Absorption spectroscopies

ver. 20191120

Absorption spectroscopies

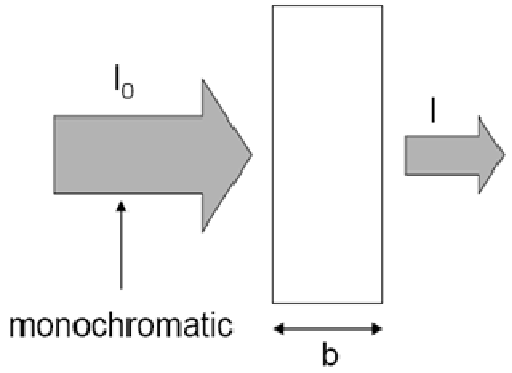
- UV spectroscopy
- Visible spectroscopy
- IR spectroscopy

Similar phenomena (Beer-Lambert's law)

and experimental techniques (spectrophotometers).

Typical experimental setup

Radiation is directed on a sample holder (cuvette) with an **incoming radiation intensity**, I_0.



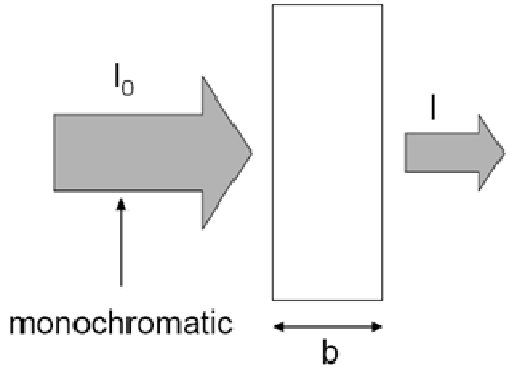
Outgoing radiation intensity, I.

The radiation intensity decreases when coming through sample (**optical path length**, b) by absorption.

We are interested in the absorbed light intensity, $I_a = I_0 - I$, but can measure only I_0 and I.

Transmittance, transmission, absorbance

Radiation is directed on a sample holder (cuvette) with an incoming radiation intensity, I_0.



Outgoing radiation intensity, I.

Transmittance (T): the fraction of the transmitted light intensity to the incident one, $T = I/I_0$

Percentage transmission (T%): $100 \cdot T$

Absorbance (A): $A = \log_{10}(I_0/I) = \log_{10}(1/T)$

When the material is transparent: $I = I_0$, therefore $T = 1$, $T\% = 100$, $A = 0$.
When the material absorbs all the incoming light: $I = 0$, therefore $T=0$, $T\% = 0$, A infinity.

Simple calculation

Problem: at 619 nm 39% of the incoming light intensity is absorbed. Give the transmittance, the percentage transmission and the absorbance!

$I_a = 39\% \cdot I$,
therefore $I = I_0 - I_a = (100\% - 39\%) \cdot I_0 = 61\% \cdot I_0$

$T = I/I_0 = 61\% \cdot I_0/I_0 = 61\% = 0.61$

$T\% = 100 \cdot T = 61$

$A = \log_{10}(I_0/(61\% \cdot I_0)) = \log_{10}(1/0.61) = 0.215$

History of the Beer-Lambert's law

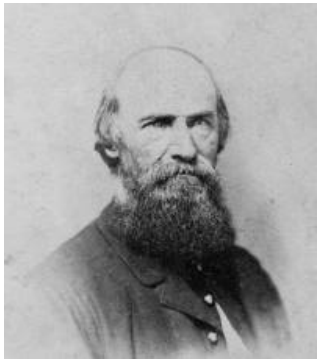
Pierre Bouguer 1729: The light intensity loss is directly proportional to the light intensity and path length (*Essai d'optique sur la gradation de la lumière* (Claude Jombert, Paris, 1729)).

Johann Heinrich Lambert just cited him in *Photometria* (1760).

August Beer found that the transmittance of a solution remains constant if the product of concentration and path length stays constant. (1852)

Pierre Bouguer
Born: 16 February 1698
Died: 15 August 1758
French mathematician, physicist, astronomer

Johann Heinrich Lambert
Born: 26 August 1728
Died: 25 September 1777
Swiss mathematician, physicist, astronomer and philosopher



August Beer
Born: 31 July 1825
Died: 18 November 1863
German mathematician, physicist, and chemist

Modern form of the Beer-Lambert's law

A common and practical expression of the Beer-Lambert law for a single absorbing species:

$$A = \varepsilon \cdot c \cdot l$$

where

ε is the molar absorbtion coefficient or absorptivity of the attenuating species
c is the molar concentration of the attenuating species
l is the optical path length

For multiple attenuating species the absorbances can be summarized:

$$A = A_1 + A_2 + \ldots + A_n = (\varepsilon_1 \cdot c_1 + \varepsilon_2 \cdot c_2 + \ldots + \varepsilon_n \cdot c_n) \cdot l$$

Note: $A \equiv \log_{10}(I_0/I)$, but this equation is not part of the BL's law.

Application of the Beer-Lambert's law

Determination of a species concentration

We know what is the absorbing species and measure the absorbance of the solution in a spectrophotometer.

Problem: Methanol has a UV absorption peak at 184 nm with ε = 150 $dm^3\ mol^{-1}\ cm^{-1}$.
Calculate the concentration of methanol in a nonabsorbing solvent for a cell of 10 cm optical length if we measure *A* = 1.574!

$A = \varepsilon \cdot c \cdot l$,
therefore $c = A / (\varepsilon \cdot l) = 1.574 / (150\ dm^3\ mol^{-1}\ cm^{-1} \cdot 10\ cm) = 0.001049333\ mol^1\ dm^{-3} \approx 0.00105\ mol^1\ dm^{-3}$

Note: ε typically has a „strange" unit, the distance kind units are not merged.

Application of the Beer-Lambert's law

Problem: We measure 0.660 absorbance for species B at 560 nm in a cuvette of 16 mm optical path. Calculate the molar concentration of B in this solution if the same species having $5.2 \cdot 10^{-4}$ mol/dm^3 concentration in another solution gives 43% transmission in a 1,0 cm cuvette at the same wavelength?

Let us calculate the molar absorption coefficient!

$\varepsilon = A / (c \cdot l) = \lg(1/T) / (c \cdot l) = \lg(1/0.43) / (5.2 \cdot 10^{-4}\ \text{mol/dm}^3 \cdot 1.0\ \text{cm}) = 704.9\ \text{dm}^3\ \text{mol}^{-1}\ \text{cm}^{-1}$

Now we can calculate the concentration of the first solution:

$c = A / (\varepsilon \cdot l) = 0.660 / (704.9\ \text{dm}^3\ \text{mol}^{-1}\ \text{cm}^{-1} \cdot 1.6\ \text{cm}) = 5.852 \cdot 10^{-4}\ \text{mol/dm}^3 \approx 5.9 \cdot 10^{-4}\ \text{mol/dm}^3$

Note: do not forget to match the units if necessary!

Derivation of the Beer-Lambert's law

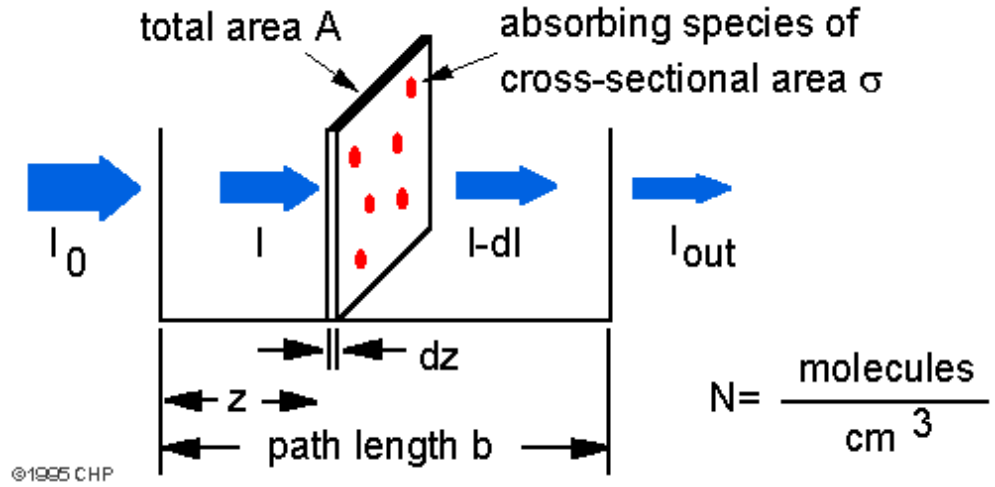
Let us approximate the absorption coefficient ~ an opaque disk whose cross-sectional area, σ, represents the effective area seen by a photon of frequency *w*.

If the frequency of the light is

- far from resonance: σ ~ 0
- close to resonance: σ maximal

Take an infinitesimal slab, dz at z!

The total opaque area on the slab due to the absorbers is $\sigma \cdot N \cdot V_{slab} = \sigma \cdot N \cdot A \cdot dz$



The absorbed light intensity (dI) to total light intensity entering this slab (I_z) ratio is equal to the total opaque area to total area ratio:

$$dI/I_z = \frac{\sigma \cdot N \cdot A \cdot dz}{A}$$

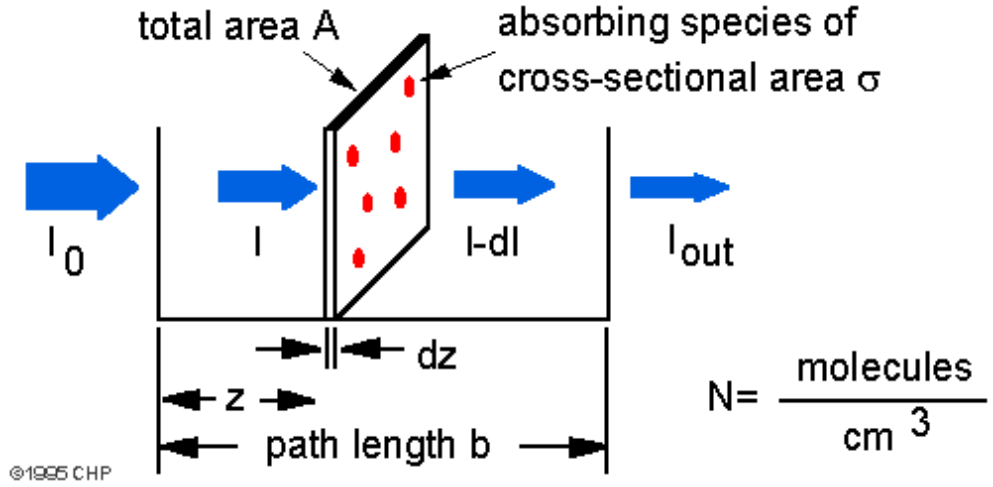
Derivation of the Beer-Lambert's law

$$\mathrm{d}I/I_z = \frac{\sigma \cdot \mathrm{N} \cdot A \cdot \mathrm{d}z}{A}$$

Integrating this equation throughout the cuvet (from $z = 0$ to $z = b$) we get:

$$\ln(I_0) - \ln(I) = \sigma \cdot \mathrm{N} \cdot \mathrm{b}$$

or $\ln(I_0/I) = \sigma \cdot \mathrm{N} \cdot \mathrm{b}$



Let us switch molecules to moles and the natural logarithm to the 10-based one:

$$\lg(I_0/I) = \frac{\sigma}{\ln(10)} \cdot \frac{1000\ \mathrm{cm^3/dm^3}}{6.023 \cdot 10^{23}\ \mathrm{molecules/mol}} \cdot N \cdot \mathrm{b} = \varepsilon \cdot c \cdot b$$

$$\lg(I_0/I) = \varepsilon \cdot c \cdot b$$

Limitations of the Beer-Lambert's law

Under certain conditions Beer–Lambert law fails to maintain a linear relationship between attenuation and concentration of analyte.

These deviations are classified into three categories:

Real deviation: fundamental deviations due to the limitations of the law itself.

Chemical deviation: deviations observed due to specific chemical species of the sample which is being analyzed.

Instrumental deviation: deviations which occur due to how the attenuation measurements are made.

Limitations of the Beer-Lambert's law

Real deviation:

- At high concentration of some materials change the charge distribution of their neighbours might cause a shift of the absorbtion wavelenth.

- High analyte concentration may change the refractive index which is not taken into account in the LB's law.

Chemical deviation:

- A concentration dependent chemical change may happens in the solution, e.g. association, dissociation or interaction with the solvent.

- Fluorescence or phosphorescence of the sample causes light emission on a different wavelength than the absorbed one.

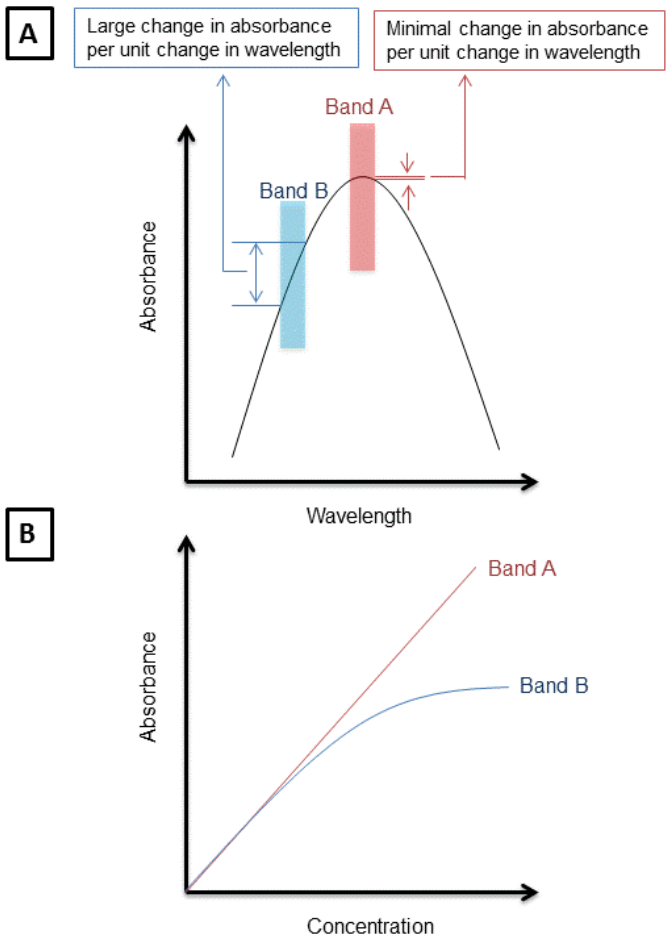
Limitations of the Beer-Lambert's law

Instrumental deviation:

- Using polychromatic light causes that we measure some kind of average absorbance.

- Scattered radiation coming and scattered from various parts of the instrument could cause non-expected absorbance.

- Mismatched cuvettes (different path lengths, unequal optical characteristics) could cause an intercept in the linear relationship.



Where to measure absorbance?

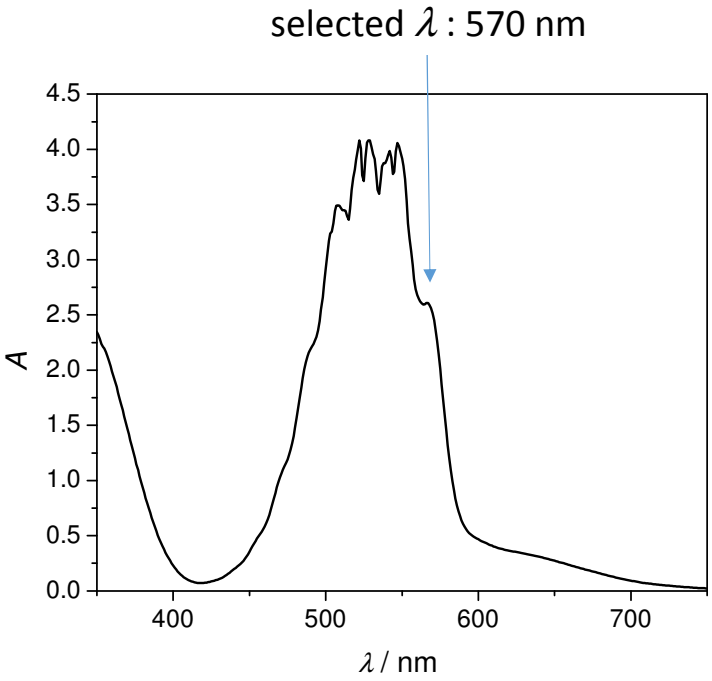
Factors should be taken into account for the most accurate measurements:

- The signal must be much larger than the noise (too low (e.g. <0.2-0.3) absorbances can not be measured accurately.
- Usage of polychromatic light causes that we measure some kind of average absorbance.
- Too high (e.g. >2-3) absorbances can not be measured accurately.
- Neglection of the absorbance of non-minor components should be avoided (we typically measure where only a single component absorbes).

Where to measure? A case study

In a lab practice we should measure the absorbance of MnO4- ions having a large absorption peak between 420-600 nm. Where to measure?

- The signal must be much larger than the noise (too low (e.g. <0.2-0.3) can not be measured accurately): **450 nm -600 nm**
- Usage of polychromatic light causes that we measure some kind of average absorbance: measure on a not very sharp peak, e.g **500 nm** or **570 nm**, with better device many more 500 nm – 550 nm
- Too high (e.g. >2-3) absorbances can not be measured accurately: **the peak at 500 nm is not good**
- Neglection of the absorbance of non-minor components should be avoided (we typically measure where only a single component absorbes): **any wavelenghs look fine**



The role of the reference (blank) system

In the LB's law we are interested in the light intensity absorbed, but can measure the light intensity on the detector. Due to other processes (reflection, scattering, absorption of the solvent) we measure larger intensity decrease which can be attributed to the absorption of the analyte.

$$I_{\text{source}} = I_{\text{solvent}} + I_0$$

$$I_{\text{source}} = I_{\text{solvent}} + I_{\text{solute}} + I$$

Measurable quantities related to the „useful" absorption: I and I_0

We can take into account these side processes using a reference (or balnk) system and measuring the light intensity (I_0) with it.

A good reference system contains all components, except the light absorbing analyte.

The absorption spectrophotometers

The absorption spectrophotometers can be classified by

- the wavelength region: UV, VIS, IR, UV/VIS, or even UV/VIS/NIR
- the principle of wavelength separation: monochromator, polychromator, Fourier transform
- the number of beams: single beam or dual beam instuments

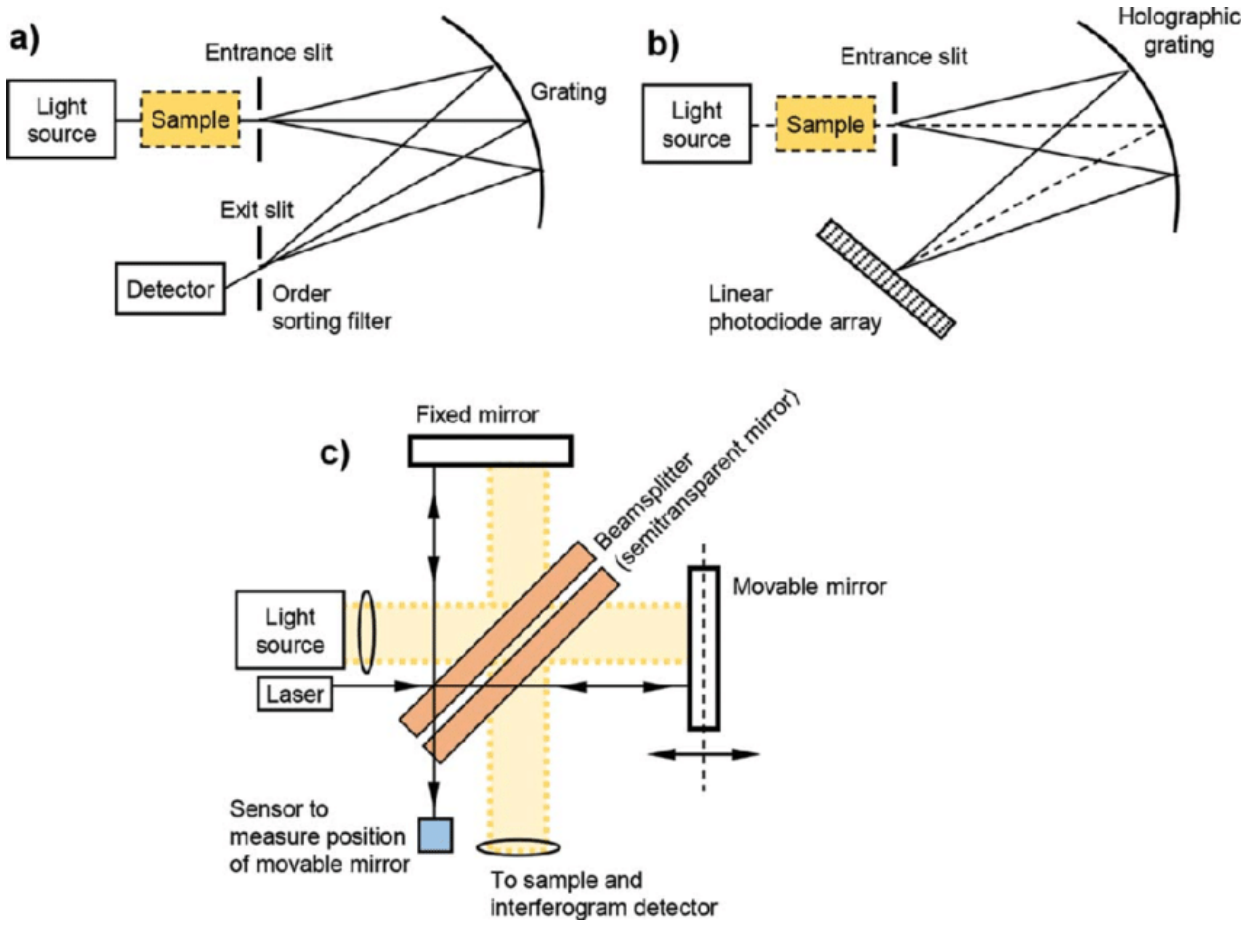
The principle of wavelength separation

(a) Scanning grating monochromator: a single wavelength is selected

(b) **Grating polychromator photodiode array**: a range of wavelengths is selected and detected in the same time

(c) **Fourier transform spectrometer**: simultaneously collects high-spectral-resolution data over a wide spectral range and transforms the information to spectrum using the mathematical process Fourier transformation

(a) and (b) called as dispersive spectrometers.

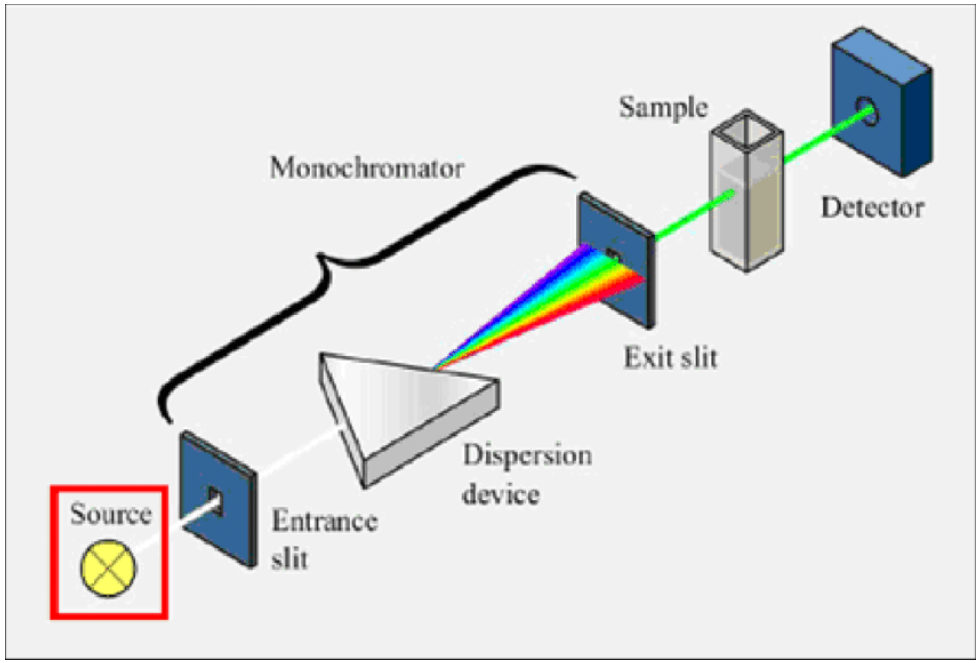


https://www.researchgate.net/profile/Jan_Skvaril2/publication/313141961/figure/fig3/AS:467141091696644@1488386523883/Principles-of-commonly-used-NIR-spectrophotometers-a-Scanning-grating-monochromator.png

Single beam spectrophotometers

We have only one place to put the solution in, therefore the reference (blank) and the sample is measured after each.

Simple, cheap instuments, but sensitive to the stability of the power supply, the light source and the detector. These could lead to problems related to the stability of zero, sensitivity, reproducibility and linearity.

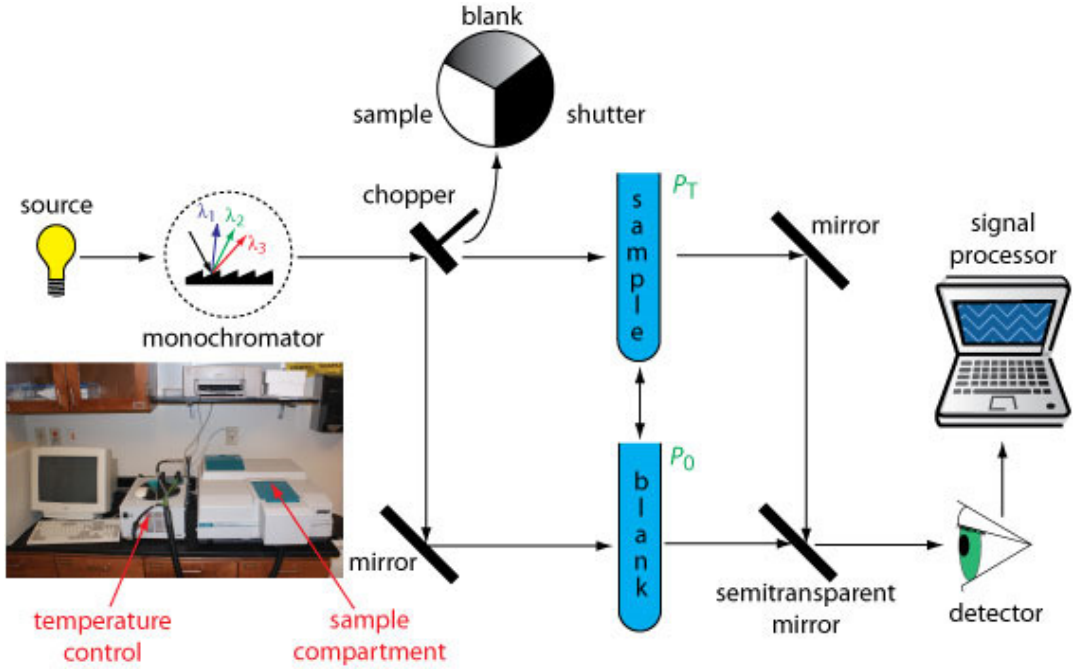


https://www.researchgate.net/profile/Neelakshi_Gohain2/publication/28358404/figure/fig5/AS:651528461352972@1532347898729/A-schematic-diagram-of-a-single-beam-UV-Visible-Spectrometer.png

Double beam spectrophotometers

We have only two ways the light passes through, therefore we can place the reference solution (blank) and the sample in the same time.

Not as cheap as the single beam instuments, but much less sensitive to the stability of the power supply, the light source and the detector, therefore more accurate ones.



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