Trilogy

Absorbance

Theory of absorbance

Light absorption occurs when atoms or molecules take up the energy of a photon of light, thereby reducing the transmission of light as it is passed through a sample. Light attenuates exponentially as it passes through clear materials or solutions. So, naturally the reduction of transmitted light is exponentially related to the concentration of the sample and path length of light traveled (Figure 1A).

*%T = [(Transmitted Light) / (Incident Light)] * 100*

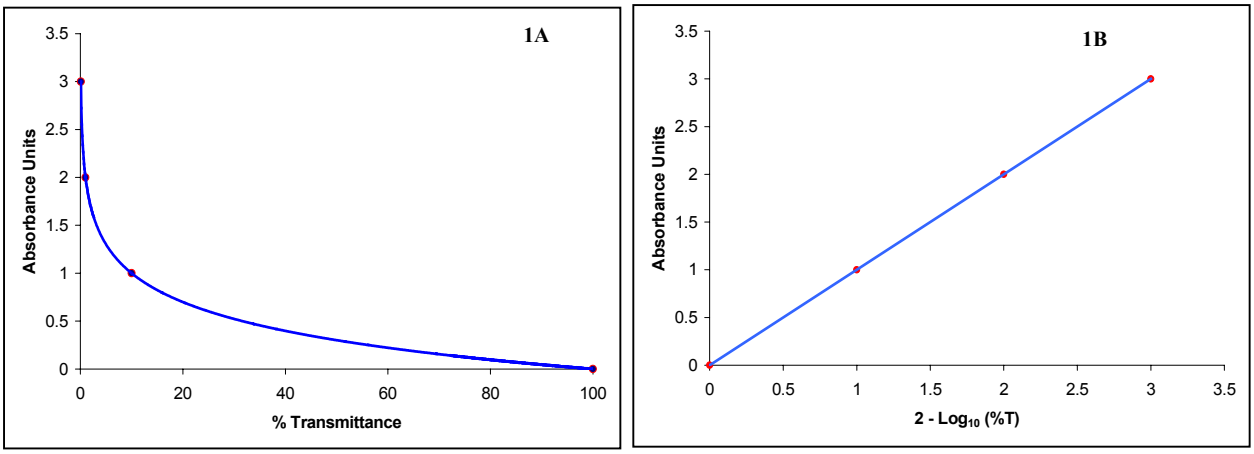

Fig. 1A) % Light transmitted per Absorbance Unit, 1B) relationship between absorbance and %T

Beer-Lambert Law

Beer-Lambert Law relates the transmittance of light to absorbance by taking the negative logarithmic function, base 10, of the transmittance observed by a sample, which results in a linear relationship to the intensity of the absorbing species and the distance traveled by light.

Absorbance = 2 - Log_{10} (T)

In short, Beer-Lambert Law states that the absorbance of a sample is directly proportional to the path length of the sample holder and the concentration of the sample.

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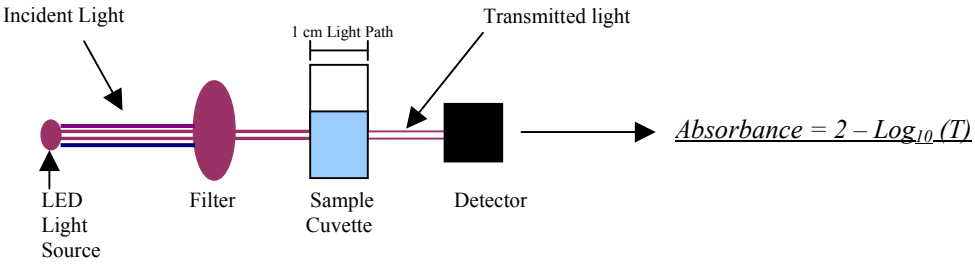

Figure 2. Absorption measurement scheme

Range and detection limits

Absorbance is measured in absorbance units (Au), which relate to transmittance as seen in figure 1. For example, ~1.0Au is equal to 10% transmittance, ~2.0Au is equal to 1% transmittance, and so on in a logarithmic trend. Because transmittance inherently decreases logarithmically, linearity breaks down at higher concentrations due to sample saturation (Figure 3). Since the transmitted light at 2.0Au is less than 1%, any increases in concentration will reflect only slight changes in absorbance. Generally, you will never reach an absorbance of 4.0Au and still maintain a linear relationship. The same applies for lower end limits.

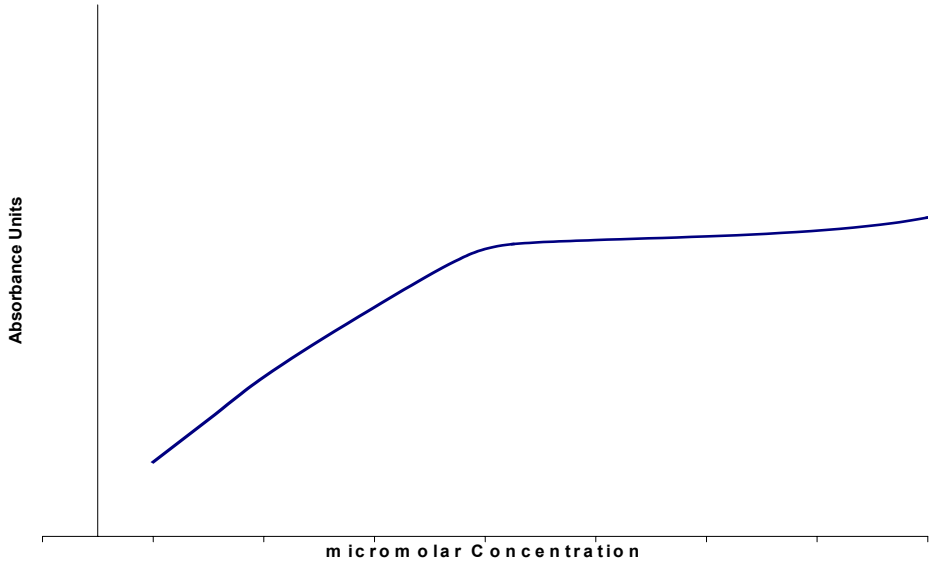

Figure 3. Theoretical response curve of absorbances at increasing concentrations

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Samples being measured for absorption, which are primarily clear solutions or close to zero, are subject to the errors associated with light refraction, reflection, or scattering. For example, scattering can decrease the transmission of light through a sample and will effect the absorbance even though no absorption is occurring. These kinds of errors can be reduced in turn maximizing resolution through particle removal, which minimizes scattering of light and using glass cuvettes, which minimizes absorption of light.

Absorbance Applications

Absorbance measurements can be used to measure the concentrations in different ways such as using absorption coefficients with pigment calculations or calibration curves as with nutrient analyses. For either method used the basic principal is to create a correlation factor between absorbance and concentration to be used for determination of absorbing species in natural samples.

Pigments

Pigment concentrations are calculated by taking the absorbance reading of the largest peak in a pure pigment spectrum, as characterized by a spectrophotometer, and dividing that absorbance value by the pigments specific absorption or molar absorption coefficients.

$$Concentration\ of\ Pigment\ (mg/L) = \left(\frac{Absorbance\ Units\ (Au)}{Specific\ Absorption\ Coefficient\ (L\ g^{-1}\ cm^{-1})} \right) * 1000$$

Pigment	Algal Group Represented	Specific Absorption Coefficient (L g^{-1} cm^{-1})	Absorbance Units (Au)	Calculated Concentrations (mg/L)
Chlorophyll a	*All Algae*	87.67	0.2519 (λ = 664nm)	2.87
Fucoxanthin	*Diatoms*	166	0.1675 (λ = 443nm)	1.009
Chlorophyll b	*Green Algae*	51.36	0.3549 (λ = 646nm)	6.91

Table 1: Example of spectrophotometric determination of pure pigment concentrations using specific absorption coefficients (Jeffrey et al 1997) for Chlorophyll a, b, and Fucoxanthin.

The derivations of these coefficients require extremely careful and highly analytical techniques but the end calculation is very simple. To obtain a specific absorption coefficient of a pigment, one would need to purify the pigment from all contaminants and either dry or crystallize the molecule. After purification, the resulting material needs to be dissolved in solvent and measured for absorbance. Keep in mind that absorbance measurements are solvent specific as well.

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The specific absorption coefficient obtained from a spectrum of the pigment would serve as the universal standard for quantifying this specific pigment's concentration from any spectrophotometric measurement. Specific absorption coefficients of pigments are usually given in volume per weight with respect to path length of the cuvette used (i.e. liter gram^{-1} centimeter^{-1}).

Nutrients

Nutrient concentrations are simpler calculations and less tedious. The simplicity of nutrient calculations comes from a basic understanding of algebraic expressions. A calibration curve is produced, which relates the absorbance to concentration of absorbing species.

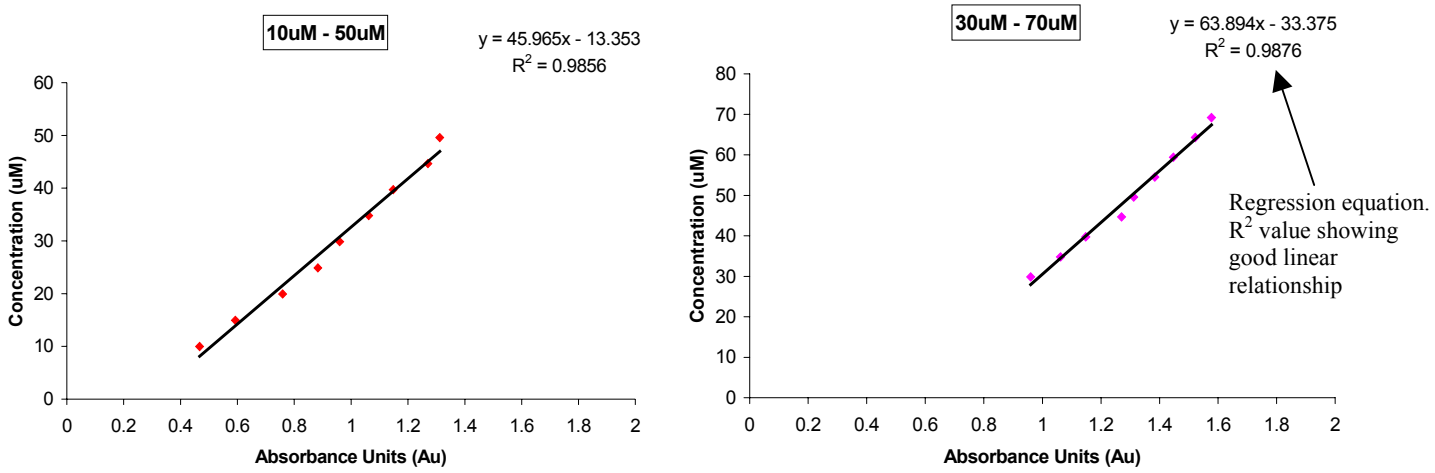

Figure 4: Example of established linear correlation between concentration of absorbing species and absorbance readings (Au) for 2 different working ranges. The equation of the regression can be used to calculate the concentrations of unknown samples by simply plugging in X values that fall within your working range.

The equation of the curve is used to calculate natural sample concentrations as long as they fit within the range of the curve (Fig. 4). Accurate concentration values can be achieved from absorbance measurements if a couple of important points are kept in mind:

1) Choosing a range of absorbance units and establishing a linear calibration curve for that range
2) If measuring samples outside the range your calibration curve, making sure your samples are diluted to fit the range calibrated
3) Following procedure for correct preparation of reagents
4) Avoid cross contamination among samples

5) Keeping the optical path constant (i.e. don't change cuvettes sizes while analyzing samples)
6) Blank correcting by running a reagent blank
7) Minimizing transmission loss, due to particle scattering, by centrifugation or other means of particle removal

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8) Minimizing transmission loss by using sample holders that will not absorb of light
9) Plugging ***x***, the absorbance value from an instrument, into the regression equation, $Y = mx+b$ will provide you with an accurate estimate of your absorbing species if you have established a good linear relationship as determined by the R^2 value or your regression (*Note: Accuracy of estimates increase as your R^2 value increases*)

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