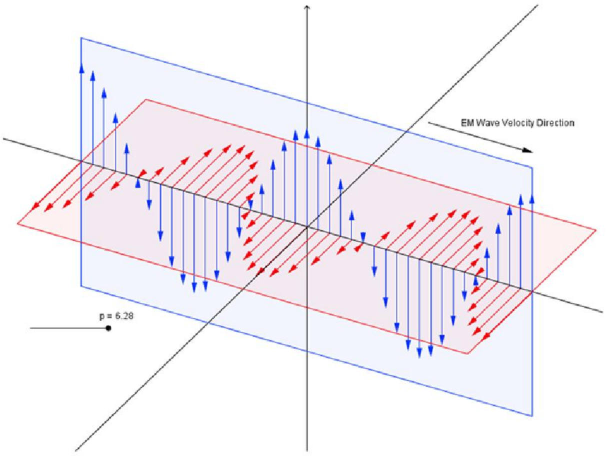


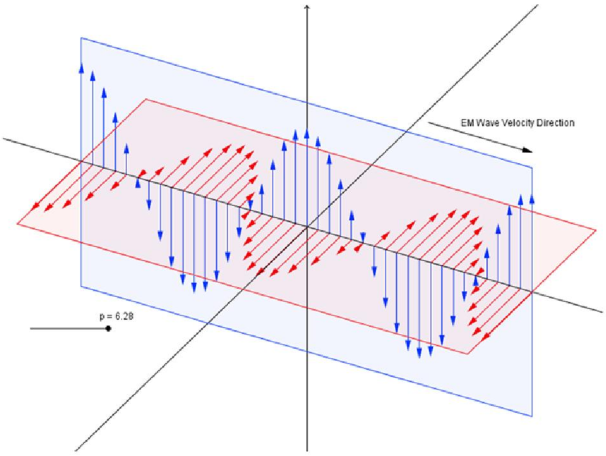
UV-vis spectroscopy

UV Spectroscopy

I. Introduction

   A. Electromagnetic waves

      1. Synchronized oscillations of electric and magnetic fields that propagate at the speed of light through a vacuum; the oscillations of the two fields are perpendicular to each other and perpendicular to the direction of wave propagation.

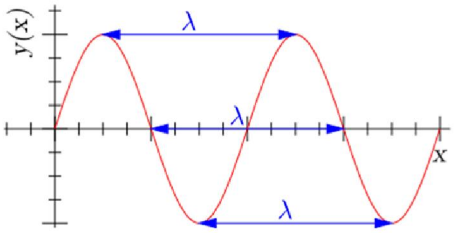


UV Spectroscopy

I. Introduction

A. Electromagnetic waves

1. The distance between two maxima is called wavelength λ.



2. The frequency is the number of oscillation made in a sec:

$$\nu = \frac{\lambda}{c}$$

3. The energy of a photon is:

$$E = h\nu = h\frac{\lambda}{c}$$

UV Spectroscopy

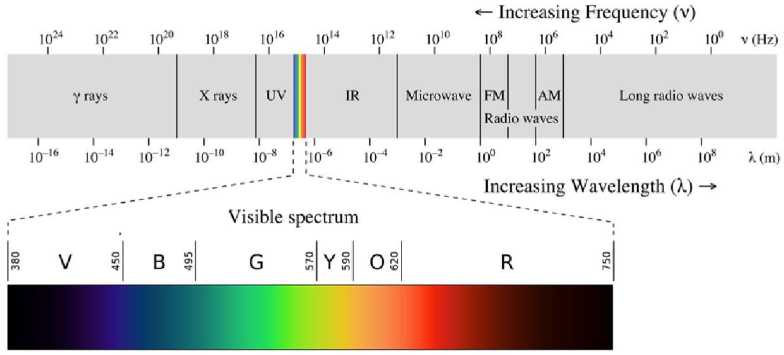
I. Introduction

B. UV radiation and Electronic Excitations

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

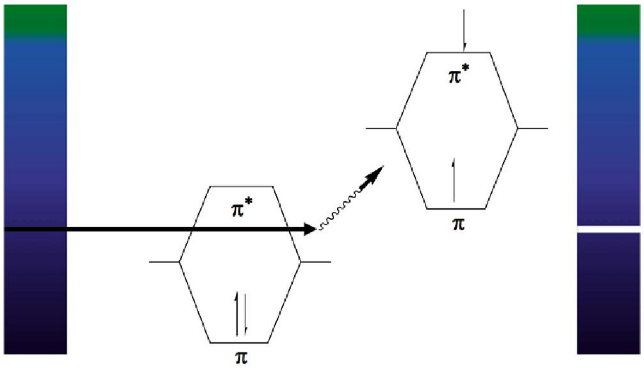
2. This energy corresponds to EM radiation in the ultraviolet (UV) region, 100-350 nm, and visible (VIS) regions 350-700 nm of the spectrum

3. For comparison, recall the EM spectrum:



UV Spectroscopy

I. Introduction
  C. The Spectroscopic Process
    1. In UV spectroscopy, the sample is irradiated with the broad spectrum of the UV radiation
    2. If a particular electronic transition matches the energy of a certain band of UV, it will be absorbed
    3. The remaining UV light passes through the sample and is observed
    4. From this residual radiation a spectrum is obtained with “gaps” at these discrete energies – this is called an **absorption spectrum**



UV Spectroscopy

I. Introduction

C. Observed electronic transitions

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

2. 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^*)

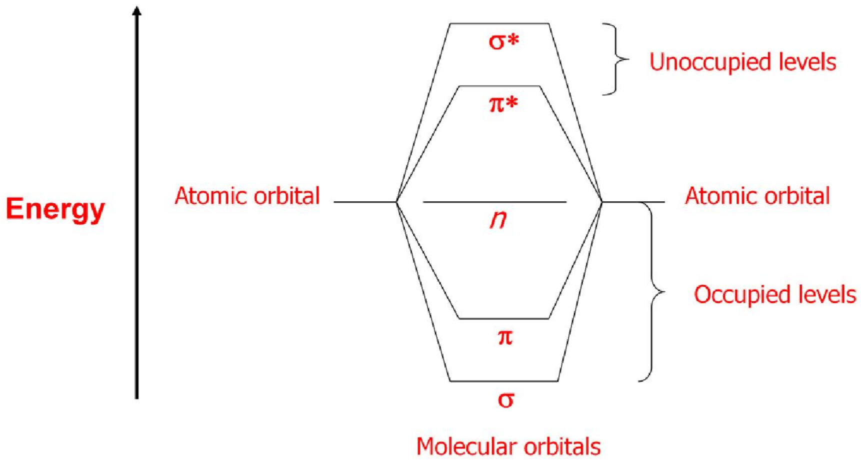
3. The lowest energy occupied orbitals are typically the σ; likewise, the corresponding anti-bonding σ^* orbital is of the highest energy

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

5. Unshared pairs lie at the energy of the original atomic orbital, most often this energy is higher than π or σ (since no bond is formed, there is no benefit in energy)

UV Spectroscopy

I. Introduction
   C. Observed electronic transitions
      6. Here is a graphical representation

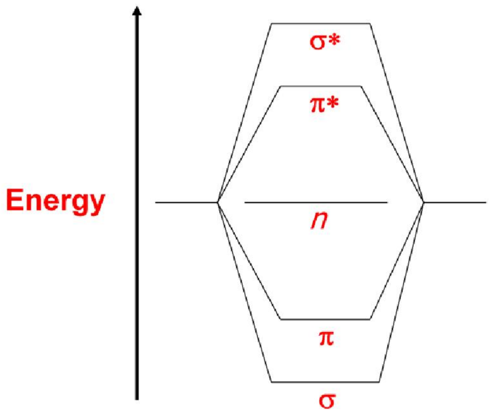


UV Spectroscopy

I. Introduction

  C. Observed electronic transitions

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



$\sigma \longrightarrow \sigma^*$	alkanes
$\sigma \longrightarrow \pi^*$	carbonyls
$\pi \longrightarrow \pi^*$	unsaturated cmpds.
$n \longrightarrow \sigma^*$	O, N, S, halogens
$n \longrightarrow \pi^*$	carbonyls

UV Spectroscopy

I. Introduction

  C. Observed electronic transitions

    7. Although the UV spectrum extends below 100 nm (high energy), oxygen in the atmosphere is not transparent below 200 nm

    8. Special equipment to study *vacuum* or *far UV* is required

    9. Routine organic UV spectra are typically collected from 200-700 nm

    10. This limits the transitions that can be observed:

$\sigma \rightarrow \sigma^*$	alkanes	150 nm	
$\sigma \rightarrow \pi^*$	carbonyls	170 nm	
$\pi \rightarrow \pi^*$	unsaturated cmpds.	180 nm	**√ - if conjugated!**
$n \rightarrow \sigma^*$	O, N, S, halogens	190 nm	
$n \rightarrow \pi^*$	carbonyls	300 nm	√

UV Spectroscopy

I. Introduction

  D. Selection Rules

    1. Not all transitions that are possible are observed

    2. For an electron to transition, certain quantum mechanical constraints apply – these are called "selection rules"

    3. For example, an electron cannot change its spin quantum number during a transition – these are "forbidden"
       Other examples include:
       - the number of electrons that can be excited at one time
       - symmetry properties of the molecule
       - symmetry of the electronic states

    4. To further complicate matters, "forbidden" transitions are sometimes observed (albeit at low intensity) due to other factors

UV Spectroscopy

I. Introduction

E. Band Structure

1. Unlike IR (or later NMR), where there may be upwards of 5 or more resolvable peaks from which to elucidate structural information, UV tends to give wide, overlapping bands

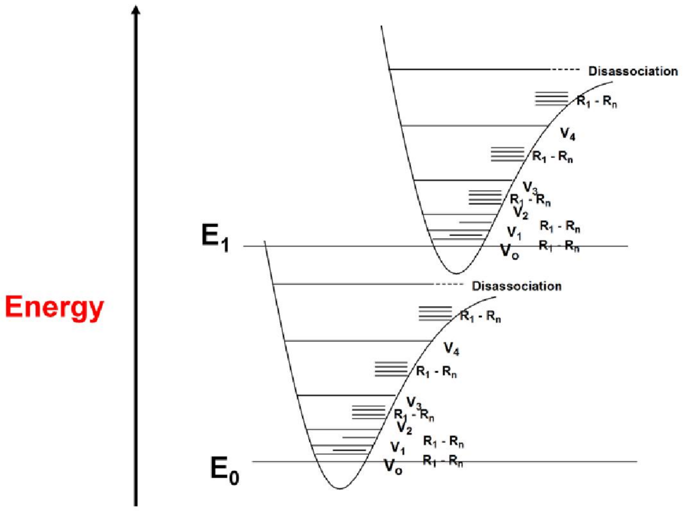
2. It would seem that since the electronic energy levels of a pure sample of molecules would be quantized, fine, discrete bands would be observed – for atomic spectra, this is the case

3. In molecules, when a bulk sample of molecules is observed, not all bonds (read – pairs of electrons) are in the same vibrational or rotational energy states

4. This effect will impact the wavelength at which a transition is observed – very similar to the effect of H-bonding on the O-H vibrational energy levels in neat samples

UV Spectroscopy

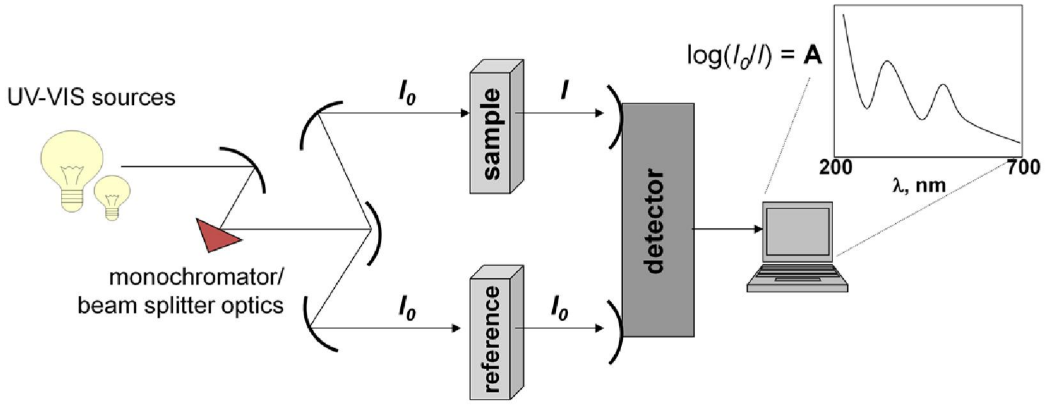
I. Introduction
  E. Band Structure
    5. When these energy levels are superimposed, the effect can be readily explained – any transition has the possibility of being observed



UV Spectroscopy

II. Instrumentation and Spectra
   A. Instrumentation
      1. The construction of a traditional UV-VIS spectrometer is very similar to an IR, as similar functions – sample handling, irradiation, detection and output are required

      2. Here is a simple schematic that covers most modern UV spectrometers:



UV Spectroscopy

II. Instrumentation and Spectra
  A. Instrumentation
    3. Two sources are required to scan the entire UV-VIS band:
      - Deuterium lamp – covers the UV – 200-330
      - Tungsten lamp – covers 330-700

    4. As with the dispersive IR, the lamps illuminate the entire band of UV or visible light; the monochromator (grating or prism) gradually changes the small bands of radiation sent to the beam splitter

    5. The beam splitter sends a separate band to a cell containing the sample solution and a reference solution

    6. The detector measures the difference between the transmitted light through the sample (I) vs. the incident light (I_0) and sends this information to the recorder

UV Spectroscopy

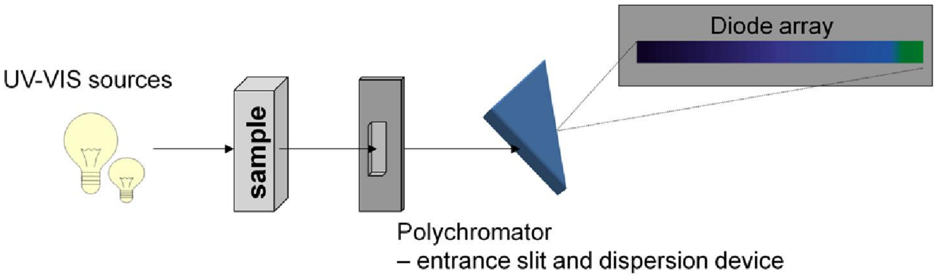
II. Instrumentation and Spectra

A. Instrumentation

7. As with dispersive IR, time is required to cover the entire UV-VIS band due to the mechanism of changing wavelengths

8. A recent improvement is the diode-array spectrophotometer - here a prism (dispersion device) breaks apart the full spectrum transmitted through the sample

9. Each individual band of UV is detected by a individual diodes on a silicon wafer simultaneously – the obvious limitation is the size of the diode, so some loss of resolution over traditional instruments is observed



UV Spectroscopy

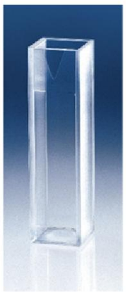
II. Instrumentation and Spectra
  B. Instrumentation – Sample Handling
    1. Virtually all UV spectra are recorded solution-phase

    2. Cells can be made of plastic, glass or quartz

    3. Only quartz is transparent in the full 200-700 nm range; plastic and glass are only suitable for visible spectra

    4. Concentration (we will cover shortly) is empirically determined

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



UV Spectroscopy

II. Instrumentation and Spectra
   B. Instrumentation – Sample Handling
      5. Solvents must be transparent in the region to be observed; the wavelength where a solvent is no longer transparent is referred to as the cutoff

      6. Since spectra are only obtained up to 200 nm, solvents typically only need to lack conjugated π systems or carbonyls

Common solvents and cutoffs:

acetonitrile	190
chloroform	240
cyclohexane	195
1,4-dioxane	215
95% ethanol	205
n-hexane	201
methanol	205
isooctane	195
water	190

UV Spectroscopy

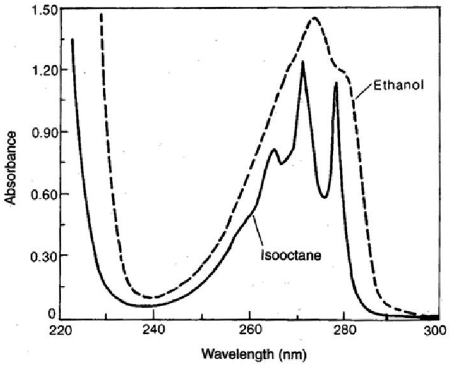
II. Instrumentation and Spectra

B. Instrumentation – Sample Handling

7. Additionally solvents must preserve the fine structure (where it is actually observed in UV!) where possible

8. H-bonding further complicates the effect of vibrational and rotational energy levels on electronic transitions, dipole-dipole interacts less so

9. The more non-polar the solvent, the better (this is not always possible)



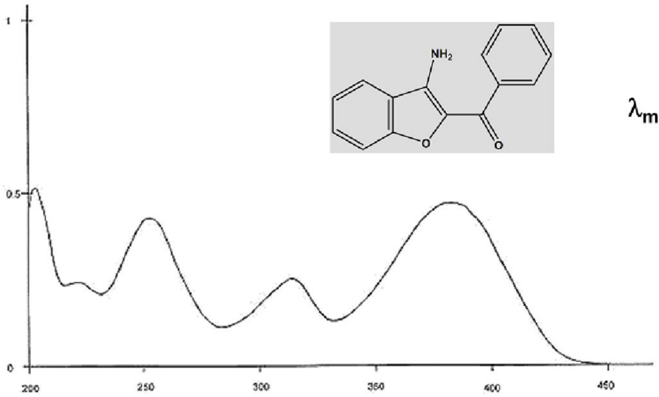
UV Spectroscopy

II. Instrumentation and Spectra

C. The Spectrum

1. The x-axis of the spectrum is in wavelength; 200-350 nm for UV, 200-700 for UV-VIS determinations

2. 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 “lamba max” values or λ_{max}



λ_{max} = 206 nm
252
317
376

UV Spectroscopy

II. Instrumentation and Spectra
  C. The Spectrum
    1. The y-axis of the spectrum is in absorbance, A

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

    3. From an experimental point of view, three other considerations must be made:
      i. a longer path length, b 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…

UV Spectroscopy

II. Instrumentation and Spectra

C. The Spectrum

4. These effects are combined into the Beer-Lambert Law: **$A = \varepsilon b c$**
   - i. for most UV spectrometers, *l* would remain constant (standard cells are typically 1 cm in path length)
   - ii. concentration is typically varied depending on the strength of absorption observed or expected – typically dilute – sub 0.001 M
   - iii. molar absorptivities vary by orders of magnitude:
     - values of 10^4-10^6 10^4-10^6 are termed high intensity absorptions
     - values of 10^3-10^4 are termed low intensity absorptions
     - values of 0 to 10^3 are the absorptions of forbidden transitions

   A is unitless, so the units for ε are $cm^{-1} \cdot M^{-1}$ and are rarely expressed

5. Since path length and concentration effects can be easily factored out, absorbance simply becomes proportional to ε, and the y-axis is expressed as ε directly or as the logarithm of ε

UV Spectroscopy

II. Instrumentation and Spectra

D. Practical application of UV spectroscopy

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

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

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

4. The most ubiquitous 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 to HPLC what mass spectrometry (MS) will be to GC

UV Spectroscopy

III. Chromophores

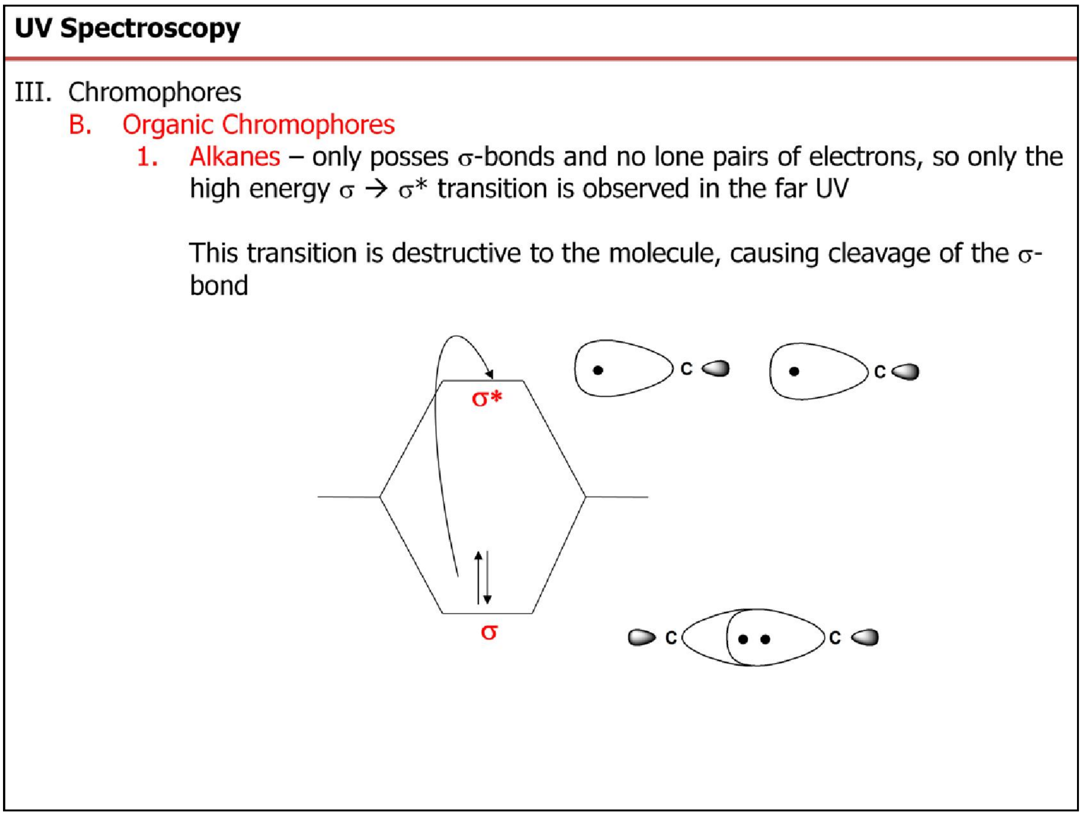
A. Definition

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

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

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

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



UV Spectroscopy

III. Chromophores
  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 σ-bond

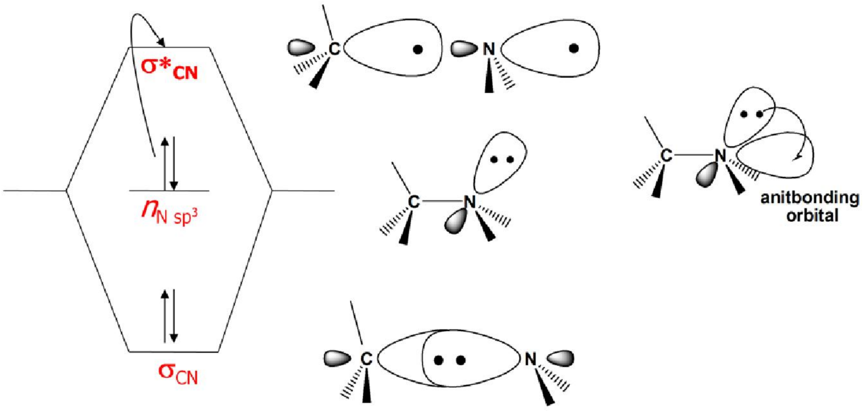
UV Spectroscopy

III. Chromophores

B. Organic Chromophores

2. Alcohols, ethers, amines and sulfur compounds – in the cases of simple, aliphatic examples of these compounds the $n \rightarrow \sigma^*$ is the most often observed transition; like the alkane $\sigma \rightarrow \sigma^*$ it is most often at shorter λ than 200 nm

   Note how this transition occurs from the HOMO to the LUMO



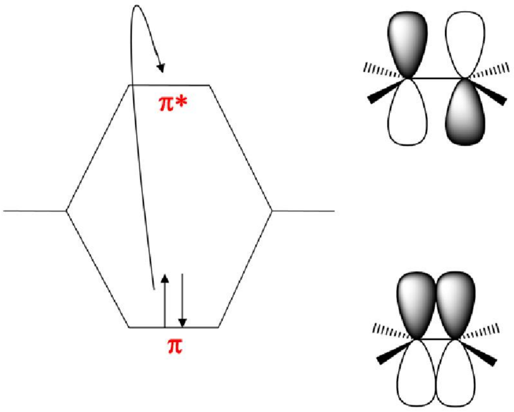
UV Spectroscopy

III. Chromophores

B. Organic Chromophores

3. Alkenes and Alkynes – in the case of isolated examples of these compounds the π → π* is observed at 175 and 170 nm, respectively

Even though this transition is of lower energy than σ → σ*, it is still in the far UV – however, the transition energy is sensitive to substitution



UV Spectroscopy

III. Chromophores
  B. Organic Chromophores
    4. Carbonyls – unsaturated systems incorporating N or O can undergo *n* → π* transitions (~285 nm) in addition to π → π*

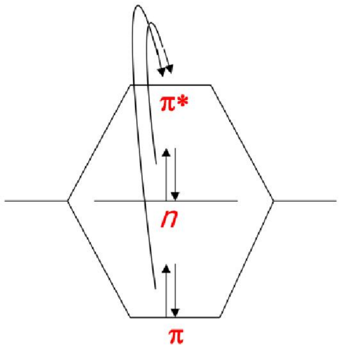
Despite the fact this transition is forbidden by the selection rules (ε = 15), it is the most often observed and studied transition for carbonyls

This transition is also sensitive to substituents on the carbonyl

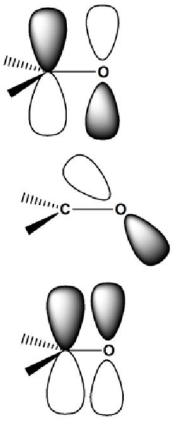
Similar to alkenes and alkynes, non-substituted carbonyls undergo the π → π* transition in the vacuum UV (188 nm, ε = 900); sensitive to substitution effects

UV Spectroscopy

III. Chromophores
  B. Organic Chromophores
    4. Carbonyls – $n \rightarrow \pi^*$ transitions (~285 nm); $\pi \rightarrow \pi^*$ (188 nm)



σ_{CO} transitions omitted for clarity



It has been determined from spectral studies, that carbonyl oxygen more approximates *sp* rather than *sp*^{2} !

UV Spectroscopy

III. Chromophores

C. Substituent Effects

General – from our brief study of these general chromophores, only the weak $n \rightarrow \pi^*$ transition occurs in the routinely observed UV

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

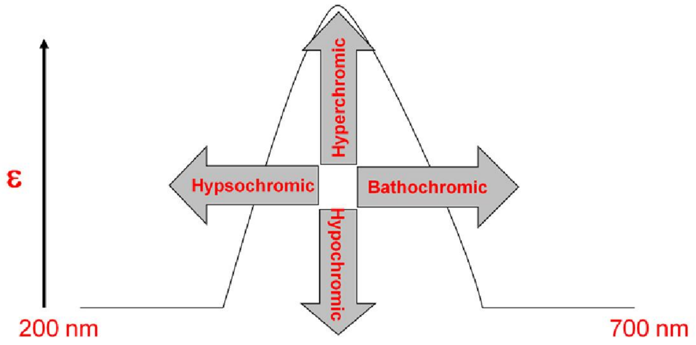
Substituents that increase the intensity and often wavelength of an absorption are called auxochromes

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

UV Spectroscopy

III. Chromophores
  C. Substituent Effects
    General – 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

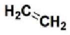
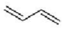
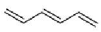
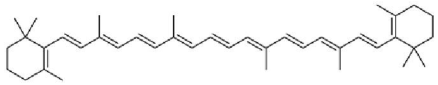
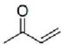


UV Spectroscopy

III. Chromophores

C. Substituent Effects

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

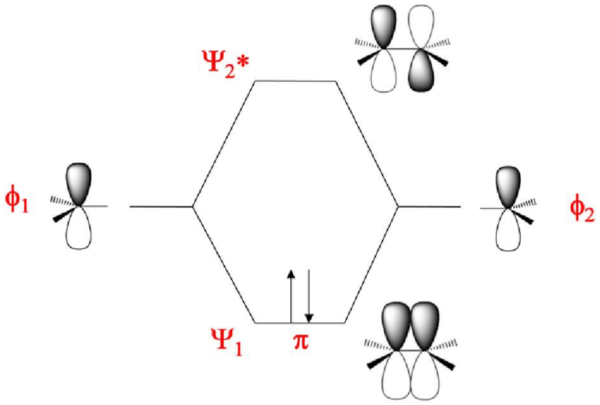
		λ_{max} nm	ε
		175	15,000
		217	21,000
		258	35,000
 β-carotene		465	125,000
	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	280 189	12 900
	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	280 213	27 7,100

UV Spectroscopy

III. Chromophores
   C. Substituent Effects
      1. Conjugation – Alkenes

The observed shifts from conjugation imply that an increase in conjugation decreases the energy required for electronic excitation

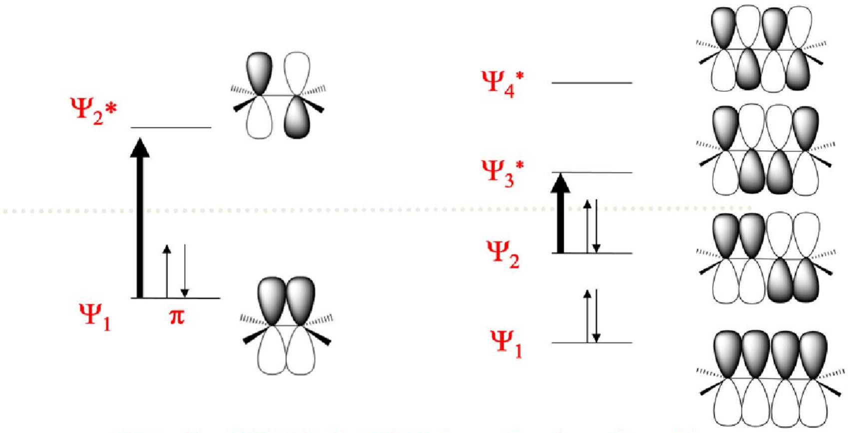
From molecular orbital (MO) theory two atomic *p* orbitals, ϕ_1 and ϕ_2 from two sp^2 hybrid carbons combine to form two MOs Ψ_1 and Ψ_2^* in ethylene



UV Spectroscopy

III. Chromophores
   C. Substituent Effects
      2. Conjugation – Alkenes

When we consider butadiene, we are now mixing 4 p orbitals giving 4 MOs of an energetically symmetrical distribution compared to ethylene

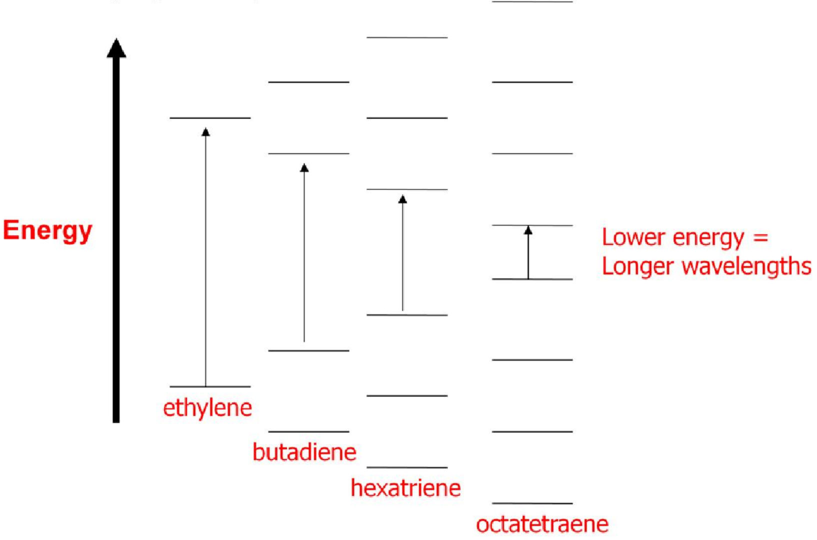


ΔE for the HOMO → LUMO transition is **reduced**

UV Spectroscopy

III. Chromophores
  C. Substituent Effects
    2. Conjugation – Alkenes

Extending this effect out to longer conjugated systems the energy gap becomes progressively smaller:

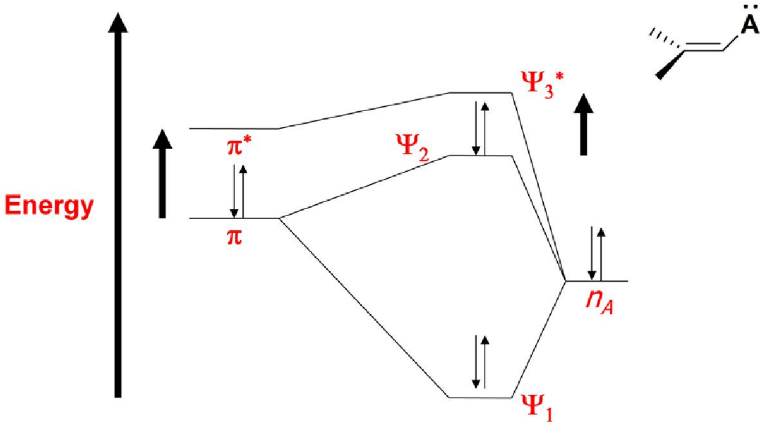


UV Spectroscopy

III. Chromophores
   C. Substituent Effects
      2. Conjugation – Alkenes

Similarly, the lone pairs of electrons on N, O, S, X can extend conjugated systems – auxochromes

Here we create 3 MOs – this interaction is not as strong as that of a conjugated π-system



UV Spectroscopy

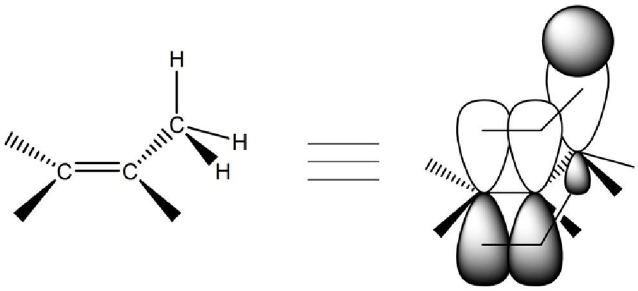
III. Chromophores

C. Substituent Effects

2. Conjugation – Alkenes

Methyl groups also cause a bathochromic shift, even though they are devoid of π- or *n*-electrons

This effect is thought to be through what is termed "hyperconjugation" or sigma bond resonance



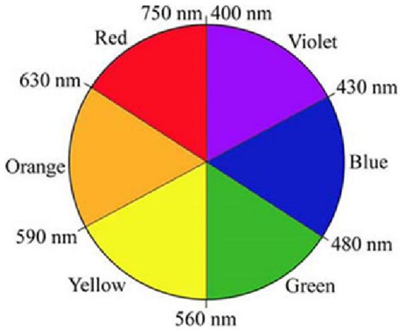
UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    1. General
      - The portion of the EM spectrum from 400-800 is observable to humans- we (and some other mammals) have the adaptation of seeing color at the expense of greater detail

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

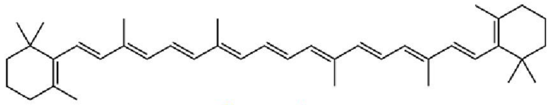
UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    1. General
      - When white (continuum of λ) light passes through, or is reflected by a surface, those λs 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"

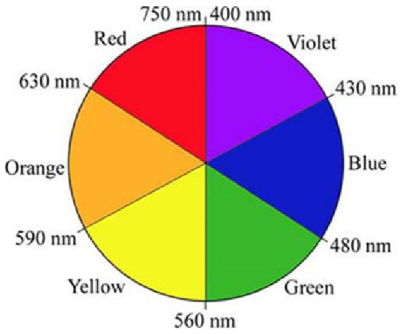


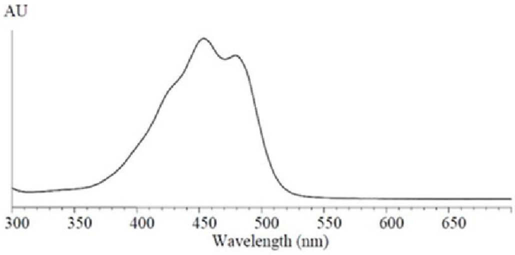
UV Spectroscopy

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



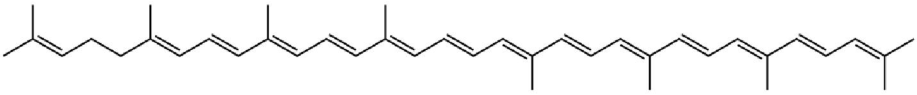
β-carotene



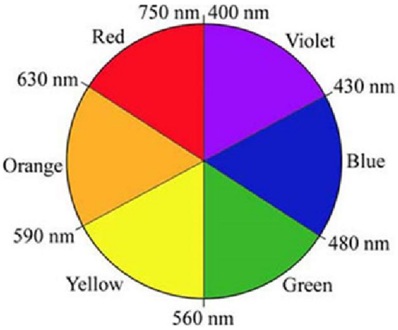


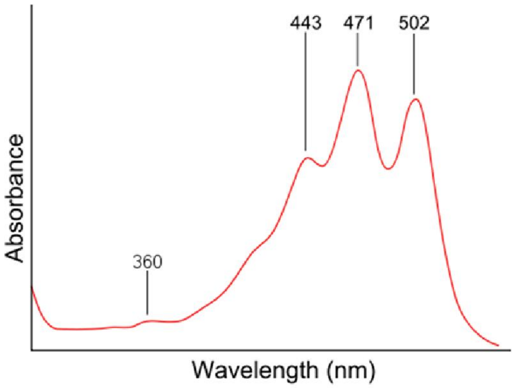
UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    1. General
      - Likewise:



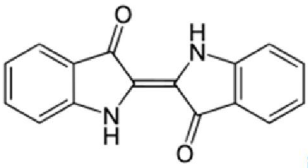
lycopene



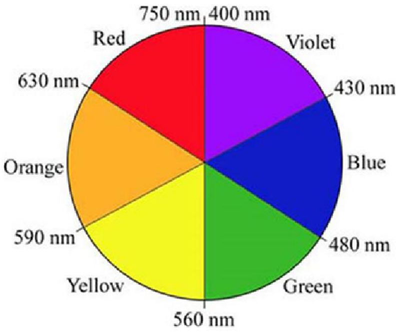


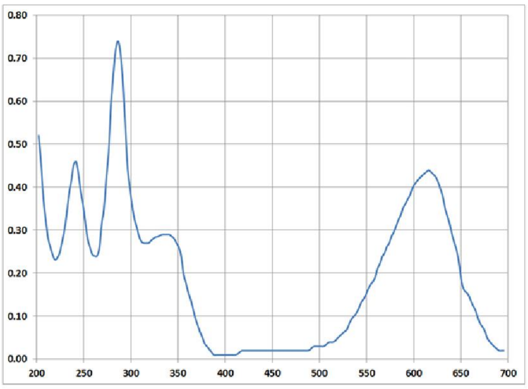
UV Spectroscopy

V. Visible Spectroscopy
   A. Color
      1. General
         - Likewise:



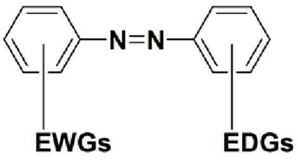
indigo





UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    2. Dyes
      - One of the most common class of colored organic molecules are the azo dyes:

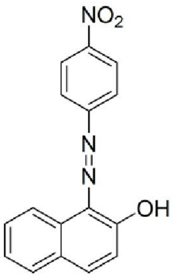


From our discussion of di-subsituted aromatic chromophores, the effect of opposite groups is greater than the sum of the individual effects – more so on this heavily conjugated system

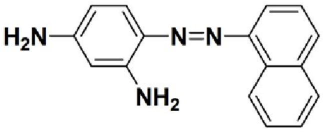
Coincidentally, it is necessary for these to be opposite for the original synthetic preparation!

UV Spectroscopy

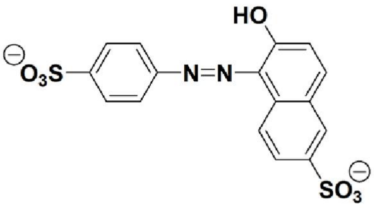
V. Visible Spectroscopy
  A. Color
    2. Dyes
      - These materials are some of the more familiar colors of our "environment"



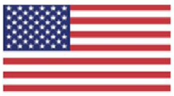
Para Red



Fast Brown



Sunset Yellow (Food Yellow 3)



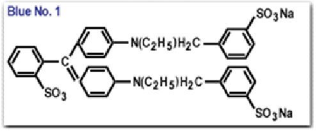
🟫

The colors of M&M's

Bright Blue

Common Food Uses

Beverages, dairy products, powders, jellies, confections, condiments, icing.

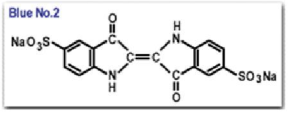


Blue No. 1

Royal Blue

Common Food Uses

Baked goods, cereals, snack foods, ice-cream, confections, cherries.

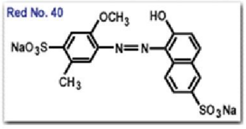


Blue No.2

Orange-red

Common Food Uses

Gelatins, puddings, dairy products, confections, beverages, condiments.

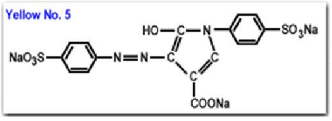


Red No. 40

Lemon-yellow

Common Food Uses

Custards, beverages, ice-cream, confections, preserves, cereals.

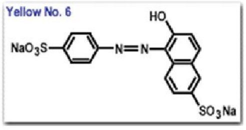


Yellow No. 5

Orange

Common Food Uses

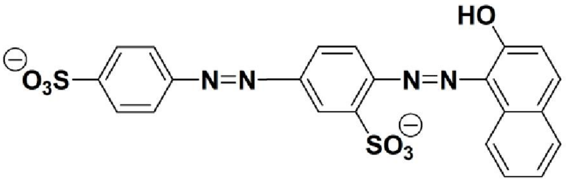
Cereals, baked goods, snack foods, ice-cream, beverages, dessert powders, confections



Yellow No. 6

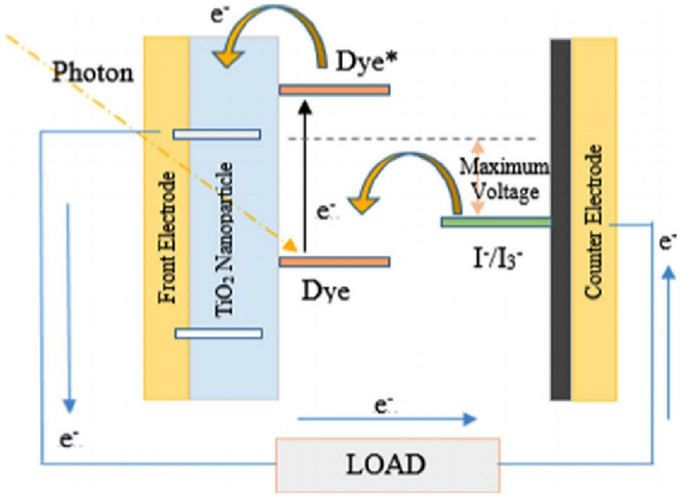
UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    2. Dyes
      - In the biological sciences these compounds are used as dyes to selectively stain different tissues or cell structures
      - Biebrich Scarlet - Used with picric acid/aniline blue for staining collagen, recticulum, muscle, and plasma. Luna's method for erythrocytes & eosinophil granules. Guard's method for sex chromatin and nuclear chromatin.



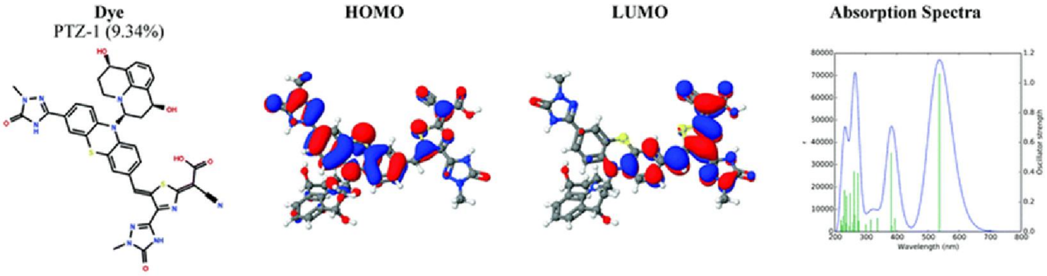
UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    2. Dyes
      - In Dye-Sensitized Solar Cells (DSSC):



UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    2. Dyes
      - In Dye-Sensitized Solar Cells (DSSC), dyes with high e are required.

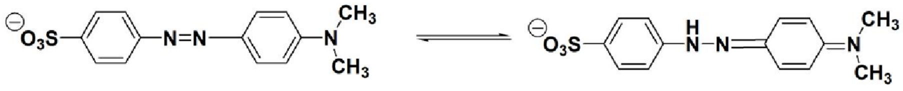


ICT
Intramolecular Charge Transfer

UV Spectroscopy

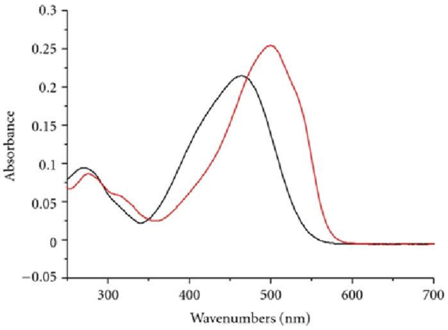
V. Visible Spectroscopy
  A. Color
    3. Indicators
      - In the chemical sciences these are the acid-base indicators used for the various pH ranges:

Methyl Orange



Yellow, pH > 4.4

Red, pH < 3.2

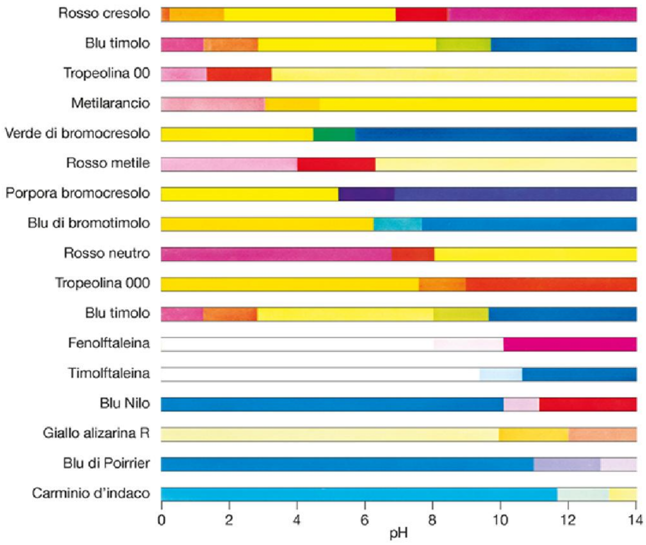


UV Spectroscopy

V. Visible Spectroscopy

A. Color

3. pH indicators



UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    3. pH indicators

$$HIn_{(aq)} + H_2O \rightleftharpoons In^-_{(aq)} + H_3O^+$$

rosso   blu

$$\frac{[HIn]}{[In^-]} = \frac{[H_3O^+]}{KIn}$$

UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    3. pH indicators

$$\mathbf{HIn_{(aq)} + H_2O \rightleftharpoons In^-_{(aq)} + H_3O^+}$$

$$\frac{[HIn]}{[In^-]} = \frac{[H_3O^+]}{KIn} \geq 10 \quad \mathrm{pH} \leq \mathrm{pKIn} - 1 \qquad \mathit{HIn}$$

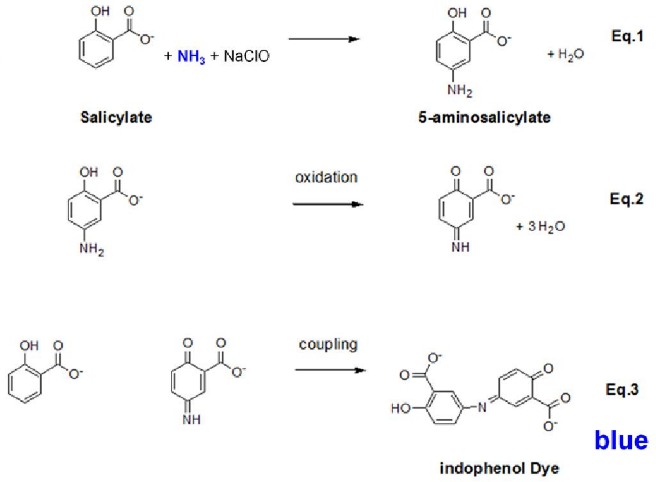
$$\frac{[HIn]}{[In^-]} = \frac{[H_3O^+]}{KIn} \leq 0.1 \quad \mathrm{pH} \geq \mathrm{pKIn} + 1 \qquad \mathit{In^-}$$

pH range $\boxed{\mathrm{pH} = \mathrm{pKIn} \pm 1}$

UV Spectroscopy

V. Visible Spectroscopy
  A. Color
    4. Quantitative analysis
      - Vis spectroscopy can be used for the quantification of particular substances (analyte).
      - The analyte must be converted into a colored compound (derivatization reaction).

Ammonia determination



UV Spectroscopy

V. Visible Spectroscopy

A. Color

4. Quantitative analysis