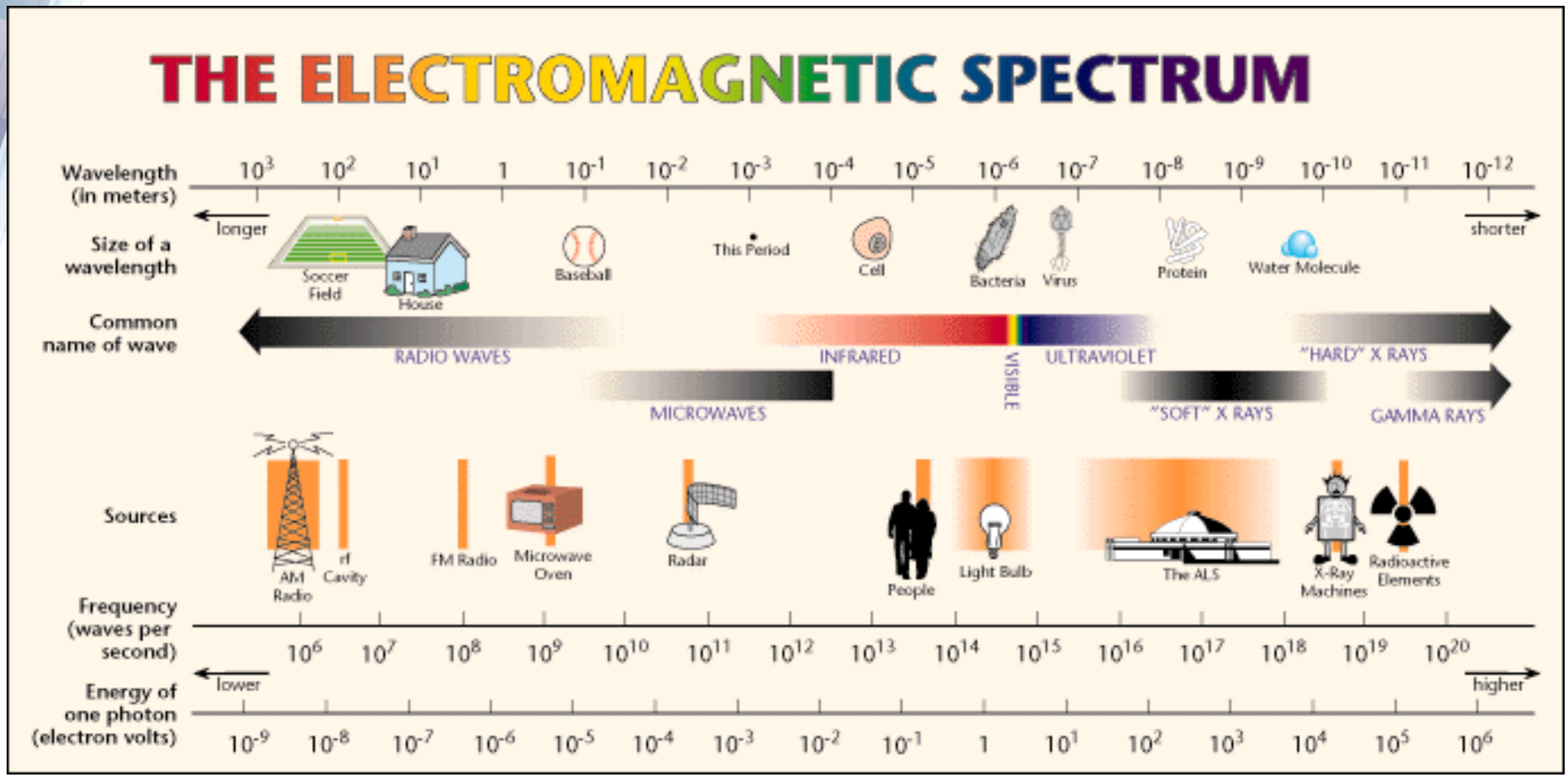
CHAPTER 2

ULTRAVIOLET-VISIBLE ABSORPTION SPECTROSCOPY

Expected Outcomes

Able to discuss the interaction of electromagnetic waves with atomic and molecular species
Describe the transmittance and absorbance
State the functions of each components of instrumentation for optical spectroscopy
Differentiate the type of optical instruments
Describe the function of ultraviolet-visible instrument

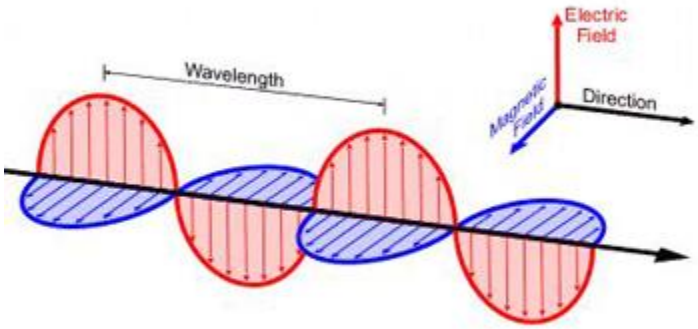
2.1 Electromagnetic spectrum



http://mail.colonial.net/~hkaiter/electromagspectrum.html

Theory of Electromagnetic Radiation

- Charged particles emit or absorb energy in a wavelike form known as electromagnetic radiation.
- This radiation consists of two components: an electric field and a magnetic field



- EM radiation travels at the speed of light, in a vacuum
- The basic unit of EM radiation is the photon,

Theory of Electromagnetic Radiation

Electromagnetic Radiation

- ***Wave properties:***

$$v = \frac{c}{\lambda}$$

c = velocity of light (3.0×10^8 ms^{-1} in vacuum)
λ = wavelength (m)
v = frequency (cycles/s, cps, Hz)

- **Particle properties (Photon energy)**

$$E = hv \qquad E = \frac{hc}{\lambda}$$

E = energy of quantum (J)

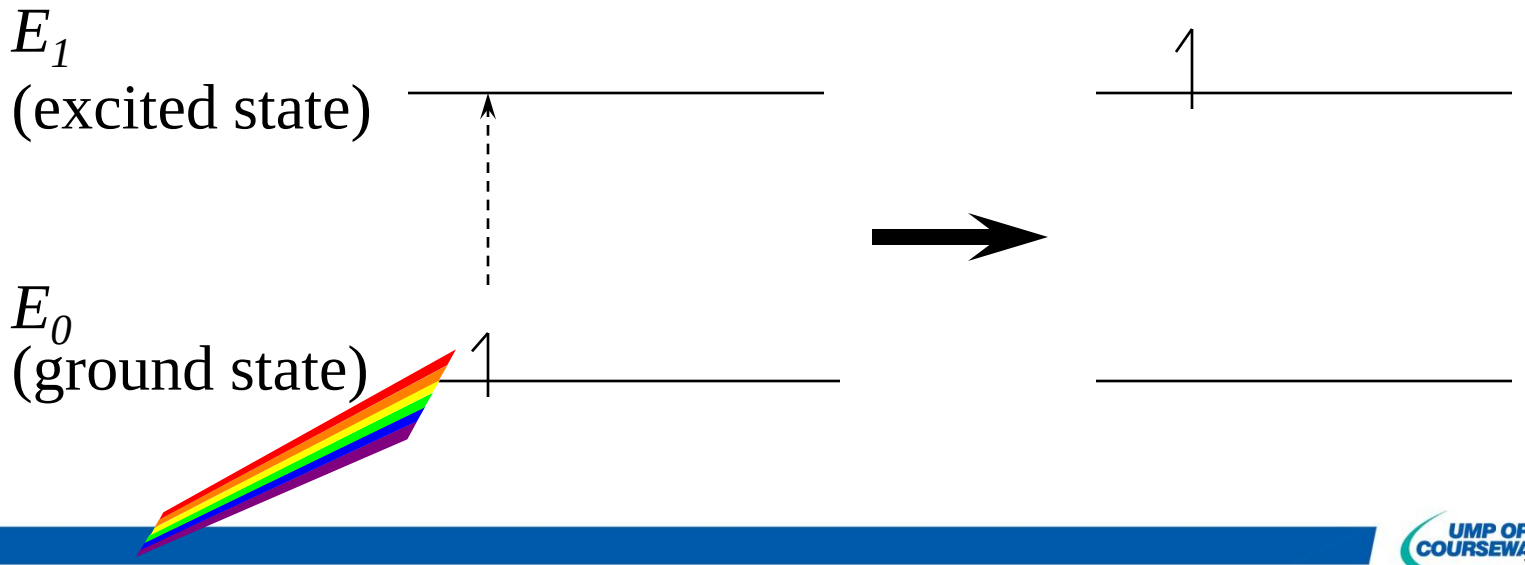
h = Planck's constant (6.63 x 10-34 J.s)

v = frequency

Theory of Electromagnetic Radiation

Absorption of Radiation

- The energy difference between the two energy states, E_1 and E_o, must be equal to the energy of the absorbed radiation ($E = hv$).



Theory of Electromagnetic Radiation

Atomic Absorption

- When atoms absorb radiation, only e^-s are excited.
- **Line spectrum** can be observed.
- **Electronic transition -** The transition of an e^- between two orbitals.

Theory of Electromagnetic Radiation

Molecular Absorption

- Molecules undergo three types of quantized transitions when excited by ultraviolet, visible, & infrared radiation:

Electronic transition

Vibrational transition

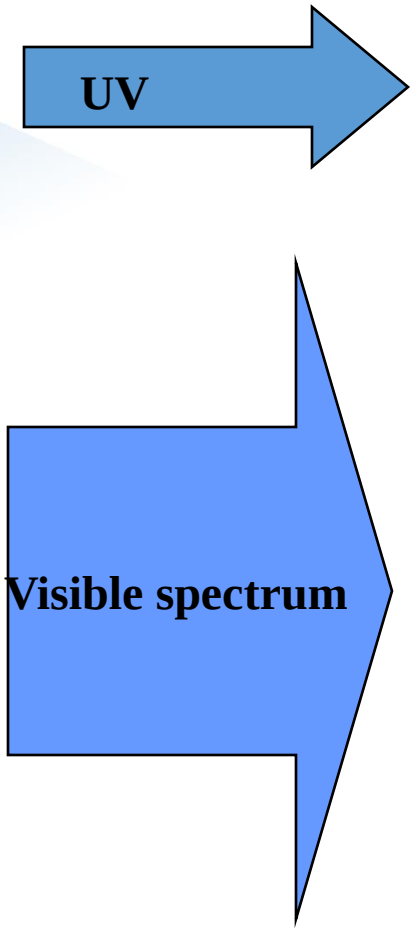
Rotational transition

Decrease in energy

Absorption and Emission

- Absorption of Radiation
  - When light passes through an object, the object will absorb certain of the wavelengths, leaving the unabsorbed wavelengths to be transmitted.
  - These residual transmitted wavelengths will be seen as a colour.
  - The absorption can be detected by suitable detectors

Absorption and Emission

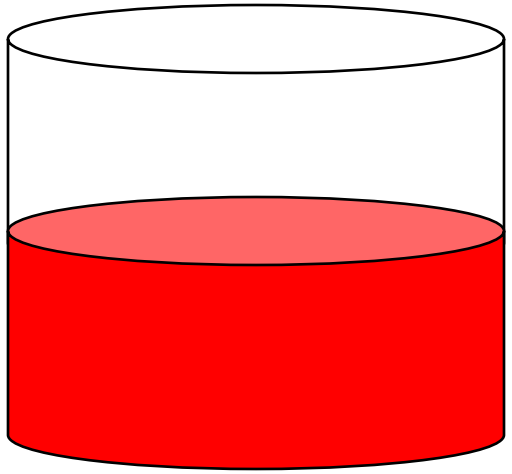


nm	Colour	Complimentary
~180-380	UV	-
400-435	Violet	Yellow-green
435-480	Blue	Yellow
480-490	Blue-green	Orange
490-500	Green-blue	Red
500-560	Green	Purple
560-580	Yellow-green	Violet
580-595	Yellow	Blue
595-650	Orange	Blue-green
650-750	Red	Green-blue

Question?

- **Why is the red solution red?**

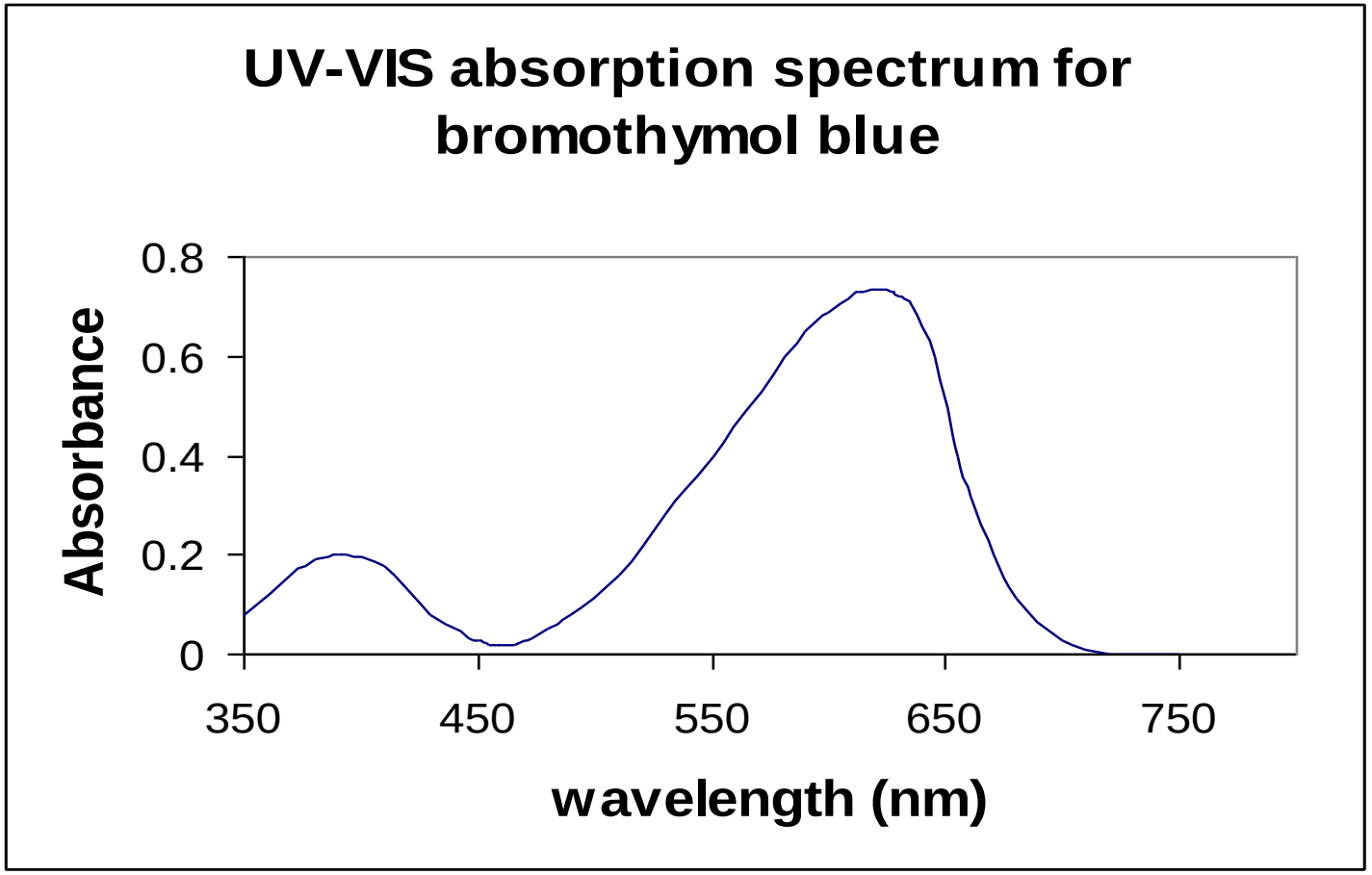
Because the object absorbs green component from the incoming white radiation and thus transmits red components.



Absorption and Emission

A plot of the amount of radiation absorbed by a sample as a function of the wavelength is called an ***absorption spectrum***

Absorption and Emission



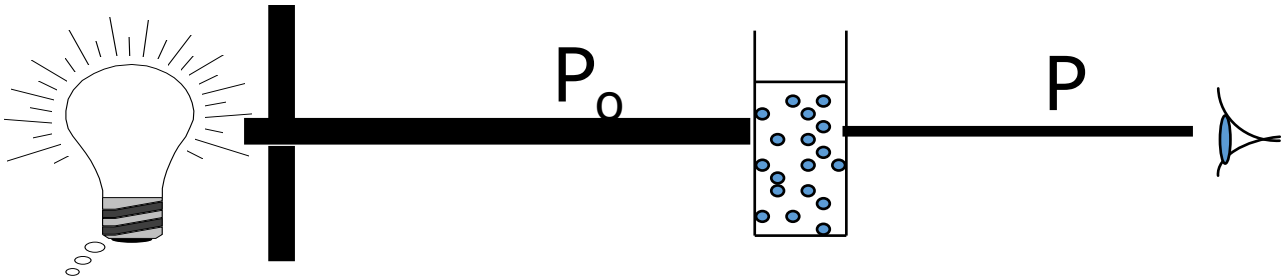
Absorption and Emission

- Emission of a photon occurs when an analyte in a higher energy state returns to a lower energy state.
- The higher energy state can be achieved by heat, photon and chemical reaction
- The emission of the sample itself provides the light and the intensity of the light emitted is a function of the analyte concentration.

- When molecules or atoms are in the excited state, they are very unstable and will lose their energy of excitation and drop back to a lower energy state or the ground state – relaxation.

- The excess energy is released in the form of electromagnetic radiation, heat, or both.

The Beer's Law



P_o = initial radiant power

P = final radiant power

Transmittance: $T = P / P_o$,

% Transmittance: $\%T = P / P_o \times 100\%$

Absorbance:

$$A = \log\left(\frac{P_o}{P}\right) = \log\left(\frac{1}{T}\right) = \varepsilon bc$$

ε: L mol^{-1} cm^{-1}; b: cm; c: mol L^{-1}

The Beer's Law

Transmittance (T): the ratio of the radiant power (P) in a beam of radiation after it has passed through a sample to the power of the incident beam (P_o).

Absorbance (A) is also known as the optical density, = log (base 10) of the reciprocal of the transmittance (T).

Exercise 2

What values of absorbance correspond to 100%T, 10%T, and 1%T?

Solution:

100%T, 10%T, and 1%T correspond to transmittances of 1.00, 0.10 and 0.010.

From the definition of A:

100%T has A = -log1 = 0

10%T has A = -log0.10 = 1.0

1%T has A = -log0.010 = 2.0

The Beer's Law

Absorption of radiant energy

When radiation of specified wavelength is passed through a solution containing only one solute which absorbs that wavelength, the absorbance (no units) can be calculated by:

$$A = \varepsilon b c$$

A = Absorbance

b = Optical path distance (cm)

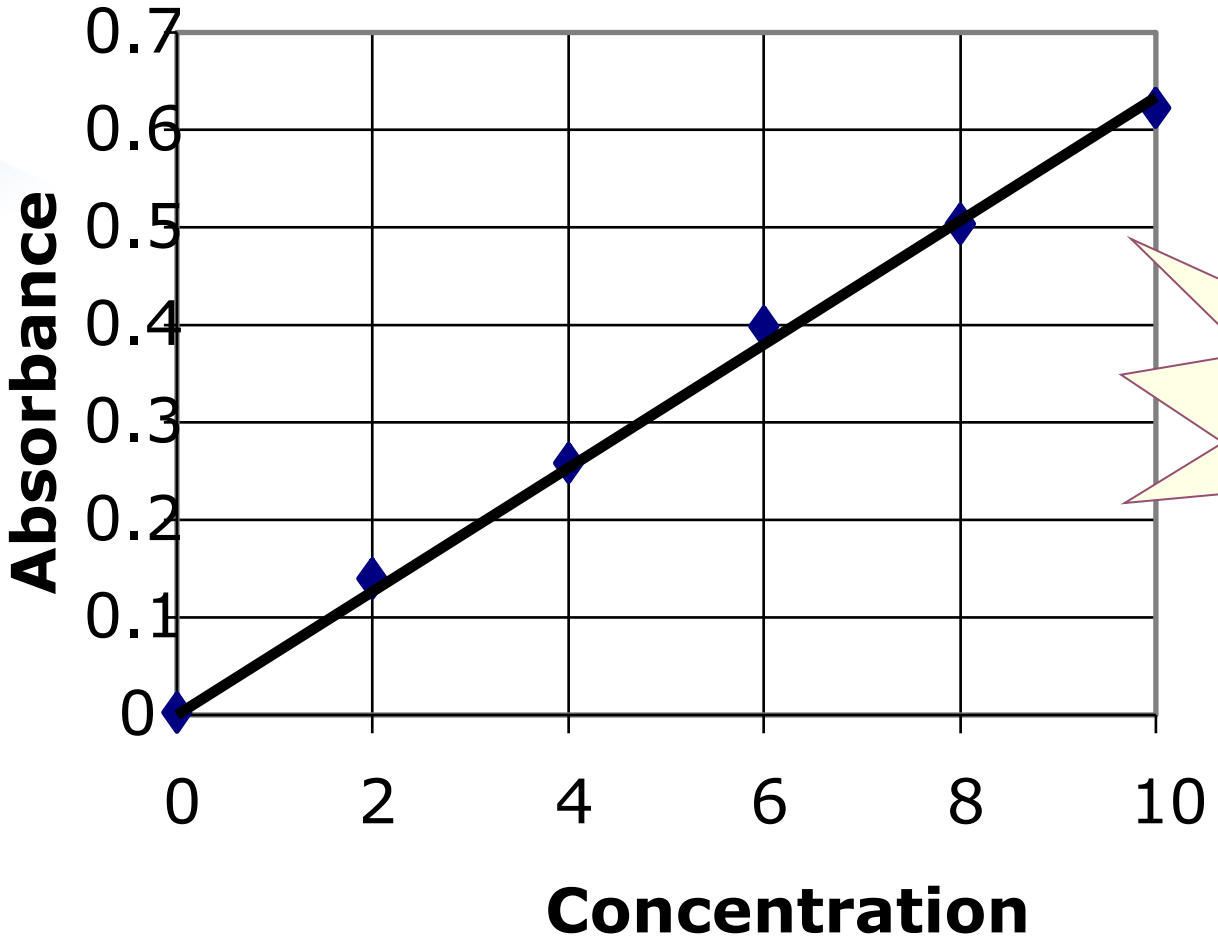
ε = Molar absorptivity ($M^{-1}cm^{-1}$)

c = Concentration (M)

Exercise 3

Monochromatic light was passed through a 1.00 cm cell containing a 0.0100M solution of a given substance. The absorbance obtained was 0.245. Calculate the molar absorptivity of the substance.

The Beer's law



$A = \varepsilon bc$

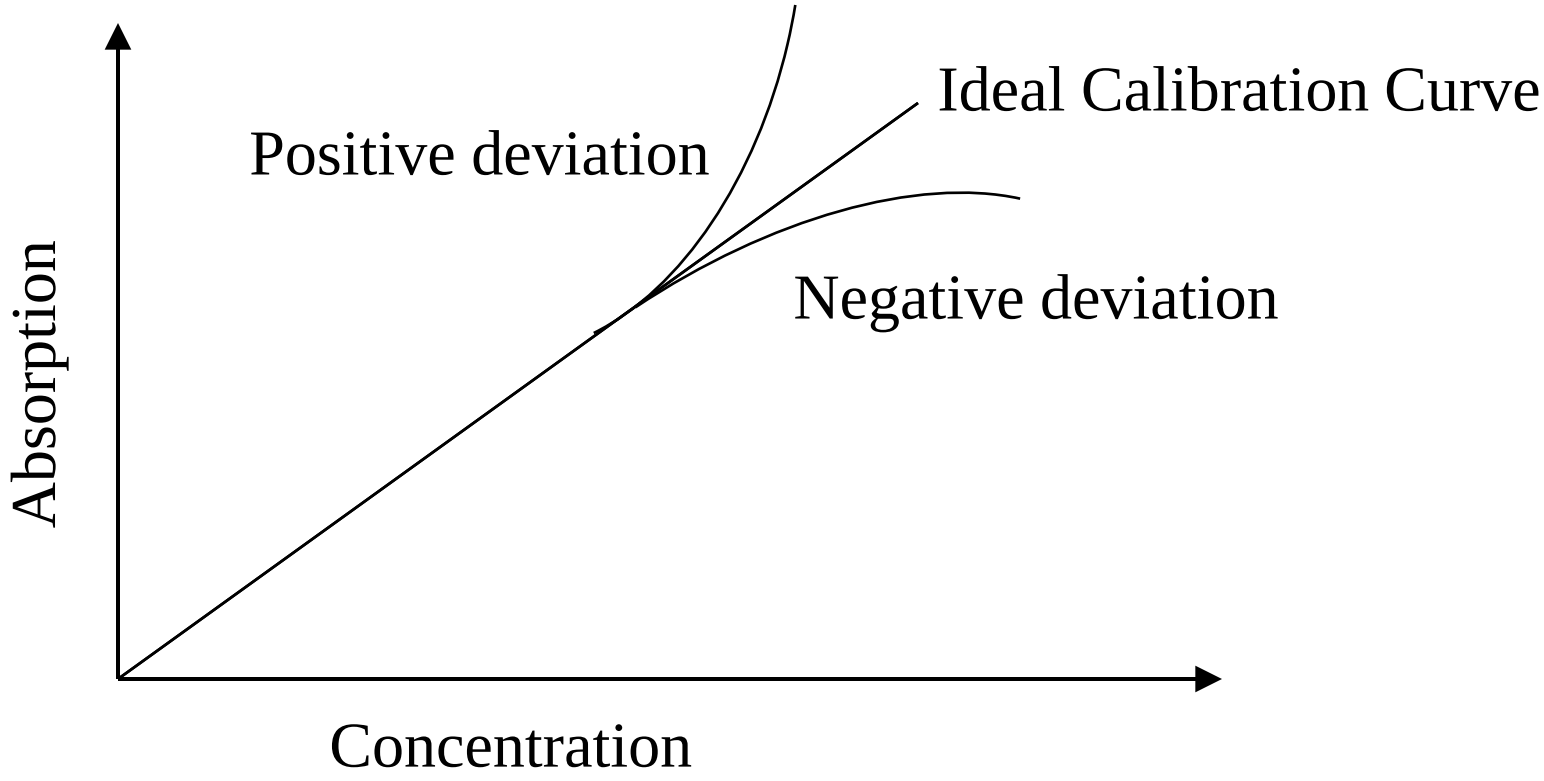
The Beer's Law

Deviation from Beer's Law

Non-linear curve may be obtained due to:

1. Fundamental limitations of Beer's law
2. Chemical deviations
3. Instrumental deviations

The Beer's Law



The Beer's Law

Fundamental limitations of Beer's law

Beer's Law is only valid for:

- **Low** concentration of analyte.
- At **high** conc. (usually >0.01M):

  → the individual particles of analyte no longer behave independently of one another

  → The absorbance changes

  - value of ε changed
  - deviation from Beer's Law

High concentration of solute may result in a shift of maximum absorption, and may also change the value of the molar absorptivity, ε.

The Beer's Law

- ε depends on sample's refractive index (RI).
- Thus, ε may change at high conc but at low conc, RI remains essential constant.
- Calibration curve will be linear.

2. Chemical limitations of Beer's law

- Occur for absorbing species that are involved in an **equilibrium** reaction.

For example in the case of weak acid and conjugate base

The Beer's Law

Example

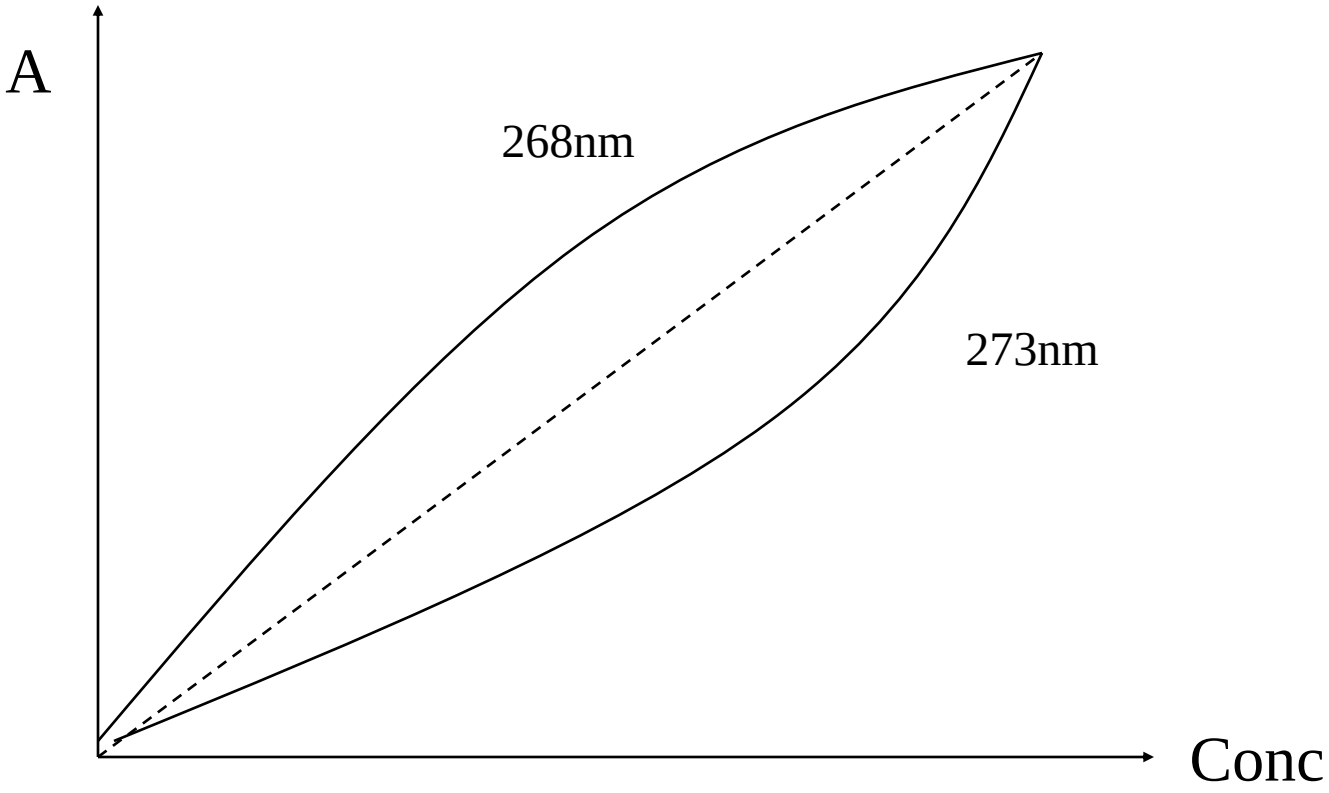
Benzoic acid exists as a mixture of ionised and unionised form:

$C_6H_5COOH + H_2O \rightleftharpoons C_6H_5COO^- + H_3O^+$

(λ_{max} = 273 nm) (λ_{max} = 268nm)

In higher dilution, or higher pH, more ionised benzoate is formed, thus the absorbance at 273nm decreases. On the other hand, at lower pH, benzoic acid remains in its unionised form whereby the absorbance at 273 nm is optimised.

The Beer's Law

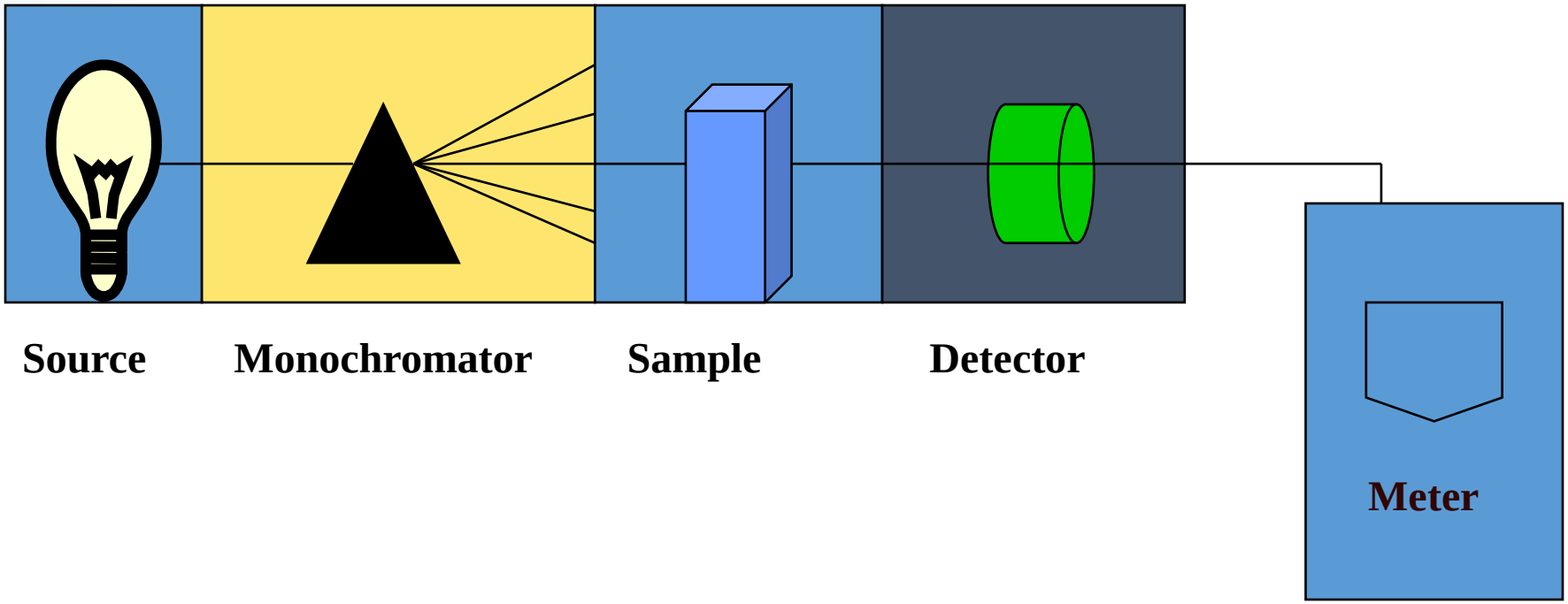


The Beer's Law

3. Instrumental deviations

- Beer's law is only valid for **monochromatic** radiation.
- The output from a continuous source (D_2 lamp) will always produce a specific band width (about ±5 nm).
- e.g. 220 nm may imply 215 to 225 nm

2.3 Components of Instrumentation for Optical Spectroscopy

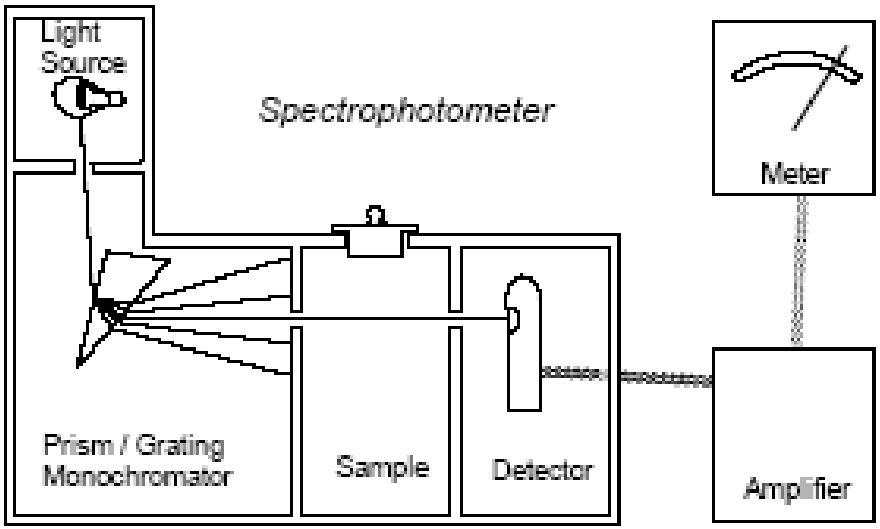


Components of Instrumentation for Optical Spectroscopy

Components

Most spectroscopic instruments are made up of :

1. source
2. wavelength selector
3. sample container
4. detector
5. signal processor and readout



http://analytical.biochem.purdue.edu/~courses/undrgrad/221/wwwboard/handouts/supplemental/spectrophotometry.pdf

Components of Instrumentation for Optical Spectroscopy

Spectroscopic sources

A good radiation source should have the following characteristics :

- the beam emits radiation over a wide range of the spectrum.
- sufficient intensity to be detectable.
- stable

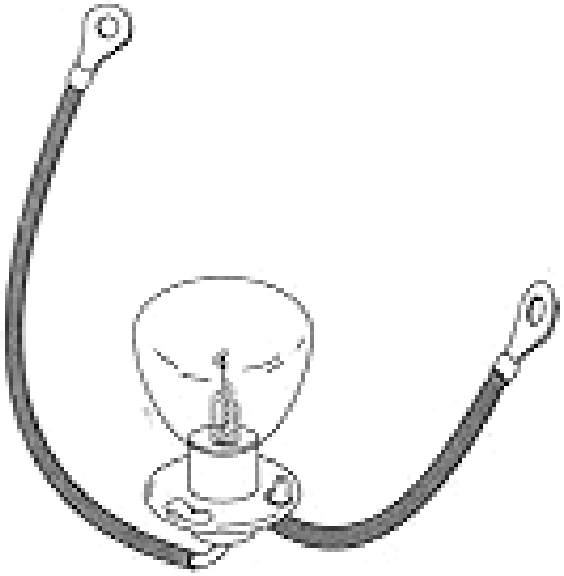
Components of Instrumentation for Optical Spectroscopy

- Line source – consists of one or more very narrow bands of radiation whose wavelengths are known exactly (Hollow cathode lamp)

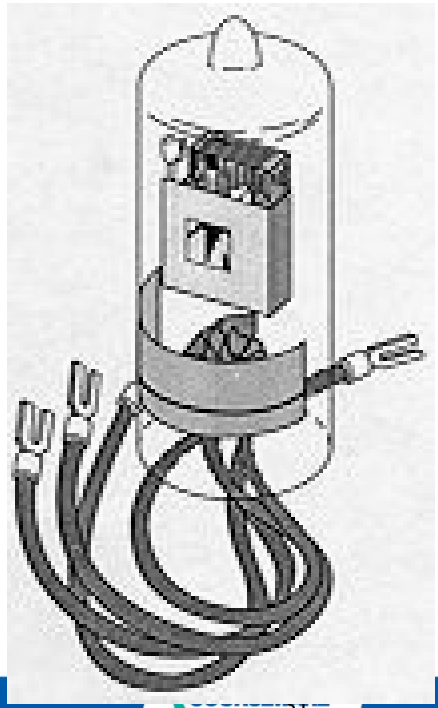
- Continuous source – produces radiation of all wavelengths within a certain spectral region (D_2 lamp)

Components of Instrumentation for Optical Spectroscopy

- Source of radiant energy for:
  - Visible region: tungsten filament lamp
  - UV region: deuterium discharge lamp (D_2)



(a) A tungsten lamp.



(a) A deuterium lamp

[Picture taken from Analytical Chemistry by Gary D. Christian Page 530 and 531]

Components of Instrumentation for Optical Spectroscopy

Wavelength selector

- **Monochromator** (Monochrome = “one colour”)

  to spread out or disperse light into its component wavelengths and select the required wavelength for analysis.

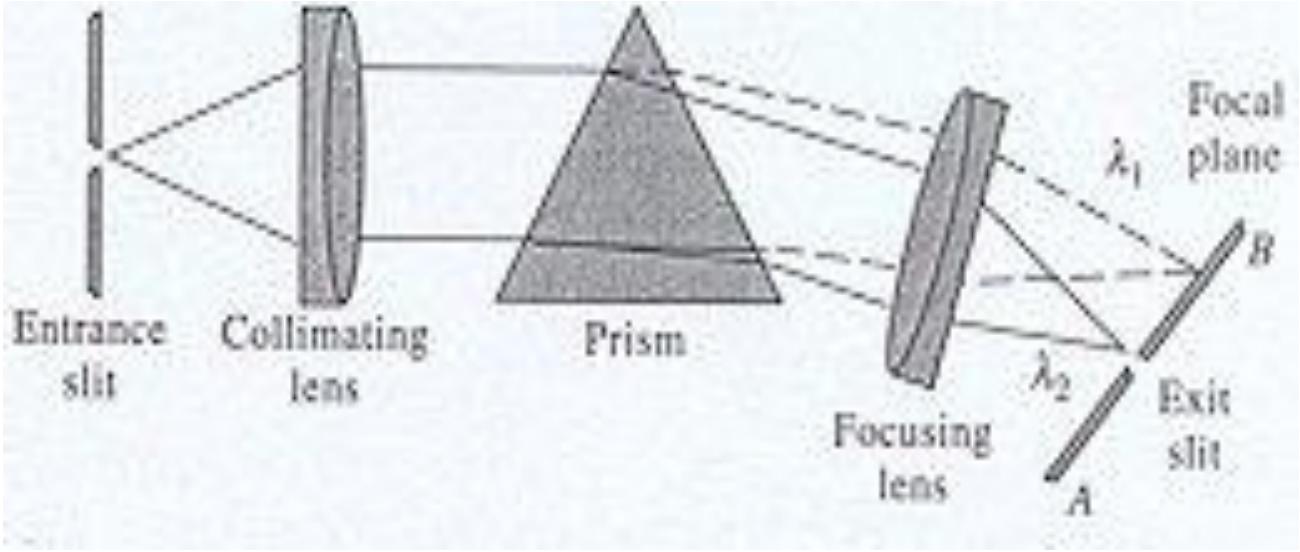
Components of Instrumentation for Optical Spectroscopy

- Function of monochromator
  - Restricts the wavelength that is to be used to a narrow band
  - Enhance the selectivity and sensitivity of an instrument
  - Narrow bandwidth => better adherence to Beer's law
  - Not possible to produce radiation of a single wavelength

Components of Instrumentation for Optical Spectroscopy

- Components of a monochromator:
  - Entrance slit (restrict unwanted radiation)
  - Dispersing element (separate the wavelengths of the polychromatic radiation)
    - prism
    - reflection grating
  - Exit slit – adjustable (control the width of the band of wavelengths)

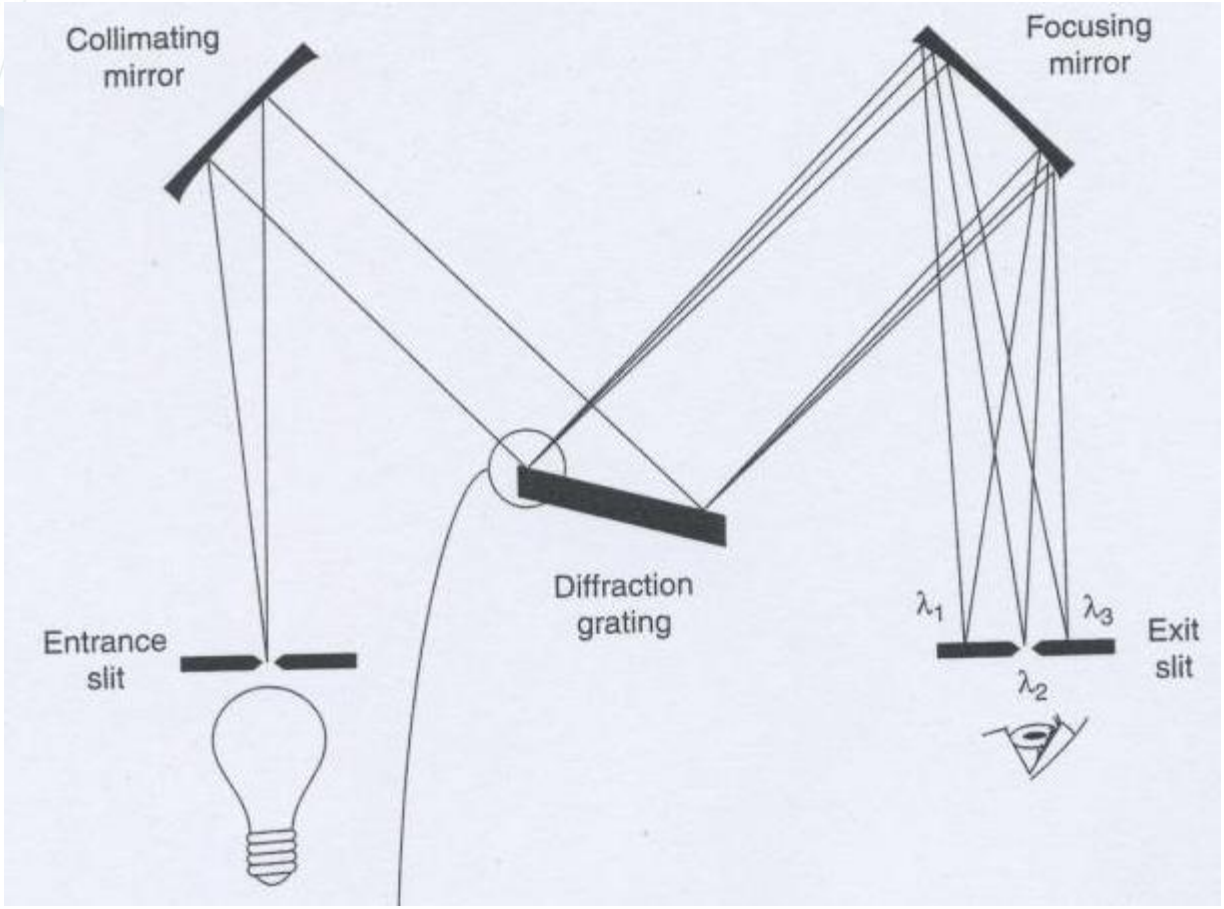
Components of Instrumentation for Optical Spectroscopy



Bunsen prism monochromator

[Picture taken from Fundamentals of Analytical Chemistry by Douglas A. Skoog, Donald M. West and F. James Holler Page 536]

Components of Instrumentation for Optical Spectroscopy



Grating Monchromator

[Pcture taken from Modern Analytical Chemistry by David Harvey, pg 378]

Components of Instrumentation for Optical Spectroscopy

Sample container

- Must be transparent in the wavelength region being measured
- UV-VIS spectroscopy
  - UV region: cell or cuvette of quartz
  - VIS region: cell or cuvette of quartz/glass/plastic

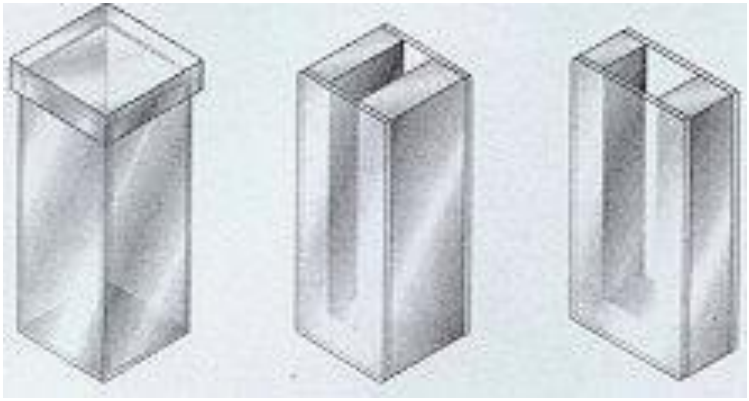
Components of Instrumentation for Optical Spectroscopy

- Cells should be optically matched (in pair).

- Selection of cells depends on:
  - wavelength of radiation used
  - amount of sample available
  - nature of sample → liquid (aqueous / organic) or gas

Components of Instrumentation for Optical Spectroscopy

- Size of curvette = 1cm x 1cm (square base)
- Volume used= 0.5ml to 2ml depending on the sample size.



[Picture taken from Analytical Chemistry by Gary D. Christian Page 427]

Some typical UV and visible absorption cells

Components of Instrumentation for Optical Spectroscopy

Detector

- Photons are detected by:
  - Photoemission or
  - Photoconduction

All photon detectors are based on the interaction of radiation with a reactive surface to produce electrons (photoemission) or to promote electrons to energy states in which they can conduct electricity (photoconduction). Only UV, visible and near-IR radiation have sufficient energy to cause these processes to occur.

Components of Instrumentation for Optical Spectroscopy

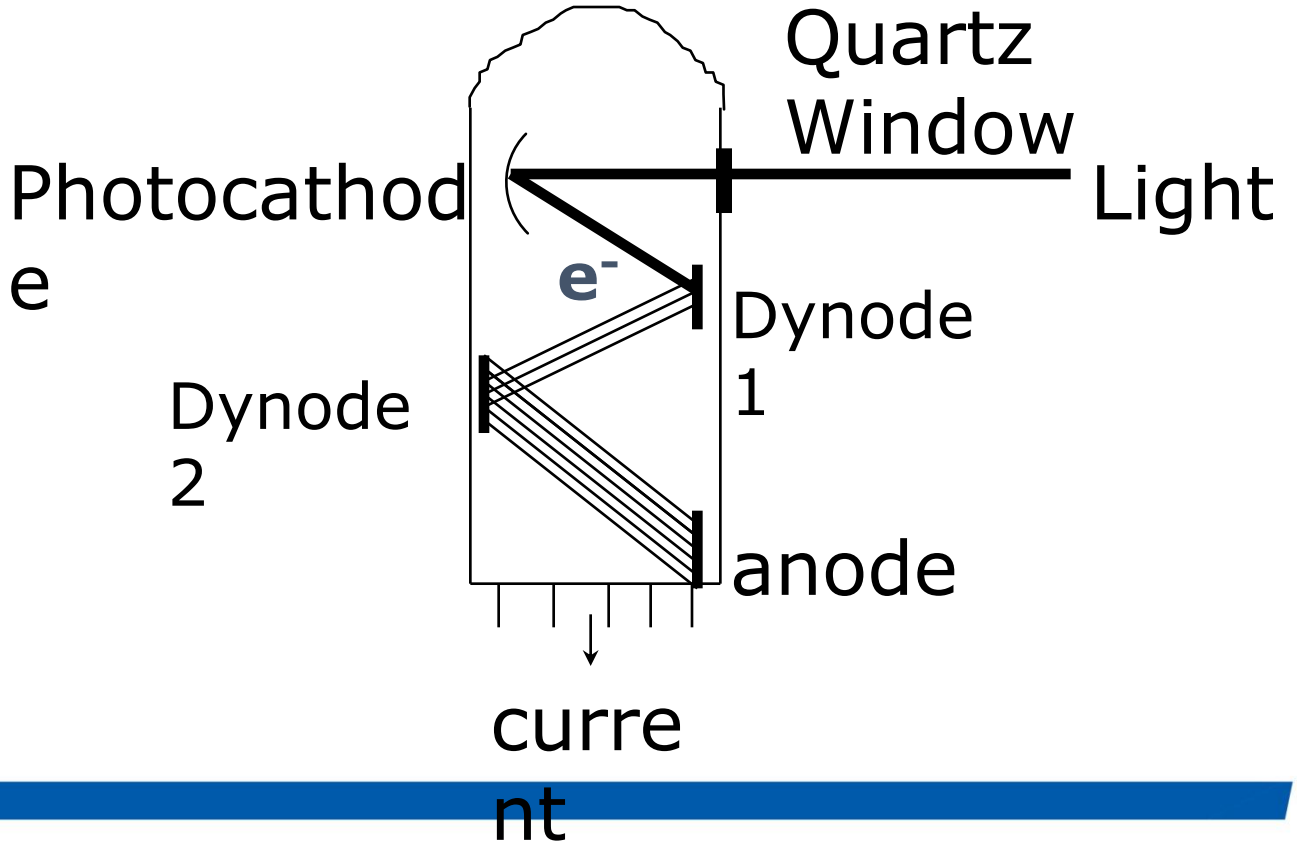
Photomultiplier tubes

A photomultiplier contains a photo-emissive cathode and several anodes (dynodes) in a vacuum. The cathode is coated with an easily ionized material such as alloys of alkali metals (K, Na, Ca, Mg) with Sb, Bi and / or Ag.

A photon falling on the surface of the cathode causes the emission of an electron, provided the photon is sufficiently energetic to ionize the material. The signal is amplified by the process of secondary emission (as shown on next slide). The electron amplification depends on the voltage.

Components of Instrumentation for Optical Spectroscopy

- ***Photomultiplier Tube***



Components of Instrumentation for Optical Spectroscopy

- ***Photomultiplier Tube***

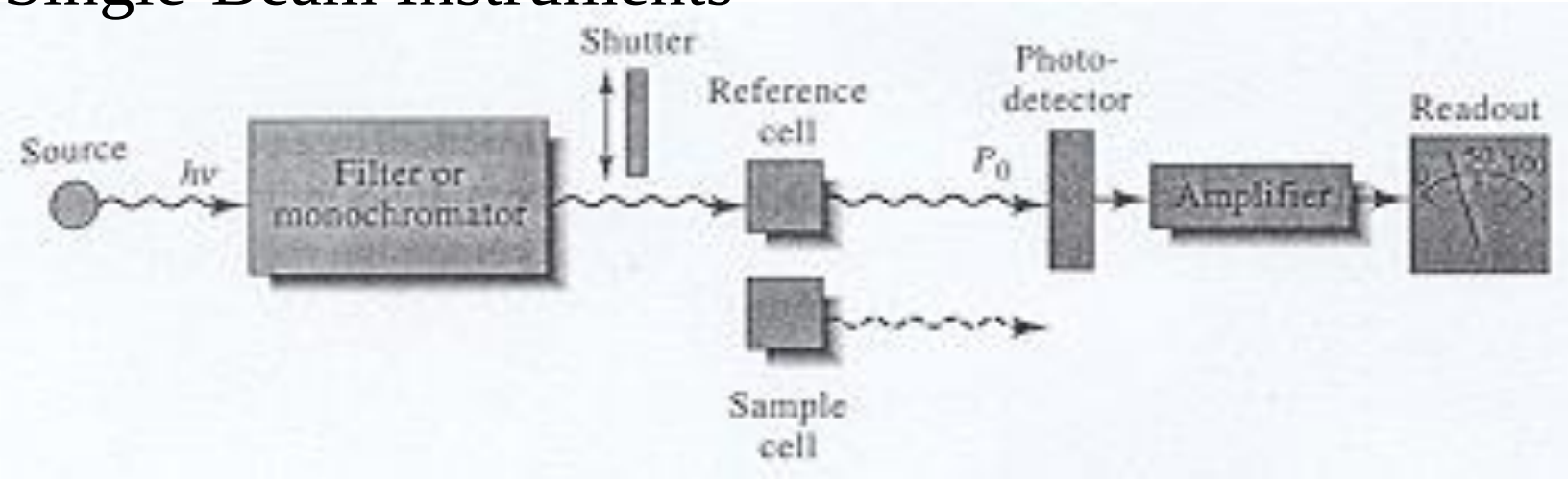
- The emitted electrons accelerate towards the dynode 1, which has higher voltage than cathode.
- Upon striking the dynode 1, each accelerated photoelectron produces more electrons. These electrons again accelerated to dynode 2, which again has a higher voltage than dynode 1. This results in electron amplification where it is finally collected at anode to measure the current electronically.

Optical Instruments

- **Single Beam** -
  - Sample and reference cells are read at **different** time.
  - Recalibration with the blank is necessary periodically due to fluctuations and wavelength changes from the radiation source.
- **Double Beam** -
  - Monochromatic beam is split into two components. (one beam for **sample**, one beam for **reference**)

2.4 Type of Optical Instruments

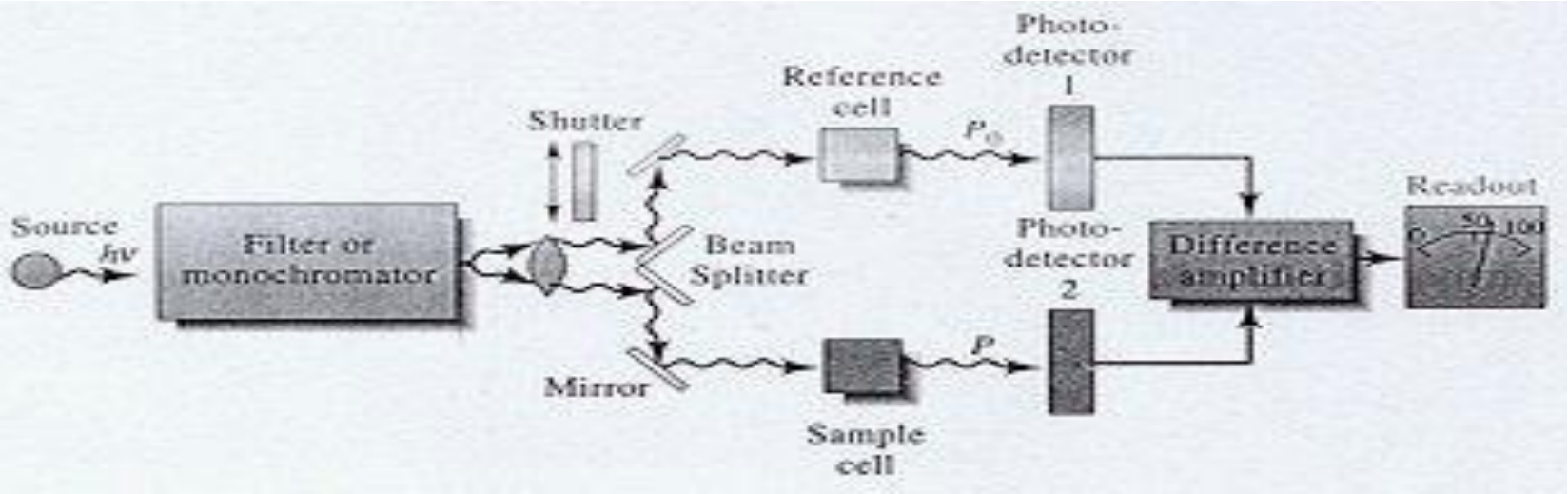
Single-Beam Instruments



[Picture taken from Fundamentals of Analytical Chemistry by Douglas A. Skoog, Donald M. West and F. James Holler Page 553]

Optical Instruments

Double-beam Instrument



[Picture taken from Fundamentals of Analytical Chemistry by Douglas A. Skoog, Donald M. West and F. James Holler Page 553]

Optical Instruments

Advantages of double - beam spectrophotometer:

1. Sample and reference cells can be read simultaneously.

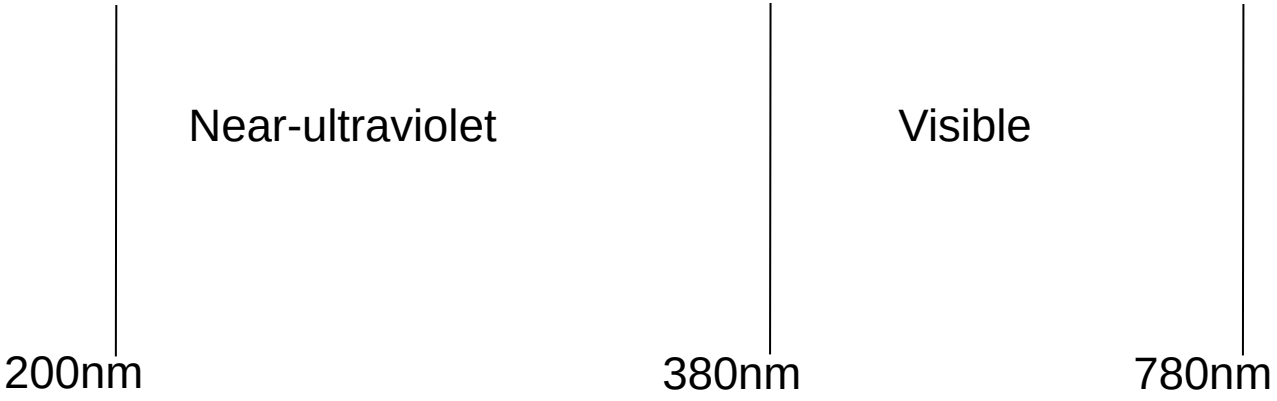
2. Compensate the fluctuations and wavelength changes from the radiation source.

3. Allows continuous recording of spectra (absorbance as a function of wavelength).

2.5 UV-VIS absorption spectrometry

Introduction

- Visible and UV Spectroscopic regions & associated energy levels :



Absorption in the UV-VIS Region

- The UV region extends from about 10 – 380nm, but the most analytically useful region is from 200 – 380nm, called the near-UV region. Below 200nm, the air absorbs substantially and the instruments are operated under vacuum conditions (vacuum-UV region).
- Absorption in the UV and visible region result in electronic transition.
- The visible region is the wavelength that can be seen by the human eyes. It extends from the near-UV region (380nm) to about 780 nm.

Absorption in the UV-VIS Region

Absorbing Species (Chromophores)

- The wavelength at which an organic molecule absorbs depends upon how tightly its electrons are bound.
- Shared electrons in single bonds of organic compounds are very firmly held => absorption at wavelengths in the vacuum UV region (<180 nm) => not used for analytical purposes.
- Electrons in double and triple bonds are not as strongly held => useful absorption peaks in the UV region.
- The absorbing groups (unsaturated organic groups) in a molecule are called **chromophores** but a *molecule containing a chromophore* is called a **chromogen.**

Absorption in the UV-VIS Region

What can be analysed?

- Chemical species that can absorb UV-VIS radiation
- Chemical species that can form soluble complexes with chromophoric reagents

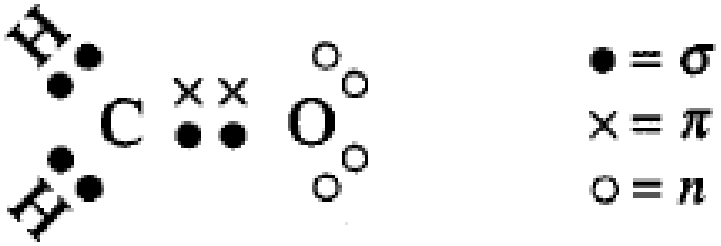
Absorption in the UV-VIS Region

- Chemical species that can absorb UV-VIS radiation
  - Organic chromophoric groups: unsaturated bonds
  - Saturated organic cpds with heteroatoms such as O, N, S or halogens
    - Containing non-bonding electrons that can be excited within 170 – 250 nm for absorption.
  - Ions of the transition metals
    - Transition between filled and unfilled d-orbitals
    - First two rows of transition metals - coloured

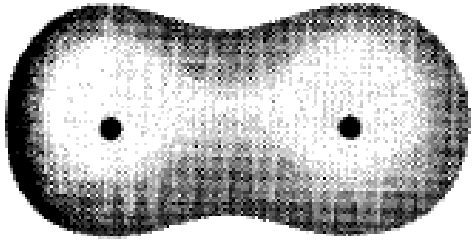
Absorbing species

- Electronic transitions

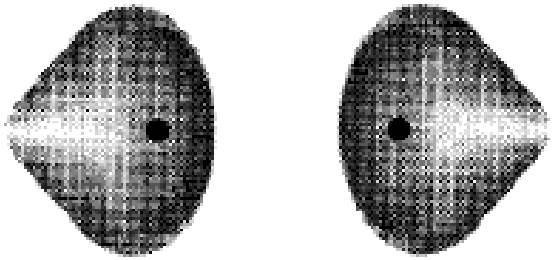
  π, σ, and n (non-bonding) electrons



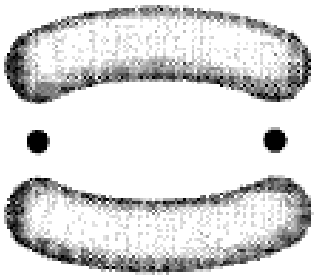
Sigma and Pi orbitals



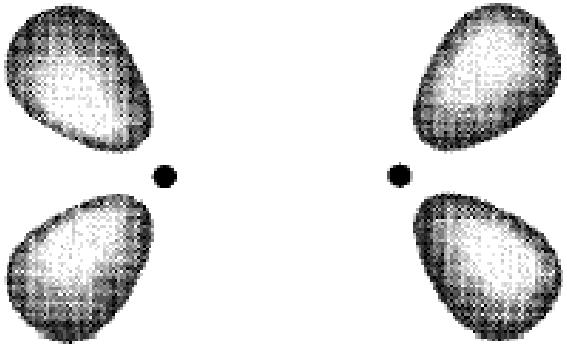
(a) σ orbital



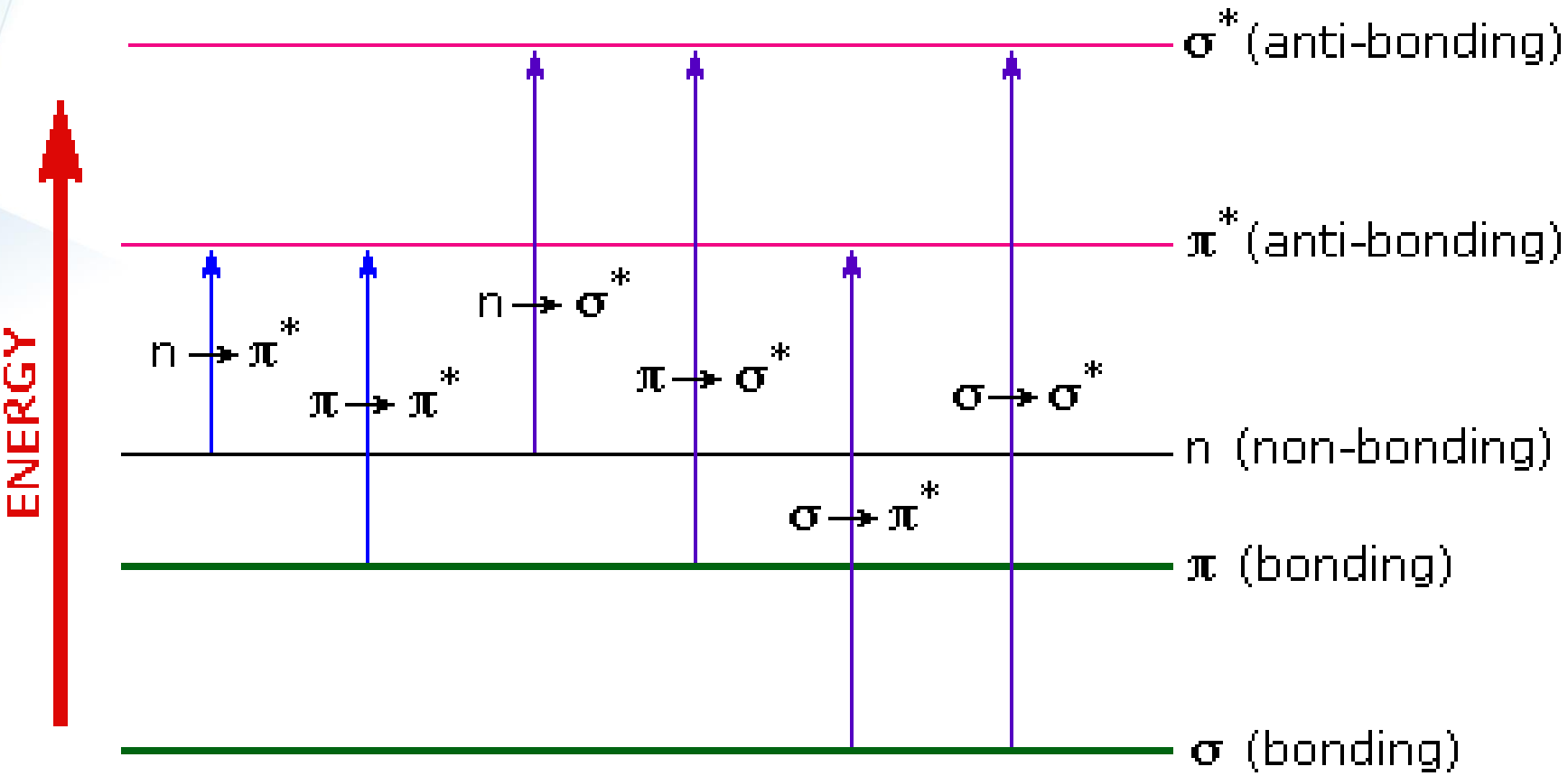
(c) σ* orbital



(b) π orbital



(d) π * orbital


σ* (anti-bonding)
π* (anti-bonding)
n → σ*
n → π*
π → π*
π → σ*
σ → σ*
n (non-bonding)
σ → π*
π (bonding)
σ (bonding)
ENERGY

Chromophore	Example	Excitation	λ_{max}, nm	ε	Solvent
C=C	Ethene	π —> π*	171	15,000	hexane
C≡C	1-Hexyne	π —> π*	180	10,000	hexane
C=O	Ethanal	n —> π* π —> π*	290 180	15 10,000	hexane hexane
N=O	Nitromethane	n —> π* π —> π*	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	Methyl bromide Methyl Iodide	n —> σ* n —> σ*	205 255	200 360	hexane hexane

Absorption in the UV-VIS Region

- Chemical species that can form soluble complexes with chromophoric reagents.

Examples

→ thiocyanate ion for iron, cobalt, & molybdenum;

→ diethyldithiocarbamate for copper;

→ diphenylthiocarbazone for lead;

→ 1,10-phenanthroline for iron.

2.5.1 Absorption in the UV-VIS Region

In spectrometric analysis, there are two major approaches:

- One is to measure the radiant energy absorbed by the ions or molecule itself. Most organic species and some inorganic species absorb ultraviolet or visible radiation and can be used for spectrophotometric analysis.
- The other approach is used on species that do not absorb significant amounts of light through the formation of **Complex Formation**. A suitable chemical reagent is added to convert them to a new species that absorbs light strongly.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

- The application of UV-VIS spectroscopy in qualitative analysis is limited due to:
  - the absorption bands tend to be broad and hence lacking in details
  - several absorbing compounds might absorb over similar wavelength ranges and the absorption spectra might overlap considerably.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

Quantitative Analysis

- Standard Curve Method
- (External Standard Method)
- Standard Addition Method

2.5.2 Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

Standard Curve Method

(External Standard Method)

The standard curve method is carried out as follows:

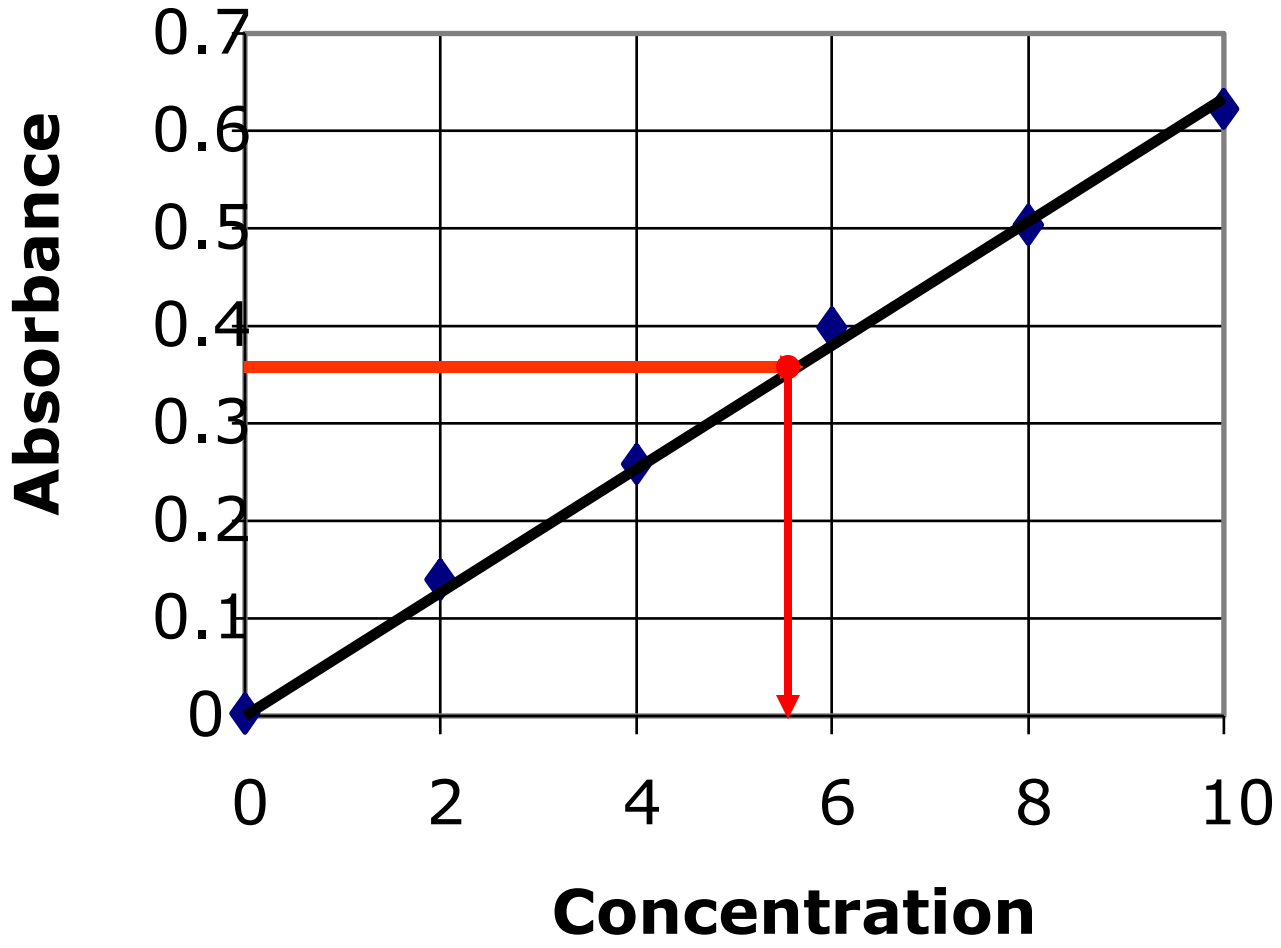
1. Ensure only the analyte in solution will absorb UV-VIS radiation strongly and not other substances in the sample.
2. Select the measurement wavelength, λ_{max}, which is most sensitive to the analyte (i.e. maximum absorbance)
3. Select an appropriate range of standard solutions.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

4. Prepare standard solutions and sample.
5. Take absorbance readings of standard solutions and plot absorbance vs. concentrations.
6. Take absorbance of sample and read off from calibration graph.

Absorbance of unknown solution must be within linear line. If it is outside range, dilution must be carried out.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy



Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

- Ideally, calibration standards should be similar to the composition of the sample to minimize effects of various other components (**matrix effect/interference**).

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

Example:

Testing of phosphate content in cola samples

$$PO_4^{3-} + \text{Reagent} \rightarrow \text{Yellow colour complex}$$

The cola colour itself will impart a darker colour as compared to the standard solutions. This background colour from the sample causes matrix (or background) interference. Thus it actually gives a higher absorbance reading.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

- To overcome this matrix interference
  - Include a “sample blank” in the standard curve method.
    - Sample Blank = contains solvent + sample (same dilution as the sample solution) except the reagent for complex reaction.
  - Use Standard-Addition method.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

Standard Addition Method

This method is used when:

1. The solid or liquid *matrix* of a sample is either *unknown* or so *complex* that an external standard cannot be used with confidence.
2. The chemistry of the preparation or of the experimental method is complex or highly variable.

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

The standard addition method is carried out as follows:

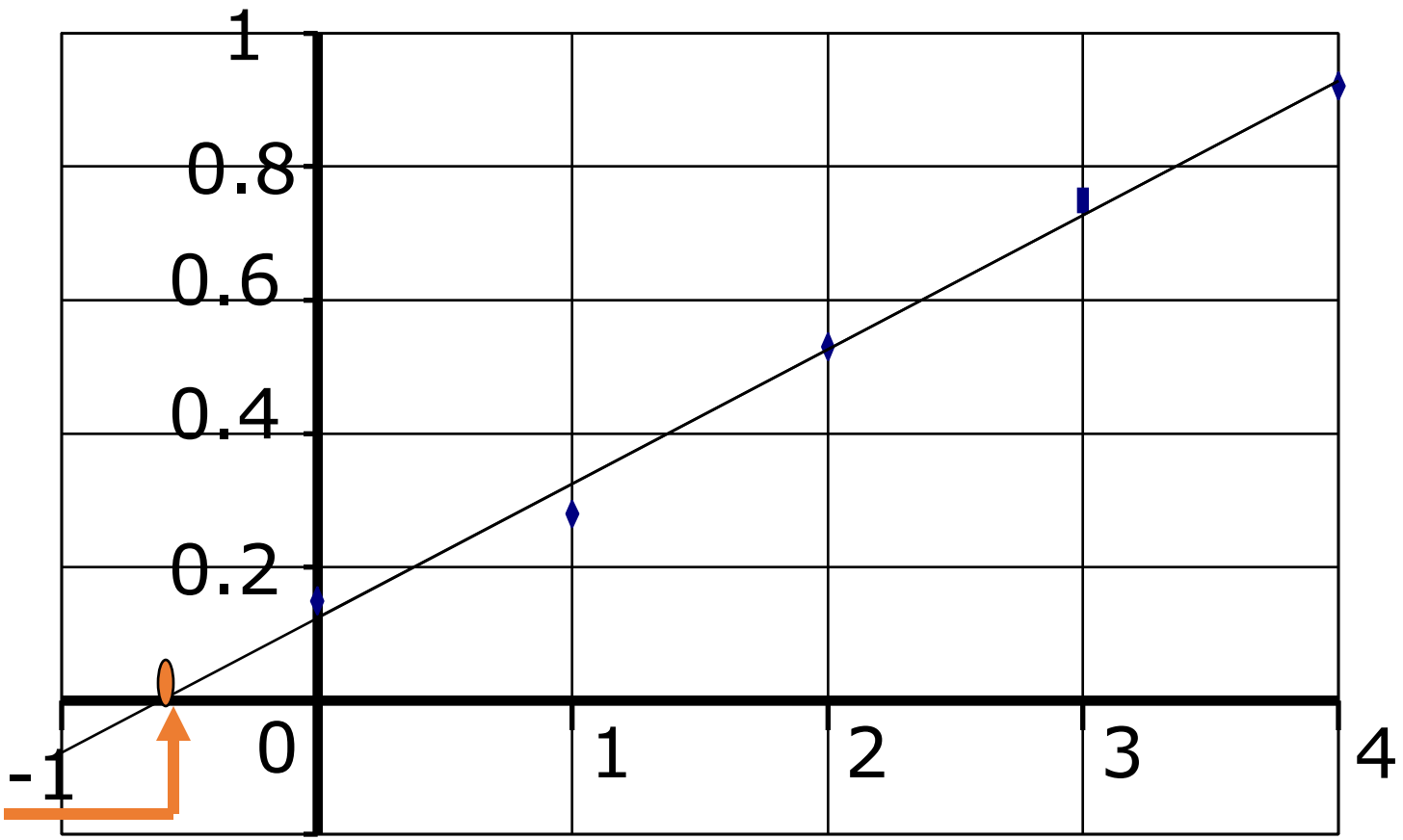
1. Fixed amounts of the unknown are added to each of the standard solutions.

Example:

Standard solution	0 mL	1 mL	2 mL	3 mL
Sample	1 mL	1 mL	1 mL	1 mL

2. Measurement is taken for each solution.
3. The absorbance is plotted against concentration.
4. The concentration of the unknown is obtained by extrapolation to zero-absorbance.

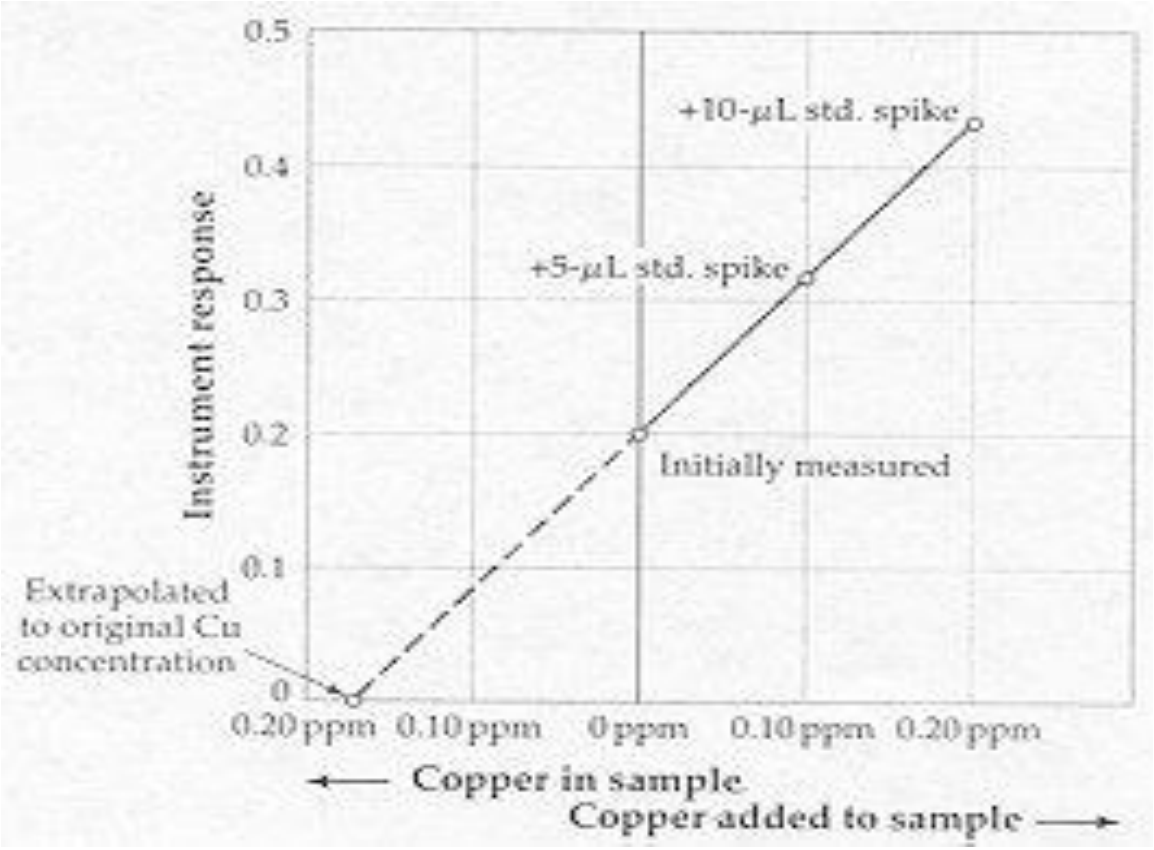
Qualitative and Quantitative Analysis by UV-VIS Spectroscopy



Conc of analyte in the sample

Qualitative and Quantitative Analysis by UV-VIS Spectroscopy

Example



[Picture taken from Contemporary Chemical Analysis by Judith F. Rubinson and Kenneth A. Rubinson, Pg119]

- Limitations are= the assay response must be selective enough so that the response only comes from the analyte.
- The concentration range over which the instrument response remains linear tends to be quite narrow (factor of 10)
- Benefit = allow compensation for quite complex effects.
- Disadvantage=tedious as a set of standard solution are to be prepared for each sample. Imagine that you have a lot of samples. How?

Applications of UV-VIS Spectroscopy

Selection of Wavelength

- Absorbance measurement are usually made at the λ_{max} of the analyte → change in absorbance per unit concentration is greatest.

λ_{max} is usually determined experimentally by measuring the spectrum (absorbance vs. wavelength) of the analyte in the selected solvent.

Applications of UV-VIS Spectroscopy

Applications

UV-VIS spectroscopy is commonly used for quantitative analysis.

Examples:

- Environmental
  - Phenol, chlorine, fluoride in water and wastewater
- Pharmaceuticals
  - Antibiotics, hormones, vitamins and analgesics
  - E.g., determine purity of aspirin tablets