INSTRUMENTAL ANALYSIS II

## Unit 4

UV-Vis Spectroscopy

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By Ermias H.

INTRODUCTION

➢UV-VIS spectroscopy is the measurement of the wavelength and intensity of absorption of near-ultraviolet and visible light by a sample.

➢Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels.

➢UV-VIS spectroscopy is usually applied to molecules and inorganic ions or complexes in solution.

• UV-VIS spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law

UV radiation and Electronic Excitations

✓ The difference in energy between molecular bonding, non-bonding and anti-bonding orbitals ranges from 125-650 kJ/mole

✓ This energy corresponds to EM radiation in the ultraviolet (UV) region, 100-350 nm, and visible (VIS) regions 350-750 nm of the spectrum

✓ For comparison, recall the EM spectrum:

| γ-rays | X-rays | UV | IR | microwave | Radio |

Visible
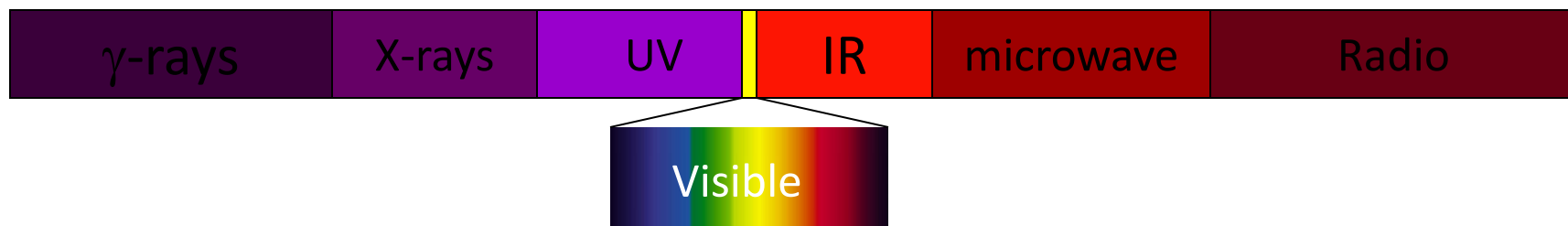
✓ Using IR will observe vibrational transitions with energies of 8-40 kJ/mol at wavelengths of 2500-15,000 nm

✓ For purposes of our discussion, we will refer to UV and VIS spectroscopy as UV

The Spectroscopic Process

➢ In UV spectroscopy, the sample is irradiated with the broad spectrum of the UV radiation

➢ If a particular electronic transition matches the energy of a certain band of UV, it will be absorbed

➢ The remaining UV light passes through the sample and is observed

➢ From this residual radiation a spectrum is obtained with "gaps" at these discrete energies-this is called an absorption spectrum
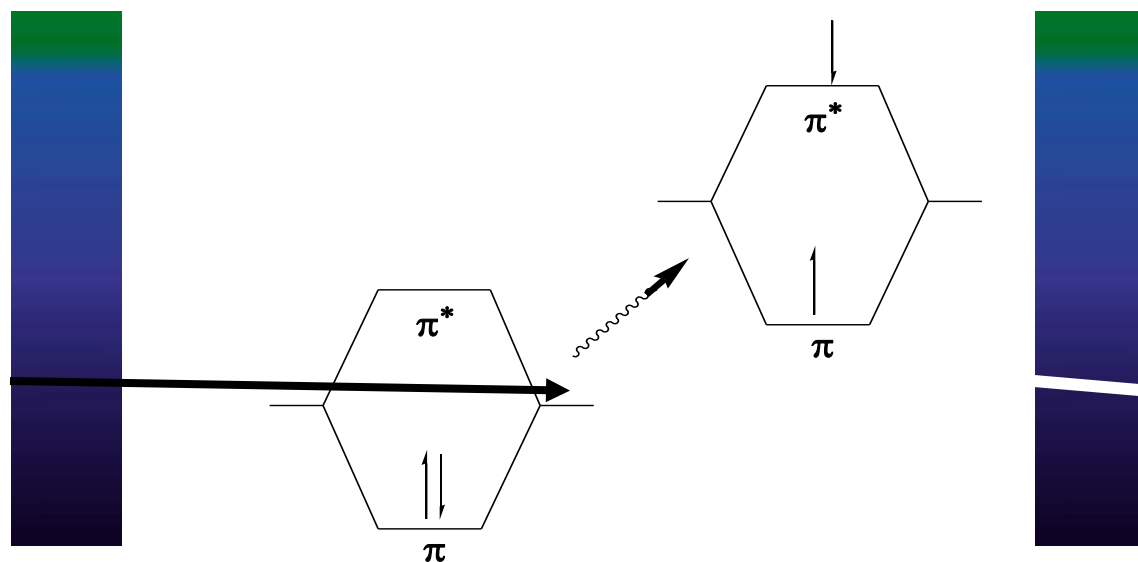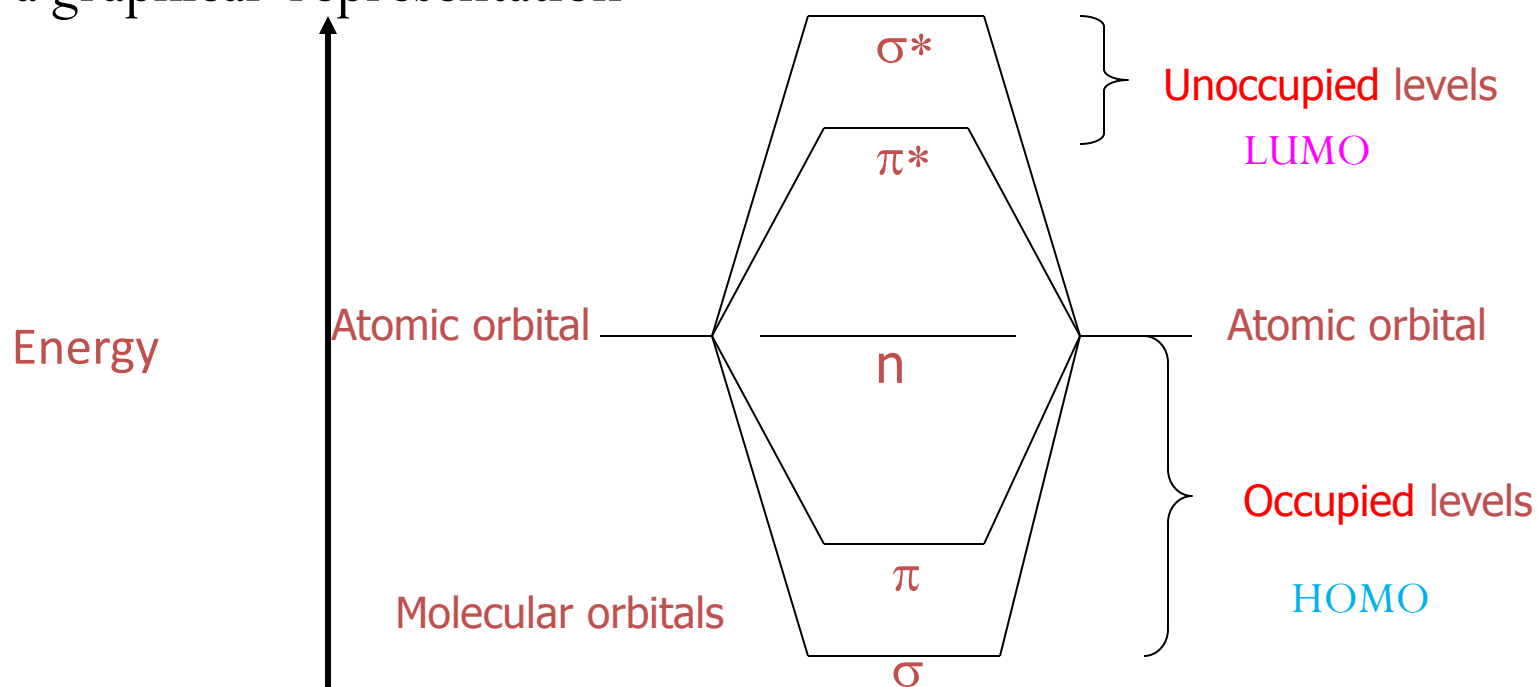
Observed electronic transitions

❖ The lowest energy transition most often observe by UV is typically that of an electron in the Highest Occupied Molecular Orbital (HOMO) to the Lowest Unoccupied Molecular Orbital (LUMO)

❑ For any bond (pair of electrons) in a molecule, the molecular orbitals are a mixture of the two contributing atomic orbitals; for every bonding orbital "created" from this mixing (σ, π), there is a corresponding anti-bonding orbital of symmetrically higher energy (σ^*, π^*)

❖ The lowest energy occupied orbitals are typically the σ; likewise, the corresponding anti-bonding σ^* orbital is of the highest energy

❖ π-orbitals are of somewhat higher energy, and their complementary anti-bonding orbital somewhat lower in energy than σ^*.

❖ Unshared pairs lie at the energy of the original atomic orbital, most often this energy is higher than π or σ.

Cont…

❑ Here is a graphical representation

❑ From the molecular orbital diagram, there are several possible electronic transitions that can occur, each of a different relative energy:

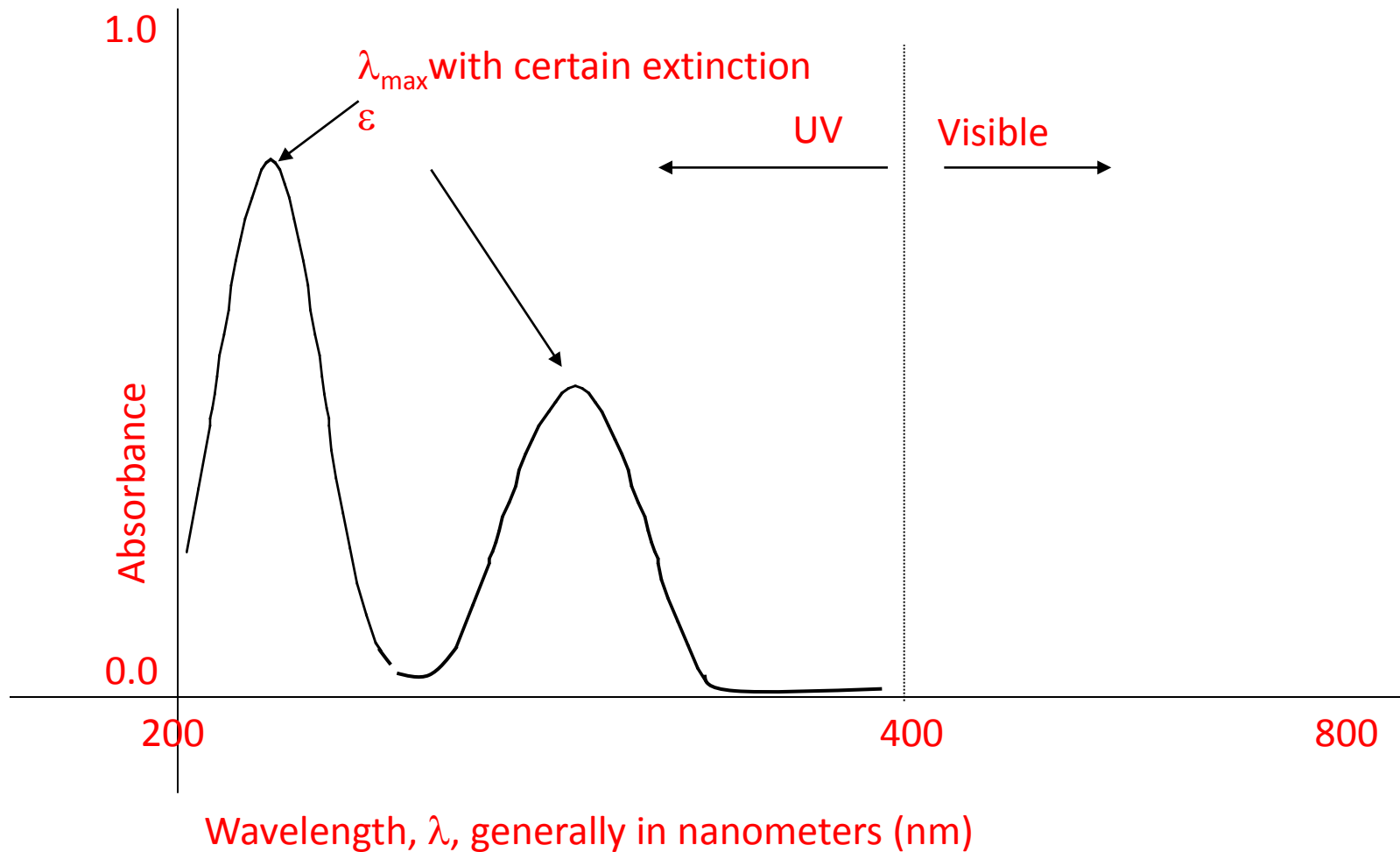$\sigma \longrightarrow \sigma^*$ alkanes

$\longrightarrow$

$\pi \longrightarrow \pi^*$ unsaturated cpds.

$n \longrightarrow \sigma^*$ O, N, S, halogens

$n \longrightarrow \pi^*$ carbonyls

By Ermias H.

An Electronic Spectrum

λ_{max} with certain extinction ε

UV   Visible

1.0

Absorbance

0.0

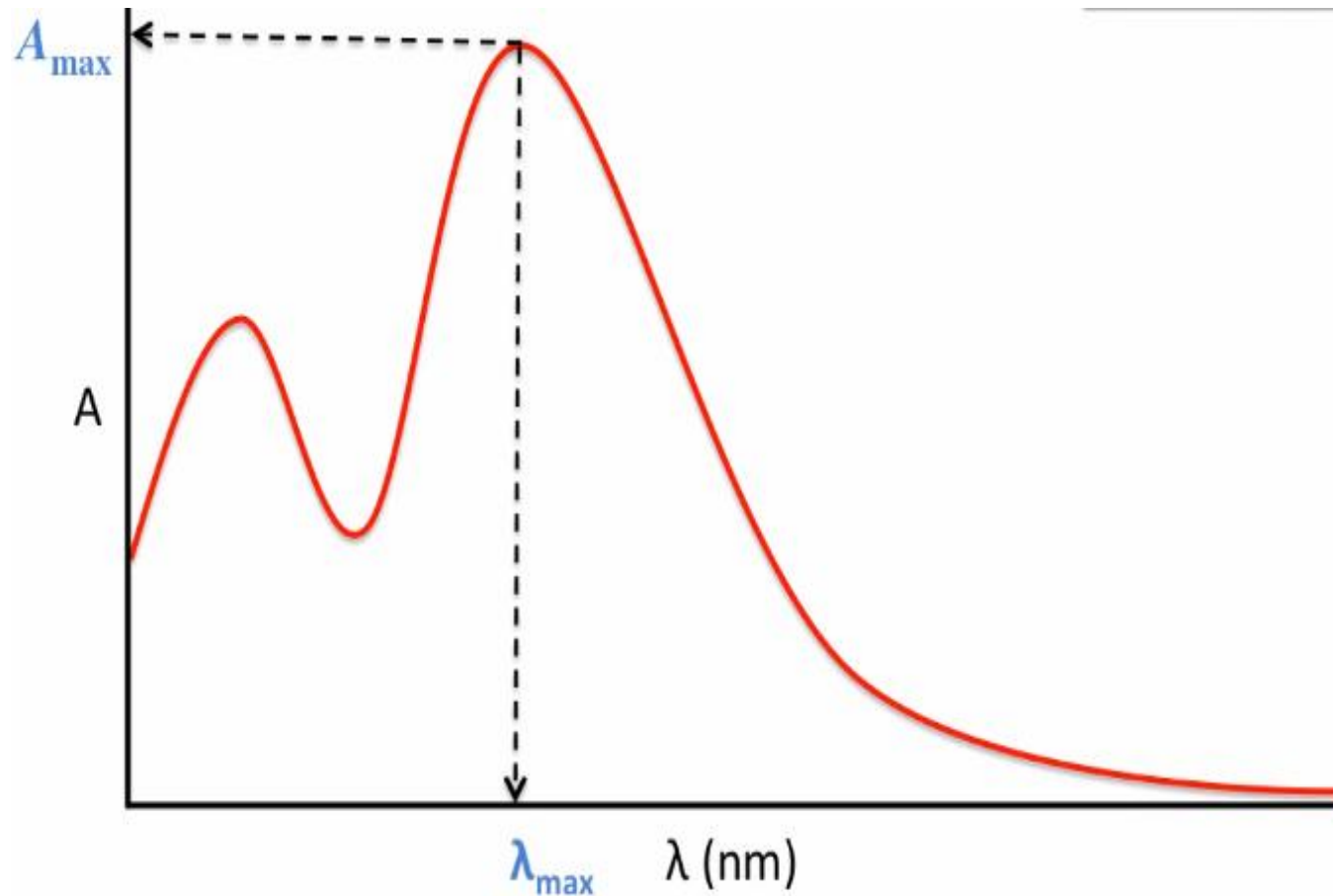200   400   800
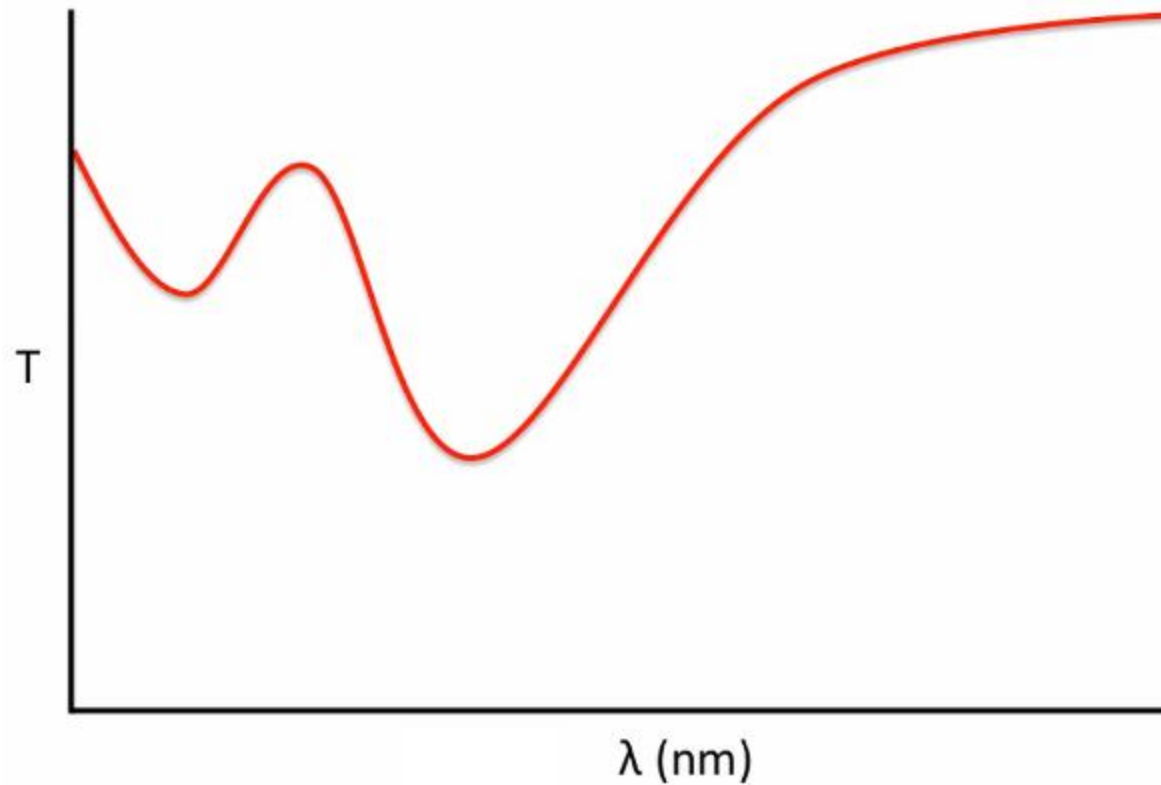
Wavelength, λ, generally in nanometers (nm)

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Absorption Spectrum.

An **absorption spectrum** is the plot of the **absorbance** versus the **wavelength** of the incident light.

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Transmission Spectrum.

A **transmittance spectrum** is the plot of the **transmittance** or %T versus the **wavelength** of the incident light.

Types of Electronic Transitions

1. Transitions involving **π, σ, and *n*** electrons

2. Transitions involving charge-transfer electrons

3. Transitions involving *d* and *f* electrons (not covered in this Unit)

❖ **Four** types of transitions are possible:

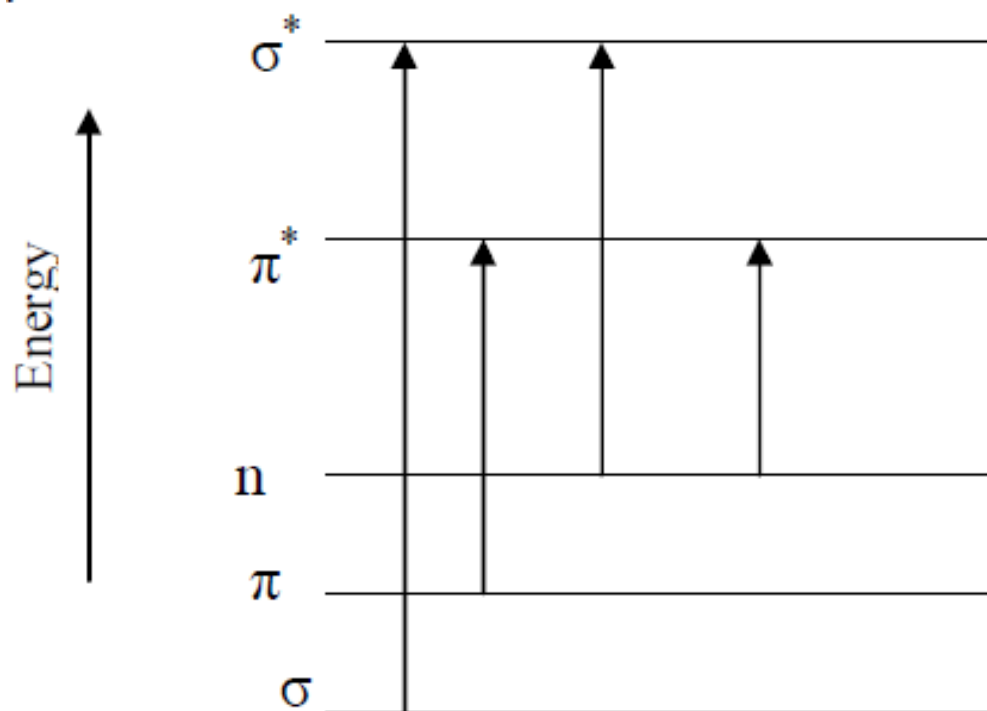$$\sigma \rightarrow \sigma^*, \quad n \rightarrow \sigma^*, \quad n \rightarrow \pi^*, \text{ and } \pi \rightarrow \pi^*.$$

Figure : Electronic molecular energy levels

Absorbing species containing π, σ, and *n* electrons

➢ Absorption of ultraviolet and visible radiation in organic molecules is restricted to certain functional groups (*chromophores*) that contain valence electrons of low excitation energy

➢ The spectrum of a molecule containing these chromophores is complex.

➢ This is because the superposition of rotational and vibrational transitions on the electronic transitions gives a combination of overlapping lines.

➢ This appears as a continuous absorption band.

σ − σ* Transitions

➢ An electron in a bonding σ orbital is excited to the corresponding antibonding orbital.

➢ The energy required is large.

➢ For example, methane (which has only C-H bonds, and can only undergo σ − σ* transitions) shows an absorbance maximum at 125 nm.

➢ Absorption maxima due to σ − σ* transitions are not seen in typical UV-Vis. spectra (200 - 700 nm)
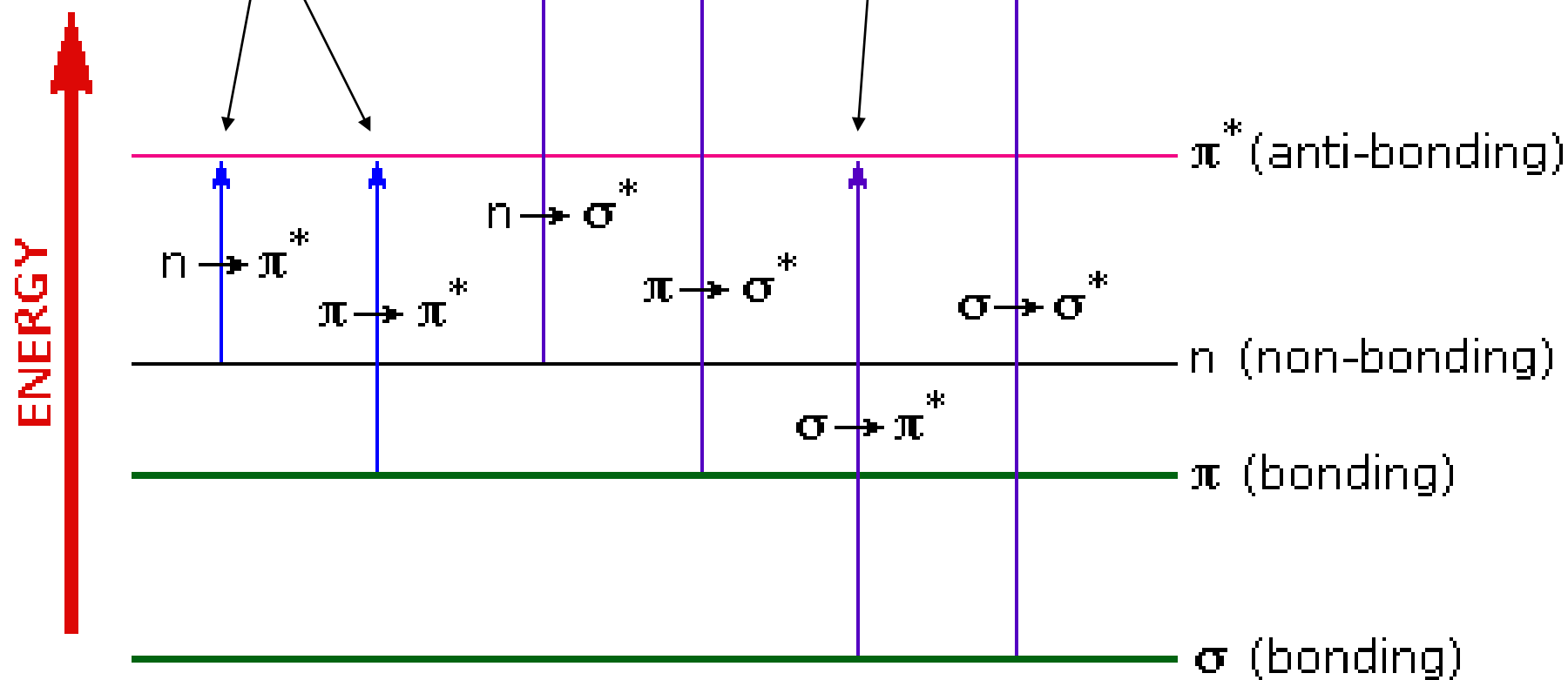
$n - \sigma^*$ Transitions

➤ Saturated compounds containing atoms with lone pairs (non-bonding electrons) are capable of $n - \sigma^*$ transitions.

➤ These transitions usually need less energy than $\sigma - \sigma^*$ transitions. They can be initiated by light whose wavelength is in the range 150 - 250 nm.

➤ The number of organic functional groups with $n - \sigma^*$ peaks in the UV region is small.

$n - \pi^*$ and $\pi - \pi^*$ Transitions

➢ Most absorption spectroscopy of organic compounds is based on transitions of n or π electrons to the π^* excited state.

➢ This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm).

➢ These transitions need an unsaturated group in the molecule to provide the π electrons.
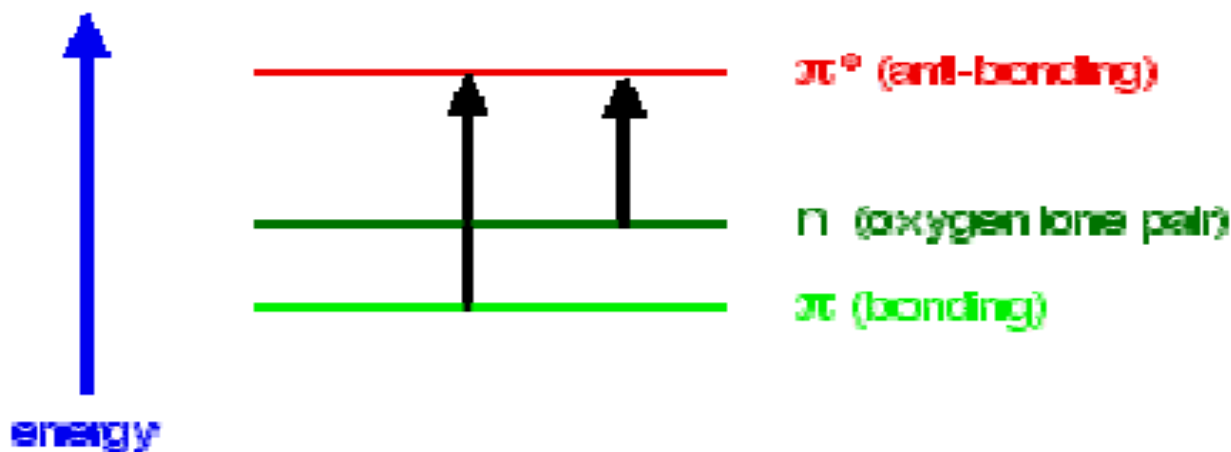
Vacuum UV or Far UV
(λ<190 nm)

UV/VIS

σ*(anti-bonding)

π*(anti-bonding)

n → π*

π → π*

n → σ*

π → σ*

σ → σ*

n (non-bonding)

σ → π*

π (bonding)

σ (bonding)

ENERGY

Example

❖ **Ethanal** can therefore absorb light of two different wavelengths:

i. the pi bonding to pi anti-bonding absorption peaks at 180 nm;

ii. the non-bonding to pi anti-bonding absorption peaks at 290 nm.

The importance of conjugation and delocalisation in what wavelength is absorbed

❖ Consider these three molecules:

$CH_2=CH_2$, $CH_2=CH-CH=CH_2$, $CH_2=CH-CH=CH-CH=CH_2$
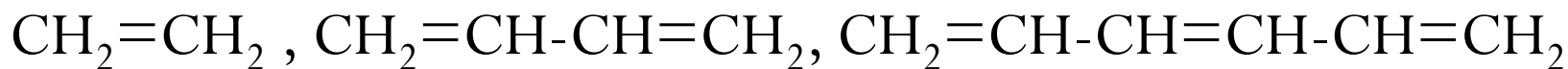
   ethene   buta-1,3-diene   hexa-1,3,5-triene

➢ Ethene contains a simple isolated carbon-carbon double bond, but the other two have conjugated double bonds.

➢ In these cases, there is delocalisation of the pi bonding orbitals over the whole molecule.

➢ The maximum absorption is moving to longer wavelengths as the amount of delocalisation increases.

molecule	wavelength of maximum absorption (nm)
ethene	171
buta-1,3-diene	217
hexa-1,3,5-triene	258

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❖ Compare ethene with buta-1,3-diene.

➤ In ethene, there is one pi bonding orbital and one pi anti-bonding orbital.

➤ In buta-1,3-diene, there are two pi bonding orbitals and two pi anti-bonding orbitals.

➤ The highest occupied molecular orbital is often referred to as the HOMO – in these cases, it is a pi bonding orbital.

➤ The lowest unoccupied molecular orbital (the LUMO) is a pi anti-bonding orbital.

ethene

π * (anti-bonding)

π (bonding)

energy

buta-1,3-diene

(not to scale)
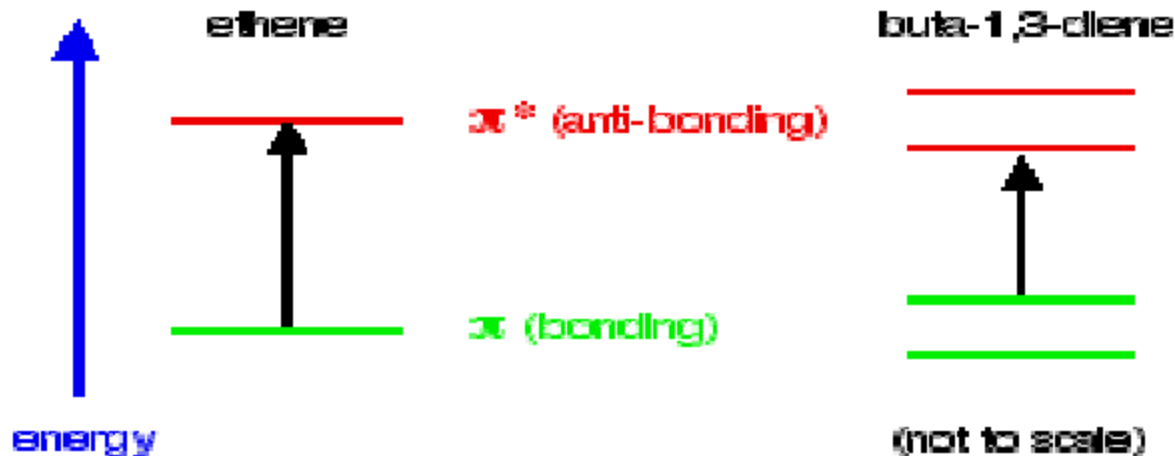
❖ In the hexa-1,3,5-triene case, it is less still.
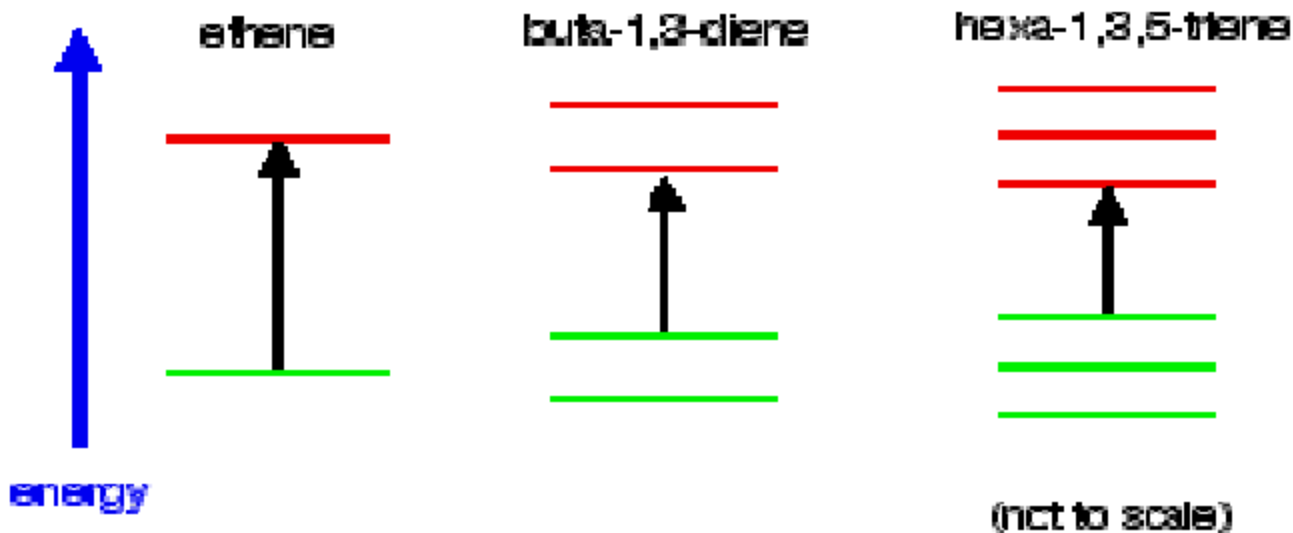
➢ If you extend this to compounds with really massive delocalization, the wavelength absorbed will eventually be high enough to be in the visible region of the spectrum, and the compound will then be seen as coloured.

The UV/visible spectrum

➢ Figure shows a simple UV-visible spectrum.

➢ Absorbance (on the vertical axis) is a measure of the amount of light absorbed.

➢ The higher the value, the more of a particular wavelength is being absorbed.

Maximum absorption at this wavelength

$\lambda_{max} = 217$ nm

absorbance

1.0
0.8
0.6
0.4
0.2
0

200 220 240 260 280 300

wavelength (nm)
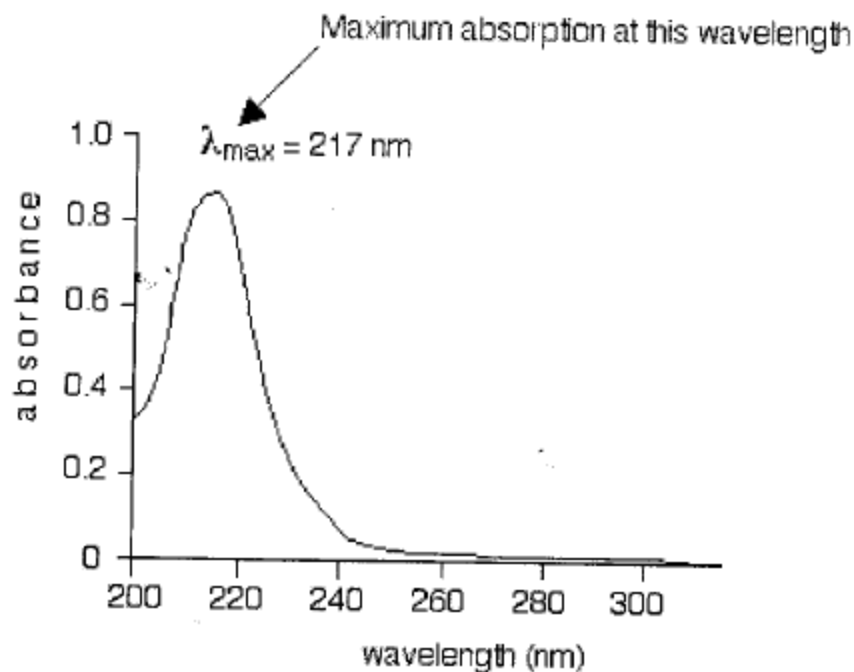
Figure : Typical absorption spectra for buta-1,3-diene (CH2=CH-CH=CH2)

Typical Absorptions of Simple Isolated Chromophores

Class	Transition	λ_{max} (nm)	log ϵ	Class	Transition	λ_{max} (nm)	log ϵ
R—OH	$n \rightarrow \sigma^*$	180	2.5	R—NO$_2$	$n \rightarrow \pi^*$	271	<1.0
R—O—R	$n \rightarrow \sigma^*$	180	3.5	R—CHO	$\pi \rightarrow \pi^*$	190	2.0
R—NH$_2$	$n \rightarrow \sigma^*$	190	3.5		$n \rightarrow \pi^*$	290	1.0
R—SH	$n \rightarrow \sigma^*$	210	3.0	R$_2$CO	$\pi \rightarrow \pi^*$	180	3.0
R$_2$C=CR$_2$	$\pi \rightarrow \pi^*$	175	3.0		$n \rightarrow \pi^*$	280	1.5
R—C≡C—R	$\pi \rightarrow \pi^*$	170	3.0	RCOOH	$n \rightarrow \pi^*$	205	1.5
R—C≡N	$n \rightarrow \pi^*$	160	<1.0	RCOOR'	$n \rightarrow \pi^*$	205	1.5
R—N=N—R	$n \rightarrow \pi^*$	340	<1.0	RCONH$_2$	$n \rightarrow \pi^*$	210	1.5

Instrumentation and Spectra

❖ The construction of a traditional UV-VIS spectrometer consists – sample handling, irradiation, detection and output

➢ Here is a simple schematic that covers most modern UV spectrometers

UV-VIS sources

$\log(I_0/I) = A$

Monochromator /
beam splitter optics

I_0 sample I

detector

I_0 reference I_0

200 λ, nm 700
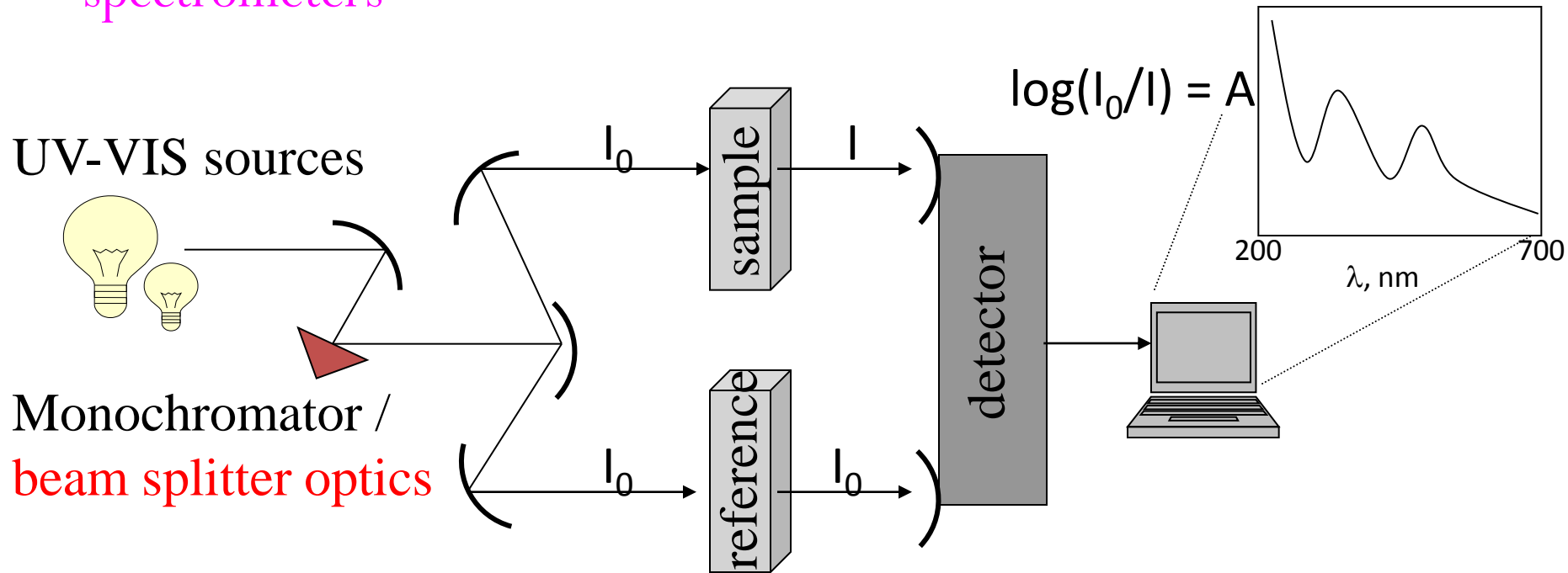
❖ Two sources are required to scan the entire UV-VIS band:

Deuterium lamp – covers the UV – 200-330

Tungsten lamp – covers 330-700

Sample Handling

➢ almost all UV spectra are recorded solution-phase

➢ Cells can be made of plastic, glass or quartz

➢ Only quartz is transparent in the full 200-700 nm range;

➢ plastic and glass are only suitable for visible spectra

➢ Concentration (we will cover shortly) is empirically determined

➢ A typical sample cell (commonly called a cuvet):

Solvents

❖ The choice of the solvent to be used in UV spec. is quite important.

➢ The first criterion for a good solvent is that it should not be absorb UV radiation(must be transparent) in the same region as the substance whose spectrum is being determined.

➢ Usually solvents that do not contain conjugated systems are most suitable for this purpose.

➢ In the UV, any solvent that does not have any π bonding can be used including water.

➢ In the visible region any solvent that is colorless can be used which also includes water.

➢ Each is transparent in the regions of UV spectrum where interesting absorption peaks from sample molecules are likely to occur.

The Spectrum

➢ The x-axis of the spectrum is in wavelength; 200-350 nm for UV, 200-700 for UV-VIS determinations, The y-axis of the spectrum is in absorbance, A

➢ Due to the lack of any fine structure, spectra are rarely shown in their raw form, rather, the peak maxima are simply reported as a numerical list of "lambda max" values or λ_{max}
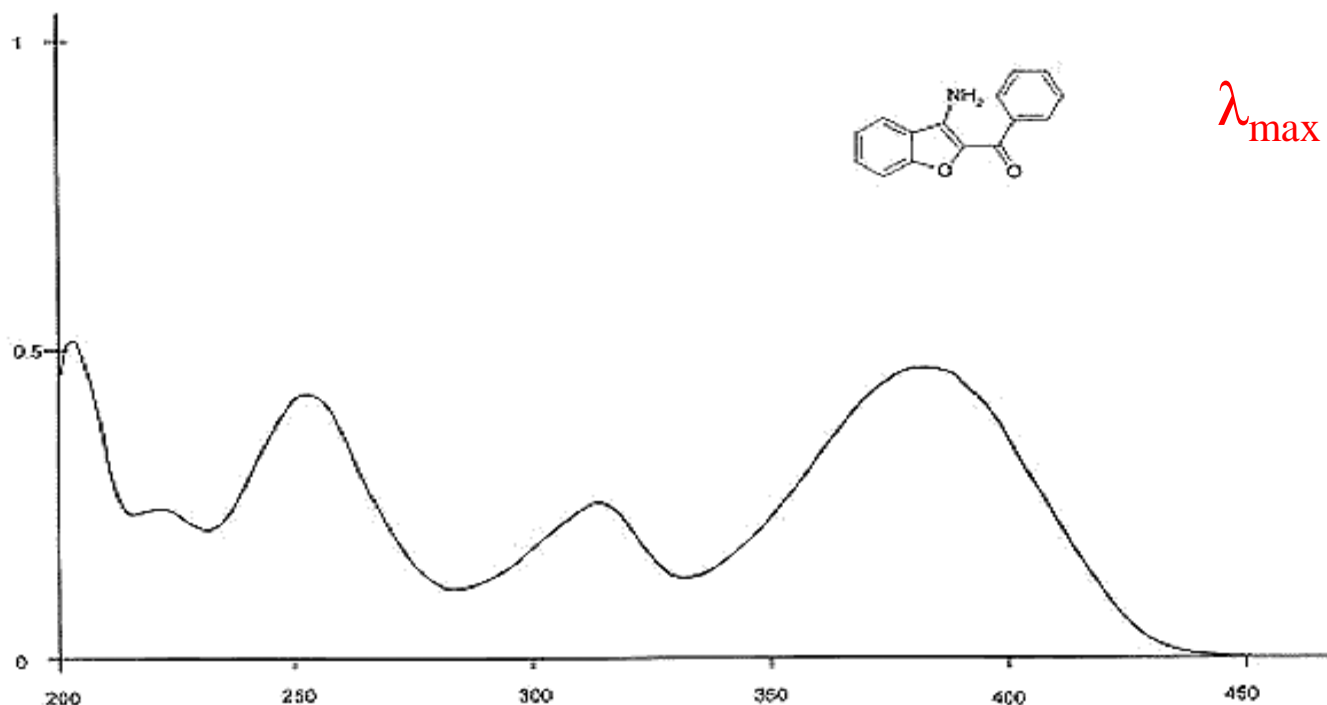
$\lambda_{max} =$ 206 nm
252
317
376

Cont…

❖ From the spectrometers point of view, absorbance is the inverse of transmittance: $A = \log_{10}(I_0/I)$

❖ From an experimental point of view, three other considerations must be made:

i.   A longer path length, l through the sample will cause more UV light to be absorbed − linear effect

ii. The greater the concentration, c of the sample, the more UV light will be absorbed − linear effect

iii. Some electronic transitions are more effective at the absorption of photon than others − molar absorptivity, ε this may vary by orders of magnitude.

❖ These effects are combined into the Beer-Lambert Law: $A = \varepsilon\, c\, l$

❖ For most UV spectrometers, l would remain constant (standard cells are typically 1 cm in path length)

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Practical application of UV spectroscopy

➢ UV spectra and visible spectra can be used to identify an unknown compound by a comparative analysis

➢ UV was the first organic spectral method, however, it is rarely used as a primary method for structure determination

➢ It is most useful in combination with NMR and IR data to elucidate unique electronic features that may be ambiguous in those methods

➢ It can be used to assay (via λ_{max} and molar absorptivity) the proper irradiation λs for photochemical experiments, or the design of UV resistant paints and coatings

➢ The most everywhere use of UV is as a detection device for HPLC; since UV is utilized for solution phase samples vs. a reference solvent this is easily incorporated into LC design

* UV is detector to HPLC as mass spectrometry (MS) to GC

Chromophores

A. Definition

➢ Remember the electrons present in organic molecules are involved in covalent bonds or lone pairs of electrons on atoms such as O/N

➢ Since similar functional groups will have es capable of discrete classes of transitions, the characteristic energy of these energies is more representative of the functional group than the es themselves

➢ A functional group capable of having characteristic electronic transitions is called a chromophore (color loving)

➢ Structural or electronic changes in the chromophore can be quantified and used to predict shifts in the observed electronic transitions

B. Organic Chromophores

1. Alkanes – only posses σ-bonds and no lone pairs of electrons, so only the high energy σ → σ * transition is observed in the far UV

➤ This transition is destructive to the molecule, causing cleavage of the s-bond
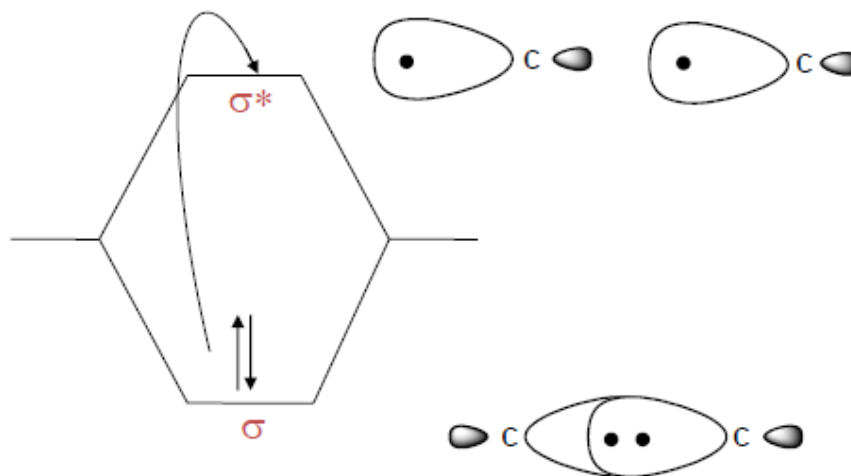
2. Alcohols, ethers, amines & sulfur cpds

➤ In the cases of simple, aliphatic examples of these cpds the n → σ* is the most often observed transition; like the alkane σ → σ* it is most often at shorter λ than 200 nm. How this transition occurs from the HOMO to the LUMO
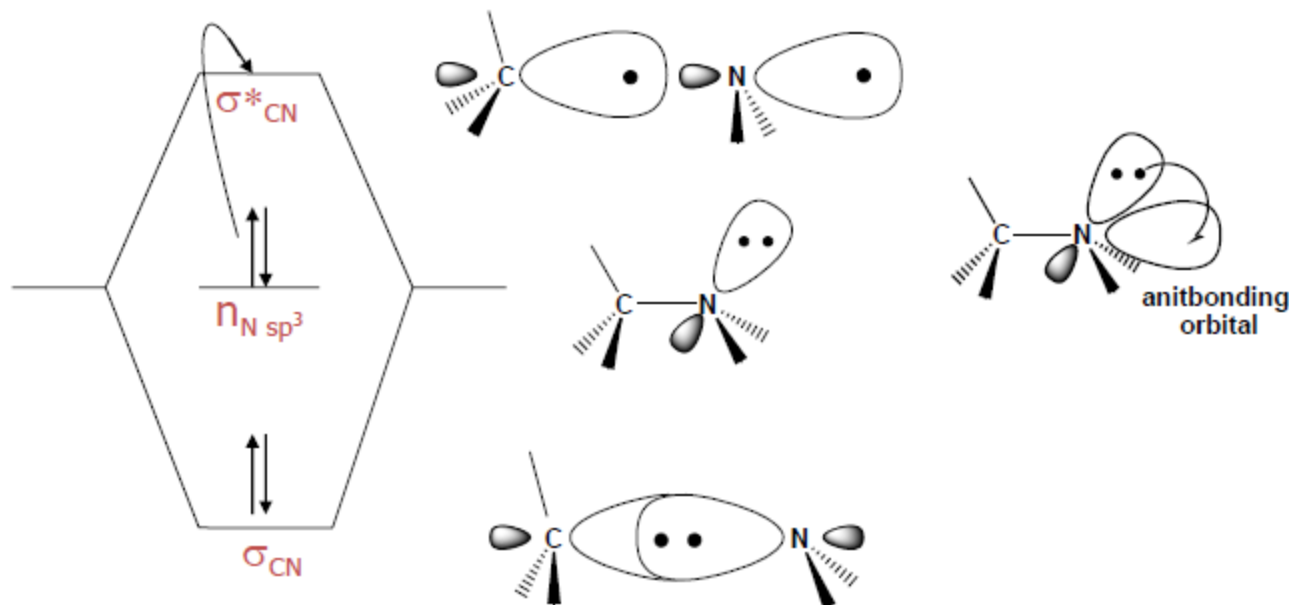
3. Alkenes & Alkynes

➤ In the case of isolated examples of these cpds the $\pi \rightarrow \pi^*$ is observed at 175 and 170 nm, respectively

➤ Even though this transition is of lower energy than $\sigma \rightarrow \sigma^*$ it is still in the far UV- however, the transition energy is sensitive to substitution

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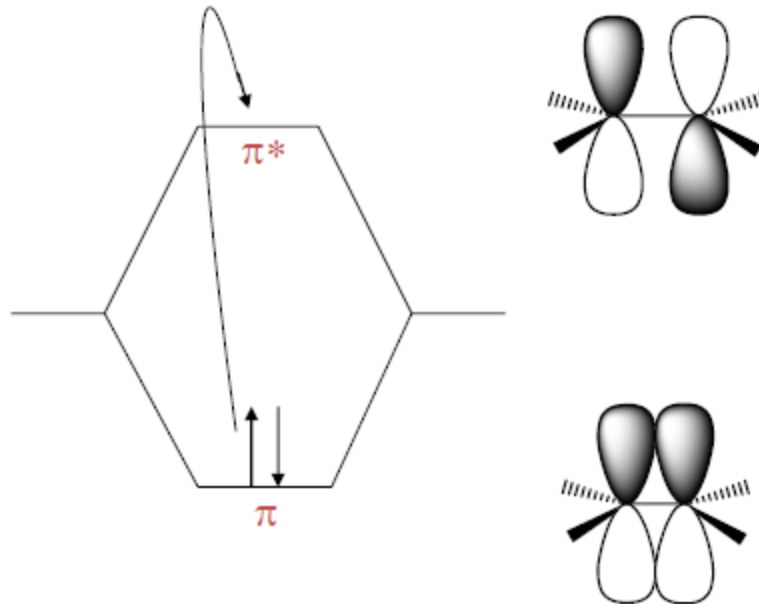
4. Carbonyls

➢ Unsaturated systems incorporating N or O can undergo $n \rightarrow \pi^*$ transitions (~285 nm) in addition to $\pi \rightarrow \pi^*$

➢ Despite the fact this transition is forbidden by the selection rules ($\varepsilon = 15$), it is the most often observed & studied transition for carbonyls

➢ This transition is also sensitive to substituents on the carbonyl

➢ Similar to alkenes and alkynes, non-substituted carbonyls undergo the $\pi \rightarrow \pi^*$ transition in the vacuum UV (188 nm, $\varepsilon = 900$); sensitive to substitution effects

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Cont…

Carbonyls – n $\rightarrow$ π^* transitions (~285 nm); π $\rightarrow$ π^* (188 nm)
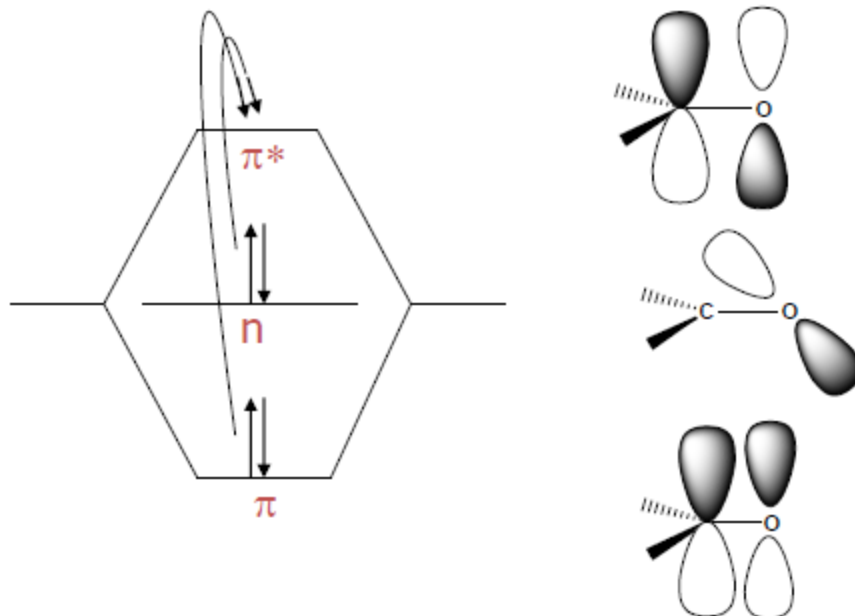
➢ It has been determined from spectral studies, that carbonyl oxygen more approximates sp rather than sp^2 !

➢ σ_{CO} transitions omitted for clarity

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C. Substituent Effects
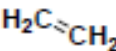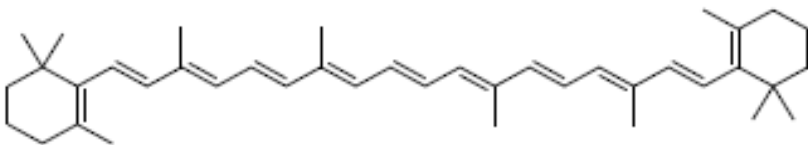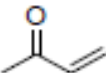
❖ General – from our brief study of these general chromophores, only the weak n → π* transition occurs in the routinely observed UV

➢ The attachment of substituent groups (other than H) can shift the energy of the transition

➢ Substituents that increase the intensity and often wavelength of an absorption are called auxochromes

➢ Common auxochromes include alkyl, hydroxyl, alkoxy and amino groups and the halogens

❖ Generally- Substituents may have any of four effects on a chromophore

i. Bathochromic shift (red shift) – a shift to longer λ; lower energy

ii. Hypsochromic shift (blue shift) – shift to shorter λ; higher energy

iii. Hyperchromic effect – an increase in intensity

iv. Hypochromic effect – a decrease in intensity

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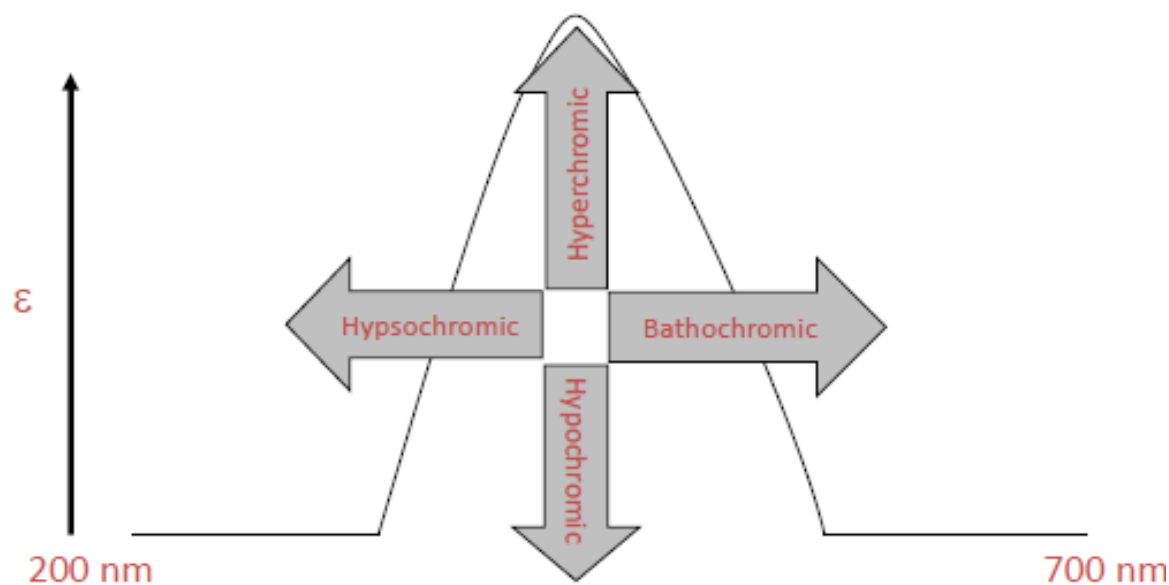
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1. Conjugation- most efficient means of bringing about a bathochromic (shift to longer λ) and hyperchromic shift of an unsaturated chromophore:

	λ_{max} nm	ε
$H_2C=CH_2$	175	15,000
	217	21,000
	258	35,000
β-carotene	465	125,000
	n → π* 280	12
	π → π* 189	900
	n → π* 280	27
	π → π* 213	7,100

Cont…

❖**Conjugation – Alkenes :** The observed shifts from conjugation imply that an increase in conjugation decreases the energy required for electronic excitation

❖ Extending this effect out to longer conjugated systems the energy gap becomes progressively smaller:
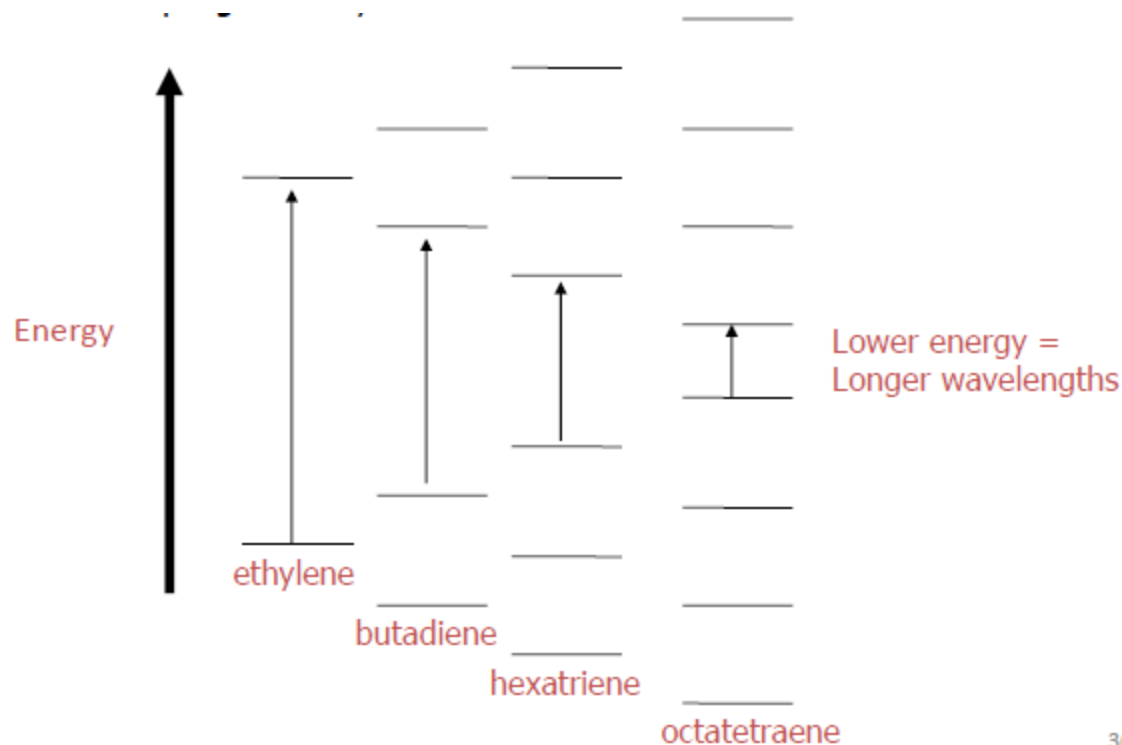
Colour and light absorption- The Chromophore concept

❖ Compounds that absorb light of wavelength between 400 and 800 nm (visible light) appear colored to the human eye.

❖When white light passes through a solution and come into view as red light, we say that the solution is red.

❖ What has actually happened is that the solution has allowed the red component of white light to pass through, whereas it has absorbed the complementary colors, yellow and blue.

❖The more concentrated the sample solution, the more yellow and blue light is absorbed and the more intensely red the solution appears to the eye.
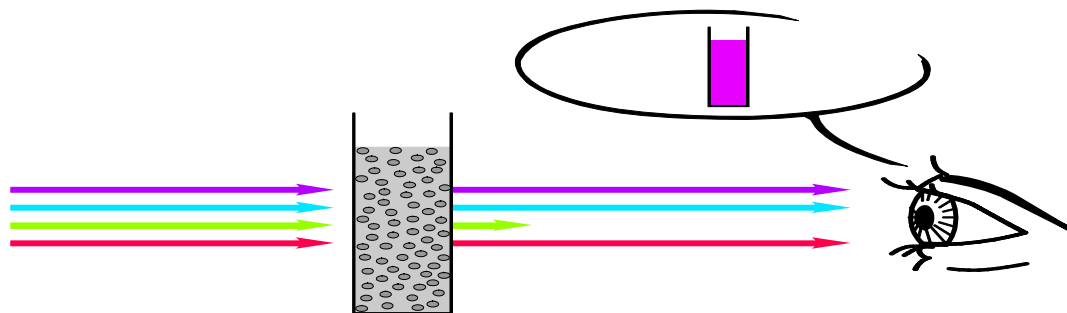
The human eye sees the complementary color to that which is absorbed

Absorbance and Complementary Colors

observed

Wavelength [nm]	Absorbed color		Complementary color
650-780	red		blue-green
595-650	orange		greenish blue
560-595	yellow-green		purple
500-560	green		red-purple
490-500	bluish green		red
480-490	greenish blue		orange
435-480	blue		yellow
380-435	violet		yellow-green
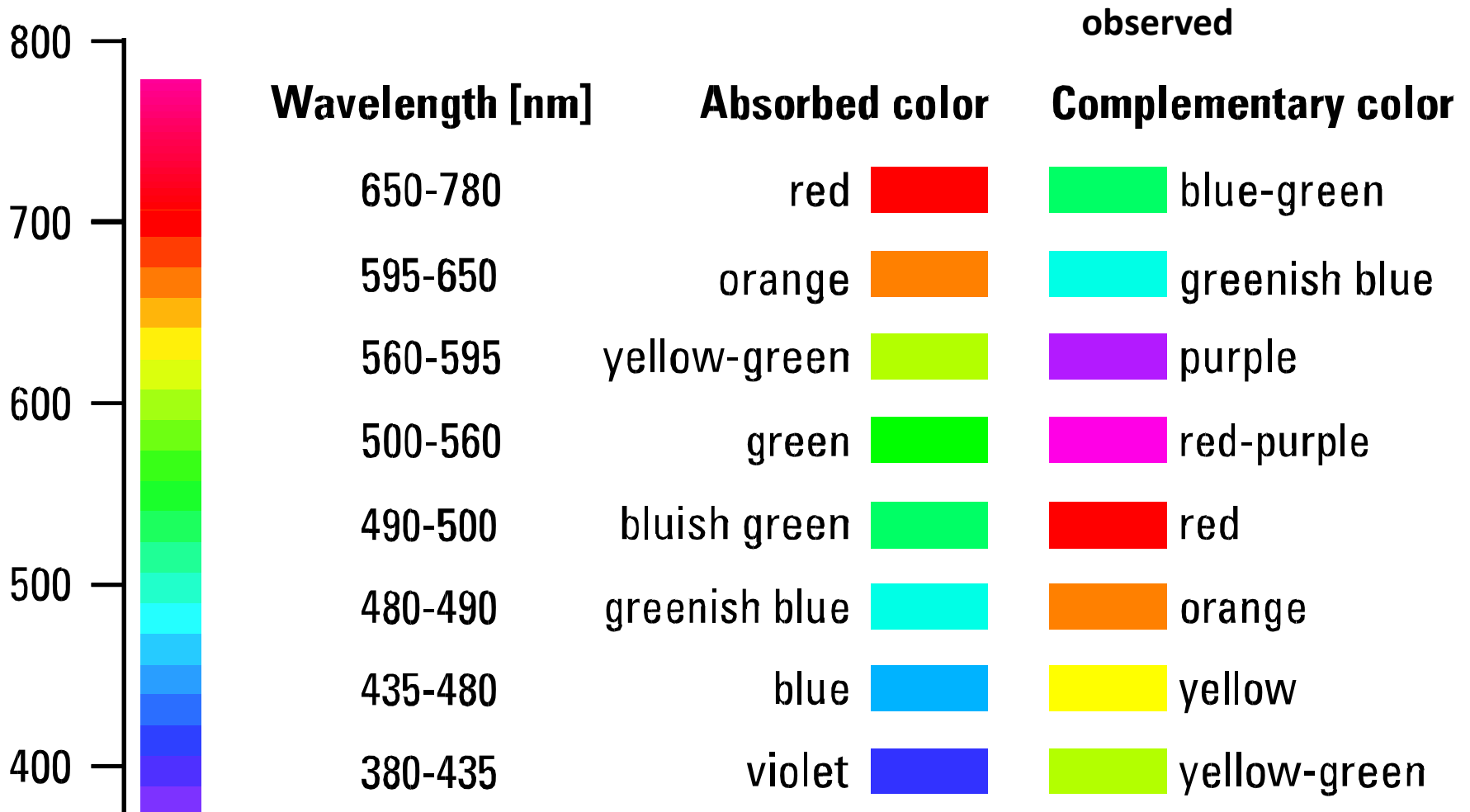
Visible Spectroscopy
Color

General:-The portion of the EMS from 400-800nm is observable to humans. We & some other mammals have the adaptation of seeing color at the expense of greater detail

400	500	600	700	800

	λ, nm
Violet	400-420
Indigo	420-440
Blue	440-490
Green	490-570
Yellow	570-585
Orange	585-620
Red	620-780

V. Visible Spectroscopy
    A. Color
        1. General

➢ When white (continuum of λ) light passes through, or is reflected by a surface, those that are absorbed are removed from the transmitted or reflected light respectively

➢ What is "seen" is the complimentary colors (those that are not absorbed)

• This is the origin of the "color wheel"
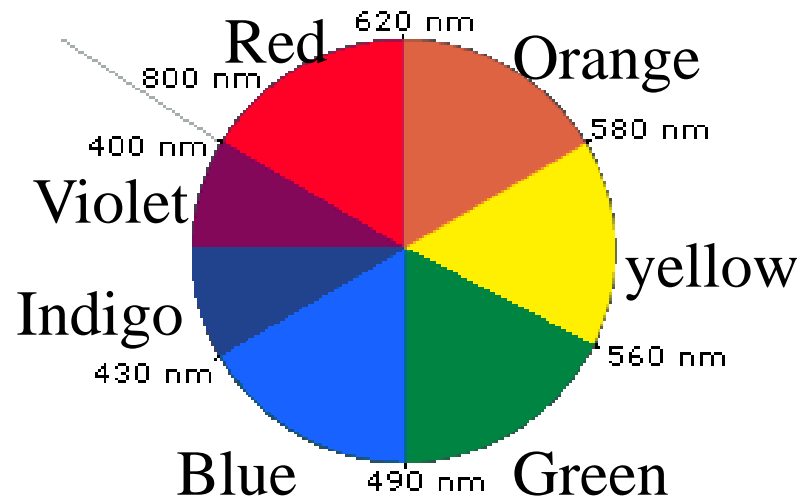
V. Visible Spectroscopy
A. Color
1. General

➢ Organic compounds that are "colored" are typically those with extensively conjugated systems (typically more than five)

• Consider β-carotene

β-carotene, λ_{max} = 455 nm
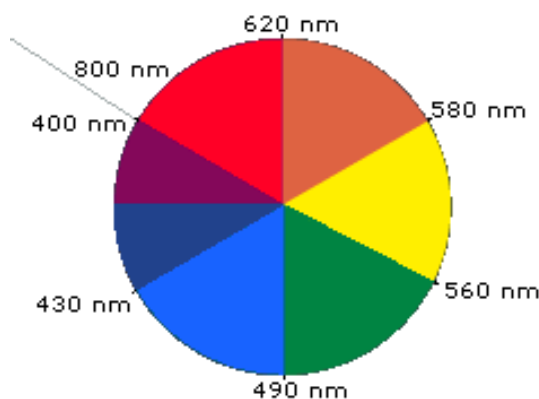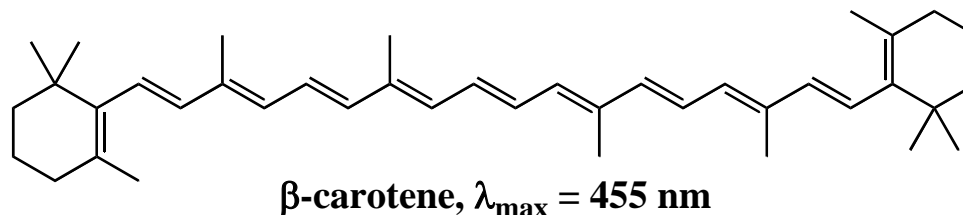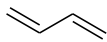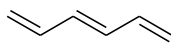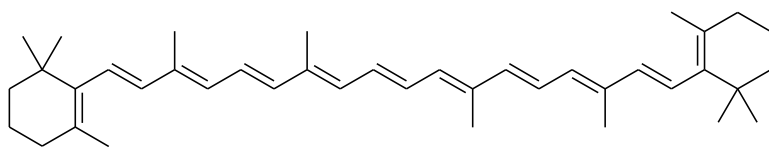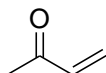
λ_{max} is at 455 – in the far blue region of the spectrum – this is absorbed

The remaining light has the complementary color of orange

Substituent Effects

1. **Conjugation** – most efficient means of bringing about a bathochromic and hyperchromic shift of an unsaturated chromophore:

	$\underline{\lambda_{max}}$ nm	$\underline{\varepsilon}$
$H_2C=CH_2$	175	15,000
	217	21,000
	258	35,000
β-carotene	465	125,000
	$n \rightarrow \pi^*$ 280	12
	$\pi \rightarrow \pi^*$ 189	900
	$n \rightarrow \pi^*$ 280	27
	$\pi \rightarrow \pi^*$ 213	7,100

IV. Structure Determination

   A. Dienes

      2. Woodward-Fieser Rules

Woodward and the Fiesers performed extensive studies of terpene and steroidal alkenes and noted similar substituents and structural features would predictably lead to an empirical prediction of the wavelength for the lowest energy $\pi \rightarrow \pi^*$ electronic transition

This work was distilled by Scott in 1964 into an extensive treatise on the Woodward-Fieser rules in combination with comprehensive tables and examples – (A.I. Scott, Interpretation of the Ultraviolet Spectra of Natural Products, Pergamon, NY, 1964)

A more modern interpretation was compiled by Rao in 1975 – (C.N.R. Rao, Ultraviolet and Visible Spectroscopy, 3rd Ed., Butterworths, London, 1975)

❖ This quantification is referred to as the **Woodward-Fieser** Rules which we will apply to three specific chromophores:

1. Conjugated dienes

2. Conjugated dienones

3. Aromatic systems

A. Dienes

1. General Features

For acyclic butadiene, two conformers are possible – s-cis and s-trans

s-trans
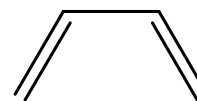
s-cis

> The s-cis conformer is at an overall higher potential energy than the s-trans; therefore the HOMO electrons of the conjugated system have less of a jump to the LUMO – lower energy, longer wavelength
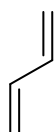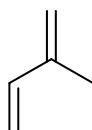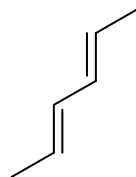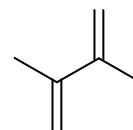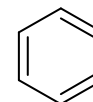
λ_{max} =   217   253   220   227   227   256   263   nm
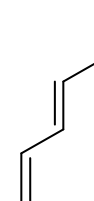
A. Dienes
  2. Woodward-Fieser Rules - Dienes

The rules begin with a base value for λ_{max} of the chromophore being observed:

          acyclic butadiene = 217 nm

The incremental contribution of substituents is added to this base value from the group tables:

Group	Increment
Extended conjugation	+30
Carbo cyclic ring	+5
Alkyl	+5
-OCOCH$_3$	+0
-OR	+6
-SR	+30
-Cl, -Br	+5
-NR$_2$	+60

A. Dienes
   2. Woodward-Fieser Rules - Dienes
     For example:
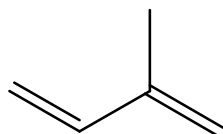
	Isoprene - acyclic butadiene =	217 nm
	one alkyl subs.	+ 5 nm
		222 nm
	Experimental value	220 nm
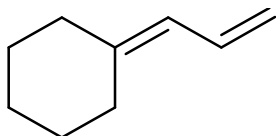
Allylidenecyclohexane

	- acyclic butadiene =	217 nm
	one exocyclic C=C	+ 5 nm
	2 alkyl subs.	+10 nm
		232 nm
	Experimental value	237 nm

A. Dienes

   3. Woodward-Fieser Rules – Cyclic Dienes

There are two major types of cyclic dienes, with two different base values

Heteroannular (transoid):

Homoannular (cisoid):
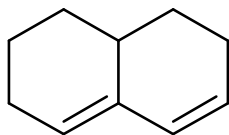
$\varepsilon = 5{,}000 - 15{,}000$
base $\lambda_{max} = 214$
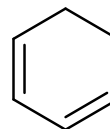
$\varepsilon = 12{,}000\text{-}28{,}000$
base $\lambda_{max} = 253$

The increment table is the same as for acyclic butadienes with a couple additions:

Group	Increment
Additional homoannular	+39
Where both types of diene are present, the one with the longer λ becomes the base	

A. Dienes
   3. Woodward-Fieser Rules – Cyclic Dienes
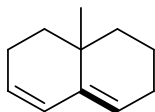   For example:
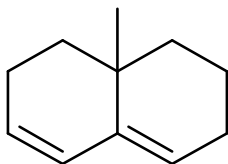
1,2,3,7,8,8a-hexahydro-8a-methylnaphthalene
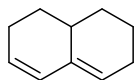
 heteroannular diene =   214 nm
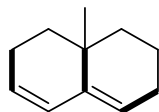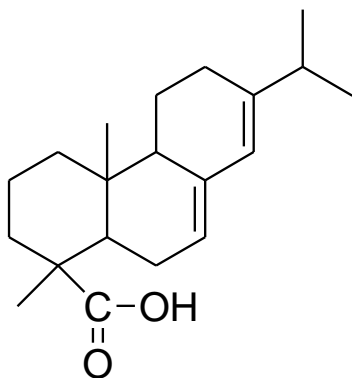
 3 alkyl subs. (3 x 5)   +15 nm

 1 exo C=C   <u>+ 5 nm</u>
234 nm

Experimental value   235 nm
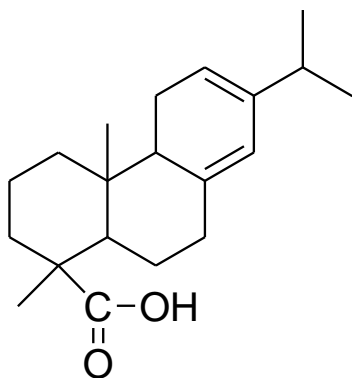
A. Dienes

3. Woodward-Fieser Rules – Cyclic Dienes

heteroannular diene =   214 nm

4 alkyl subs. (4 x 5) +20 nm
1 exo C=C   + 5 nm

239 nm

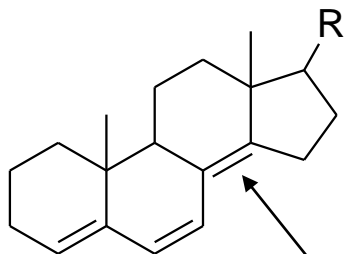homoannular diene =   253 nm

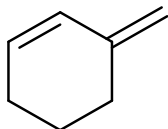4 alkyl subs. (4 x 5) +20 nm
1 exo C=C   + 5 nm

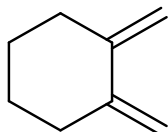278 nm

A. Dienes
   3. Woodward-Fieser Rules – Cyclic Dienes
      Be careful with your assignments – three common errors:

This compound has three exocyclic double bonds; the indicated bond is exocyclic to two rings

This is not a heteroannular diene; you would use the base value for an acyclic diene

Likewise, this is not a homooannular diene; you would use the base value for an acyclic diene