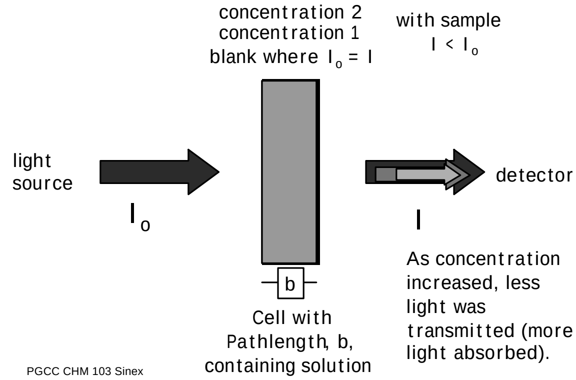
Spectrophotometry:

An Analytical Tool

The process of light being absorbed by a solution



PGCC CHM 103 Sinex

Some terminology

- I – intensity where I_o is initial intensity
- T – transmission or %T = 100 x T

  (absorption: Abs = 1 – T or %Abs = 100 - %T)

  $$T = I / I_o$$
- A – absorbance

  $$A = -\log T = -\log I / I_o$$

PGCC CHM 103 Sinex

Beer's Law

$$A = abc$$

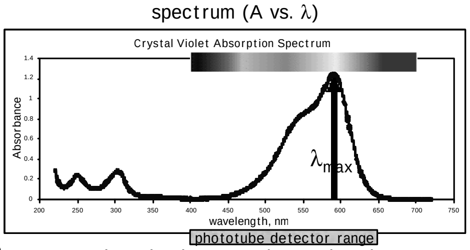
where a – molar absorptivity, b – pathlength, and c – molar concentration

See the Beer's Law Simulator

PGCC CHM 103 Sinex

Analyze at what wavelength?

Scan visible wavelengths from 400 – 650 nm (detector range) to produce an absorption spectrum (A vs. λ)



λ_{max} - wavelength where maximum absorbance occurs

PGCC CHM 103 Sinex

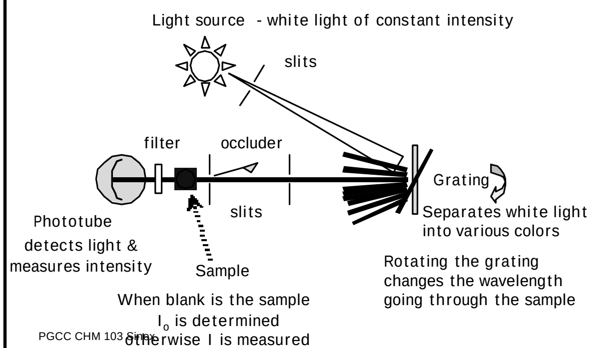
The BLANK

- ✓ The blank contains all substances expect the analyte.
- ✓ Is used to set the absorbance to zero:

  $$A_{blank} = 0$$
- ✓ This removes any absorption of light due to these substances and the cell.
- ✓ All measured absorbance is due to analyte.

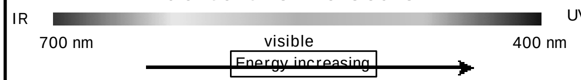
PGCC CHM 103 Sinex

The components of a Spec-20D



PGCC CHM 103 Sinex

What does the absorbed light (electromagnetic radiation) do to the molecule?



- high energy UV – ionizes electrons
- low energy UV and visible – promotes electrons to higher energy orbitals
  (absorption of visible light leads to a colored solution)
- IR – causes molecules to vibrate (more later)

PGCC CHM 103 Sinex

UV/visible light absorption

Valence electrons

- In organic molecules, electronic transitions to higher energy molecular orbitals – double bonds: $\pi \rightarrow \pi^*$
- In transition metals, hydrated ions as Cu^{++} have splitting of d orbital energies and electronic transitions – weak absorption
- In complexed transition metals, charge transfer of electrons from metal to ligand as $Cu(NH_3)_4^{++}$ – strong absorption

PGCC CHM 103 Sinex

Uses of visible spectrophotometry

- ✓ Analysis of unknowns using Beer's Law calibration curve (Been there, done that!)
- ✓ Absorbance vs. time graphs for kinetics
- ✓ Single-point calibration for an equilibrium constant determination
- ✓ Spectrophotometric titrations – a way to follow a reaction if at least one substance is colored – sudden or sharp change in absorbance at equivalence point, a piece-wise function

PGCC CHM 103 Sinex

Kinetics of Crystal Violet Reaction

$CV^+ + OH^- \rightarrow CV\text{-}OH$

purple colorless colorless

Follow concentration of crystal violet over time as it reacts by measuring its absorbance.

How will absorbance change with time?

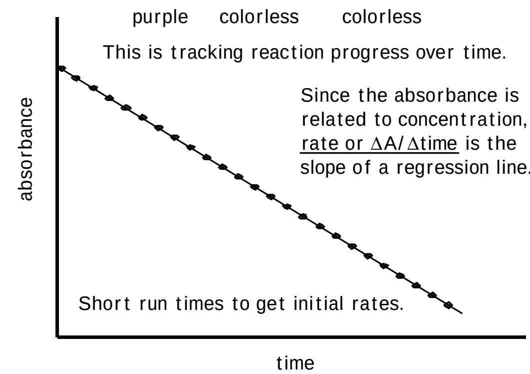
For a absorbance vs. time plot, how will you determine the rate of the reaction?

PGCC CHM 103 Sinex Chime structures

$CV^+ + OH^- \rightarrow CV\text{-}OH$

purple colorless colorless

This is tracking reaction progress over time.



PGCC CHM 103 Sinex STELLA model

Single-point calibration

- Standard with measured absorbance

$$A_{std} = abc_{std}$$

- Unknown with measured absorbance

$$A_{unk} = abc_{unk}$$

Ratio the two equations

$$A_{unk} / A_{std} = abc_{unk} / abc_{std}$$

$$A_{unk} / A_{std} = c_{unk} / c_{std}$$

- Solve for c_{unk}

Equilibrium Constant Determination

Fe^{+3} + SCN^- = $Fe(SCN)^{++}$
colorless colorless orange

$$K = (Fe(SCN)^{++}) / (Fe^{+3})(SCN^-)$$

Using the reactants, shift reaction based on Le Chatelier's principle.

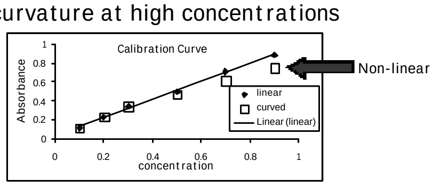
$$Fe(SCN)^{++} + SCN^- = Fe(SCN)_2^+$$

We start with a high concentration of Fe^{+3} and lower its value by dilution.

Interactive Excel spreadsheet

When calibration curves go bad!

- The linear Beer's Law relationship starts to show curvature at high concentrations



- Single-point calibration assumes a linear calibration curve

Spectrophotometric titration

- Let's consider the analysis of hydrogen peroxide with potassium permanganate in an acidic solution.
- The potassium permanganate or MnO_4^- is the <u>only colored substance</u> in the reaction. (It can serve as its own indicator.)
- How would the absorbance change as titrant was added?

$$5H_2O_2 + 2MnO_4^- + 6H^+ \rightarrow 5O_2\,(g) + 2Mn^{+2} + 8H_2O$$

purple