VISIBLE SPECTROSCOPY

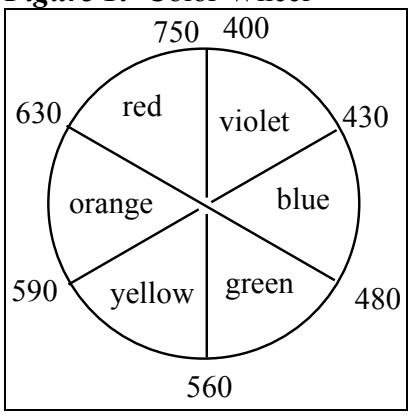
Visible spectroscopy is the study of the interaction of radiation from the visible part (λ = 380 - 720 nm) of the electromagnetic spectrum with a chemical species. Quantifying the interaction of visible light with a chemical sample allows for the determination of an unknown solution concentration, the monitoring of reaction progress as a function of time, and many other quantitative uses.

Understanding visible spectroscopy requires understanding visible light. Light travels in packets of energy called photons. Each photon has a specific energy related to a certain frequency or wavelength ($E = h\nu = hc/\lambda$). Visible light consists of wavelengths ranging from 380 nm (blue violet) to 720 nm (red). When all wavelengths of visible light are present, the light appears "white" to our eyes. If any wavelength is removed (absorbed), we perceive the remaining combination of wavelengths of light as the "complimentary" color (*Table 1, Figure 1*).

Table 1: Absorbed & Perceived Colors

Absorbed Wavelength (nm)	**Absorbed Color**	**Perceived (Transmitted) Color**
400	violet	green - yellow
450	indigo	yellow
480	blue	orange
490	blue-green	red
530	green	purple
570	yellow-green	dark blue
600	orange	blue
650	red	green

Figure 1: Color Wheel



Colored compounds are colored because of the absorption of visible radiation. The color is a result of the compound absorbing a certain color of light, leading to the perception of the compound being the complimentary color. For example, if white light passes through a test tube containing a solution of copper (II) sulfate ($CuSO_4$), the solution will be blue because the Cu^{2+} ions strongly absorb “orange” photons of light (photons of light with $\lambda \sim 600$ nm).

When a photon of colored light is absorbed by a compound an electron transitions from lower energy orbital to higher energy orbital. The energy of absorbed radiation is equal to the energy difference between the highest energy electronic occupied orbital (OO) and the closest unoccupied orbital (UO). Many transition metal complexes and large conjugated organic molecules are brightly colored because this energy difference is equal to an energy within the visible region of the electromagnetic spectrum. Mathematically, this relationship is expressed by *equation 1*:

(1) $E_{light} = h\nu_{light} = hc/\lambda_{light} = \Delta E = E_{UO} - E_{OO}$

Before the absorption of a photon, the electrons within the compound are in the lowest energy orbitals possible. Such an electron configuration is called the **ground state**. When a photon of visible radiation is absorbed by a compound an electron is promoted from an occupied orbital to an unoccupied orbital. The result is a higher energy compound in an **excited state**. (*Figure 2*)

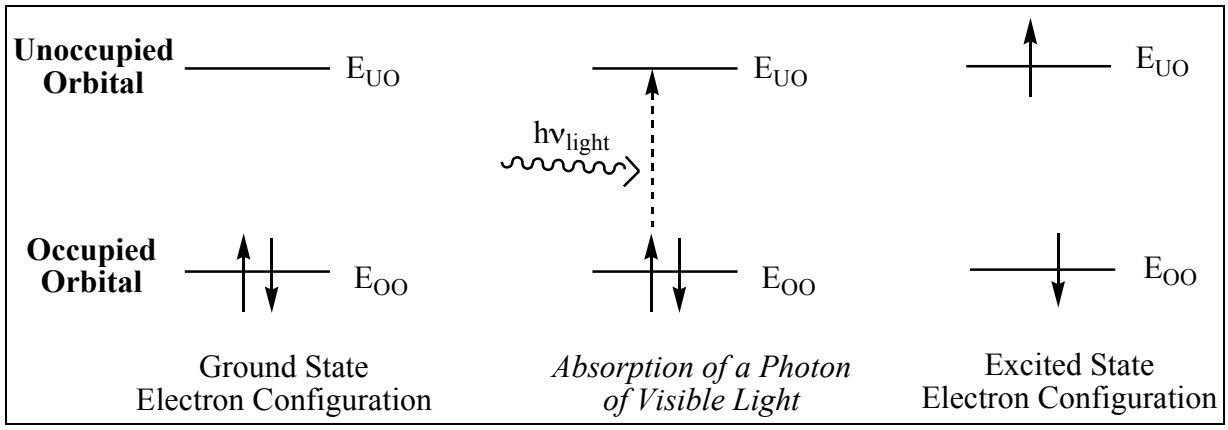


Figure 2. Absorption of light resulting in the excitation of an electron

The wavelength (i.e., frequency, energy, or color) of light required to promote an electron from the ground to the excited state is specific to each chemical, just as the energy difference between E_{OO} and E_{UO} is dependent on chemical identity.

Most methods of measuring absorbance required that the compound be in the liquid form or dissolved in a liquid solution. Once in solution, the amount of a particular energy of light passing (transmitted) through that solution is quantified as **transmittance**. Transmittance is

calculated by taking the ratio of the intensity (amount) of light *leaving* the chemical sample (I) to the intensity (amount) of light *entering* the chemical sample (I_o). (*Figure 3*)

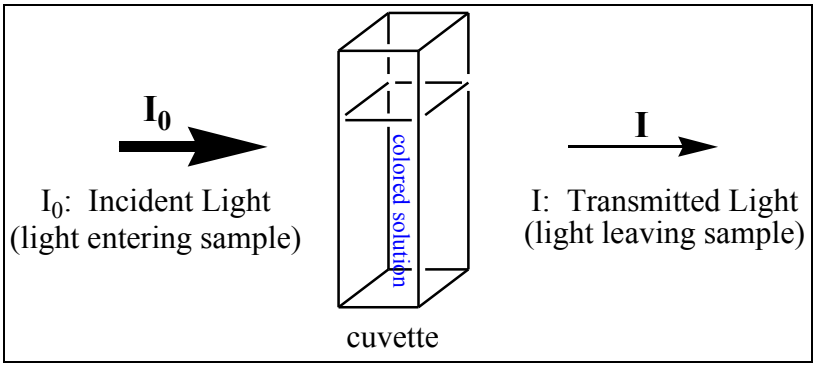


Figure 3. Transmittance ($T = I/I_0$) of Light by a Sample

Absorbance (A) (the amount of light absorbed by the chemical sample) is calculated from the transmittance (2):

(2) $A = \log(1/T) = -\log T$ or $A = 2.000 - \log \%T$

Absorbances are measured by an instrument called a spectrophotometer or **spectrometer**. A spectrometer contains a light source, focusing lenses, a diffraction grating or prism to split light into different wavelengths, a sample holder or "cell", a photosensitive detector which measures the light passing through the sample, an amplifier, and an output device such as a meter or recorder.

The solution to be analyzed is poured into a special vessel called a **cuvette**. A cuvette is a rectangular box with two opposing clear walls. Typically, two cuvettes are used when measuring a sample. One contains the **blank** (typically, the solvent only) and the other contains the sample dissolved in the same solvent.

To prevent spilling and insure a good measurement, fill a cuvette with solution to about 75% of its total volume. Wipe the outside of a cuvette with a tissue before placing it in the sample holder. Beads of water, fingerprints, or bubbles in the solution interfere with transmission of light through the sample. Gently insert a cuvette into the sample holder so that the light passes

through its opposing clear smooth walls (take care not to spill solutions into the spectrometer). Replace scratched cuvettes.

Absorption Spectrum

The absorption spectrum is a plot of the absorbance of a sample as a function of wavelength. This plot can be used to help identify an unknown sample since some compounds have characteristic absorption spectra. Figure 6 shows the absorbance spectrum in the visible region for a complex metal ion. Maximum absorption for this ion occurs at a wavelength of approx. 560 nm.

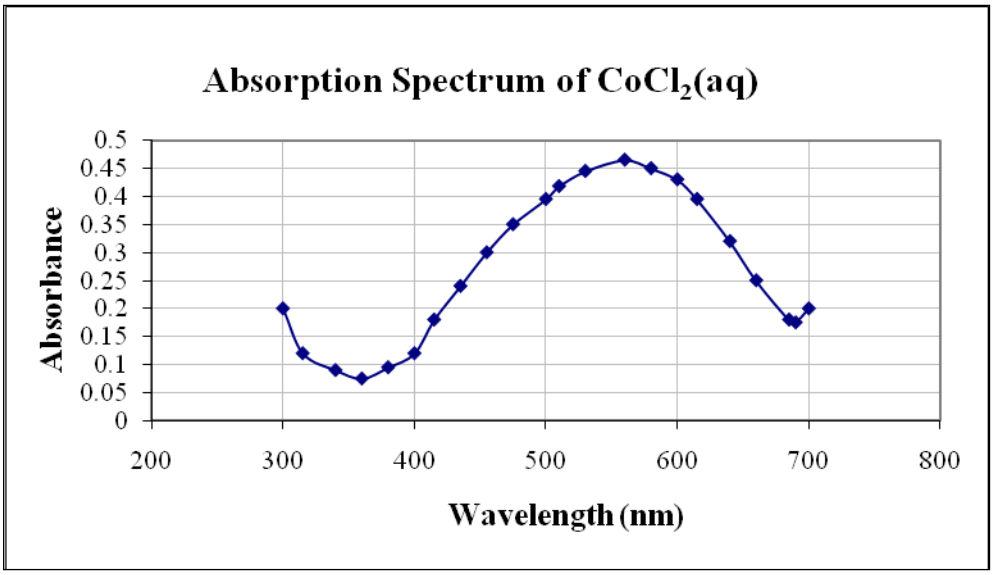


Figure 6: A Plot of Absorbance vs. Wavelength for a Metal Ion Complex

Beer's Law Plot

For dilute solutions, the amount of light absorbed at a specific wavelength is directly proportional to the concentration of the solution. This relationship is called Beer's Law (3).

(3) $A = \varepsilon C \ell$

A = absorbance (no units)

ε = molar absorptivity coefficient (units = L/mol-cm)

C = concentration of absorbing species (units = mol/L)

ℓ = path length (units = cm)

A Beer's Law Plot is a **calibration curve** of absorption plotted as a function of concentration. An absorption spectrum must be acquired first to determine the wavelength of maximum absorbance, λ_{max}, for the compound being studied. All absorbances are acquired at this wavelength setting because the signal is the strongest and least likely to be obscured by instrument fluctuations. To create the plot, the absorbances of at least three solutions of known concentration are measured. A graph of absorbance versus concentration is constructed and a best fit straight line is drawn through the data points. Then the absorbance of a solution of unknown concentration is measured and its concentration is determined by comparison to the Beer's Law Plot. The "Plot" must be used in two ways to determine the unknown's concentration. The first way is visual: a horizontal line is drawn from the value of the experimentally found absorbance on the y-axis to the calibration curve; a vertical line is then drawn to the x-axis to determine the value of the independent variable (the unknown solution concentration). The second way is mathematical, utilizing the line equation for the calibration curve. This equation is easily generated by a graphing program (See "Graphing Techniques" for instructions). Plug in the experimentally found value for y and solve for x to find the solution concentration. Both methods must be utilized.

Example: Absorbance readings are taken for seven standard cobalt (II) chloride solutions and a Beer's Law Plot is created with the equation: $y = 4.8571x + 0.0038$. A cobalt (II) chloride solution of unknown concentration is found to have an absorbance of 0.28. The unknown concentration can be calculated by setting $y = 0.28$ and solving for x. Also, by a visual comparison to the plot (*Figure 7*), the concentration of the unknown is 0.057 mol/L.

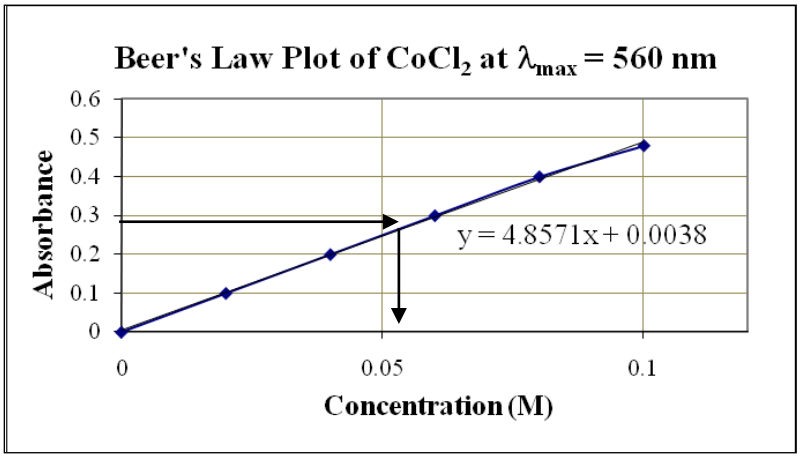


Figure 7: A Beer's Law Plot for a $CoCl_2$(aq) at 560 nm.

Notice that the best-fit line in the plot appears to pass through the origin; that is, absorbance equals zero when concentration equals zero, as expected. (As shown, the y-intercept is very close to zero, 0.0038. What experimental error might have resulted from the deviation?) However, Beer's Law is linear only for very dilute solutions and may deviate from linearity at higher concentrations. The range of dilution for a particular compound must be determined by experiment. (For some compounds the range may be 0.01-0.1 M, for others it may be closer to 10^{-5} to 10^{-6} M.)

Interferences

Because absorption is dependent on concentration, identity of the absorbing species, and path length, experiments need to be conducted carefully so that there is no error caused by changes in the path length of light during the experiment or interferences caused by other absorbing species in the solution.

The path length of light can be held constant if the same cuvette or identical cuvettes are used for each measurement. The cuvettes provided to you will have standard, reproducible diameters and volumes.

The solvent, impurities in the solvent, and/or the cuvette glass can absorb light in the selected region. These interferences can be corrected or eliminated by calibrating the spectrometer with the blank in the sample compartment. In effect, this act instructs the instrument to ignore any absorbance from materials in the glass or solvent and to detect only the absorbance from the particular species to be measured in the sample. (A similar analogy is the taring of a balance.)

Review Questions:

- Define: absorbance, transmittance, blank, path length, Beer's Law, cuvette.
- If a solution is blue-colored, what wavelength of light (in nm) is being absorbed?
- What is absorbance when percent transmittance is equal to 58%?

- When absorbance is infinite, what is %T?
- If fingerprints are left on the cuvette, how does it affect A and %T?
- A student rinses a cuvette with DI water and then pours his solution into the wet cuvette. How does this affect A and %T?
- How does a student correct for an absorbing impurity present in the solvent?
- How does the student correct for any stray light that might enter the sample holder?
- What is the purpose of the mark on the edge of the sample holder?
- What is being measured in an "absorption spectra"?
- What is being measured in a Beer's Law Plot? What two factors are held constant when constructing the plot?
- Why is the wavelength of light set at maximum absorbance when making a Beer's Law Plot? Does the wavelength change while doing the experiment?
- Which solution is expected to deviate from the straight line in a Beer's Law plot: the more concentrated or more dilute solution?
- Is the straight line of a Beer's Law plot expected to pass through the origin of the graph or at some point above the origin?