Absorbance Overview

Why measure absorbance?

For calculation of pigment concentrations

- Chlorins
- Carotenoids
- Phycobilins

For determining nutrient concentrations

What is absorbance?

Measurement of light absorption by liquid, solid or gas

An analysis that quantifies absorbing species in a substance

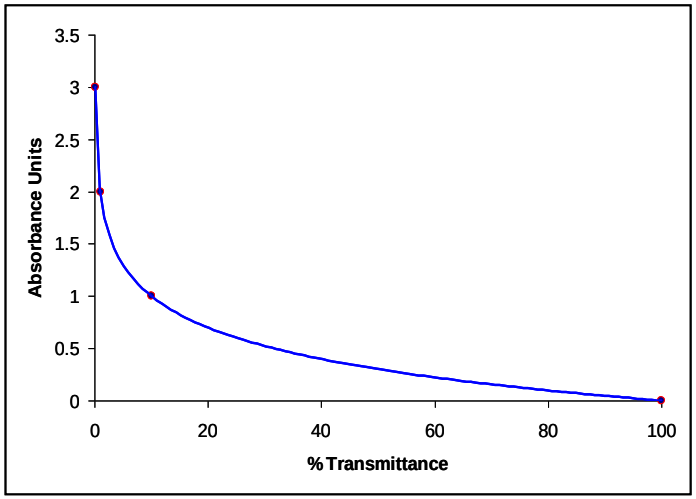
Absorbance Theory

Definition:

Absorption of energy of a photon of light by molecules or atoms

Light is attenuated exponentially as it passes through substances

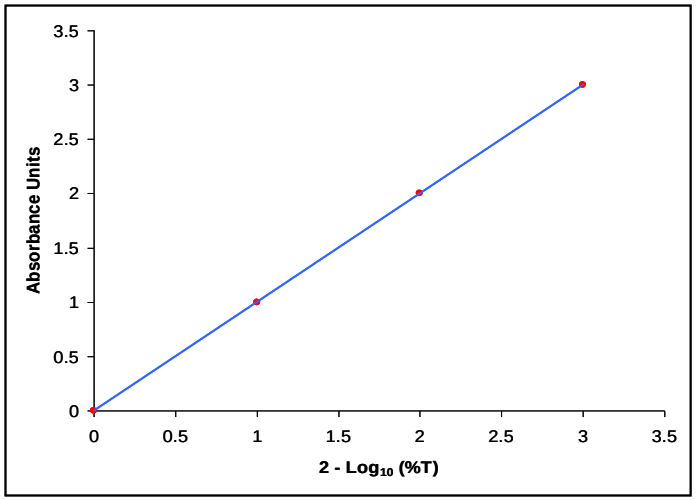
*%T = [(Transmitted Light) / (Incident Light)] * 100*



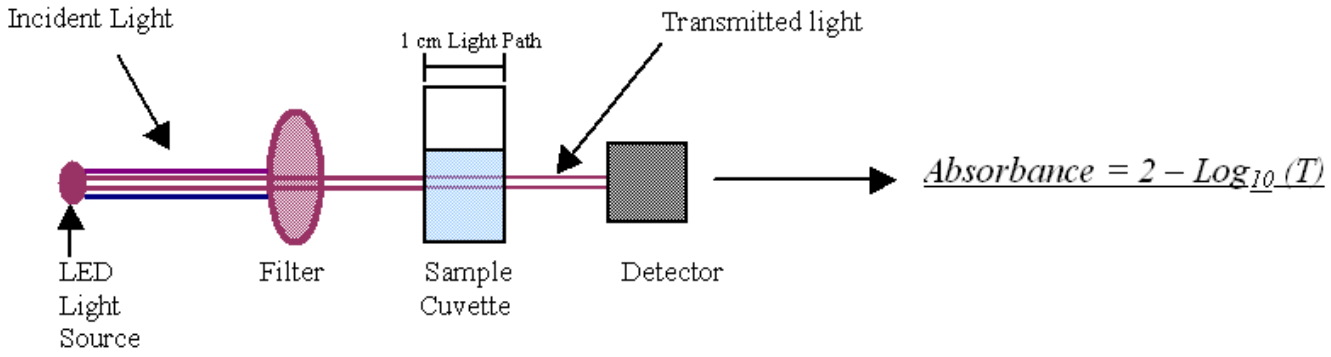
Beer's Law:

Reduction of light transmitted as it passes through the substance is exponentially related to the concentration of absorbing species and path length light has traveled

Absorbance = 2 - Log_{10} (T)

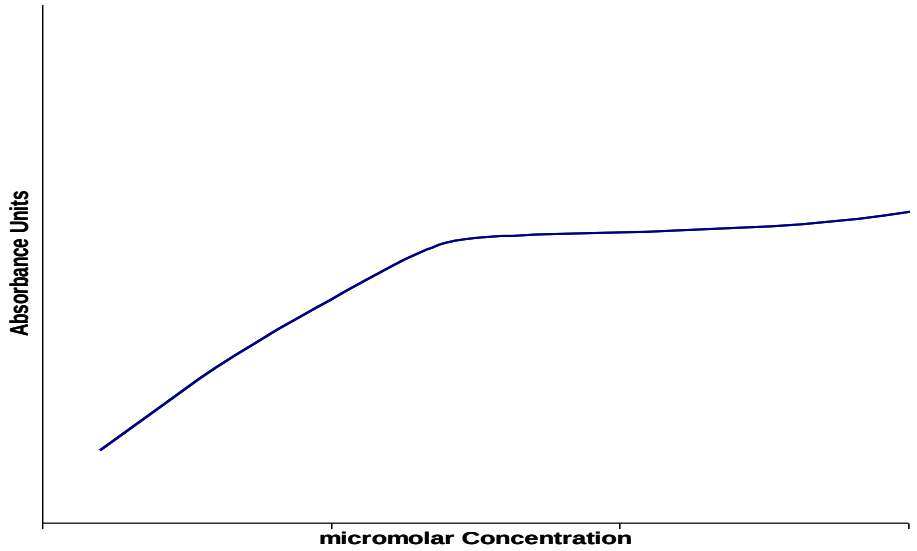


Considerations



Transmittance Table:

Absorbance Units	% Transmittance
0	100%
1.0	10%
2.0	1%
3.0	0.1%
4.0	0.01%



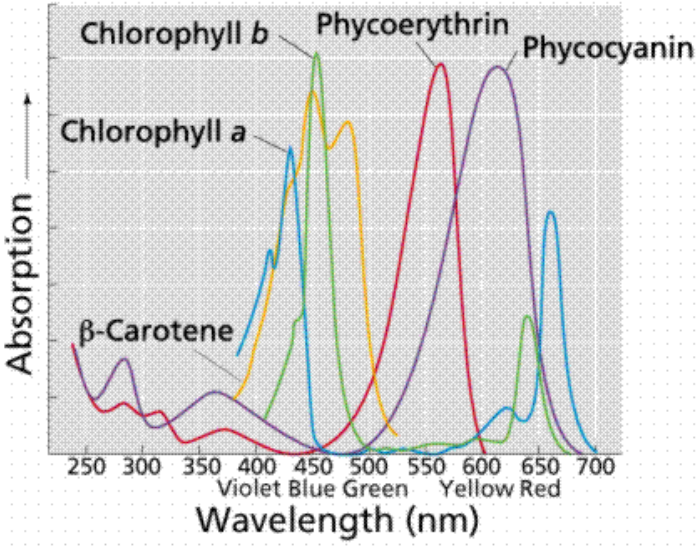
Absorbance Applications

Pigments

Pigment's extinction (absorption) coefficients represent the quantitative relationship between the absorption of light at a specific wavelength of a known amount of pigment in a known volume of solvent (Jeffrey et al, 1997)

Created by purifying pigments into solid state, diluting & measuring absorption

Can determine abundances of groups using group specific pigment analyses



Universal calculation

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

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 = 664 nm)	2.87
Fucoxanthin	Diatoms	166	0.1675 (λ = 443 nm)	1.009
Chlorophyll b	Green Algae	51.36	0.3549 (λ = 646 nm)	6.91

Absorbance Applications

Nutrients

Nutrient analysis is important in determining primary production and the health of aquatic systems

Turner Designs, Inc. has developed a cost efficient way to accurately estimate nutrient concentrations in water

Turner Designs Trilogy Absorbance Modules use combinations of Light Emitting Diodes (LED's) and bandpass filters to select nutrient specific wavelengths

Colorimetric method

Involves chemical reactions to create absorbing complexes (colored solutions)

Uses wavelength absorption specific to absorbing species

Simple calculation for determining concentrations of nutrients in samples

$$Y = mx + b$$

Phosphate

Phosphate

Inorganic Phosphate (PO_4^{3-}) can be found:

as a free ion in water systems

as a salt in terrestrial environments

used in detergents as water softeners

in rock deposits

Inorganic phosphate, which is typically limiting, is one of the three necessary nutrients used for primary production by terrestrial and aquatic plants

It is used in fertilizers to enhance agricultural growth and production and when introduced into aquatic systems can cause algal blooms

So, why measure phosphate?

Measuring phosphate in aquatic environments can be a very important tool in understanding the health of a system or its "water quality"

Monitoring of phosphate levels in water can help identify possible sources for phosphate introduction to aquatic systems

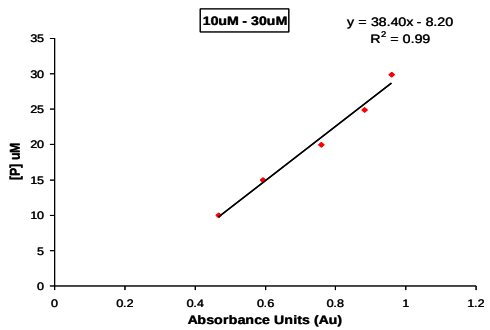
Precautions can be taken to minimize these inputs and prevent any harmful effects

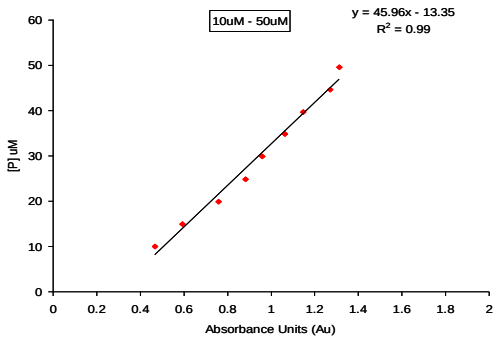
Phosphate

Setting range:

The graphs show examples of a calibration curve determined for the specific ranges

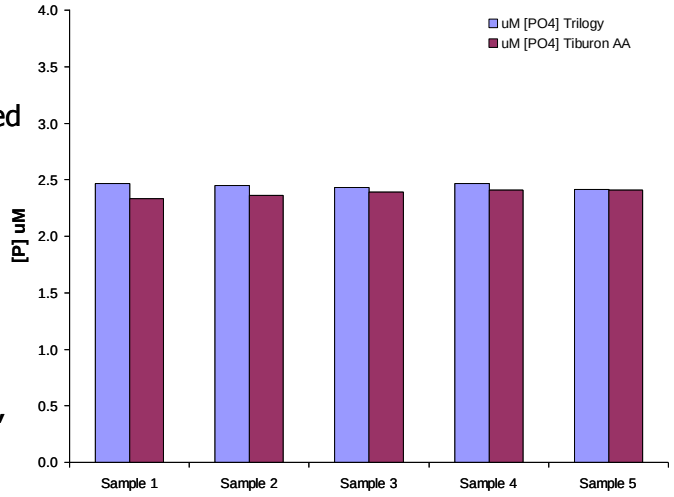
The equation from the regression can be used to calculate concentrations which are within the limits of the set range





Comparison with other platforms:

- Comparison data showing split water phosphate concentrations determined using a Bran & Luebbe Auto Analyzer operated by Al Marchi at Romberg Tiburon Lab and a Turner Designs Trilogy.
- The Auto Analyzer uses a lamp/pmt platform, whereas the Trilogy uses LED/photodiode
- The Auto Analyzer is a really sensitive instrument which can detect phosphate concentrations at really low levels.
- Again, results show less than a 3% difference from Auto Analyzer values, which has greater sensitivity



Silicate

Silicate

Silica can be found in many forms such as:

- sand
- quartz
- opal
- rock
- sea floor sediments

It also is an important element for building structural materials and growth in animal and plant life

Primary producers such as Diatoms extract silica from water allowing blooms to occur and ultimately increasing the energy flow from lower to higher trophic levels in an ecosystem.

Microorganisms called radiolarians use silica to make glass tests, or structures, which are deposited after blooming events and can be used as markers to track past events such as volcanic eruptions and storms.

Sponges are larger organisms that use silica from water to produce their housing structures and are important filter feeders that help balance ecosystems.

Silicate

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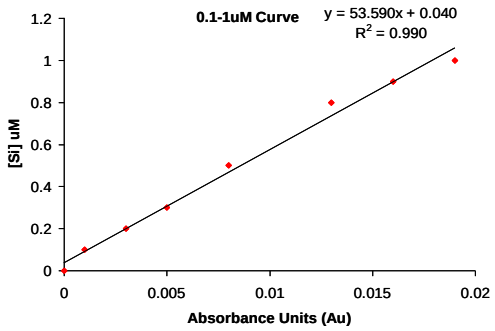

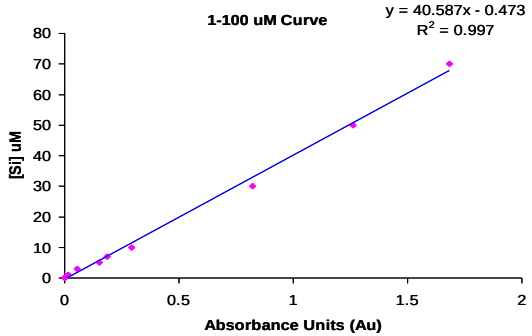

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