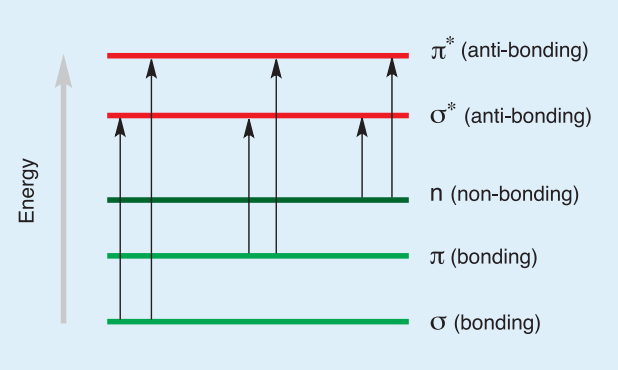
Introduction to Ultraviolet - Visible Spectroscopy[1] (UV)

Background Theory

Absorption of ultraviolet and visible radiation

Absorption of visible and ultraviolet (UV) radiation is associated with **excitation of electrons**, in both atoms and molecules, from lower to higher energy levels. Since the energy levels of matter are quantized, only light with the precise amount of energy can cause transitions from one level to another will be absorbed.

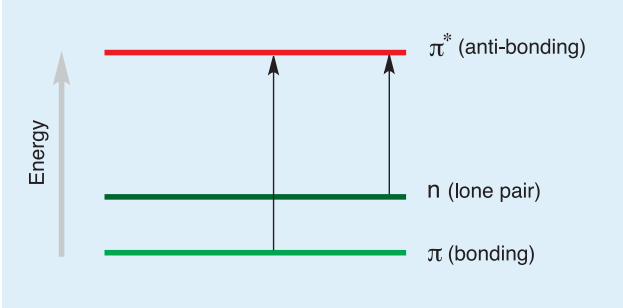
The possible electronic transitions that light might cause are[2]:



In each possible case, an electron is excited from a full (low energy, ground state) orbital into an empty (higher energy, excited state) anti-bonding orbital.[2] Each wavelength of light has a particular energy associated with it. If that particular amount of energy is just right for making one of these electronic transitions, then that wavelength will be absorbed.

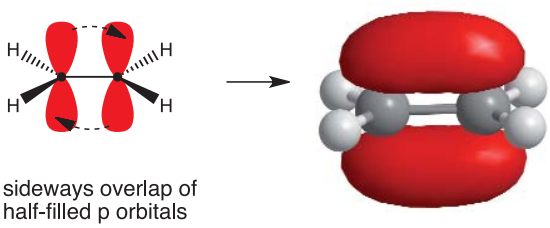
The larger the gap between the energy levels, the greater the energy required to promote the electron to the higher energy level; resulting in light of higher frequency, and therefore shorter wavelength, being absorbed.

All molecules will undergo electronic excitation following absorption of light, but for most molecules very high energy radiation (in the vacuum ultraviolet, <200 nm) is required. Consequently, absorption of light in the UV-visible region will only result in the following transitions:



Therefore in order to absorb light in the region from 200 - 800 nm (where spectra are measured), the molecule must contain either π bonds or atoms with non-bonding orbitals. A non-bonding orbital is a lone pair on, say, oxygen, nitrogen or a halogen.

π bonds are formed by sideways overlap of the half-filled p orbitals on the two carbon atoms of a double bond. The two red shapes shown in the diagram below for ethene are part of the same π bonding orbital. Both of the electrons are found in the resulting π bonding orbital in the ground state.



sideways overlap of half-filled p orbitals

1.Also known as UV-visible spectrophotometry
2.For further reading about bonding refer to http://www.chemguide.co.uk/analysis/uvvisiblemenu.html#top

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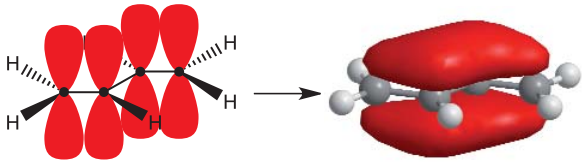
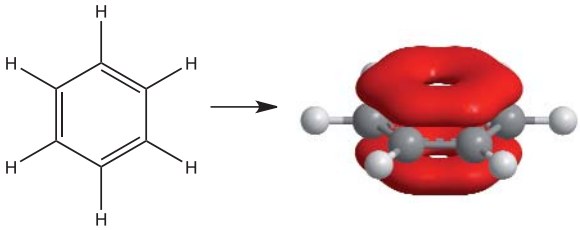
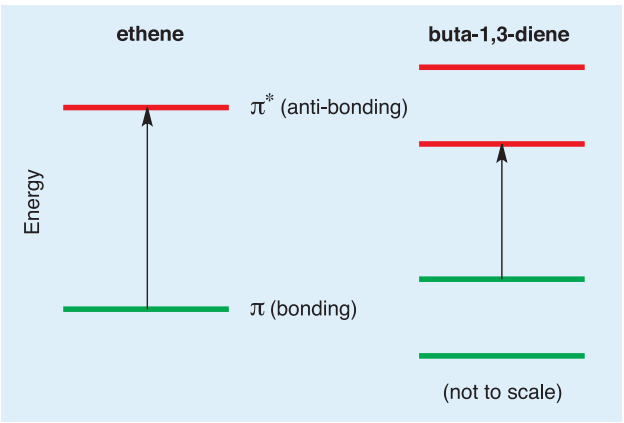
Molecules that contain **conjugated systems**, i.e. **alternating single and double bonds**, will have their electrons delocalised due to overlap of the p orbitals in the double bonds. This is illustrated below for buta-1,3-diene.



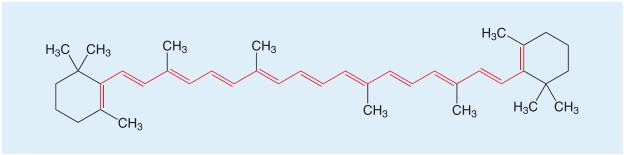
Benzene is a well-known example of a conjugated system. The Kekulé structure of benzene consists of alternating single double bonds and these give rise to the delocalised π system above and below the plane of the carbon – carbon single bonds.



As the amount of delocalisation in the molecule increases the energy gap between the π bonding orbitals and π anti-bonding orbitals gets smaller and therefore light of lower energy, and longer wavelength, is absorbed.



Although buta-1,3-diene absorbs light of a longer wavelength than ethene it is still absorbing in the UV region and hence both compounds are colourless. However, if the delocalisation is extended further the wavelength absorbed will eventually be long enough to be in the visible region of the spectrum, resulting in a highly coloured compound. A good example of this is the orange plant pigment, beta-carotene – which has 11 carbon-carbon double bonds conjugated together.

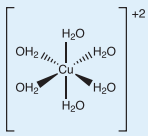


Beta-carotene absorbs throughout the UV region but particularly strongly in the visible region between 400 and 500 nm with a peak at 470 nm.

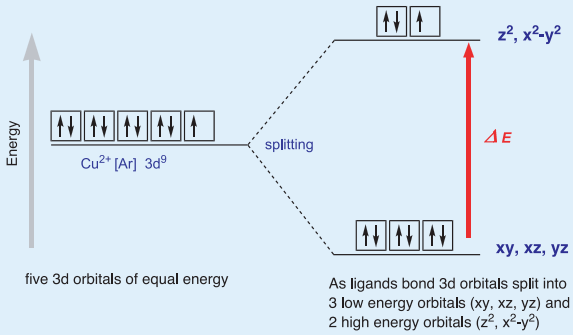
Groups in a molecule which consist of alternating single and double bonds (conjugation) and absorb visible light are known as **chromophores**.

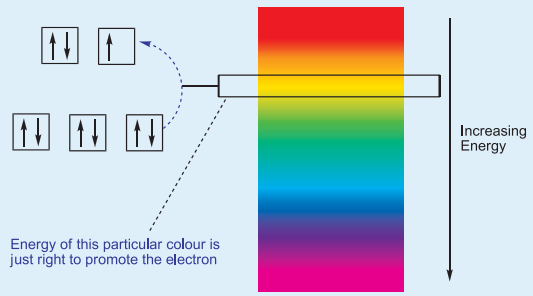
Transition metal complexes are also highly coloured, which is due to the splitting of the d orbitals when the ligands approach and bond to the central metal ion. Some of the d orbitals gain energy and some lose energy. The amount of splitting depends on the central metal ion and ligands.

The difference in energy between the new levels affects how much energy will be absorbed when an electron is promoted to a higher level. The amount of energy will govern the colour of light which will be absorbed.



For example, in the octahedral copper complex, $[Cu(H_2O)_6]^{2+}$, yellow light has sufficient energy to promote the d electron in the lower energy level to the higher one.





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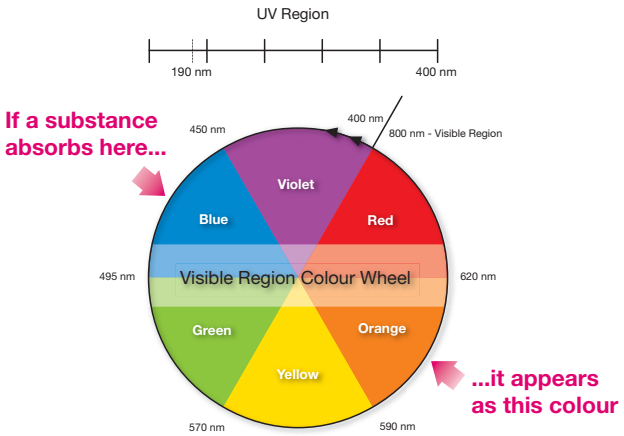
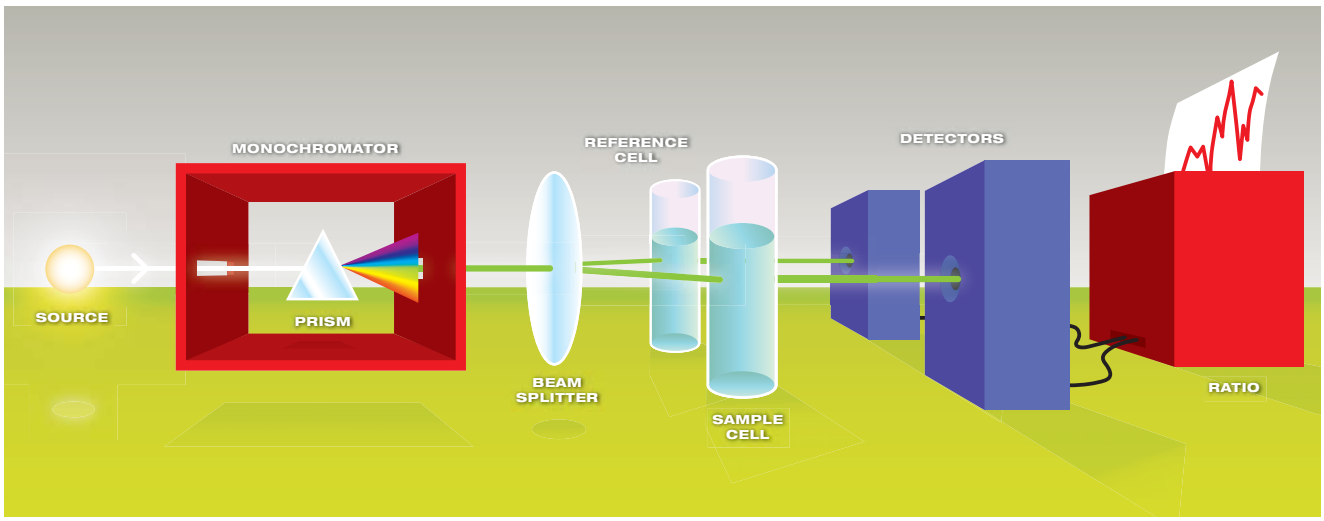


Red	620-750 nm
Orange	590-620 nm
Yellow	570-590 nm
Green	496-570 nm
Blue	450-495 nm
Violet	380-450 nm

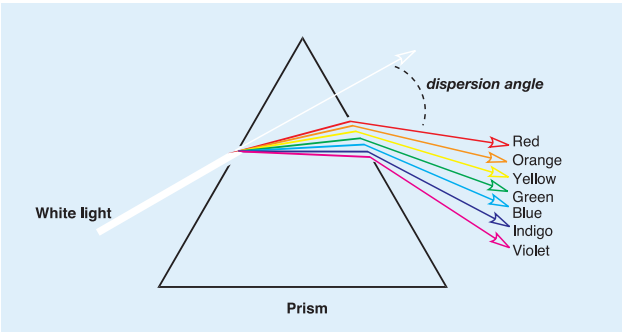
It is possible to predict which wavelengths are likely to be absorbed by a coloured substance. When white light passes through or is reflected by a coloured substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary colour to the wavelength(s) absorbed. This relationship is demonstrated by the colour wheel shown on the right. Complementary colours are diametrically opposite each other.

UV-Visible Spectrometer



UV-visible spectrometers can be used to measure the absorbance of ultra violet or visible light by a sample, either at a single wavelength or perform a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm. The technique can be used both quantitatively and qualitatively. A schematic diagram of a UV-visible spectrometer is shown above.

The light source (a combination of tungsten/halogen and deuterium lamps) provides the visible and near ultraviolet radiation covering the 200 – 800 nm. The output from the light source is focused onto the diffraction grating which splits the incoming light into its component colours of different wavelengths, like a prism (shown below) but more efficiently.



For liquids the sample is held in an optically flat, transparent container called a **cell** or **cuvette**. The reference cell or cuvette contains the solvent in which the sample is dissolved and this is commonly referred to as the **blank**.

For each wavelength the intensity of light passing through both a reference cell (I_o) and the sample cell (I) is measured. If I is less than I_o, then the sample has absorbed some of the light.

The absorbance (A) of the sample is related to I and I_o according to the following equation:

$$A = \log_{10} \frac{I_o}{I}$$

The detector converts the incoming light into a current, the higher the current the greater the intensity. The chart recorder usually plots the absorbance against wavelength (nm) in the UV and visible section of the electromagnetic spectrum. *(Note: absorbance does not have any units).*

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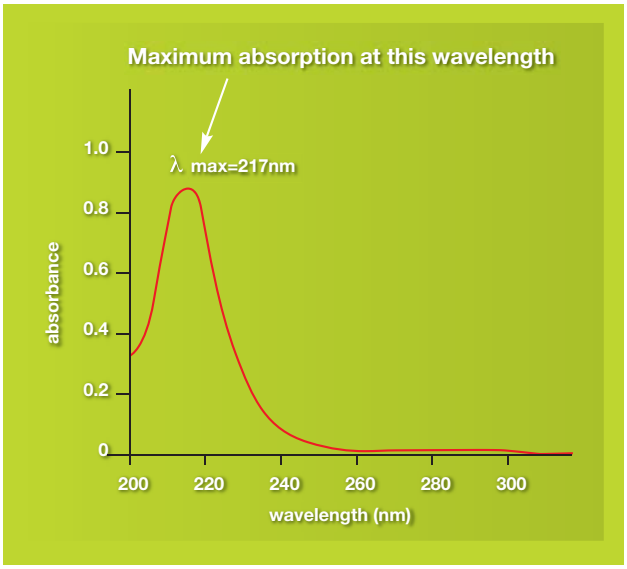
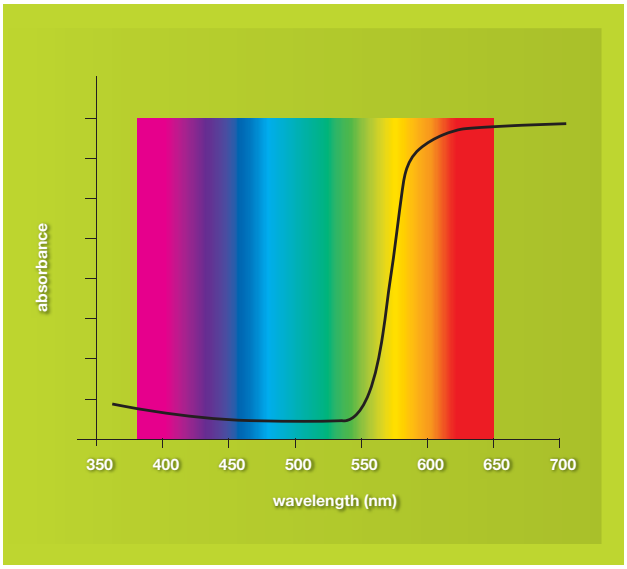
UV-Visible Spectrum

The diagram below shows a simple UV-visible absorption spectrum for buta-1,3-diene. Absorbance (on the vertical axis) is just a measure of the amount of light absorbed. One can readily see what wavelengths of light are absorbed (peaks), and what wavelengths of light are transmitted (troughs). The higher the value, the more of a particular wavelength is being absorbed.



The absorption peak at a value of 217 nm, is in the ultra-violet region, and so there would be no visible sign of any light being absorbed making buta-1,3-diene colourless. The wavelength that corresponds to the highest absorption is usually referred to as "**lambda-max**" (λmax).

The spectrum for the blue copper complex shows that the complementary yellow light is absorbed.



The Beer-Lambert Law

According to the Beer-Lambert Law the **absorbance** is **proportional** to the **concentration** of the substance in solution and as a result UV-visible spectroscopy can also be used to measure the concentration of a sample.

The Beer-Lambert Law can be expressed in the form of the following equation:

$$A = \varepsilon c l$$

Where

A = absorbance

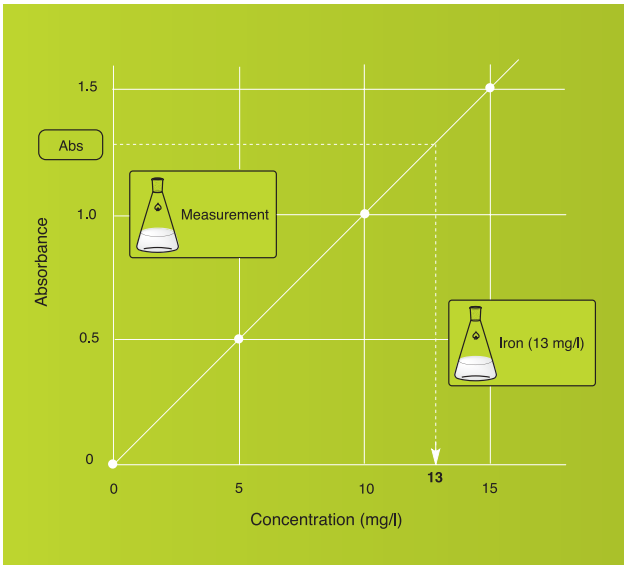
l = optical path length, i.e. dimension of the cell or cuvette (cm)

c = concentration of solution (mol dm^{-3})

ε = molar extinction, which is constant for a particular substance at a particular wavelength (dm^3 mol^{-1} cm^{-1})

If the absorbance of a series of sample solutions of known concentrations are measured and plotted against their corresponding concentrations, the plot of **absorbance versus concentration** should be linear if the Beer-Lambert Law is obeyed. This graph is known as a **calibration graph**.

A calibration graph can be used to determine the concentration of unknown sample solution by measuring its absorbance, as illustrated below.



Since the absorbance for dilute solutions is directly proportional to concentration another very useful application for UV-visible spectroscopy is studying reaction kinetics. The rate of change in concentration of reactants or products can be determined by measuring the increase or decrease of absorbance of coloured solutions with time. Plotting absorbance against time one can determine the orders with respect to the reactants and hence the rate equation from which a mechanism for the reaction can be proposed.

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Modern Applications of UV Spectroscopy

UV-visible spectroscopy is a technique that readily allows one to determine the concentrations of substances and therefore enables scientists to study the rates of reactions, and determine rate equations for reactions, from which a mechanism can be proposed. As such UV spectroscopy is used extensively in teaching, research and analytical laboratories for the quantitative analysis of all molecules that absorb ultraviolet and visible electromagnetic radiation.

Other applications include the following:

- In clinical chemistry UV-visible spectroscopy is used extensively in the study of enzyme kinetics.
  Enzymes cannot be studied directly but their activity can be studied by analysing the speed of the reactions which they catalyse. Reagents or labels can also be attached to molecules to permit indirect detection and measurement of enzyme activity. The widest use in the field of clinical diagnostics is as an indicator of tissue damage.
  When cells are damaged by disease, enzymes leak into the bloodstream and the amount present indicates the severity of the tissue damage. The relative proportions of different enzymes can be used to diagnose disease, say of the liver, pancreas or other organs which otherwise exhibit similar symptoms.
- UV-visible spectroscopy is used for dissolution testing of tablets and products in the pharmaceutical industry. Dissolution is a characterisation test commonly used by the pharmaceutical industry to guide formulation design and control product quality. It is also the only test that measures the rate of *in-vitro* drug release as a function of time, which can reflect either reproducibility of the product manufacturing process or, in limited cases, *in-vivo* drug release.
- In the biochemical and genetic fields UV-visible spectroscopy is used in the quantification of DNA and protein/enzyme activity as well as the thermal denaturation of DNA.
- In the dye, ink and paint industries UV-visible spectroscopy is used in the quality control in the development and production of dyeing reagents, inks and paints and the analysis of intermediate dyeing reagents.
- In environmental and agricultural fields the quantification of organic materials and heavy metals in fresh water can be carried out using UV-visible spectroscopy.

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