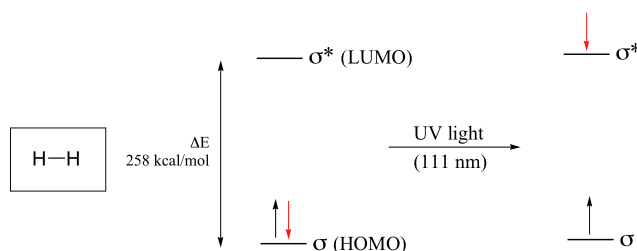


## 20.3: Excited Electronic States: Electronic Spectroscopy of Molecules

While interaction with infrared light causes molecules to undergo vibrational transitions, the shorter wavelength, higher energy radiation in the UV (200-400 nm) and visible (400-700 nm) range of the electromagnetic spectrum causes many organic molecules to undergo **electronic transitions**. What this means is that when the energy from UV or visible light is absorbed by a molecule, one of its electrons jumps from a lower energy to a higher energy molecular orbital.

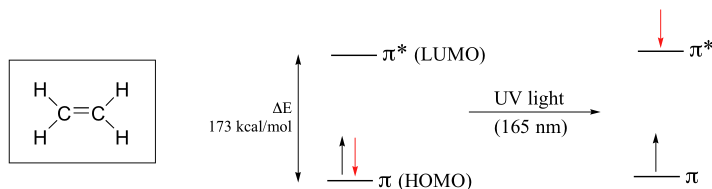
### Electronic transitions

Let's take as our first example the simple case of molecular hydrogen,  $H_2$ . As you may recall from section 2.1A, the molecular orbital picture for the hydrogen molecule consists of one bonding  $\sigma$  MO, and a higher energy antibonding  $\sigma^*$  MO. When the molecule is in the ground state, both electrons are paired in the lower-energy bonding orbital – this is the Highest Occupied Molecular Orbital (HOMO). The antibonding  $\sigma^*$  orbital, in turn, is the Lowest Unoccupied Molecular Orbital (LUMO).



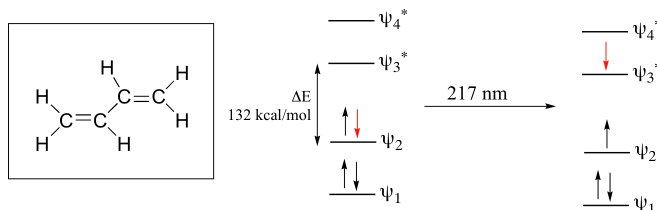
If the molecule is exposed to light of a wavelength with energy equal to  $\Delta E$ , the HOMO-LUMO energy gap, this wavelength will be absorbed and the energy used to bump one of the electrons from the HOMO to the LUMO – in other words, from the  $\sigma$  to the  $\sigma^*$  orbital. This is referred to as a  **$\sigma - \sigma^*$  transition**.  $\Delta E$  for this electronic transition is 258 kcal/mol, corresponding to light with a wavelength of 111 nm.

When a double-bonded molecule such as ethene (common name ethylene) absorbs light, it undergoes a  **$\pi - \pi^*$  transition**. Because  $\pi - \pi^*$  energy gaps are narrower than  $\sigma - \sigma^*$  gaps, ethene absorbs light at 165 nm - a longer wavelength than molecular hydrogen.



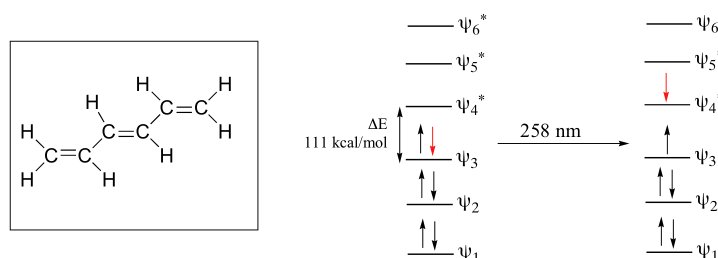
The electronic transitions of both molecular hydrogen and ethene are too energetic to be accurately recorded by standard UV spectrophotometers, which generally have a range of 220 – 700 nm. Where UV-vis spectroscopy becomes useful to most organic and biological chemists is in the study of molecules with conjugated  $\pi$  systems. In these groups, the energy gap for  $\pi - \pi^*$  transitions is smaller than for isolated double bonds, and thus the wavelength absorbed is longer. Molecules or parts of molecules that absorb light strongly in the UV-vis region are called **chromophores**.

Let's revisit the MO picture for 1,3-butadiene, the simplest conjugated system. Recall that we can draw a diagram showing the four  $\pi$  MO's that result from combining the four  $2p_z$  atomic orbitals. The lower two orbitals are bonding, while the upper two are antibonding.

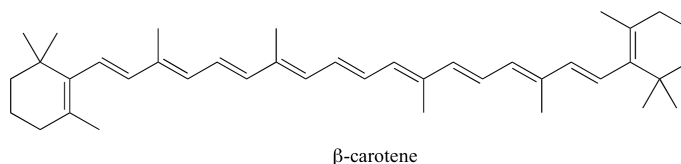


Comparing this MO picture to that of ethene, our isolated pi-bond example, we see that the HOMO-LUMO energy gap is indeed smaller for the conjugated system. 1,3-butadiene absorbs UV light with a wavelength of 217 nm.

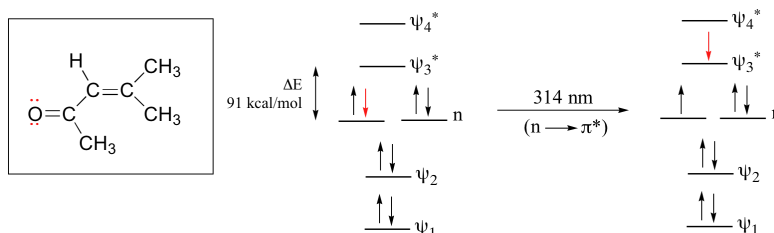
As conjugated pi systems become larger, the energy gap for a  $\pi - \pi^*$  transition becomes increasingly narrow, and the wavelength of light absorbed correspondingly becomes longer. The absorbance due to the  $\pi - \pi^*$  transition in 1,3,5-hexatriene, for example, occurs at 258 nm, corresponding to a  $\Delta E$  of 111 kcal/mol.



In molecules with extended pi systems, the HOMO-LUMO energy gap becomes so small that absorption occurs in the visible rather than the UV region of the electromagnetic spectrum. Beta-carotene, with its system of 11 conjugated double bonds, absorbs light with wavelengths in the blue region of the visible spectrum while allowing other visible wavelengths – mainly those in the red-yellow region - to be transmitted. This is why carrots are orange.



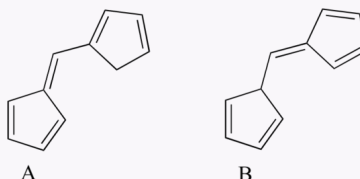
The conjugated pi system in 4-methyl-3-penten-2-one gives rise to a strong UV absorbance at 236 nm due to a  $\pi - \pi^*$  transition. However, this molecule also absorbs at 314 nm. This second absorbance is due to the transition of a non-bonding (lone pair) electron on the oxygen up to a  $\pi^*$  antibonding MO:



This is referred to as an  **$n - \pi^*$  transition**. The nonbonding (n) MO's are higher in energy than the highest bonding p orbitals, so the energy gap for an  $n \rightarrow \pi^*$  transition is smaller than that of a  $\pi - \pi^*$  transition – and thus the  $n - \pi^*$  peak is at a longer wavelength. In general,  $n - \pi^*$  transitions are weaker (less light absorbed) than those due to  $\pi - \pi^*$  transitions.

**Exercise 4.9:** What is the energy of the photons (in kJ/mol) of light with wavelength of 470 nm, the  $\lambda_{\text{max}}$  of b-carotene?

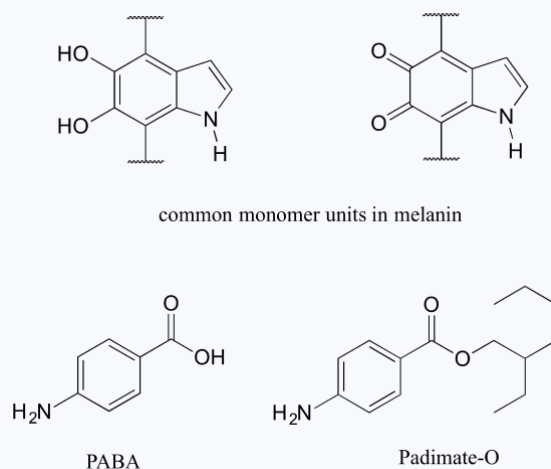
**Exercise 4.10:** Which of the following molecules would you expect absorb at a longer wavelength in the UV region of the electromagnetic spectrum? Explain your answer.



[Solutions](#)

## Protecting yourself from sunburn

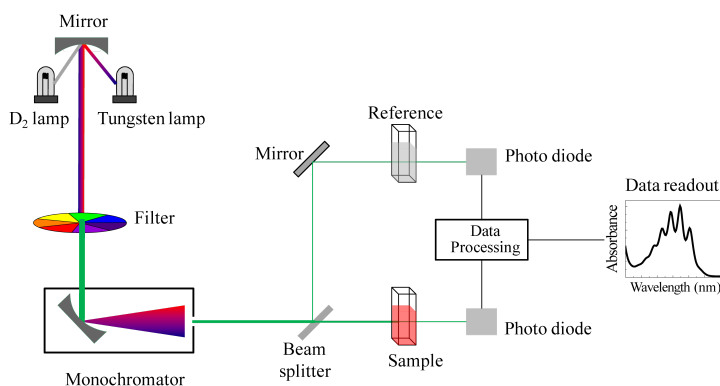
Human skin can be damaged by exposure to ultraviolet light from the sun. We naturally produce a pigment, called melanin, which protects the skin by absorbing much of the ultraviolet radiation. Melanin is a complex polymer, two of the most common monomer units of which are shown below.



Overexposure to the sun is still dangerous, because there is a limit to how much radiation our melanin can absorb. Most commercial sunscreens claim to offer additional protection from both UV-A and UV-B radiation: UV-A refers to wavelengths between 315-400 nm, UV-B to shorter, more harmful wavelengths between 280-315 nm. PABA (*para*-aminobenzoic acid) was used in sunscreens in the past, but its relatively high polarity meant that it was not very soluble in oily lotions, and it tended to rinse away when swimming. Many sunscreens today contain, among other active ingredients, a more hydrophobic derivative of PABA called Padimate O.

## Looking at UV-vis spectra

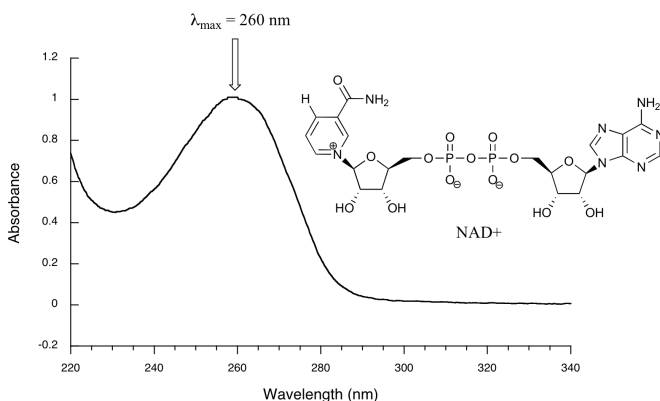
We have been talking in general terms about how molecules absorb UV and visible light – now let's look at some actual examples of data from a UV-vis absorbance spectrophotometer. The basic setup is the same as for IR spectroscopy: radiation with a range of wavelengths is directed through a sample of interest, and a detector records which wavelengths were absorbed and to what extent the absorption occurred.



## Schematic for a UV-Vis spectrophotometer

(Image from [Wikipedia Commons](#))

Below is the absorbance spectrum of an important biological molecule called nicotinamide adenine dinucleotide, abbreviated NAD<sup>+</sup>. This compound absorbs light in the UV range due to the presence of conjugated pi-bonding systems.



You'll notice that this UV spectrum is much simpler than the IR spectra we saw earlier: this one has only one peak, although many molecules have more than one. Notice also that the convention in UV-vis spectroscopy is to show the baseline at the bottom of the graph with the peaks pointing up. Wavelength values on the x-axis are generally measured in nanometers (nm) rather than in  $\text{cm}^{-1}$  as is the convention in IR spectroscopy.

Peaks in UV spectra tend to be quite broad, often spanning well over 20 nm at half-maximal height. Typically, there are two things that we look for and record from a UV-Vis spectrum. The first is  $\lambda_{\text{max}}$ , which is the wavelength at maximal light absorbance. As you can see, NAD<sup>+</sup> has  $\lambda_{\text{max}} = 260 \text{ nm}$ . We also want to record how much light is absorbed at  $\lambda_{\text{max}}$ . Here we use a unitless number called **absorbance**, abbreviated 'A'. This contains the same information as the 'percent transmittance' number used in IR spectroscopy, just expressed in slightly different terms. To calculate absorbance at a given wavelength, the computer in the spectrophotometer simply takes the intensity of light at that wavelength *before* it passes through the sample ( $I_0$ ), divides this value by the intensity of the same wavelength *after* it passes through the sample ( $I$ ), then takes the  $\log_{10}$  of that number:

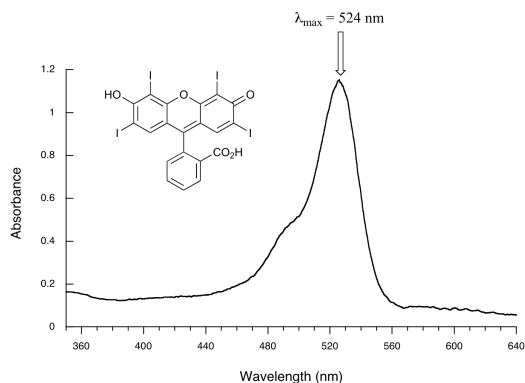
$$A = \log \frac{I_0}{I} \quad (4.3.1)$$

You can see that the absorbance value at 260 nm ( $A_{260}$ ) is about 1.0 in this spectrum.

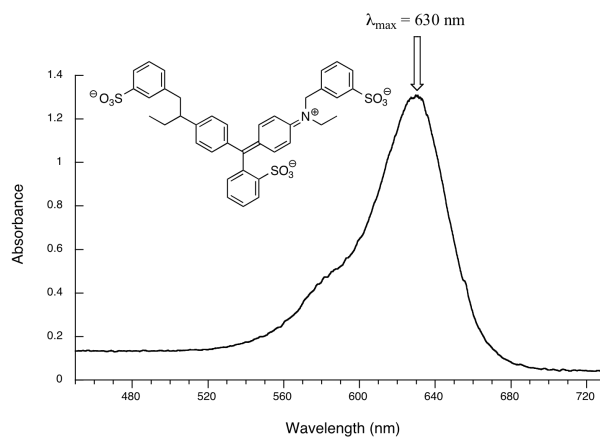
**Exercise 4.11:** Express  $A = 1.0$  in terms of percent transmittance (%T, the unit usually used in IR spectroscopy (and sometimes in UV-vis as well).

[Solutions](#)

Here is the absorbance spectrum of the common food coloring Red #3:



Here, we see that the extended system of conjugated pi bonds causes the molecule to absorb light in the visible range. Because the  $\lambda_{\text{max}}$  of 524 nm falls within the green region of the spectrum, the compound appears red to our eyes. Now, take a look at the spectrum of another food coloring, Blue #1:



Here, maximum absorbance is at 630 nm, in the orange range of the visible spectrum, and the compound appears blue.

### Applications of UV spectroscopy in organic and biological chemistry

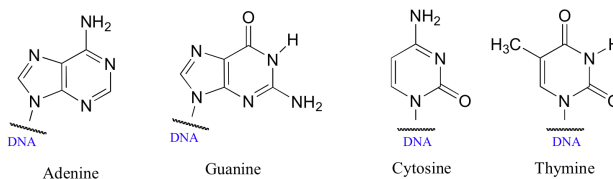
UV-vis spectroscopy has many different applications in organic and biological chemistry. One of the most basic of these applications is the use of the **Beer - Lambert Law** to determine the concentration of a chromophore. You most likely have performed a Beer – Lambert experiment in a previous chemistry lab. The law is simply an application of the observation that, within certain ranges, the absorbance of a chromophore at a given wavelength varies in a linear fashion with its concentration: the higher the concentration of the molecule, the greater its absorbance.

If we divide the observed value of  $A$  at  $\lambda_{\max}$  by the concentration of the sample ( $c$ , in mol/L), we obtain the **molar absorptivity**, or **extinction coefficient** ( $\epsilon$ ), which is a characteristic value for a given compound.

$$\epsilon = \frac{A}{c} \quad (4.3.2)$$

The absorbance will also depend, of course, on the **path length** - in other words, the distance that the beam of light travels through the sample. In most cases, sample holders are designed so that the path length is equal to 1 cm, so the units for molar absorptivity are  $L \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . If we look up the value of  $\epsilon$  for our compound at  $\lambda_{\max}$ , and we measure absorbance at this wavelength, we can easily calculate the concentration of our sample. As an example, for  $\text{NAD}^+$  the literature value of  $\epsilon$  at 260 nm is  $18,000 L \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . In our  $\text{NAD}^+$  spectrum we observed  $A_{260} = 1.0$ , so using equation 4.4 and solving for concentration we find that our sample is  $5.6 \times 10^{-5} \text{ M}$ .

The bases of DNA and RNA are good chromophores:



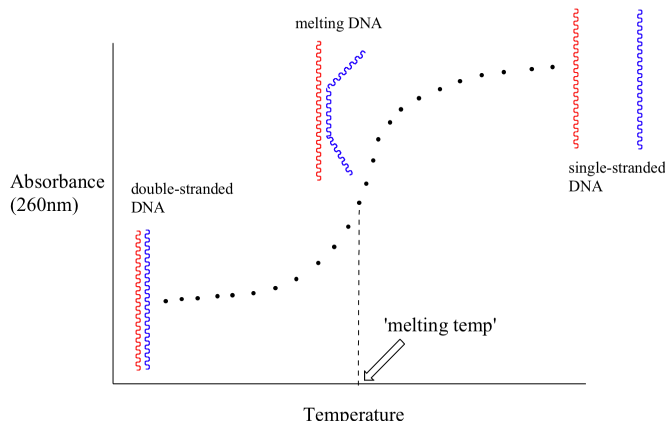
Biochemists and molecular biologists often determine the concentration of a DNA sample by assuming an average value of  $\epsilon = 0.020 \text{ ng}^{-1} \cdot \text{mL}$  for double-stranded DNA at its  $\lambda_{\max}$  of 260 nm (notice that concentration in this application is expressed in mass/volume rather than molarity: ng/mL is often a convenient unit for DNA concentration when doing molecular biology).

**Exercise 4.12:** 50 microliters of an aqueous sample of double stranded DNA is dissolved in 950 microliters of water. This diluted solution has a maximal absorbance of 0.326 at 260 nm. What is the concentration of the original (more concentrated) DNA sample, expressed in micrograms per microliter?

[Solutions](#)

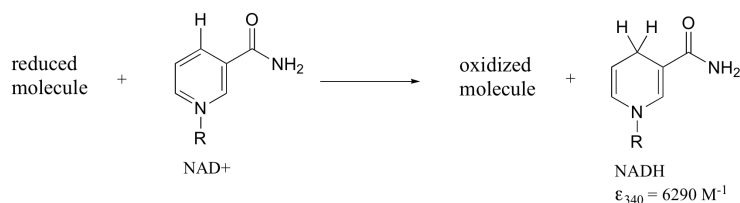
Because the extinction coefficient of double stranded DNA is slightly lower than that of single stranded DNA, we can use UV spectroscopy to monitor a process known as DNA melting. If a short stretch of double stranded DNA is gradually heated up, it will

begin to 'melt', or break apart, as the temperature increases (recall that two strands of DNA are held together by a specific pattern of hydrogen bonds formed by 'base-pairing').

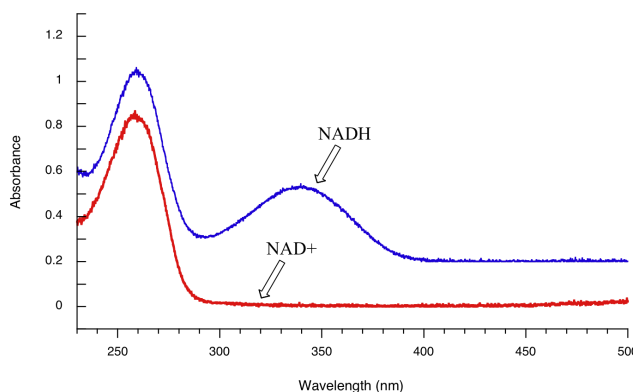


As melting proceeds, the absorbance value for the sample increases, eventually reaching a high plateau as all of the double-stranded DNA breaks apart, or 'melts'. The mid-point of this process, called the 'melting temperature', provides a good indication of how tightly the two strands of DNA are able to bind to each other.

Later we will see how the Beer - Lambert Law and UV spectroscopy provides us with a convenient way to follow the progress of many different enzymatic redox (oxidation-reduction) reactions. In biochemistry, oxidation of an organic molecule often occurs concurrently with reduction of nicotinamide adenine dinucleotide ( $\text{NAD}^+$ , the compound whose spectrum we saw earlier in this section) to  $\text{NADH}$ :

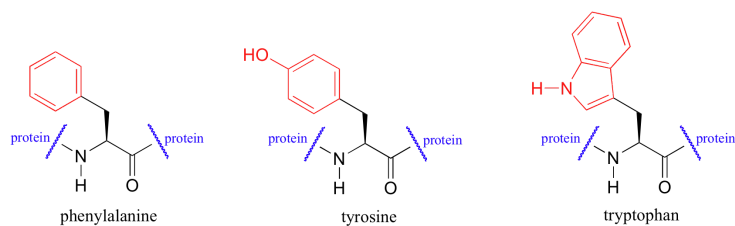


Both  $\text{NAD}^+$  and  $\text{NADH}$  absorb at 260 nm. However  $\text{NADH}$ , unlike  $\text{NAD}^+$ , has a second absorbance band with  $\lambda_{\text{max}} = 340 \text{ nm}$  and  $\epsilon = 6290 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . The figure below shows the spectra of both compounds superimposed, with the  $\text{NADH}$  spectrum offset slightly on the y-axis:



By monitoring the absorbance of a reaction mixture at 340 nm, we can 'watch'  $\text{NADH}$  being formed as the reaction proceeds, and calculate the rate of the reaction.

UV spectroscopy is also very useful in the study of proteins. Proteins absorb light in the UV range due to the presence of the aromatic amino acids tryptophan, phenylalanine, and tyrosine, all of which are chromophores.



Biochemists frequently use UV spectroscopy to study conformational changes in proteins - how they change shape in response to different conditions. When a protein undergoes a conformational shift (partial unfolding, for example), the resulting change in the environment around an aromatic amino acid chromophore can cause its UV spectrum to be altered.

## Kahn Academy video tutorials on UV-Vis spectroscopy

[Organic Chemistry With a Biological Emphasis](#) by [Tim Soderberg](#) (University of Minnesota, Morris)

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