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Subject: Practical Biochemistry

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Lab 3: Spectrophotometer

مختبر3: جهاز الطيف الضوئي

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Spectrophotometry

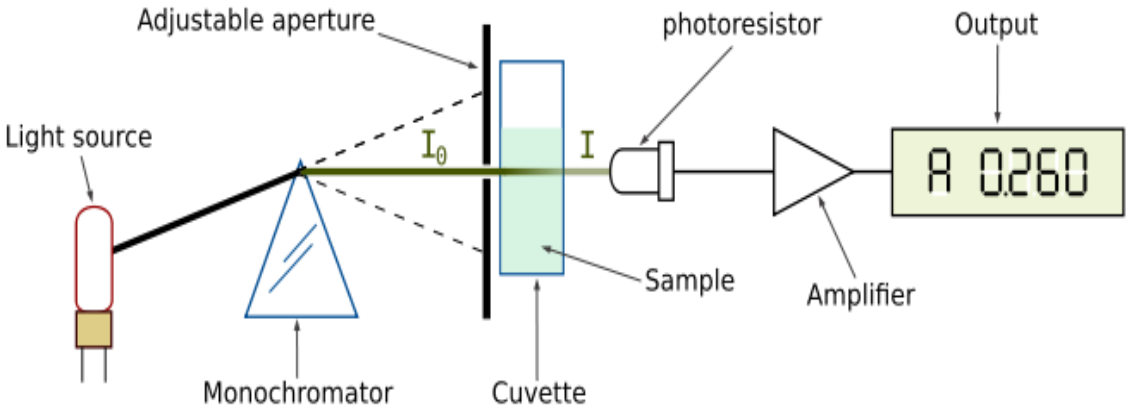
In chemistry, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear rangeabsorption or reflectance measurement

In short, the sequence of events in a modern spectrophotometer is as follows:

1. The light source is imaged upon the sample
2. A fraction of the light is transmitted or reflected from the sample
3. The light from the sample is imaged upon the entrance slit of the monochromator

The monochromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially



Beer-Lambert Law

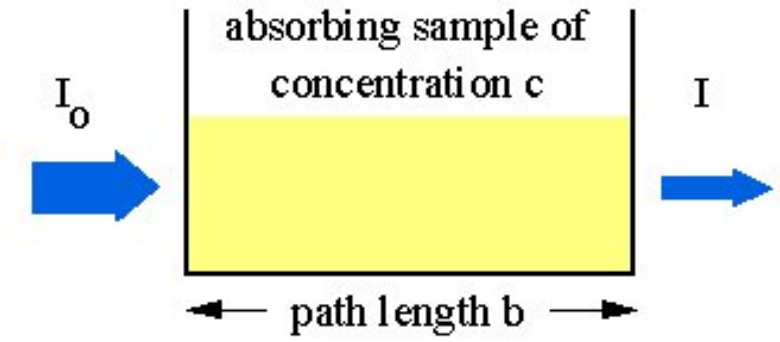
Introduction

The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:
$A = a(\lambda) * b * c$
where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. When working in concentration units of molarity, the Beer-Lambert law is written as:
$A = \varepsilon * b * c$
where ε is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}.

Instrumentation

Experimental measurements are usually made in terms of transmittance (T), which is defined as:
$T = I / I_o$
where I is the light intensity after it passes through the sample and I_o is the initial light intensity. The relation between A and T is:
$A = -\log T = -\log (I / I_o)$.

Absorption of light by a sample



Modern absorption instruments can usually display the data as either transmittance, %-transmittance, or absorbance. An unknown concentration of an analyte can be determined by measuring the amount of light that a sample absorbs and applying Beer's law. If the absorptivity coefficient is not known, the unknown concentration can be determined using a working curve of absorbance versus concentration derived from standards.