Applications of Spectrophotometry

Determining the concentration of substances in solution is the most common use of the spectrophotometer. Exact concentrations can be determined in cases where ε are known and the measurement is carried out under the rescribed
conditions. The substance being measured does not necessarily need to absorb radiation if it can scatter radiation. For example, measuring the A_{600} is a quick and easy way to monitor bacterial growth and determine the number of bacteria in cultures. In addition, since compounds exhibit unique absorption profiles, spectrophotometry can also be used to identify unknown compounds. Spectrophotometry is also a convenient method to measure enzyme activity in cases where the substrate and the product exhibit different $\boldsymbol{\lambda_{max}}$. Either the disappearance of substrate or the appearance of product over time is measured. The change in the A_λ per unit time (generally per minute) is calculated. The change in $\boldsymbol{A_\lambda}$ of a blank (= identical sample without enzyme source) is subtracted from this value. The enzyme activity in terms of amount of product formed per unit time per mg protein can be calculated by factoring in the amount of enzyme, dilution factors and the extinction coefficient (see example in appendix). A typical example of a formula for the calculation of enzyme activity is:

$$\textbf{activity} = (\Delta\boldsymbol{\lambda}_{\textbf{sample}} - \Delta\boldsymbol{\lambda}_{\textbf{blank}})\cdot\textbf{volume}\cdot 10^6/\boldsymbol{\varepsilon}\cdot\Delta\textbf{time}\cdot\textbf{mg protein},$$

where activity is expressed as μ moles product formed/min/mg protein; $\Delta\boldsymbol{\lambda_{sample}}$ is the change in absorbance of the sample containing enzyme; $\Delta\boldsymbol{\lambda_{blank}}$ is the change in absorbance of a sample containing everything except the enzyme; the volume in the cuvette expressed in the same units as **ε**; $\mathbf{10^6}$ **μ** moles per mole (assuming **ε** is expressed in moles); **ε** is the molar extinction coefficient; Δ**time** is the time in minutes the reaction was measured; and mg protein in the cuvette. In deriving such formulas it is important to match the units. The units of **ε** may also include the 1 cm thickness of the cuvette which is ignored in the calculations.

Factors Affecting Absorption

Although the absorption spectrum is primarily determined by the chemical structure of the chromophore, environmental factors can affect $\boldsymbol{\lambda_{max}}$ and ***ε***. The **pH** determines the ionization of chromophore which in many cases will affect the absorption properties of a chromophore. Indicator dyes and **pH** paper are examples of this phenomenon. The polarity of the solvent or neighboring molecules can also affect absorption. Because of this effect, spectrophotometry can also be used to determine structural features of macromolecules. For example, whether particular amino acid residues are buried within the internal portion of proteins or exposed on the aqueous solvent at the protein surface can be determined by spectrophotometry. The relative orientation of neighboring chromophores also affects absorption. Hypochromism of nucleic acids is an example of orientation effects. The absorbance (i.e., **ε**) decreases as free nucleotides are polymerized into single-stranded **DNA** and decreases further in double-stranded **DNA**.

Variations in Spectrophotometry

It is also possible to use other spectra be sides UV/visible range. For example, vibrations between the atoms of molecules can be analyzed using infrared (**IR**) and Raman spectrophotometries. Many molecules will exhibit characteristic 'signatures' and therefore can be identified. Other Forms of Spectrophotometry

Spectrophotometry	Comment
Infrared (IR)	vibrational levels
Raman	
Optical Rotary Dispersion (ORD	polarize light
Circular Dichroism (CD)	
Nuclear Magnetic Resonance (NMR	magnetic moments
Electron Spin Resonance (ESR)	

Light can be polarized so that all of the waves are in the same orientation. The study of the absorption of polarized light can yield more information about the structure of molecules if the chromophores have optically active centers. Circular dichroism (CD) measures the ability of chromophores to differentially absorb left and right circularly polarized light. Optical rotary dispersion (ORD) measures the ability of a optically active chromophore to rotate plane polarized light. Both CD and ORD are useful in structural studies or proteins and nucleic acids. For example, it is possible to approximate the amount of α-helix, β-sheet and random coil in proteins.

The effects of molecules on the magnetic component of radiation can also be analyzed. Nuclear magnetic resonance (NMR) is a spectros copic method capable of yielding information on the structure of molecules, interactions between molecules and molecular motion. This method is based upon the principle that a spinning charge (i.e., the nucleus) generates a magnetic field. Similarly, an electron also possesses a spin magnetic moment which can be analyzed by electron spin resonance (ESR). A common use for ESR in biological sciences is to monitor the fluidity of membranes.