**Appendix A3: An Introduction to Ultraviolet-Visible (UV-VIS) Spectroscopy**
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**Electromagnetic radiation** is made up of packets of energy called **photons**. A photon is a particle of electromagnetic radiation having zero mass and energy proportional to its frequency of radiation. This radiation, ranging from the high frequency gamma rays to the low frequency radio waves comprises the **electromagnetic spectrum** (**Figure 1**). The human eye can only see a tiny portion of this spectrum, in the range from 700-400 nm.

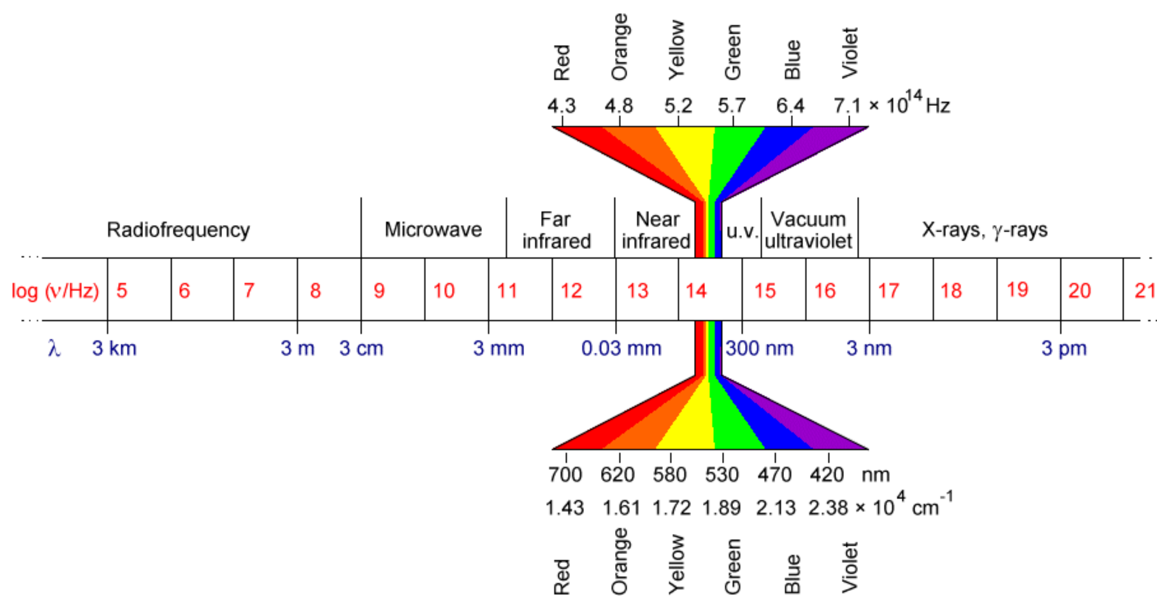


**Figure 1: The electromagnetic spectrum. The region detected by the human eye covers the range between 700-400 nm. Other regions can be perceived by our senses or detected by means of analytical instrumentation.**

Interaction of electromagnetic radiation with matter may induce a redirection of the radiation or transitions between **quantized energy levels** in atoms or molecules. This redirection, called **scattering**, occurs when radiation propagates between different transmission media, e.g. air-glass. It may or may not occur with the transfer of energy, i.e., the scattered radiation may have the same or a slightly different wavelength. A transition from a lower to a higher energy level involves an energy transfer from the radiation source to the atom or molecule. This phenomenon is called **absorption** of radiation. The release of a photon to produce a transition from a higher level to a lower level is called emission.

Ultraviolet-Visible Spectroscopy comprises a series of analytical methods in which absorption, emission, scattering, fluorescence, phosphorescence, or chemiluminescence are used to study electronic transitions in atoms and molecules in the 190-800 nm region of the electromagnetic spectrum. These studies can be used to obtain either qualitative or quantitative information about a sample. For the purpose of this course, we will focus our attention to **Molecular UV-VIS Absorption Spectroscopy** for both qualitative and quantitative analysis in aqueous solutions.

The process in which the energy of the radiation emitted by a source matches the energy required to promote a transition from a lower to a higher energy level in an atom or molecule is known as **resonance**. UV-VIS Spectroscopy involves the absorption of resonant UV-VIS radiation by atoms or molecules to promote electronic transitions from their ground state to quantum excited states.

Several instruments for measuring the absorption of ultraviolet and visible radiation are commercially available (double beam, single beam, diode array, etc.). In this manual, we will focus our attention to the basic components of single beam spectrophotometers. Typically, they consist of a radiation source, a wavelength selector, a sample container, a radiation detector, a signal processor and a readout device (**Figure 2**).

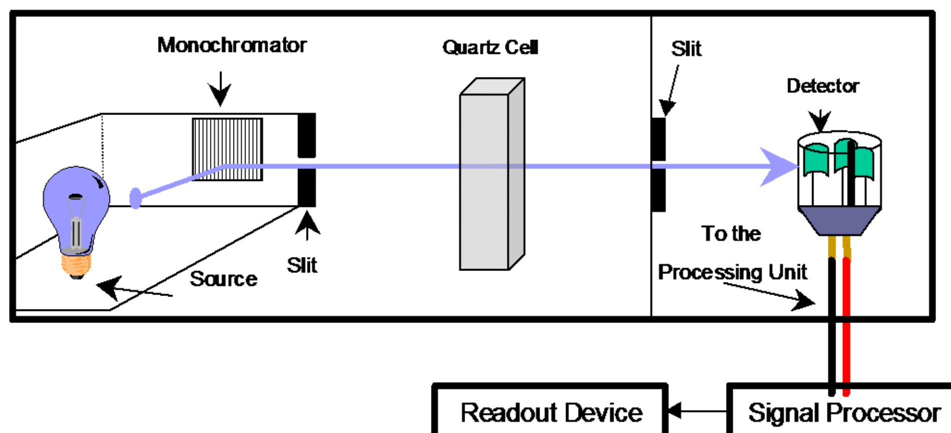


**Figure 2: Basic components of an UV-VIS spectrophotometer: radiation source, wavelength selector (monochromator), sample container (Quartz Cell), radiation detector, signal processor and readout device.**

In molecular absorption measurements, a continuous **light source**, whose power does not change sharply over a considerable range of wavelengths, is required. The light source is usually a deuterium or xenon lamp, for UV measurements, and a tungsten lamp for visible measurements. The wavelengths of these “continuous” light sources are selected with a wavelength separator such as a prism or a **grating monochromator**. The latter consists of a hard, optically flat, polished surface upon which a large number of closely spaced grooves are ruled.

Samples are placed in **cuvettes (cells)** that hold them in the light path of the source. Since the energy of UV-VIS radiation is relatively high, **the sample component is placed after all of the other optical components of the instrument to prevent possible photodecomposition of the sample**. The cuvette and the solvent used for sample preparation must be **transparent** (non-absorbing) in the spectral region of interest. Typically, **quartz cells** are **used for** studies **in the UV-VIS region**. Less expensive **glass and plastic cells can only be employed in the visible region**. The precision of the analysis is critically dependent upon the proper selection of the cells. UV-VIS cells must be handled with care. Fingerprints, grease or other deposits on the walls markedly alter the transmission characteristics of a cell. **Therefore, to obtain accurate spectrophotometric data, it is imperative to use clean cells.**

**CLEANING AND HANDLING OF UV CELLS/CUVETTES:**

**To clean and handle the cells properly, follow these guidelines:**

a. **Clean the cell with a mild cleaning agent, as soon as possible, after each use.** Always start with distilled water for aqueous solutions, or use a suitable organic solvent for organic materials. Mild detergents, such as Trace-Klean™, may be used only if they are true solutions and do not contain particulate matter. For hard-to-remove deposits, a solution of 0.1 N HCl may be used followed by distilled water. Remember that non-spectrograde reagents may leave deposits on the cell window after evaporation.
b. **A final rinse with distilled water should be made prior to drying.**
c. **Never touch the transparent walls of the cells, use the opaque faces instead.**
d. **Whenever possible, rinse the cell with the sample solution before filling and measuring.**

When light is absorbed by a sample, the ***radiant power* (P°)** of the source beam decreases (**Figure 3**).

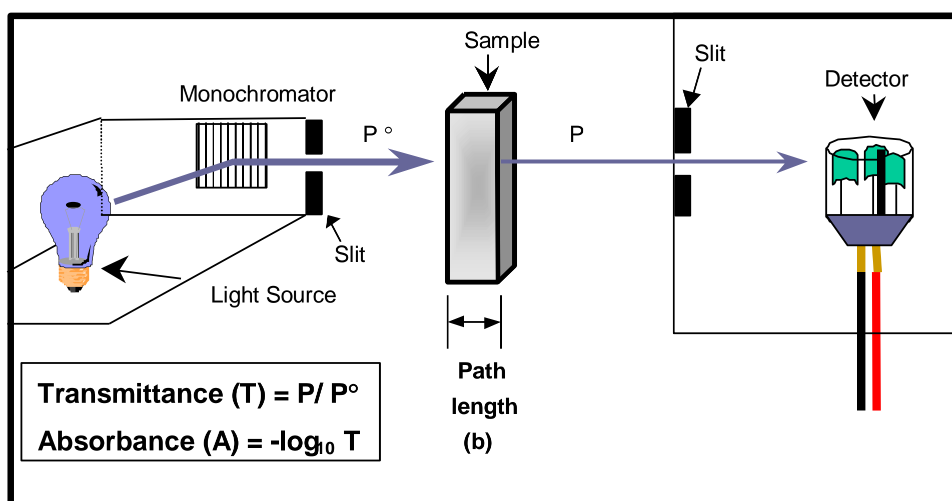


**Figure 3: Schematic representation of the attenuation of UV-VIS radiation by a sample containing an absorbing solute of C concentration and a path length of b cm. The ratio of the incident and transmitted light is termed as the transmittance (T). The absorbance is defined as the negative logarithm of T.**

The radiant power is the transmitted energy per unit area in one second. The decrease in radiant power occurs due to the absorption of resonant radiation by sample components. Light scattering and reflection also contribute to the decrease in radiant energy incident onto the sample. When radiant energy reaches the sample, a fraction of the molecules absorbs the radiation that exactly matches the energy of an electronic transition. A direct consequence of this process is the attenuation of the incident radiation from P° to P. The transmitted radiation is defined as the **Transmittance (T):**

$$\mathbf{T = \frac{P}{P^{\circ}}} \qquad \textbf{(1)}$$

where: P° = radiant power; P = transmitted radiation

Another useful expression for the reduction in radiant energy is the **absorbance (A)**:

$$\mathbf{A = -\log_{10} T} \qquad \textbf{(2)}$$

The absorbance is directly related to concentration by the **Beer's-Lambert Law:**

$$\mathbf{A = \varepsilon bc} \qquad \textbf{(3)}$$

where: $\varepsilon$ = is a constant called the **absorptivity coefficient**
**b** = the **path length** of the cell
**C** = is the concentration of the absorbing species.

Absorbance is directly proportional to the path length (**b**) in cm, and the concentration (**C**) of the absorbing species. When the concentration of the sample is expressed as moles per liter, the absorptivity coefficient ($\varepsilon$) is known as the **molar absorptivity coefficient**. The molar absorptivity coefficient is a constant, characteristic of the chemical nature of the absorbing substance, and has units of $M^{-1}cm^{-1}$.

Beer's-Lambert Law also applies when the sample medium contains more than one absorbing substance. Provided there is no interaction among components, the total absorbance of the system is given by:

$$\mathbf{A_{total} = \Sigma A_I = \Sigma \varepsilon_i b C_I} \qquad \textbf{(4)}$$

Beer's-Lambert Law successfully describes the absorption behavior of samples containing relatively low analyte concentrations. Usually, at concentrations greater than 0.01 M, serious deviations occur due to an increase in solute-solute interactions.

The absorption of ultraviolet and visible radiation by atomic or molecular species involves the excitation of electronic states. The electrons that contribute to the absorption of UV-VIS radiation in an organic molecule are those that directly participate in the formation of a bond, and the nonbonding or unshared electrons typically present in highly electronegative atoms (e.g. oxygen). The lifetime of this excitation is relatively brief ($10^{-8}$-$10^{-9}$ s). It is terminated when the absorbed energy is released from the excited molecule to the solvent or to other molecules or atoms. This phenomenon is known as relaxation. The most common type of relaxation involves the conversion of excitation energy into heat.

Since absorption of UV-VIS radiation is related to the excitation of bonding electrons, the wavelength of the absorption peaks can be correlated to the types of bonds present on the species under study. Therefore, UV-VIS spectroscopy is a valuable tool for the qualitative and quantitative identification of compounds containing absorbing groups.

All organic compounds are capable of absorbing UV-VIS radiation. Most of the absorption takes place via σ, π, and n electrons. As shown on **Figure 4**, four types of transitions are possible: σ→σ*, n→σ*, n→π*, π→π*.

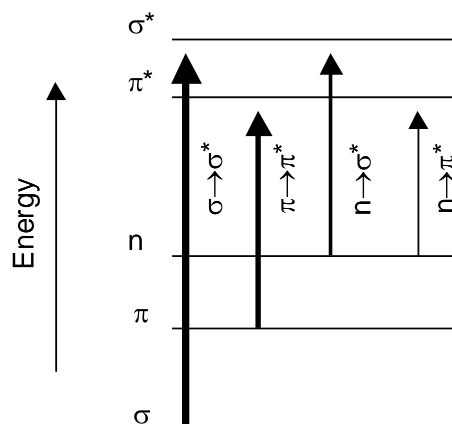


**Figure 4: Permitted transitions in electronic molecular orbitals. Four types of transitions are possible: σ→σ*, n→σ*, n→π*, π→π*.**

> **σ→σ* Transition**: Involves the transition of an electron from a bonding σ orbital to the corresponding antibonding σ* orbital. The energy required is larger than for any other transition; it exhibits an absorption maximum around 125 nm.
> **n→σ* Transition**: Occurs in saturated compounds with unshared electron pairs. It requires less energy than the σ →σ* and occurs in the region of 150-250 nm. This transition tends to shift to shorter wavelengths when the sample is prepared in a polar solvent.
> **n→π* and π→π* Transitions:** Mostly responsible for all the absorption spectroscopy of organic compounds. Lower in energy, these absorption peaks occurs in the region of 200-700 nm. These transitions require the presence of unsaturated functional groups, called **chromophores**, which induce absorption of UV-VIS radiation at longer wavelengths. Another series of functional groups, called **auxochromes**, do not absorb UV-VIS radiation, but their presence in a molecule containing a chromophore induces a shifting of its peaks to longer wavelengths, as well as an increase in their intensity.

The transmitted light, or that, which is not absorbed by the molecules, reaches the detector of the spectrophotometer. Several types of detectors (phototubes, photomultipliers and diode arrays detectors)

are commercially available for UV-VIS spectrophotometers. The most commonly used is the **photomultiplier tube (PMT)** (Figure 5 and Figure 6).

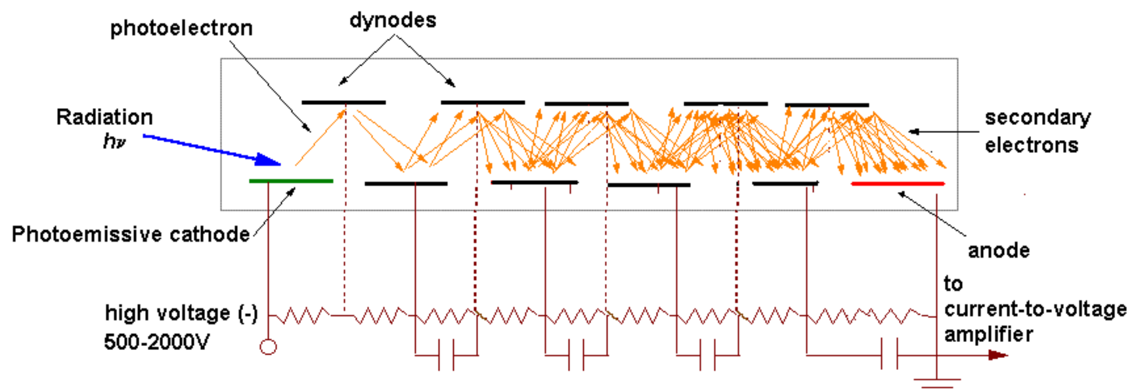


**Figure 5: Schematic representation of a photomultiplier tube.**

**Figure 6: Picture of two types of photomultiplier tubes.**

A **photomultiplier tube** (Figure 6), consists of a vacuum-sealed photocathode that emits electrons when exposed to UV-VIS radiation. The tube also contains a series of nine additional electrodes called dynodes. These electrodes are located in a sequential array, each of them at a potential ≈90 V more positive than the previous one. As a consequence, when a photon reaches the cathode the emitted electrons are accelerated toward the next dynode inducing an additional emission of about $10^6$-$10^7$ electrons. This cascade of electrons is finally collected at the anode of the photomultiplier. The resulting current is electronically amplified and processed as an absorption spectrum, which is a plot of the amount of light absorbed as a function of wavelength. Photomultiplier tubes are highly sensitive and have extremely fast response times. They are limited to measurements of low power radiation, and so care should be taken to prevent damage to the photoelectric surface.

Absorption spectroscopy is one of the most useful tools for quantitative analysis due to its wide applicability, relatively high sensitivity and moderate to high selectivity. In a spectrophotometric analysis, the analyst must select the experimental conditions for the preparation of a calibration curve, based on the Beer-Lambert's relationship. The optimum wavelength ($\lambda_{max}$) for the analysis is that at which a maximum is observed for the samples absorbance. The sensitivity of the determination, based on the change in absorbance per unit concentration, is greatest at this wavelength The value of $\lambda_{max}$ must be determined experimentally.. Several calibration methods such as direct calibration and standard additions can be used in UV-VIS spectroscopy. A validation must be performed in order to certify the precision and accuracy of the analytical method.