Spectroscopy

Biogeochemical Methods
OCN 633

Rebecca Briggs

Definitions of Spectrometry

Defined by the method used to prepare the sample

1. **Optical spectrometry**
   - **Elements are converted to gaseous atoms or elementary ions via atomization**
2. **Mass spectrometry**
   - **Sample is also atomized by also converted to positive ions and separated based on mass-to-charge ratio**
3. **X-ray spectrometry**
   - **Atomization not required, analysis based on chemical reaction of sample**

Definitions of Spectrometry

Measurement is conducted using ultraviolet/visible absorption, emission, or fluorescence

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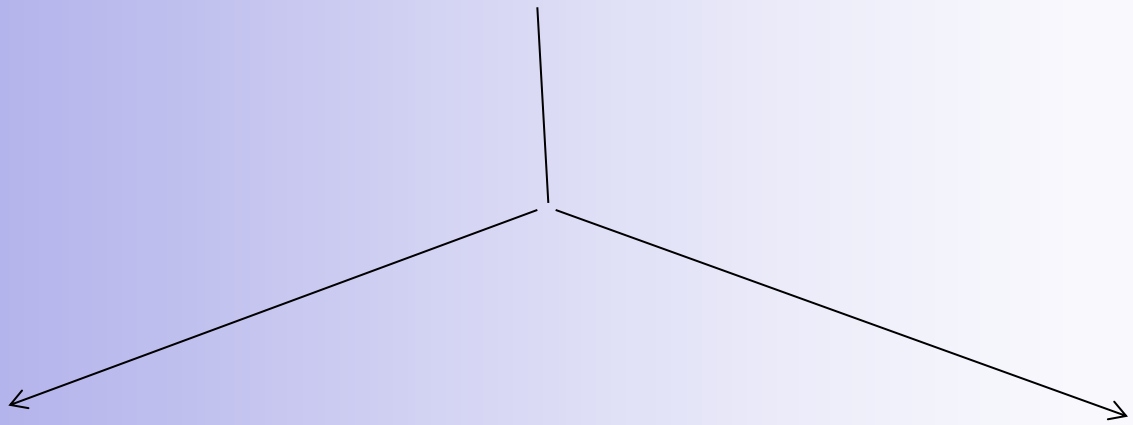
1. **Optical spectrometry**
   - **AA**
   - **ICP OES**
   - **FT-IR**
2. **Mass spectrometry**
   - **ICP MS**
   - **GC MS**
   - **LC MS**
3. **X-ray spectrometry**
   - **Spectrophotometer**
   - **Colorimeter**
   - **Fluorometer**

Definitions of Spectrometry

Defined by the measurement/detector

Measurement is conducted using ultraviolet/visible absorption, emission, or fluorescence

Spectrometric Methods of Analysis



Absorption Spectrometry

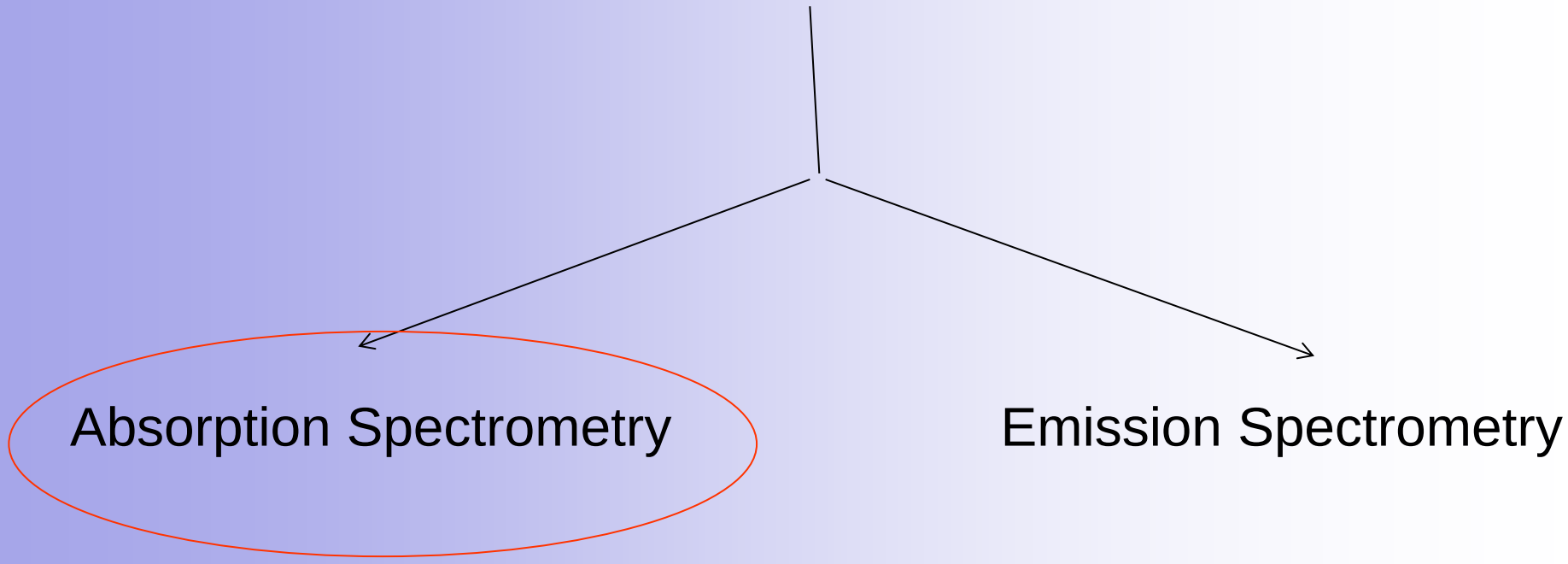
Emission Spectrometry

Definitions of Spectrometry

Definition by measurement/detector:

Measurement is conducted using ultraviolet/visible absorption, emission, or fluorescence

Spectrometric Methods of Analysis



Definitions of Spectrometry

Absorption Spectrometry

1- Molecular UV Absorption Spectrometry

2- Molecular Visible Absorption Spectrometry

3- Infrared Spectrometry

4- Nuclear Magnetic Resonance(NMR)

5- Atomic Absorption Spectrometry

Emission Spectrometry

1- Fluorimetry

2- Emission Spectrography (Arc/Spark Emission Spectrometry)

3- Atomic Emission Spectrometry

(Flame photometry)

4- Atomic Fluorescence Spectrometry

5- X-ray Fluorescence Spectrometry

6- Radiochemical Methods of Analysis

Definitions of Spectrometry

Many optical instruments share similar design

1. **(1) stable radiation source**
2. **(2) transparent sample holder**
3. **(3) wavelength selector**
4. **(4) radiation detector**
5. **(5) signal processor and readout**

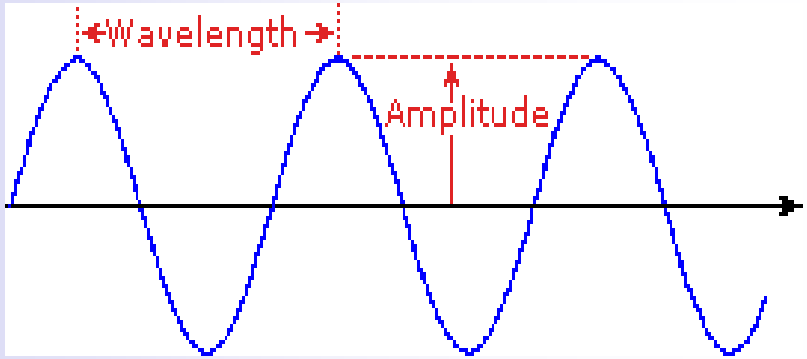
Electromagnetic radiation

Electromagnetic radiation is a type of energy that is transmitted through space as a transverse wave at enormous velocity.

It takes numerous forms known as **electromagnetic** spectrum. The electromagnetic spectrum include gamma ray, X-ray, ultraviolet (UV), visible, infrared (IR), microwave and radio-wave radiation.

1- Wave Properties

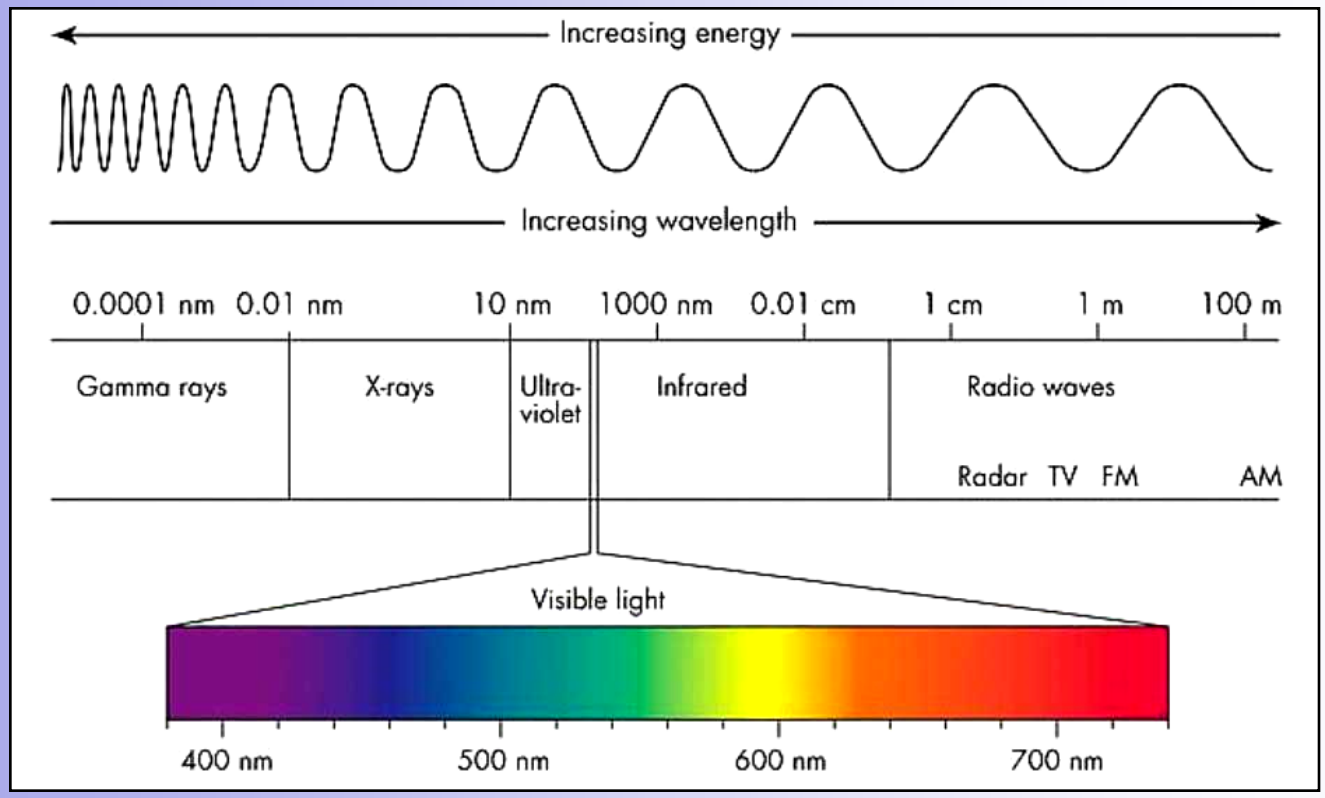
The wave is described either in terms of its **wavelength (λ)**,the distance between successive maxima or minima of a wav(nm), or in terms of the **frequency(ν)**, the number of oscillation of the field per second.



The velocity of light, c, is given by the equation:

C= ν λ

Spectroscopic Light Spectrum



Wavelengths used in spectroscopy:

- Ultra-violet: 175 – 380 nm
- Visible light: 380 – 900 nm
- Infrared: 900 – 3300 nm

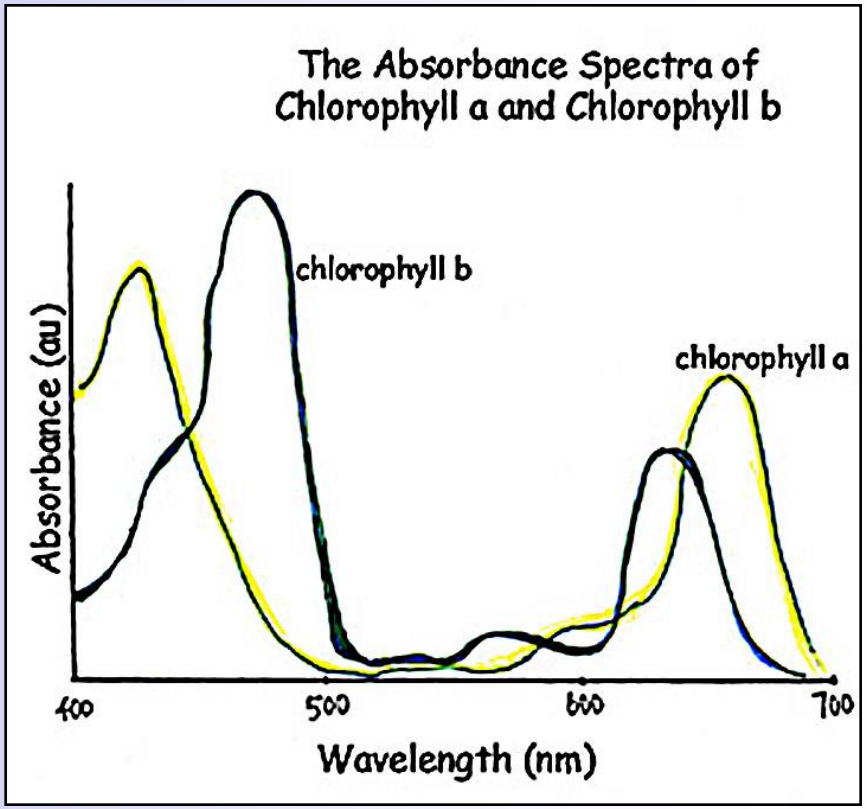
Spectrophotometric analysis

- **Spectrophotometric techniques** are used to measure the concentration of solutes in solution by measuring the amount of light that is absorbed by the solution in a cuvette placed in the spectrophotometer.

- **The spectrophotometer** can measure the amount of light or electromagnetic radiation (of certain frequency) transmitted or absored by the solution.

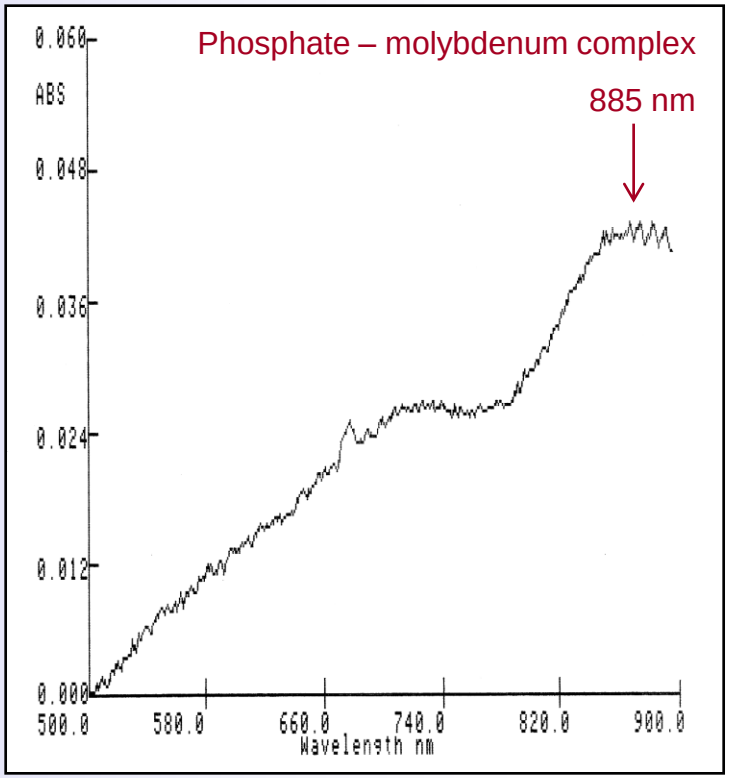
Absorbance Scanning

- Shows bands of light absorption by pure compounds
- Use to identify peaks for quantitation

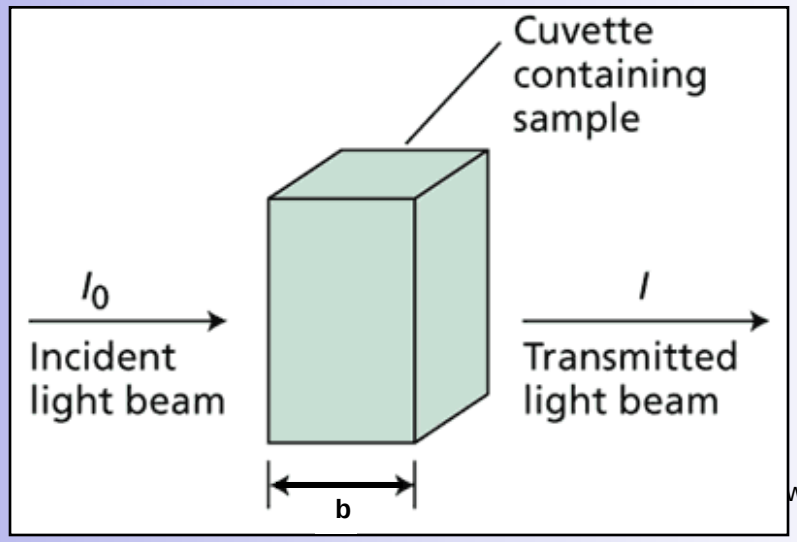


Colorimetric Analyses

- Analyte (chemical species to be measured) + reagent → colored product
- Measure color intensity (absorbance) of colored product to determine the analyte's concentration



Light Absorbance



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Absorbance (A) ≡ log I_o / I

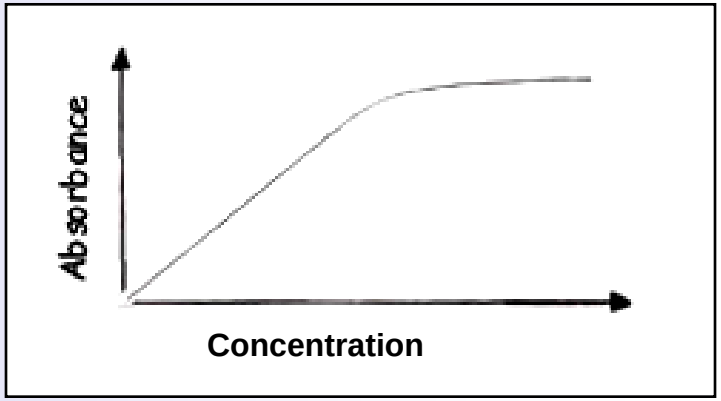
(*i.e.*, the smaller "I" is, the larger the absorbance)

Beer's Law: A = a · b · c

a = absorbtivity (cm^{-1} M^{-1})

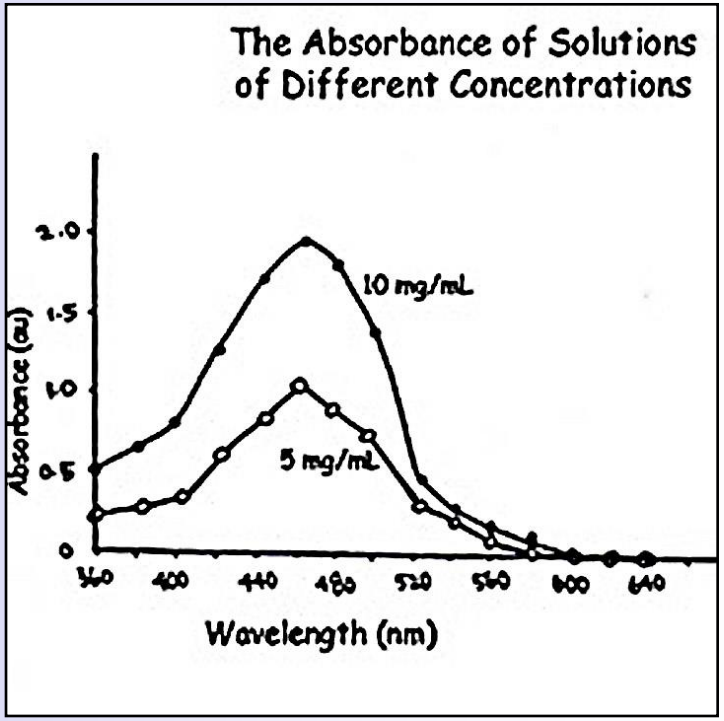
b = pathlength (cm)

c = concentration (M)



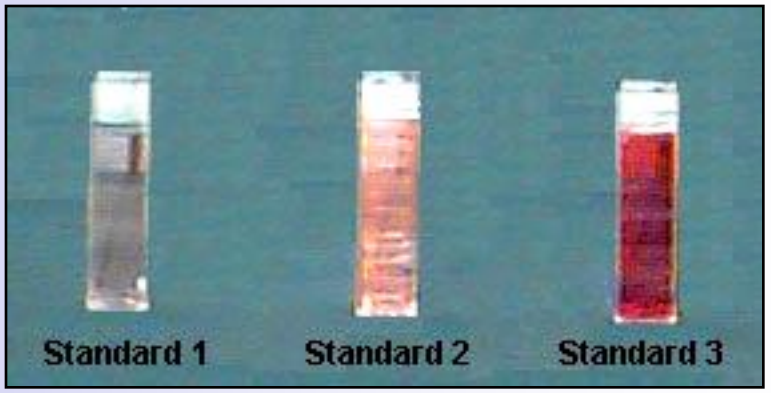
Beer's Law

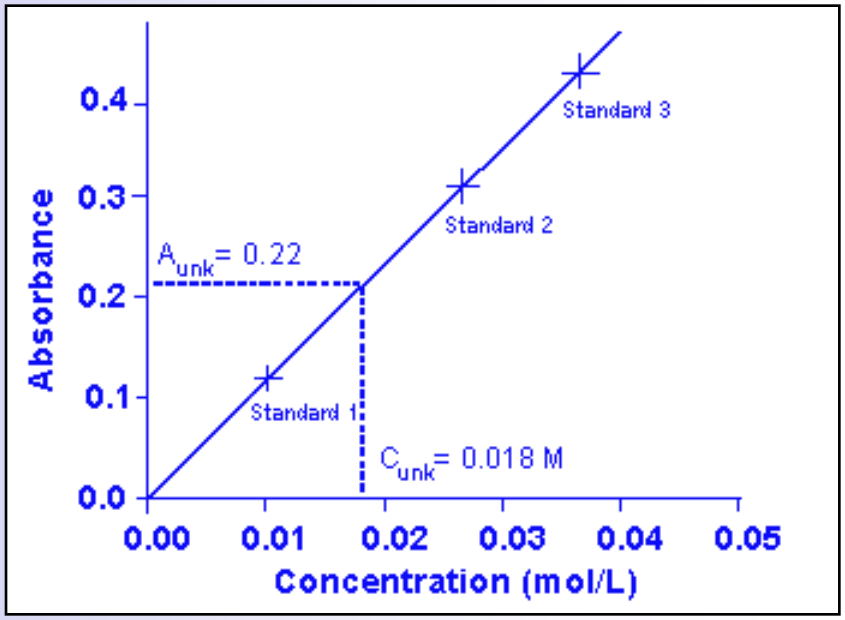
- Concentration difference result in proportional absorbance change



Linear standard curve indicates conformity with Beer's Law

- Prepare standards of known concentration
- Measure absorbance at λmax
- Plot A vs. concentration
- Obtain slope
- Use slope (and intercept) to determine the concentration of the analyte in the unknown





Quantitative methods:

Specificity: **the ability of a method to distinguish the analyte from others in the sample** Check resolution

Linearity: **How well a calibration curve follows a straight line.** Square of correlation coefficient

Accuracy: **nearness to truth**, check with different methods and spiking

Precision: **reproducibility,** standard deviation

Range: **concentration interval over which linearity, accuracy and precision are all good**

Detection Limits: **defined by signal detection limit: 3s (standard deviation), minimum concentration: 3s/m, m is the slope of the linear curve.**

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Radiation source

Single –beam, double-beam in space, double-beam in time and multichannel detectors

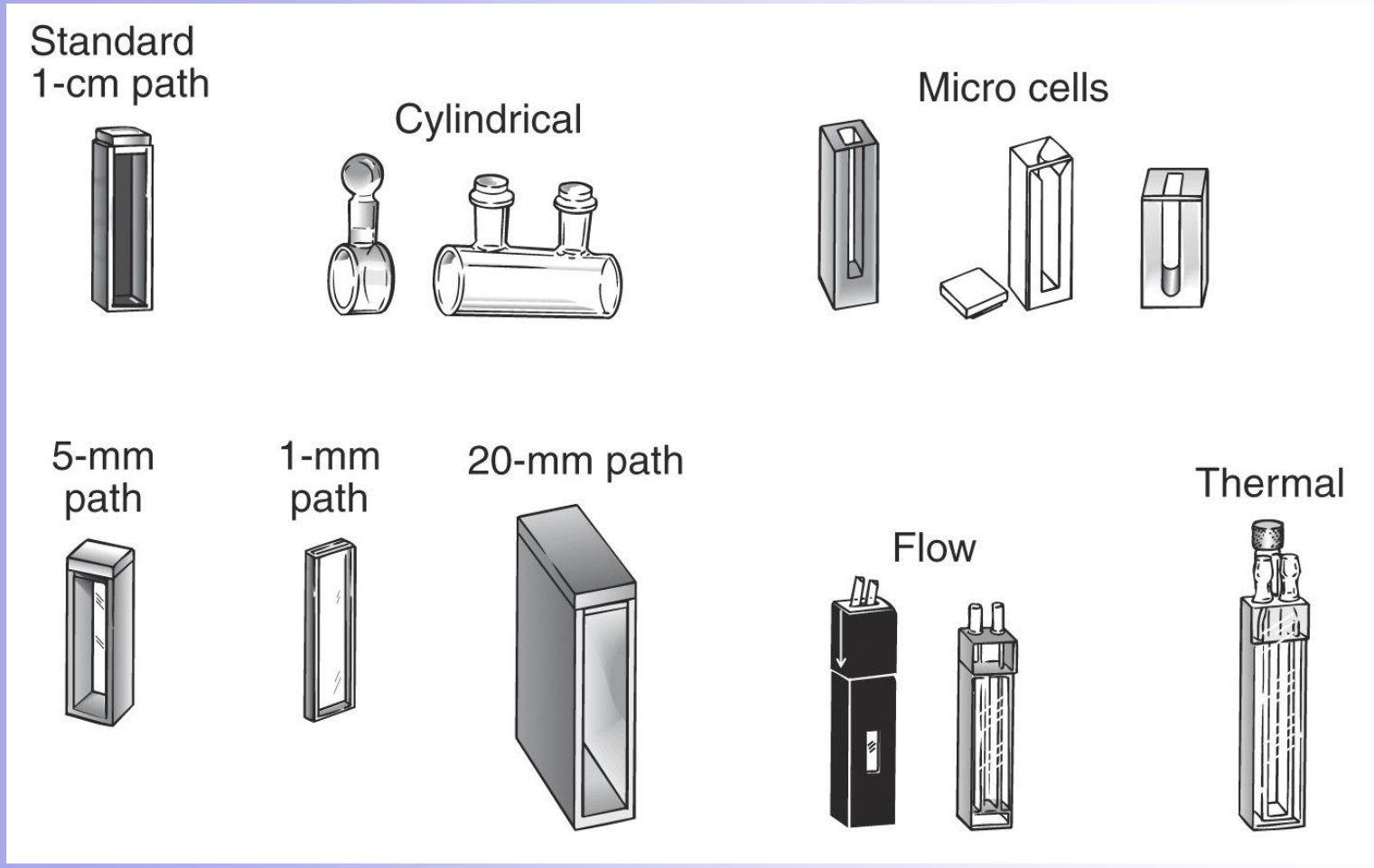
Deuterium or hydrogen arc lamp

Low pressure gas discharge light source, used when a full spectrum source of illumination in the UV region is needed (160-375nm)

Tungsten filament lamp

Used for the visible region (350-2500nm). Voltage to lamp must be stable for energy output to be stable, thus constant voltage transformer is needed.

Sample holders: Cuvettes



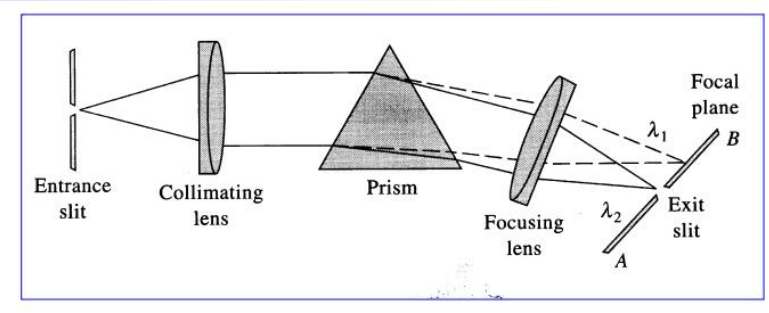
Wavelength Selectors

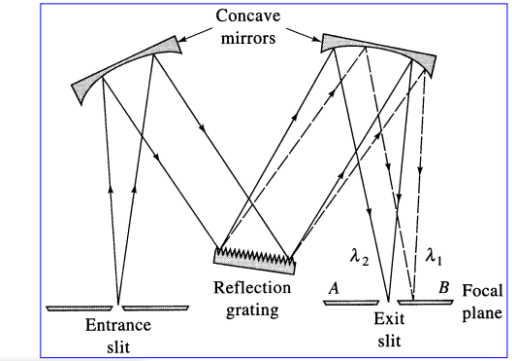
Monochromator

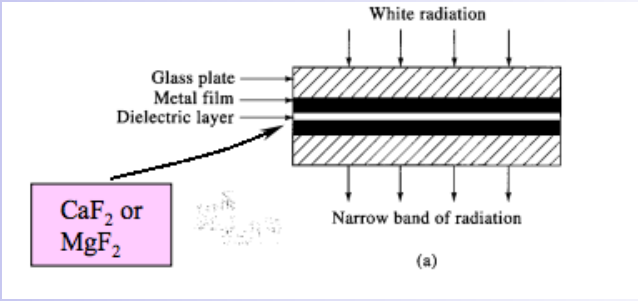
Prism monochromator
Grating monochromator
Echelle grating
Concave grating
Holographic grating

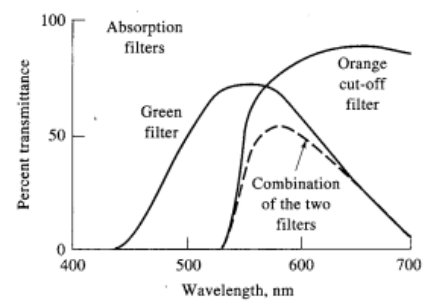
Filter

Interference filters
Interference wedges
Absorption filters









Radiation detector

Photomultiplier

A vacuum phototube that is sensitive to detector of light in the UV, vis, and IR ranges. Multiplies the current produced by light in multiple dynode stages, enabling individual photons to be detected when the incident flux of light is low

Linear Photodiode array

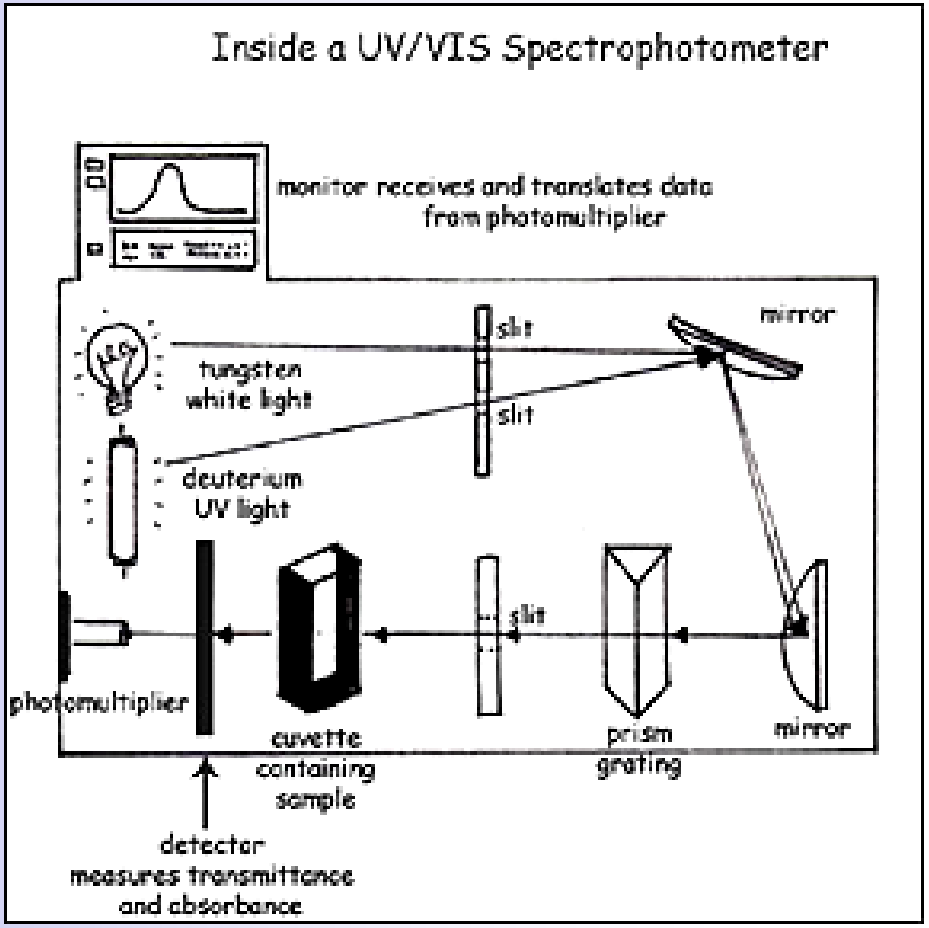
Multichannel photon detector, comprised of small silicon photodiodes, capable of measuring all elements of a beam of dispersed radiation

Charge-coupled devices

Similar to a diode array detector but consist of photo capacitors instead of diodes

Conventional UV-Vis Spectrophotmeter

- Uses prism or grating to select one wavelength at a time
- A wavelength scan requires one to several minutes



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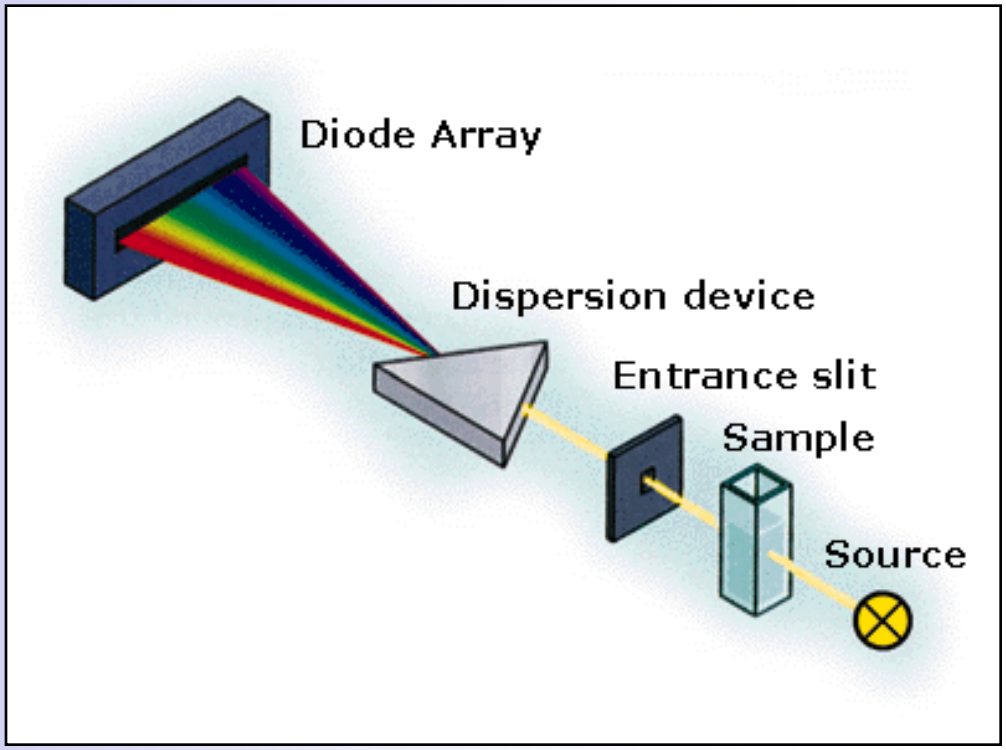
Diode Array UV-Vis Spectrophotmeter

- Diode array photo-sensor eliminates need for moveable wavelength-dispersive grating or prism
- Much more rugged – fewer moving parts
- Nearly instantaneous wavelength scans
- Not good very very detailed (high resolution) wavelength scans



Dipping Colorimeter

- Uses filter instead of prism/grating for wavelength selection
- Relatively low sensitivity
- High ease-of-use – just dip it in
- Relatively cheap
- Ok for estuarine nutrient analyses