Lambert-Beer's law

The law was first developed by Pierre Bouguer before 1729. It was later attributed to Johann Heinrich Lambert who cited Bouguer's findings. The law included path length as a variable that affected absorbance. Later, Beer extended in 1852 the law to include the concentration of solutions, thus giving the law its name Beer-Lambert Law.

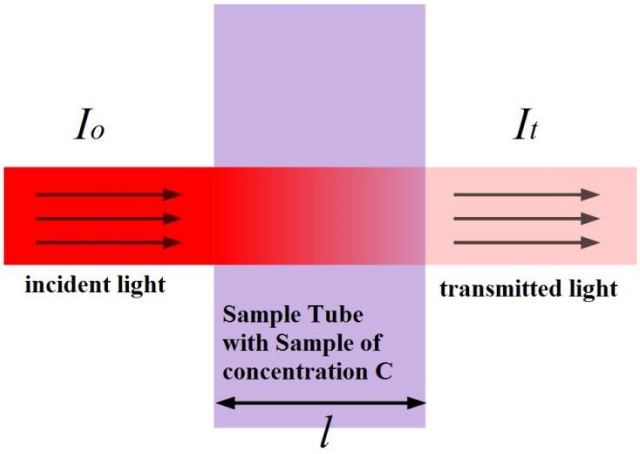
Definition & Equation

- The Beer-Lambert law states that the quantity of light absorbed by a substance dissolved in a fully transmitting solvent is directly proportional to the concentration of the substance and the path length of the light through the solution.

  What is Beer's Law?
  Beer law states that concentration and absorbance are directly proportional to each other and it was stated by August Beer.

  What is Lambert Law?

  Lambert law states that absorbance and path length are directly proportional and it was stated by Johann Heinrich Lambert.



- Because Beer's law states this, it means we can both calculate the concentration of a solution by using the absorbencies, or plot a graph of various concentrations, align them to their correct absorbencies, and use a colorimeter to find the concentration of an unknown solution

- **Equation:** The law states that:

  $A(\lambda) = \varepsilon(\lambda)\, l\, c$.

  The proportionality constant $\varepsilon(\lambda)$ is called the absorptivity of the substance at the wavelength λ. $\varepsilon(\lambda)$ is called the molar absorptivity if the concentration is measured in moles/liter.

  - The absorbance is inversely proportional to the transmittance of the solution

Derivation of Law

A spectrophotometer is an apparatus that measures the intensity, energy carried by the radiation per unit area per unit time, of the light entering a sample solution and the light going out of a sample solution. The two intensities can be expressed as transmittance: the ratio of the intensity of the exiting light to the entering light or percent transmittance (%T). Different substances absorb different wavelengths of light. Therefore, the wavelength of maximum absorption by a substance is one of the characteristic properties of that material. A completely transparent substance will have $I_t = I_0$ and its percent transmittance will be 100. Similarly, a substance which allows no radiation of a particular wavelength to pass through it will have $I_t = 0$, and a corresponding percent transmittance of 0.

Transmittance

$T = I_t / I_0$

% Transmittance: $\%T = 100\ T$

Absorbance

$A = \log10\ (I_0/I_t)$

$A = \log10\ (1/T) = -\log10\ (T)$

$A = \log10\ (100/\%T)$

$A = 2 - \log10\ (\%T)$

Deviations to the law

The Beer-Lambert law maintains linearity under specific conditions only. The law will make inaccurate measurements at high concentrations because the molecules of the analyte exhibit stronger intermolecular and electrostatics interactions which is due to the lesser amount of space between molecules. This can change the molar absorptivity of the analyte. Not only does a high

concentration change molar absorptivity, but it also changes the refractive index of the solution causing departures from the Beer-Lambert law.

Applications

- Quantitative analysis by determination of concentration through absorbance maxima.
- Qualitative analysis by value of absorbance maxima.
- Detectors for separation in HPLC.
- Determination of reaction kinetics.

Limitations of the Beer-Lambert law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity
- scattering of light due to particulates in the sample
- fluoresecence or phosphorescence of the sample
- changes in refractive index at high analyte concentration
- shifts in chemical equilibria as a function of concentration
- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
- stray light

Limitations and Deviations of Beer-Lambert Law with respect to Ultraviolet-Visible (UV-Vis) Spectroscopy

Beer-Lambert's law proves a direct correlation between the absorbance (A) of a molecule to the concentration (c) and the path length (l) of the sample. This relationship is a linear for the most part. However, under certain circumstances the Beer Lambert relationship breaks down and gives a non-linear relationship. These deviations from the Beer Lambert law can be classified into three categories:

1. **Real Deviations** – These are fundamental deviations due to the limitations of the law itself.

2. **Chemical Deviations**– These are deviations observed due to specific chemical species of the sample which is being analyzed.

3. **Instrument Deviations** – These are deviations which occur due to how the absorbance measurements are made.

Real Limitation and Deviation of Beer-Lambert Law

Beer law and Lambert law is capable of describing absorption behavior of solutions containing relatively low amounts of solutes dissolved in it (<10mM). When the concentration of the analyte in the solution is high (>10mM), the analyte begins to behave differently due to interactions with the solvent and other solute molecules and at times even due to hydrogen bonding interactions.

1. At high concentrations, solute molecules can cause different charge distribution on their neighboring species in the solution. Since UV-visible absorption is an electronic phenomenon, high concentrations would possibly result in a shift in the absorption wavelength of the analyte. At times, even electrolyte concentrations (such as those present in buffers) play an important role in altering the charge distributions and affecting UV-visible absorbance. Some large ions or molecules show deviations even at very low concentrations. For e.g. methylene blue absorptivity at 436 nm fails to observe Beer Lambert law even at concentrations as low as 10μM.

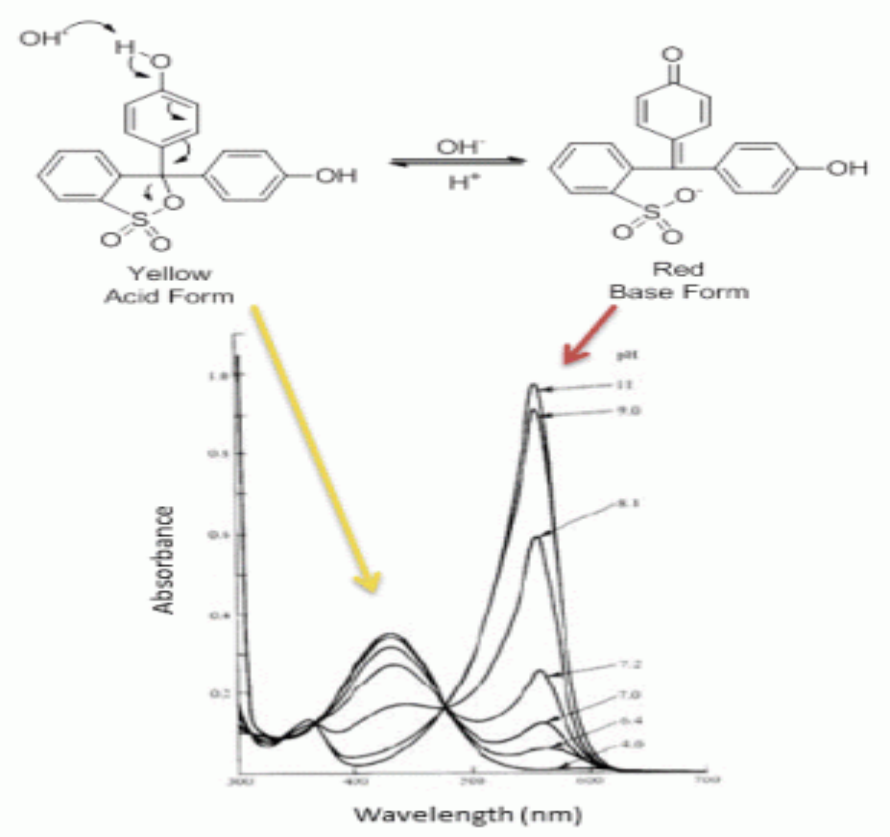
2. High analyte concentrations can also possibly alter the refractive index (η) of the solution which in turn could affect the absorbance obtained. If the addition of solute causes a significant change in the refractive index of the solution a correction to the Beer Lambert formula can be placed as:

   $$A = \varepsilon bc\,(\eta^2 + 2)^2$$

   This correction is normally not required below concentrations of 10mM.

Chemical Deviations and Limitations to Beer-Lambert Law

Chemical deviations occur due to chemical phenomenon involving the analyte molecules due to association, dissociation and interaction with the solvent to produce a product with different absorption characteristics. For example, phenol red undergoes a resonance transformation when moving from the acidic form (yellow) to the basic form (red). Due to this resonance, the electron distribution of the bonds of molecule changes with the pH of the solvent in which it is dissolved. Since UV-visible spectroscopy is an electron-related phenomenon, the absorption spectrum of the sample changes with the change in pH of the solvent.



Acid and Base forms of phenol red along with their UV spectra at different pH demonstrates chemical deviations of Beer-Lambert law in UV-Visible spectroscop

Instrumental Deviations and Limitations to Beer-Lambert Law

A] Due to Polychromatic Radiation (Also the reason why absorbance measurements are taken at the wavelength of maximum absorbance λ_{max})

Beer-Lambert law is strictly followed when a monochromatic source of radiation exists. In practice, however, it is common to use a polychromatic source of radiation with continuous distribution of wavelengths along with a filter or a grating unit (monochromators) to create a monochromatic beam from this source. For example (see figure below), consider a molecule having molar absorptivities ε' and ε" at wavelengths λ' and λ". The absorbance (A_m) for such a species can be calculated as:

$$A_m = log \frac{(I_0' + I_0'')}{(I_0' 10^{-\varepsilon' bc} + I_0'' 10^{-\varepsilon'' bc})}$$

Equation to calculate absorbance of a sample with polychromatic light source.

When the molar absorptivities are the same at both wavelengths (i.e. ε' = ε") , the relationship between absorbance and concentration follows Beer-Lambert law to obtain a straight line.

However, as the difference between ε' and ε" increases, the deviations from linearity also increases.

❖ Why absorption measurements are taken at wavelength of maximum absorbance λ_{max}?

If the band of wavelength selected on the spectrometer is such that the molar absorptivities of the analyte is essentially constant, deviations from Beer-Lambert law are minimal. However, if a band is chosen such that the molar absorptivity of the analyte at these wavelengths changes a lot, the absorbance of the analyte will not follow Beer-Lambert law. It is observed (as demonstrated in the figure below) that the deviations in absorbance over wavelengths is minimal when the wavelength observed is at the λ_{max}. Due to this reason absorption measurements are taken at wavelengths.

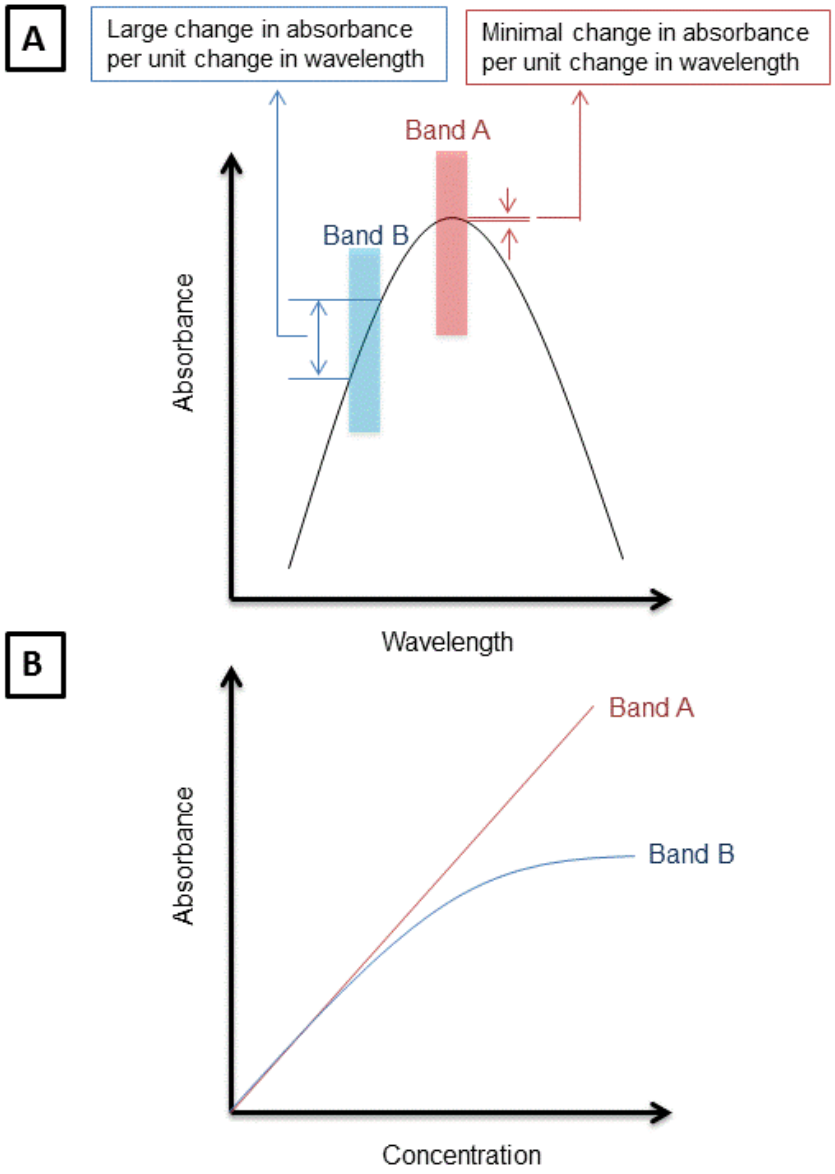


Figure A: Shows the difference in deviations in absorbance when values are obtained at maximum wavelength of absorbance (band A) vs other wavelengths of absorbance (band B). Figure B: shows the deviations in Beer-Lambert law due to observations made at wavelengths other than lambda max.

B] Due to Presence of Stray Radiation

Stray radiation or scattered radiation is defined as radiation from the instrument that is outside the nominal wavelength band selected. Usually the wavelength of the stray radiation is very different from the wavelength band selected. It is known that radiation exiting from a monochromator is often contaminated with minute quantities of scattered or stray radiation. Usually, this radiation is due to reflection and scattering by the surfaces of lenses, mirrors, gratings, filters and windows. If the analyte absorbs at the wavelength of the stray radiation, a deviation from Beer-Lambert law is observed similar to the deviation due to polychromatic radiation.

C] Due to Mismatched Cells or Cuvettes

If the cells holding the analyte and the blank solutions are having different path-lengths, or unequal optical characteristics, it is obvious that there would be a deviation observed in Beer-Lambert law. In such cases when a plot of absorbance versus concentration is made, the curve will have an intercept k and the equation will be defined as:

$$A = \varepsilon bc + k$$

In today's instrument this problem is generally not observed, however if it is present, appropriate linear regression to quantify this deviation must be made.

References

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