>
<th></th>
<th>$\pi$</th>
<th>$\pi^*$</th>
<th>$\nu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x$-axis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>$y$-axis</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$\pi$</th>
<th>$\pi^*$</th>
<th>$\nu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x$</td>
<td>odd</td>
<td>even</td>
<td>even</td>
</tr>
<tr>
<td>$y$</td>
<td>odd</td>
<td>even</td>
<td>odd</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$\pi$</th>
<th>$\pi^*$</th>
<th>$\nu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x^2$</td>
<td>even</td>
<td>odd</td>
<td>even</td>
</tr>
</tbody>
</table>

The integrals of the transition dipole moments must be evaluated over all space. If the integrand is odd when reflected through any three planes, the integral will vanish.

Represent the operator by the projection along the axes.

Symmetry of the operator

Dipole operator $\mu$

<table>
<thead>
<tr>
<th>plane</th>
<th>$xy$</th>
<th>$xz$</th>
<th>$yz$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_x$</td>
<td>even</td>
<td>even</td>
<td>odd</td>
</tr>
<tr>
<td>$\mu_y$</td>
<td>even</td>
<td>odd</td>
<td>even</td>
</tr>
<tr>
<td>$\mu_z$</td>
<td>odd</td>
<td>even</td>
<td>even</td>
</tr>
</tbody>
</table>
Evaluate the integral $\langle \pi | \mu | \pi^* \rangle$

| $\langle \pi | \mu_x | \pi^* \rangle \neq 0$ | Because the product of the symmetries of the $\pi$, $\pi^*$ and $\pi$ is even for all planes (xy and xz) |
| $\langle \pi | \mu_y | \pi^* \rangle = 0$ | Because is odd for the xz and yz plane |
| $\langle \pi | \mu_z | \pi^* \rangle = 0$ | Because is odd for the xy and yz plane |

In conclusion

$\langle \pi | \mu | \pi^* \rangle = \langle \pi | \mu_x | \pi^* \rangle$

This is an allowed transition when the electric vector of the light is parallel to the x-axis of the molecule. The transition is polarized along the C = O bond.
For the n- $\pi^*$ transition we have

\[ <n|\mu_x|\pi^*> = 0 \]
Because is odd for the xy and xz plane

\[ <n|\mu_y|\pi^*> = 0 \]
Because is odd for the xy

\[ <n|\mu_z|\pi^*> = 0 \]
Because is odd for the xz

In conclusion

\[ <n|\mu|\pi^*> = 0 \]

The transition is symmetry forbidden

The extinction coefficient of the $\pi - \pi^*$ transition is 100 times larger than the n- $\pi^*$ transition
Peptide absorption

The $\pi$ electrons are delocalized over the O=C-N bond

The lowest energy transition is $n-\pi^*$. The $n$ electrons are localized on the O atom.

This is a symmetry forbidden transition ($\varepsilon=50-100$)

The wavelength of this transition is 210-220 nm

The main observable peptide absorbance is the $\pi-\pi^*$ ($\varepsilon = 7000$).

This transition is not strictly polarized.
Acetone in n-hexane

$\pi \rightarrow \pi'$

$\mu \rightarrow \pi^*$
<table>
<thead>
<tr>
<th>Type of bond</th>
<th>Transition of lowest energy</th>
<th>Shape of molecular orbitals</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_3\text{C} \equiv \text{H}$</td>
<td>$\sigma \rightarrow \sigma^*$</td>
<td><img src="image1" alt="Ground state" /> <img src="image2" alt="Excited state" /></td>
</tr>
<tr>
<td>$\text{R}_2\text{C} \equiv \text{CR}_2$</td>
<td>$\pi \rightarrow \pi^*$</td>
<td><img src="image3" alt="Ground state" /> <img src="image4" alt="Excited state" /></td>
</tr>
<tr>
<td>$\text{R}_2\text{C} \equiv \text{O}$</td>
<td>$n \rightarrow \pi^*$</td>
<td><img src="image5" alt="Ground state" /> <img src="image6" alt="Excited state" /></td>
</tr>
</tbody>
</table>

$\pi$ electron system perpendicular to plane of page
<table>
<thead>
<tr>
<th>Chromophore</th>
<th>$\lambda_{max}$ [nm]</th>
<th>$\varepsilon$ [M$^{-1}$ cm$^{-1}$]</th>
<th>Transitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{-COO-}R$</td>
<td>205</td>
<td>50</td>
<td>$n \rightarrow n$</td>
</tr>
<tr>
<td></td>
<td>165</td>
<td>$4 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td>$\text{C=O}$</td>
<td>280</td>
<td>20</td>
<td>$n \rightarrow \pi$</td>
</tr>
<tr>
<td></td>
<td>190</td>
<td>$2 \cdot 10^3$</td>
<td>$n \rightarrow \sigma$</td>
</tr>
<tr>
<td></td>
<td>190</td>
<td></td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td>$\text{C=S}$</td>
<td>500</td>
<td>10</td>
<td>$n \rightarrow n$</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>$9 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td>$\text{S-S}$</td>
<td>250-330</td>
<td>$10^3$</td>
<td>$n \rightarrow \sigma$</td>
</tr>
<tr>
<td>$\text{C=C}$</td>
<td>190</td>
<td>$9 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td>$\text{C≡C}$</td>
<td>175</td>
<td>$8 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td>N</td>
<td>300</td>
<td>325</td>
<td>$n \rightarrow \pi$</td>
</tr>
<tr>
<td></td>
<td>245</td>
<td>$2 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>$3 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
<tr>
<td></td>
<td>265</td>
<td>$8 \cdot 10^3$</td>
<td>$\pi \rightarrow \pi$</td>
</tr>
</tbody>
</table>
Aromatic side chain absorption in proteins
Absorption maxima: The importance of conjugation

The wavelength value of the absorption maximum and the molar absorbivity are determined by the degree of Conjugation of \( \pi \)-bonds.

*Increasing the number of double bonds shifts the absorption to lower energy*

\[\begin{align*}
N=5 & \quad 5 \text{ \( \pi \)-bonds, 10 electrons} \\
N=4 & \quad 4 \text{ \( \pi \)-bonds, 8 electrons} \\
N=3 & \quad 3 \text{ \( \pi \)-bonds, 6 electrons}
\end{align*}\]
As the degree of conjugation increases
(i.e. the number of electrons involved in the delocalized π-orbitals)

the absorption energy decreases (>\(\lambda\), the energy between the ground and excited state decreases)

the absorption becomes more intense (>\(\varepsilon\), increased probability of absorption)

Benzene < Naphthalene < Anthracene < naphthacene < pentacene

<table>
<thead>
<tr>
<th></th>
<th>Abs. Max</th>
<th>Log (\varepsilon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>262 nm</td>
<td>3.84</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>275 nm</td>
<td>3.75</td>
</tr>
<tr>
<td>Anthracene</td>
<td>375 nm</td>
<td>3.90</td>
</tr>
<tr>
<td>naphthacene</td>
<td>475 nm</td>
<td>4.05</td>
</tr>
<tr>
<td>pentacene</td>
<td>580 nm</td>
<td>4.20</td>
</tr>
</tbody>
</table>

275 nm 375 nm 475 nm absorption wavelength
What can be learned from electronic spectra?

1. We are just at the stage that we can start to calculate from first principles the energy levels of a biomolecule. At present, it is impossible to calculate from first principles the entire spectra of a biomolecule.

2. A semi-empirical approach can be used
   
   a. From the spectra it is possible to have information on the composition of the biomolecule
   
   b. Spectral changes upon external perturbation can provide information on the localization (exposure) of the absorbing residue.

Spectrum sensitivity to solvent polarity

In general, the effect of the solvent is to decrease the average energy of the electronic level. The more polar the solvent the stronger the interaction will be.

In the absorption measurement what counts is the difference between energy levels.

A quantitative analysis of the effect of the solvent is very difficult.

When the absorbing molecule is in the ground state, solvent molecules will assume a configuration to minimize the ground state energy.
The excitation takes place in about $10^{-15}$ seconds.

For the energy of the excited state what counts is the configuration of the solvent corresponding to the ground state. If the solvent has a permanent dipole moment (such as that of water), no changes in orientation can occur in $10^{-15}$ seconds. However the electronic polarizability of the solvent can change rapidly enough.

If the polarizability effect dominates, because the excited state has a larger dipole moment than the ground state, the interaction energy will be larger in the excited state and red shift of the absorption transition will occur.

This is generally true for the $\pi--\pi^*$ transition. However for the $n-\pi^*$ transition, the $n$ electron can interact with the solvent (for example in hydrogen bond formation) in the ground state. In this case a blue shift will occur.
If dipolar effects dominate, the results are not so simple