Inorganic ions

Table 3: Stretching frequencies of common inorganic ions in cm^{-1}.

Ion	ν(ion)/cm^{-1}
NH_4^+	3300-3030, 1485-1390
CO_3^{2-}	1450-1410, 880-800
SO_4^{2-}	1130-1080, 680-610
NO_3^-	1380-1350, 860-800
NO_2^-	1250-1230
PO_4^{3-}	1100-1000
NC^-, NCS^-, SCN^-	2200-2000
SiO_4^{2-}	1100-900
MnO_4^-	920-890, 850-840

Student Corner

UV-visible Spectroscopy

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Molecules have different energy states: electronic, vibrational, rotational, and nuclear. Quantized energy is responsible for each transition (*e.g.*, rotational - microwaves, infrared - vibrational) within the molecule. UV-visible spectroscopy is one of the principle analytical tools used by chemists to find out absorption properties of molecules in ultraviolet (200 - 400 nm) and visible (400 - 700 nm) regions of the electromagnetic (EM) spectrum. The absorption of UV-*vis* radiation corresponds to electronic transitions of outer electrons.

An electron of a molecule in the ground state absorbs energy and moves to the excited state. **Energy gap** (ΔE) between these two states is related to the absorbed energy. The maximum wavelength ($\boldsymbol{\lambda_{max}}$) is measured where, ΔE = hν = hc/λ, h = Plank's constant (6.626×10^{-34} Js), ν = frequency, c = speed of light.

There are 3 types of **electrons** in organic molecules; **sigma** (σ), **pi** (π) and **lone-pair** (n), which are in **non-bonding** orbitals. Electrons in σ and π orbitals have lower energies. Anti-bonding orbitals (σ* and π* have higher energies and are usually empty. Main electron transitions ($\mathbf{T_1}$-$\mathbf{T_4}$) between these energy levels are shown in Fig. 1, which depend on the availability of σ, π and n-electrons (see Table 1).

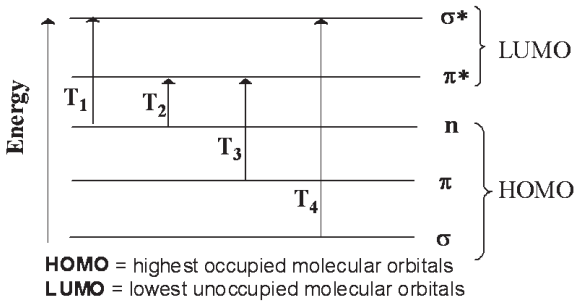


Figure 1: Main electronic transitions

HOMOs of propane, propene, acetone and ethanol are σ, π, n & n, respectively. LUMOs of propane, propene, acetone and ethanol are σ*, π*, π* & σ*, respectively. Table 1 gives some details with respect to transitions (T_1-T_4).

Table 1: Details of electronic transitions

	Transition	λ_{max}/nm	Compound
$\mathbf{T_1}$	n → σ*	> 185	compounds with O, S, N, halogen
$\mathbf{T_2}$	n → π*	> 275	carbonyls

T_3	$\pi \rightarrow \pi^*$	> 165	alkenes, alkynes, carbonyls, azo-compounds, *etc.*
T_4	$\sigma \rightarrow \sigma^*$	< 165	alkanes

Note that selection rules predict whether the transition is allowed or forbidden (*e.g.*, $n \rightarrow \pi^*$). Forbidden transitions are less intense or not observed.

UV-visible Spectrophotometer

The UV-visible spectrophotometer has three main components: (i) light source (deuterium and tungsten lamps to generate UV and visible light), (ii) monochromator (a prism) to split the light into its components (or wavelengths), and (iii) detector to collect and process the data, and finally the spectrum is recorded.

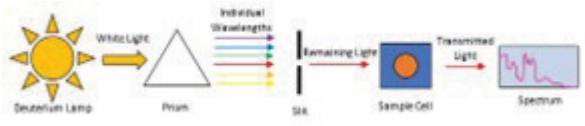


Figure 2: Diagram depicting the processes involved in the spectrophotometer

Normally, each component of the light beam is split into two halves: one half is sent through the cell containing the sample and other half is sent through the cell containing only the solvent (reference).

The recorded spectrum (λ vs Absorbance) shows broad bands due to the rotational and vibrational sub-levels associated with each energy level, and the λ_{max} value with the highest absorbance is reported.

Beer-Lambert Law

Absorbance of a particular λ is determined by Beer-Lambert Law as given below.

$$A = \log (I_o/I) = \varepsilon cl$$

A = Absorbance, I_o = Intensity of the incident light, I = Intensity of the emitted light, c = molar concentration of the sample, l = length of sample cell, ε = molar absorptivity/molar extinction coefficient, which depends on the chromophore of the molecule.

Chromophore is the part of a molecule responsible for its color. It can absorb UV-visible light, exciting an electron from its ground state into the excited state. They have conjugated double bonds and/or unsaturated group such as azo, keto, nitro, nitroso etc.

Auxochrome is a group attached to a chromophore which modifies its ability to absorb more light, *i.e.*, it intensifies the color of the molecule, *e.g.*, halogens, alkyl, hydroxyl, alkoxy, amino groups, *etc.*

The effects of auxochrome are as follows:

a) Bathochromic shift (red shift) - increase in conjugation/substitution causes a shift to longer wavelength

b) Hypsochromic shift (blue shift) - a shift of spectral colors towards shorter wavelength

c) Hyperchromic effect - increase in absorbance

d) Hypochromic effect - decrease in absorbance.

Table 2 gives the mode of transition (**T**) of some organic groups and their λ_{max} values in nm.

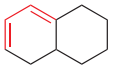
Table 2: Mode of transition (**T**) of organic groups and their λ_{max} values

Class	T	λ_{max} (nm)	Class	T	λ_{max} (nm)
R-OH	T_1	180	RNO_2	T_3	271
R-OR	T_1	180	R-CHO	T_3	190
				T_2	290
$R-NH_2$	T_1	190	R_2CO	T_3	180
				T_2	280
R-SH	T_1	210	RCOOH	T_2	205
$R_2C=CR_2$	T_3	175	$RCOOR^/$	T_2	205
RC≡CR	T_3	180	$RCONH_2$	T_2	210
RC≡N	T_3	160	RN=NR	T_3	340

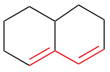
The λ_{max} values of complicated organic molecules can be calculated by using Woodward-Fieser rules. First, we consider the base values of some chromophores.

Base values for conjugated dienes

The base values of ***cisoid*** (homoannular) and ***transoid*** (heteroannular) conjugated diene systems are **253** nm and **214** nm.



Cisoid
(Homoannular)
λ_{max} = **253**+20 nm

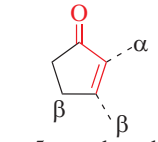


Transoid
(Heteroannular)
λ_{max} = **214**+20 nm

If both systems are present in a molecule, only the base value of the cisoid confirmation is considered as the base value.

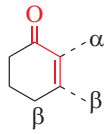
Base values for enones

The base values for cyclic 5- and 6-membered **enones** are **202** and **215** nm, respectively.



5-membered enone

λ_{max} = **202**+12 nm

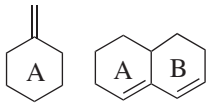


6-membered enone

λ_{max} = **215**+12 nm

Increments due to EDBs

Exocyclic double bonds (EDB) are double bonds which lie outside the ring. If there are EDBs, the increment due to each EDB is 5 nm.



(**1**) (**2**)

The compound (**1**) has one bond exocyclic to the ring A. The compound (**2**) has one bond exocyclic to the ring B.

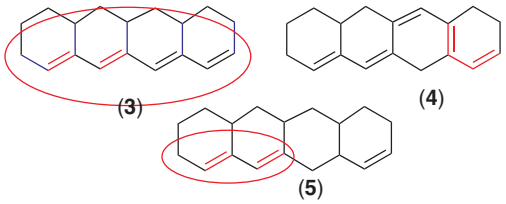
For (**2**), the base value = 214 nm

Alkyl residues 3 x 5 = 15 nm

The double bond in the ring A is exocyclic to the ring B; thus, the increment due to one EDB is 5 nm.

The λ_{max} of (**2**) = 214 + 15 + 5 = 234 nm

Double bond extending conjugation (DBEC) – the increment = 30 nm



(**3**) (**4**) (**5**)

For (**3**) & (**5**), base value = 214 nm

For (**4**), base value = 253 nm

For (**3**), DBEC = 2 × 30 nm = 60 nm

For (**4**), DBEC = 3 × 30 nm = 90 nm

For (**5**), DBEC = 0 × 30 nm = 0 nm

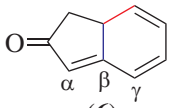
Ring Residues (RRs) - These are the alkyl groups attached to the unsaturated system (including the extended conjugation).

For diene base system: RR = 5 nm

For (**3**), RR = 5 bonds x 5 nm = 25 nm

For (**5**), RR = 4 bonds x 5 nm = 20 nm

For enone base system: RR = 10, 12 and 18 nm for substituents at α, β and γ (and higher) positions, respectively.



(**6**)

The molecule (**6**) has two substituents,

RR = 12 (β) + 18 (γ and higher) = 30 nm

Homoannular diene component

(HADC) = 39 nm

This increment is added to the components consisting of an enone base {*e.g.*, for 6-membered diene ring system in (**6**)}.

Polar groups (PG) - When the polar groups are in the unsaturated base system, the following increments are added.

For a molecule with a homoannular and heteroannular base system: PG values are OAc = 0 nm, OR = 6 nm, SR = 30 nm, Cl and Br = 5 nm, and NR_2 = 60 nm.

The summaries of rules for **diene** and **enone** based systems are given in Table 3 and Table 4.

Table 3: Summary of rules for a diene base system

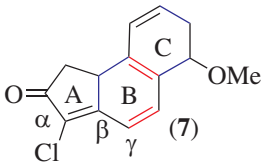
Base value for heteroannular or *transoid* diene	214 nm
Base value for homoannular or *cisoid* diene	253 nm
DBEC	+30 nm
RR or an alkyl substituent	+5 nm
EDB	+5 nm
-OAc	0 nm
-OAlkyl	+6 nm
-SAlkyl	+30 nm
Cl, Br	+5 nm
$N(Alkyl)_2$	+60 nm
Solvent correction	0 nm

Table 4: Summary of rules of an enone base system

Base value for ring or acyclic parent enone			215 nm
Base value: parent enone with a 5-membered ring			202 nm
DBEC			30 nm
EDB			5 nm
HADC			39 nm
Groups at	**α**	**β**	**γ** and higher nm
RR	10	12	18
OH	35	30	50
OC(=O)Me	6	6	6
OMe	35	30	17
Cl	15	12	12
Br	25	30	25
NR_2		95	

Solvent correction: water = –8; methanol/ethanol = 0; ether = +7; hexane/cyclohexane = +11

Let us calculate the λ_{max} value for the hypothetical molecule (**7**).



Five membered enone = 202 nm

DBEC = 90 nm (3 × 30 nm)

EDB = 5 nm (A is exocyclic to B)

RR= 12 nm + (3 × 18 nm) = 66 nm

Polar groups (Cl) = 15 nm

HADC = 39 nm

Theoretical λ_{max} for (**7**) = 417 nm

Applications

UV-visible spectrometry is used in fields of natural, environmental and forensic sciences, biomedicine, geology, analytical chemistry, *etc*. It is widely used in both quantitative and qualitative analysis.

Spectrometric quantitative analysis

In spectrometric titrations either titrand, titrant or the product should absorb UV-visible radiation.

The titration curve is a plot of absorbance vs titrant volume. Four types of graphs can be obtained (Fig. 3).

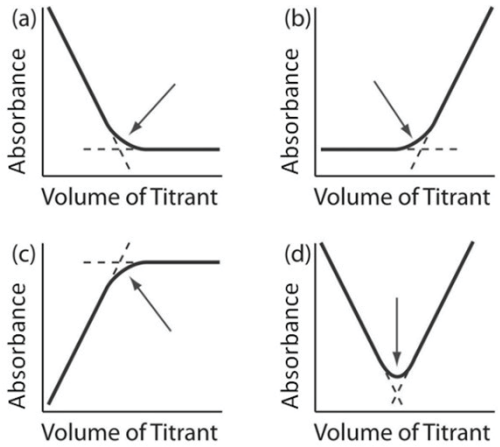


Figure 3: The plots of Absorbance vs volume of titrant

a) Titrand absorbs UV radiation, while titrant and the product does not absorb the radiation, *e.g.*, ferric salicylate as titrand and EDTA as titrant.

In this case, absorbance decreases with the addition of titrant to the solution of ferric salicylate. Fe^{III}-EDTA complex does not absorb light and when the reaction is complete, the plot becomes constant even though excess EDTA is being added.

b) Titrant absorbs UV radiation while titrand and the product do not absorb the radiation, *e.g.*, a solution containing $KBrO_3$ and KBr as the titrant and $AsCl_3$ as the titrand. After the end point, the excess titrant enhances the absorbance.

c) Only the product absorbs radiation, *e.g.*, $CuSO_4$ as the titrand and EDTA as the titrant. Absorbance of the solution increases as the formation of the Cu-EDTA complex increases.

d) Both titrand and titrant absorb UV radiation while the product does not, *e.g.*, red dye and LiBr strongly absorb UV radiation.

Single component quantitative analysis

In this method, Beer-Lambert law is applied to find the concentration of an analyte, after plotting a graph (absorbance vs concentration) using standard solutions.

Qualitative & Quantitative Analysis

UV-visible spectroscopy is a valuable technique to analyze biological samples.

It is now being used to study (i) NAD^+ to NADH

conversion, (ii) to determine the purity of proteins and DNA, and the melting point of DNA. The UV-visible spectra of NAD^+ and NADH are given in Fig. 4.

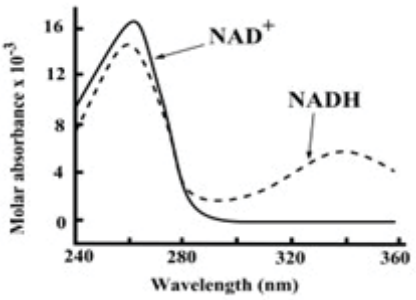
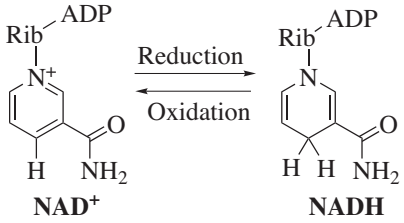


Figure 4: UV-vis spectra of NAD^+ & NADH

Both species give strong absorption bands around 260 nm for the adenine moiety. During reduction, NAD^+ accepts electrons and becomes NADH as shown below.



NAD^+ **NADH**

Importantly, NADH gives another absorption peak around 350 nm due the availability of the lone pair electrons on nitrogen, which lowers the energy gap between HOMO and LUMO. By measuring the absorption at 350 nm, we can study the reaction and determine the amount of NAD^+ and NADH present in the solution.

Proteins containing aromatic amino acid residues such as tyrosine, histidine and tryptophan can be analyzed by UV-vis spectroscopy. UV-vis spectrum of such a protein is given in Fig. 5.

The amide -CONH- group of proteins absorbs around 220 nm. Protein samples containing aromatic amino acid give a broad peak around 260-280 nm. Thus, UV spectroscopy can be used to study proteins and to determine the purity of proteins.

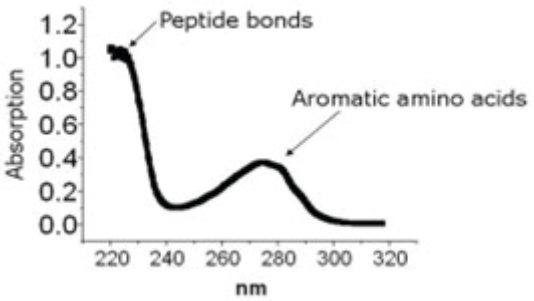


Figure 5: UV-vis spectrum of a protein with aromatic amino acid residues

Manuka honey produced in New Zealand contains dihydroxyacetone, methylglyoxal and hydroxymethyl furfural. Quality of this very expensive honey is determined by UV spectroscopy.

UV-vis spectroscopy is especially very useful for "plasmonic" materials such as gold and silver nanoparticles because they have distinctive absorbance spectra. These spectra provide us with information such as the size of the nanoparticles and the quality of the sample. We also can check whether the sample has changed or if it has started to aggregate.