Spektrofotometer UV-Vis

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UV - Vis Spectrophotometry

- What is it ?
- What for is it ?
- How is it work ?

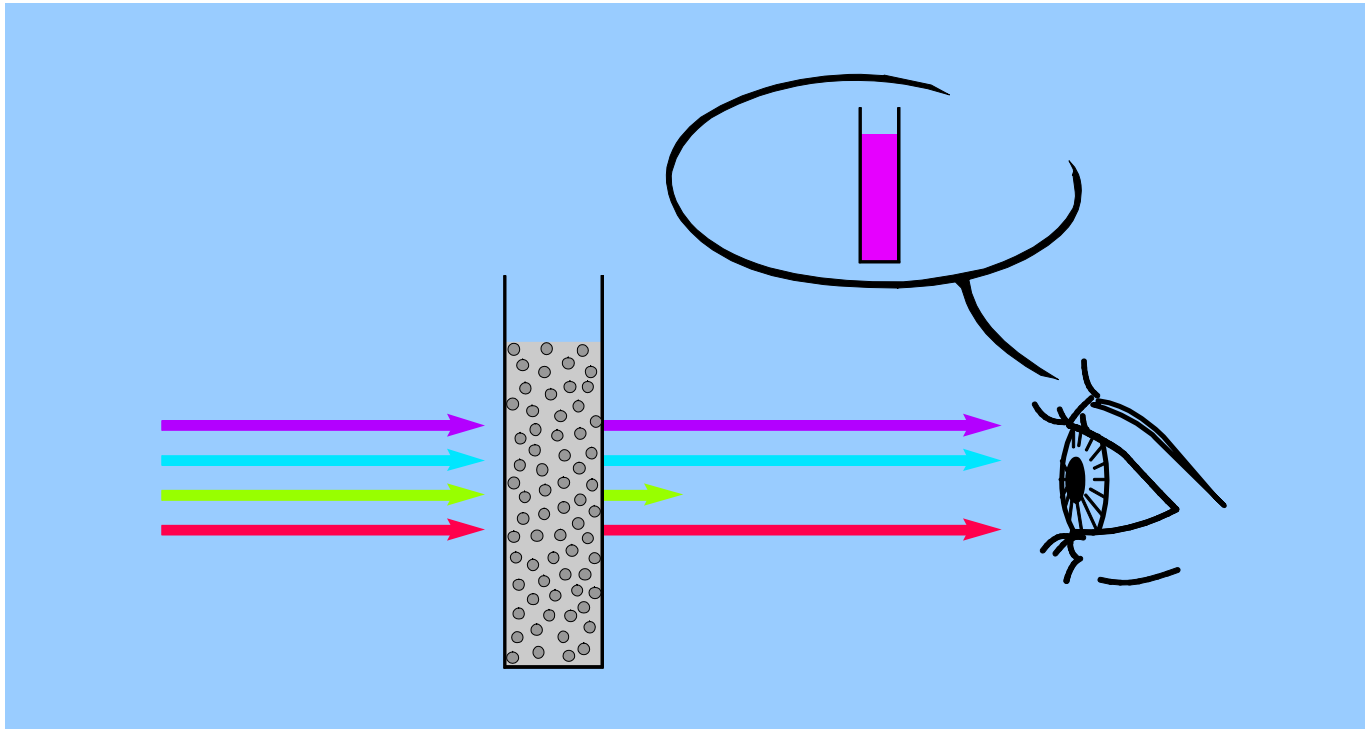
A spectrophotometer is a photometer
(a device for measuring light intensity)
that can measure intensity as a function of the color, or more specifically, the wavelength of light.

Ultraviolet-Visible Spectrophotometry (**UV/ VIS**) involves the spectroscopy of photons (spectrophotometry). It uses light in the visible and near ultraviolet (UV) ranges as sources of electromagnetic radiation (sources of energy)

Applications of Spectrophotometry

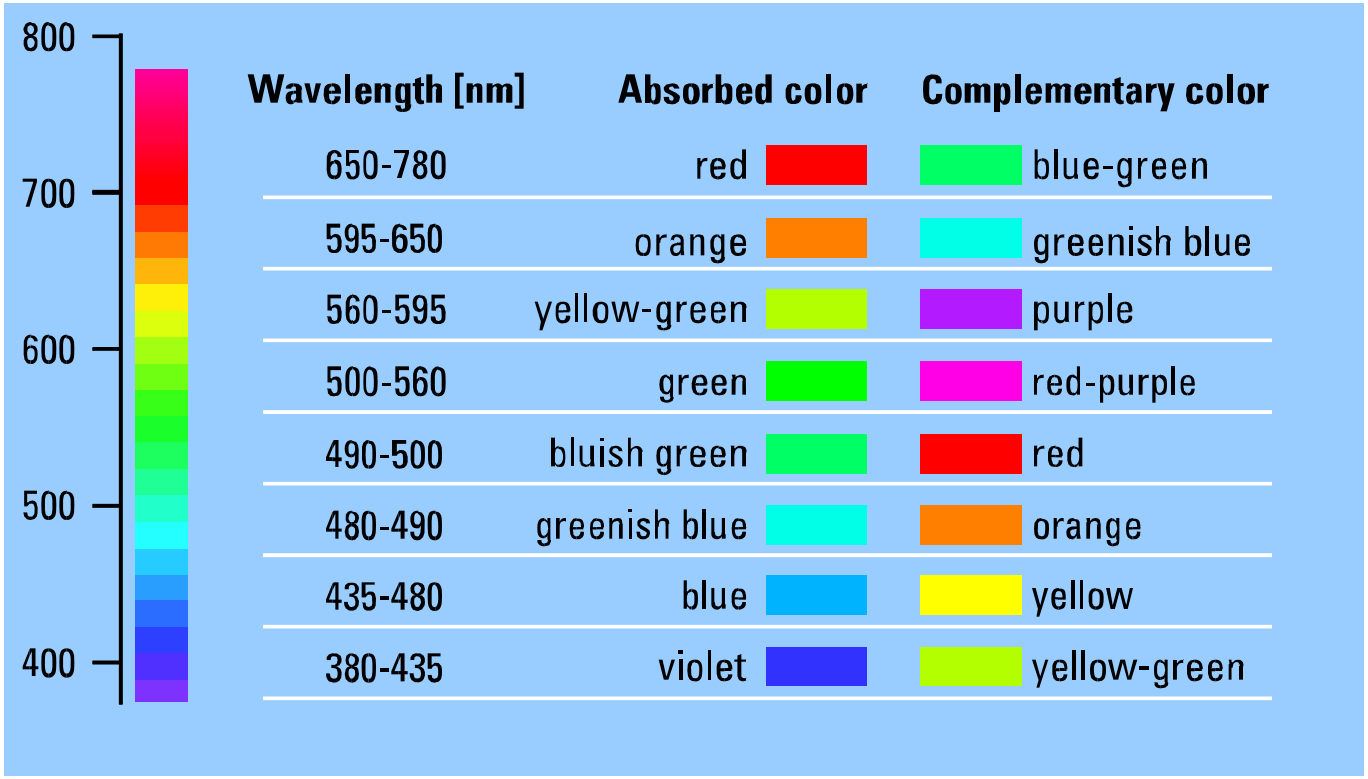
- Quantitatif Analysis
- Analysis of a mixture
- Stoichiometry with Continous Variation Method
- Complex formation identification
- etc

Interaction of Photon and Matter

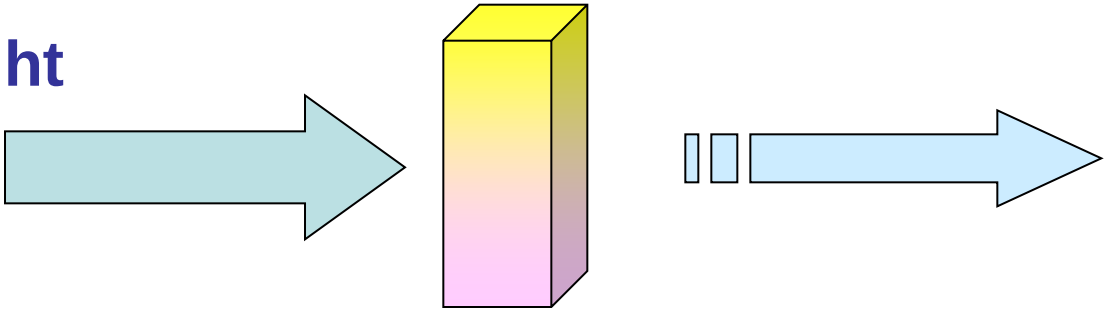


The human eye sees the complementary color to that which is absorbed

Absorbance and Complementary Colors



Colours of Visible light



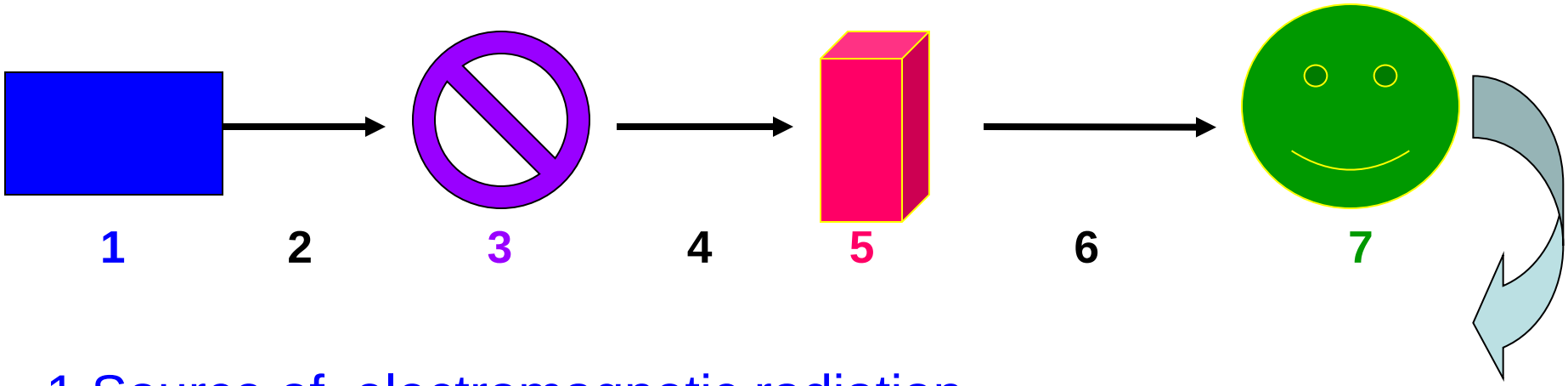
Wavelengths of max absorption (nm)	Colour absorbed	Colour observed
380-420	Violet	Green-yellow
420-440	Violet-blue	Yellow
440-470	Blue	Orange
470-500	Blue-green	Red
500-520	Green	Purple
520-550	Yellow-green	Violet
550-580	Yellow	Violet-blue
580-620	Orange	Blue
620-680	Red	Blue-green
680-780	Purple	Green

(Harris, 2004)

There are two major classes of spectrophotometers single beam and double beam,

- A single beam spectrophotometer measures the absolute light intensity
- A double beam spectrophotometer measures the ratio of the light intensity on two different light paths

Diagram Block of Spectrophotometer



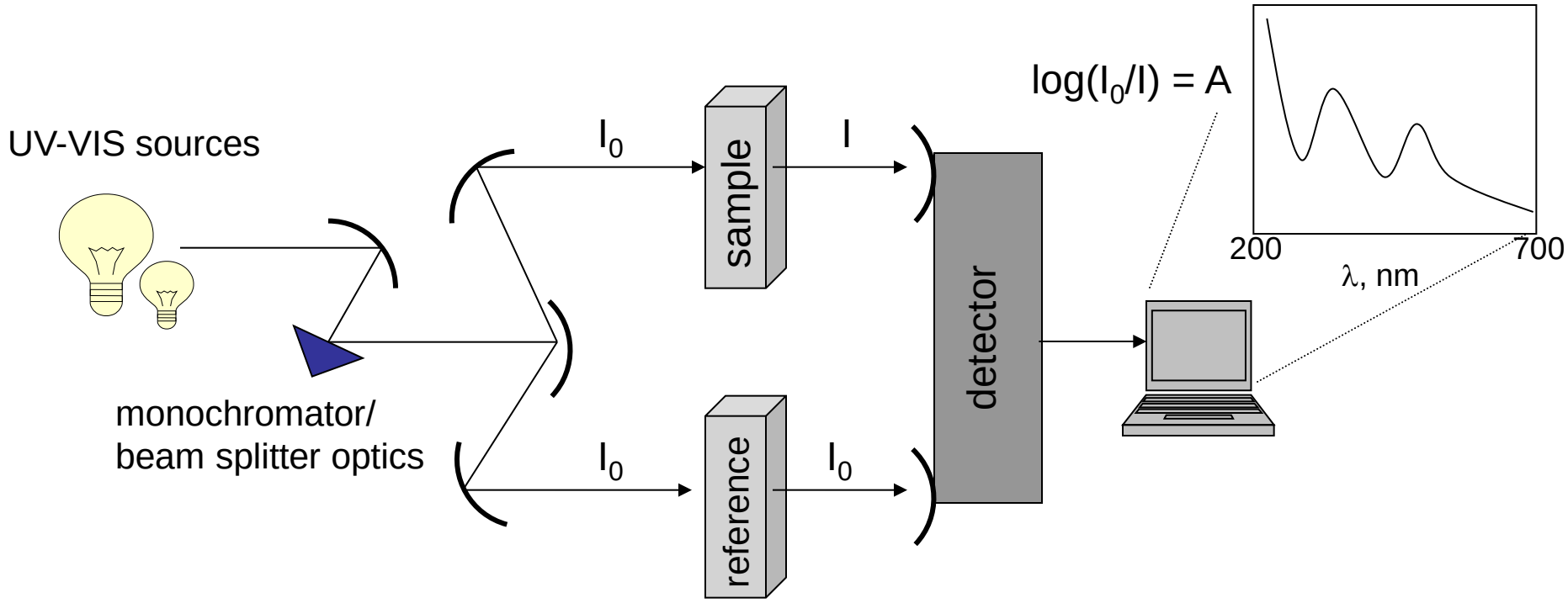
1 Source of electromagnetic radiation
2 Polychromatic rays
3 Wavelength selector
4 Monochromatic rays with Io
5 Matter (atom or molecules or substances)
6 Monochromatic ray with I_t (transmitancy)
7 Photon detector

II. Instrumentation and Spectra

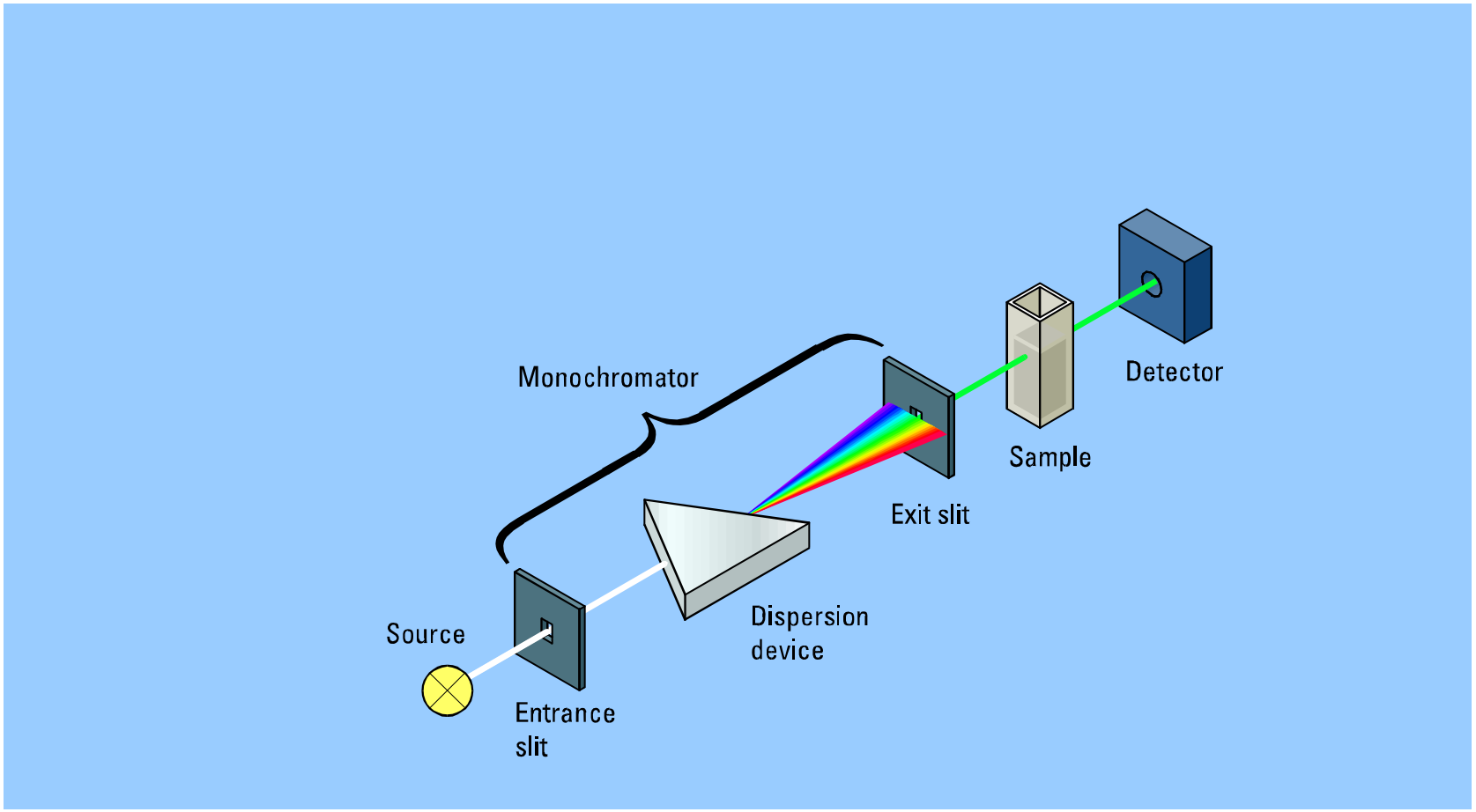
A. Instrumentation

1. The construction of a traditional UV-VIS spectrometer is very similar to an IR, as similar functions – sample handling, irradiation, detection and output are required

2. Here is a simple schematic that covers most modern UV spectrometers:

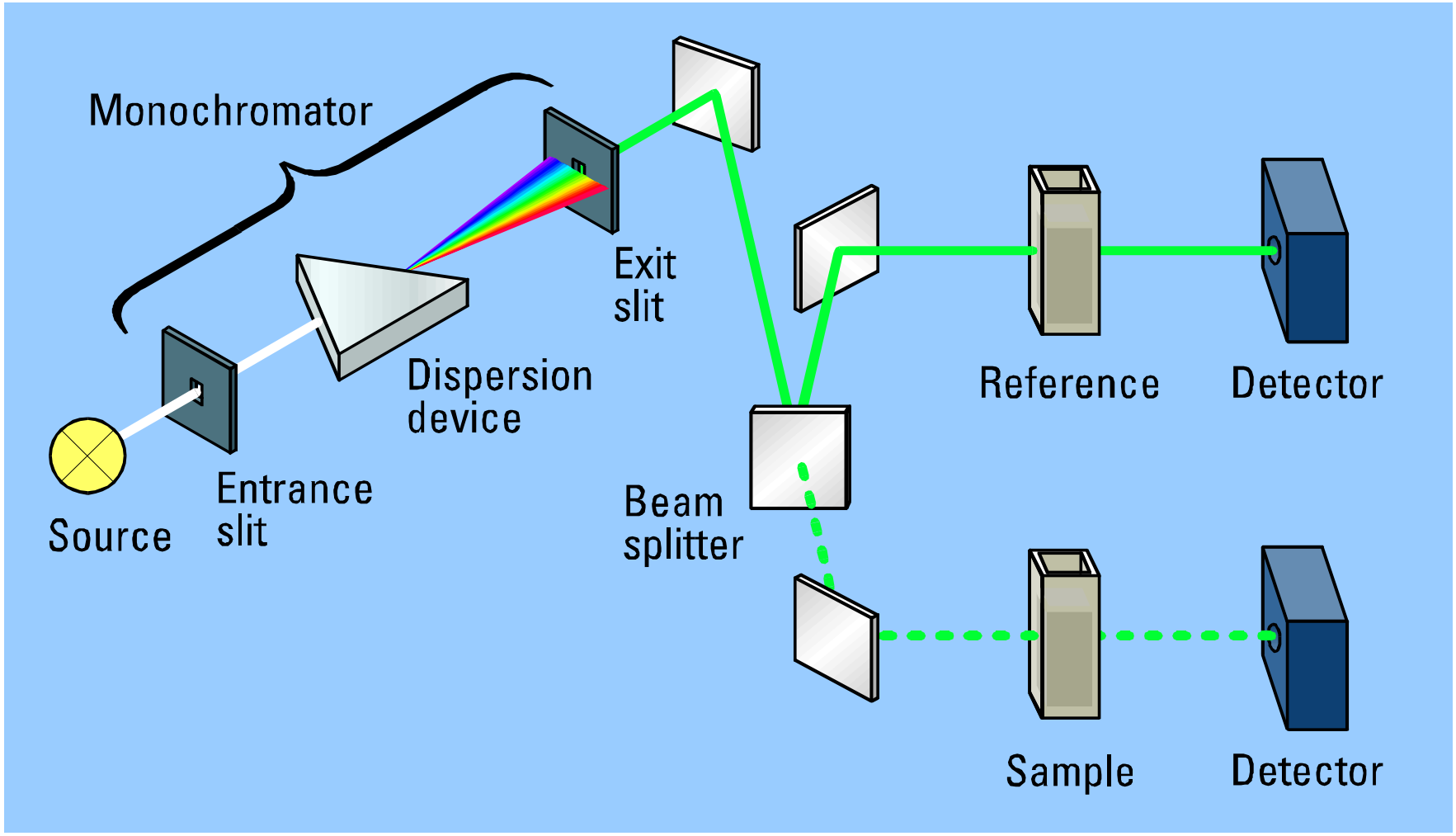


Conventional Spectrophotometer



Schematic of a conventional single-beam spectrophotometer

Double-beam spectrophotometer



Light Sources

UV Spectrophotometer emited 200 – 350 nm

1. **Hydrogen Gas Lamp**
2. **Mercury Lamp**

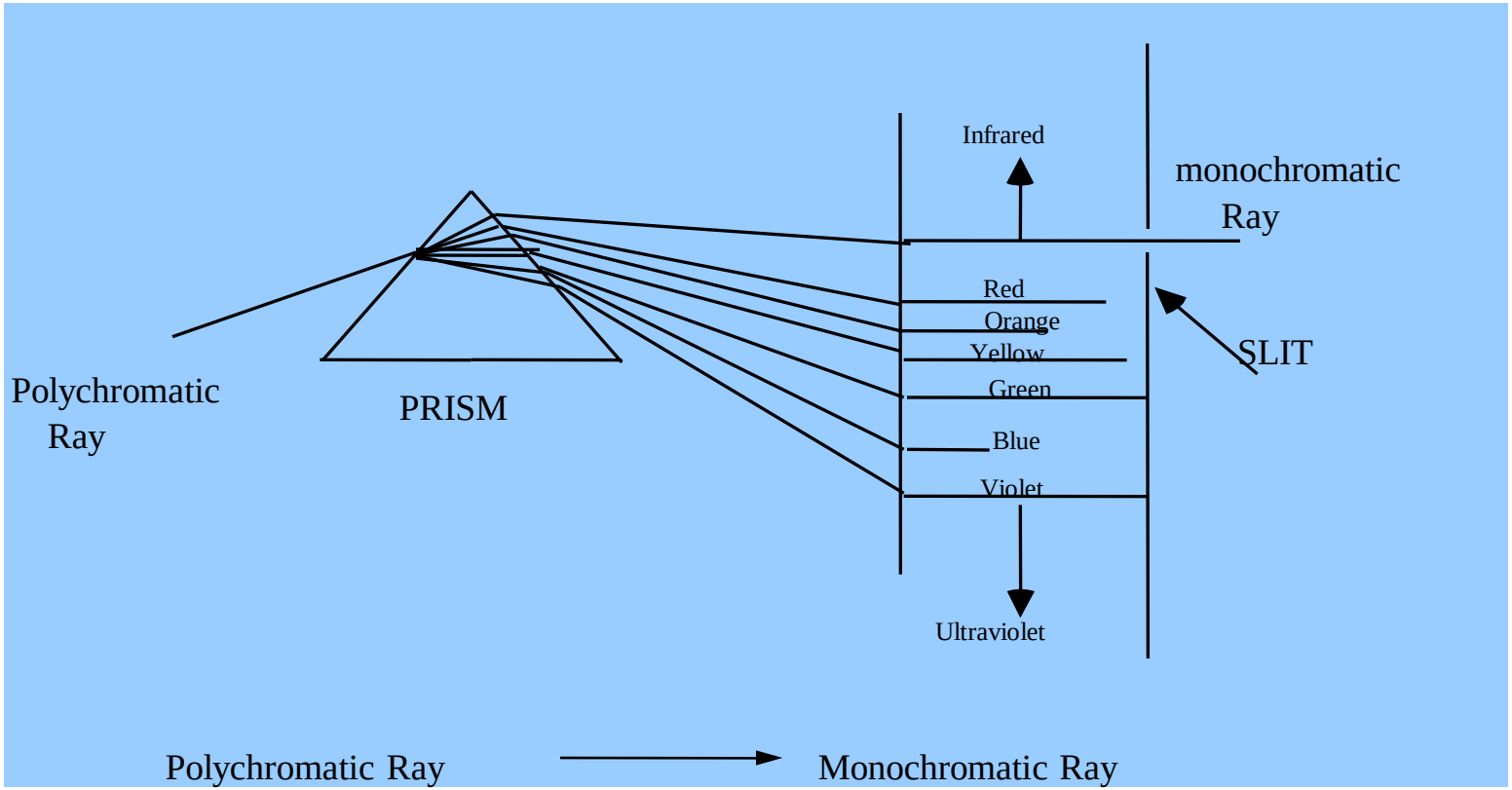
Visible Spectrophotometer

emited 350 – 850 nm

1. **Tungsten Lamp**

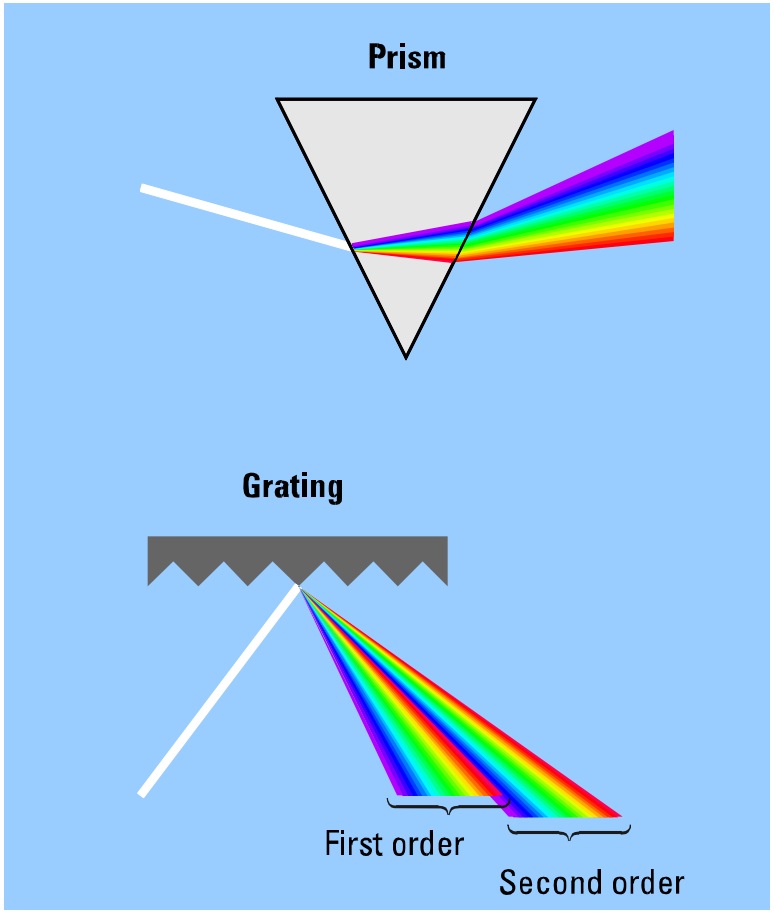
Dispersion of polychromatic light with a prism

Prism - spray out the spectrum and choose the certain wavelength (l) that you want by moving the slit.



Dispersion Devices

- **Non-linear dispersion**
- **Linear Dispersion**



Cells

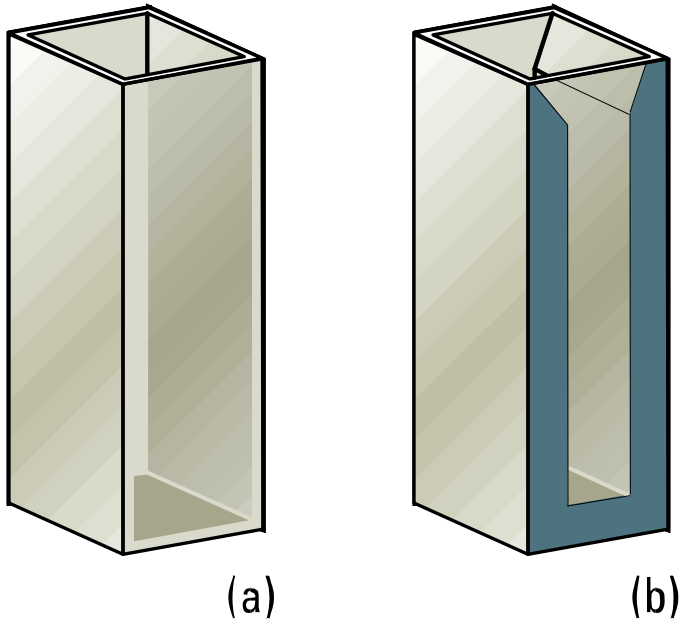
UV Spectrophotometer

Quartz (crystalline silica)

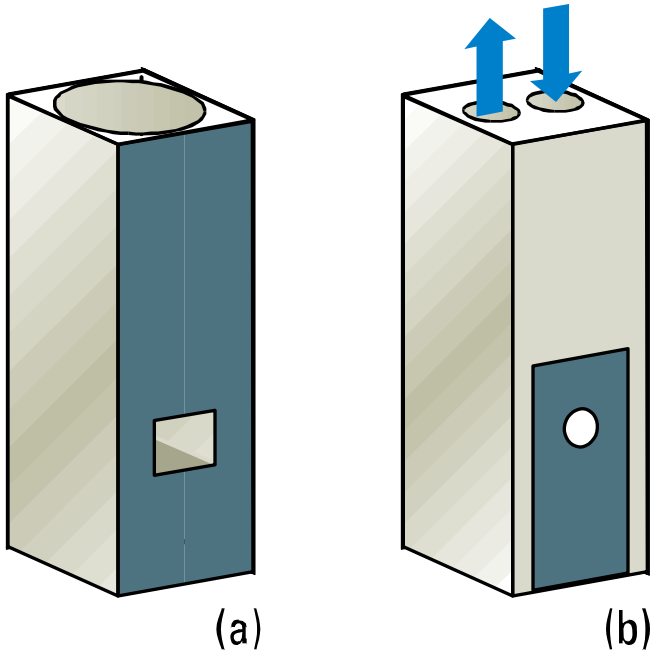
Visible Spectrophotometer

Glass

Cell Types

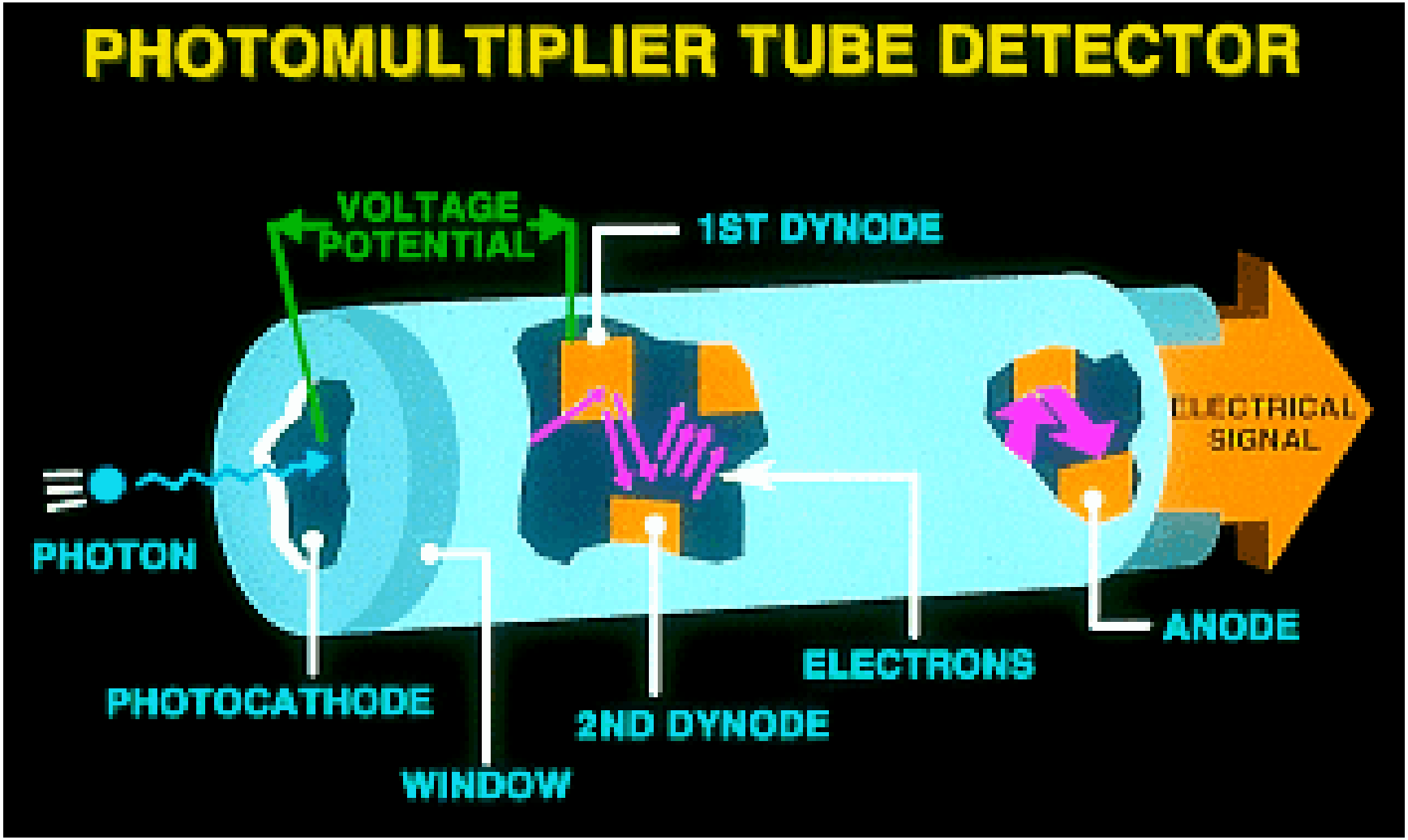


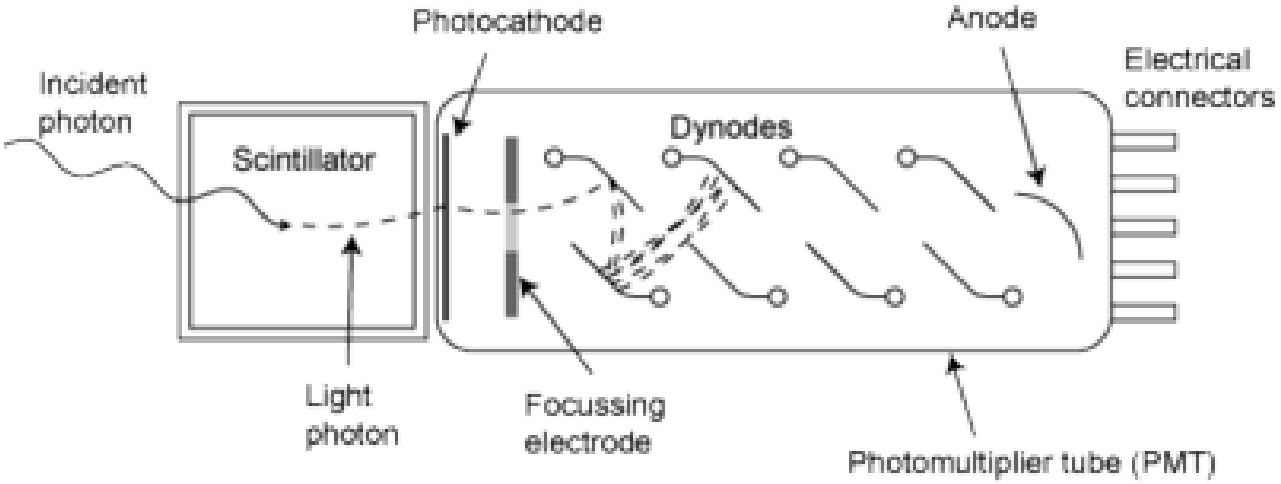
Open-topped rectangular standard cell (a) and apertured cell (b) for limited sample volume



Micro cell (a) for very small volumes and flow-through cell (b) for automated applications

Detector


PHOTOMULTIPLIER TUBE DETECTOR
VOLTAGE POTENTIAL
1ST DYNODE
ELECTRICAL SIGNAL
PHOTON
ANODE
ELECTRONS
PHOTOCATHODE
2ND DYNODE
WINDOW



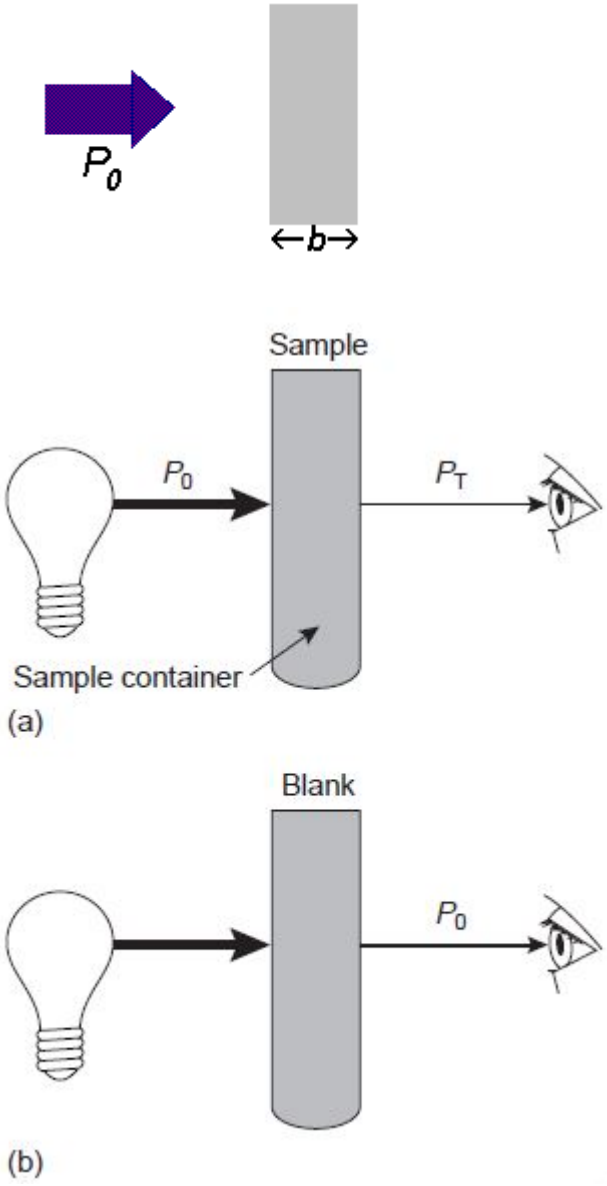
Photomultipliers, or photomultiplier tubes (PMT), are extremely sensitive detectors of light in the ultraviolet, visible and near infrared. These detectors multiply the signal produced from the incident light from which single photons are detectable.

Pengertian Transmitansi dan Absorbansi

- Saat senyawa kimia menyerap ultraviolet (UV) atau visible (Vis), maka akan terjadi proses absorbansi.
- Saat radiasi elektromagnetik dari sumber radiasi (P_O) dilewatkan ke sampel maka radiasi tersebut akan melewati sampel tersebut dan keluar sebagai P_T.
- Rasio dari sumber radiasi (P_O) dan radiasi keluar (P_T) disebut dengan transmitansi.

$$T = P_T / P_O \quad (1)$$

- Jika transmitansi itu dikalikan dengan 100, maka akan memberikan persen transmitansi (%T), dimana diartikan sebagai 100% (tidak ada absorbansi) dan 0% (absorbansi sempurna).
- Semua metode deteksi, baik itu mata manusia atau transducer fotoelektrik modern mengukur transmitansi dari radiasi elektromagnetik ini.



(a) Ilustrasi yang menggambarkan radiasi yang melewati sampel, P_O adalah radiasi dari sumber dan P_T radiasi yang ditransmisikan oleh sampel.

(b) Skema yang menggambarkan $P_O$didefinisikan ulang sebagai tenaga radiasi yang ditransmisikan oleh blank, mengoreksi transmisi pada (a) karena pengurang yang disebabkan oleh scattering, reflection, atau absorpsi oleh kuvet dan absorpsi oleh matrik sampel

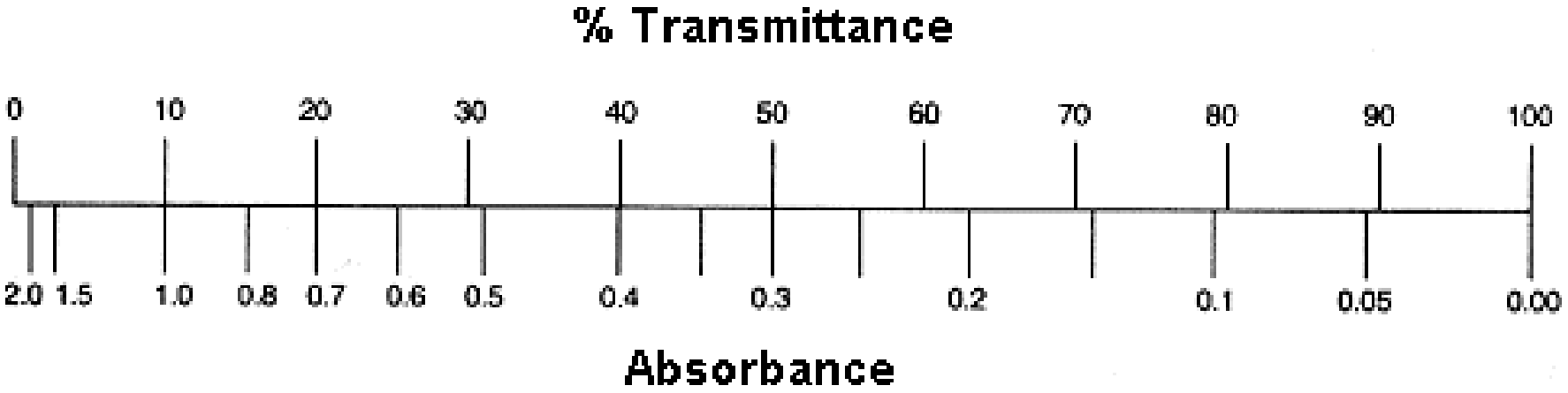
- Saat kita mengukur absorbansi senyawa kimia menggunakan spectrometer UV-Vis pastinya ada wadah untuk menampung senyawa kimia tersebut. Wadah tersebut dinamakan kuvet.
- Proses absorbansi bisa dipengaruhi oleh hal lainnya seperti scattering dan refleksi dan juga oleh kuvetnya.
- Untuk mengatasi hal ini digunakanlah metode blank yang berfungsi untuk mengoreksi proses pengukuran.

Untuk mengetahui konsentrasi dari suatu senyawa kimia dengan menggunakan spektrofotometer UV-Vis biasanya kita menggunakan absorbansi. Absorbansi ini dapat dikalkulasi menggunakan formula berikut:

$$A = -\log T = -\log P_T / P_O = \log P_T / P_O \quad (1)$$

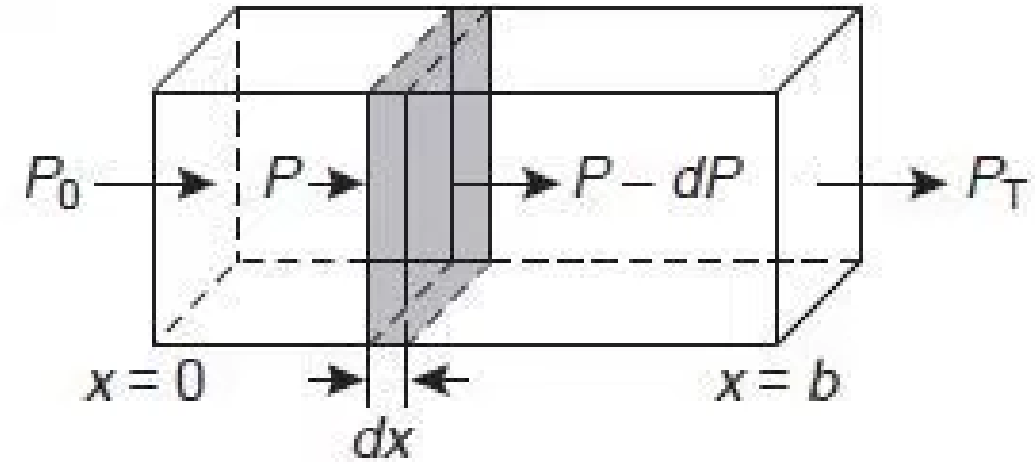
Absorbansi merupakan satuan yang sering digunakan untuk mengekspresikan radiasi, karena absorbansi mempunyai fungsi linier dengan konsentrasi analit (sampel).

Transmittance vs absorbance



- So, if all the light passes through a solution *without* any absorption, then absorbance is zero, and percent transmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite.

Absorbansi dan Konsentrasi: Hukum Lambert-Beer



- Faktor untuk menentukan hukum Lambert-Beer

- Ketika radiasi elektromagnetik monokromatik melewati lapisan tipis dari sampel, ketebalan (dx) dari lapisan tersebut akan mengurangi kekuatan dari dP

Hubungan antara pengurangan kekuatan akibat ketebalan lapisan dan konsentrasi analit C sebagai berikut:

$$-dP/P = \alpha C\, dx \qquad (2)$$

Dimana P adalah kekuatan dari lapisan tipis sampel, α adalah konstanta proporsional.

Jika persamaan tersebut diintegralkan dari x=0 ke x=b, dimana b adalah ketebalan sampel secara umum, maka

$$-\int_{P=P_0}^{P=P_T} \frac{dP}{P} = \alpha C \int_{x=0}^{x=b} dx \qquad (3)$$

$$\mathrm{Ln}(P0 / P_T) = \alpha\ b\ C \qquad (4)$$

Konversi ln ke log, dan substitusi dari persamaan (1) ke (4), memberikan persamaan (5):

$$A = \alpha\, b\, C \qquad (5)$$

Dimana a adalah absorptivitas analit dengan satuan cm-1 kons-1.

Apabila konsentrasi diekspresikan dengan molaritas, maka absorptivitas diubah menjadi absorptivitas molar, ε dengan satuan cm-1 M-1.

$$A = \varepsilon\, b\, C \qquad (6)$$

- Nilai dari α dan ε bergantung pada panjang gelombang dari radiasi elektromagnetik.
- Persamaan tersebut memiliki persamaan yang linier antara absorbansi dan konsentrasi, dan persamaan ini kita kenal dengan hukum lambert-beer, atau secara umum sebagai hukum Beer.
- Kurva kalibrasi berdasarkan hukum lambert-beer secara rutin sering digunakan dalam analisa kuantitatif.

Hukum Lambert-Beer

$$A = \varepsilon \, . \, b. \, C$$

A = Absorban (serapan)

ε = koefisien ekstingsi molar ($M^{-1} cm^{-1}$)

b = tebal kuvet (cm)

C = konsentrasi (M)

Pada beberapa buku ditulis juga:

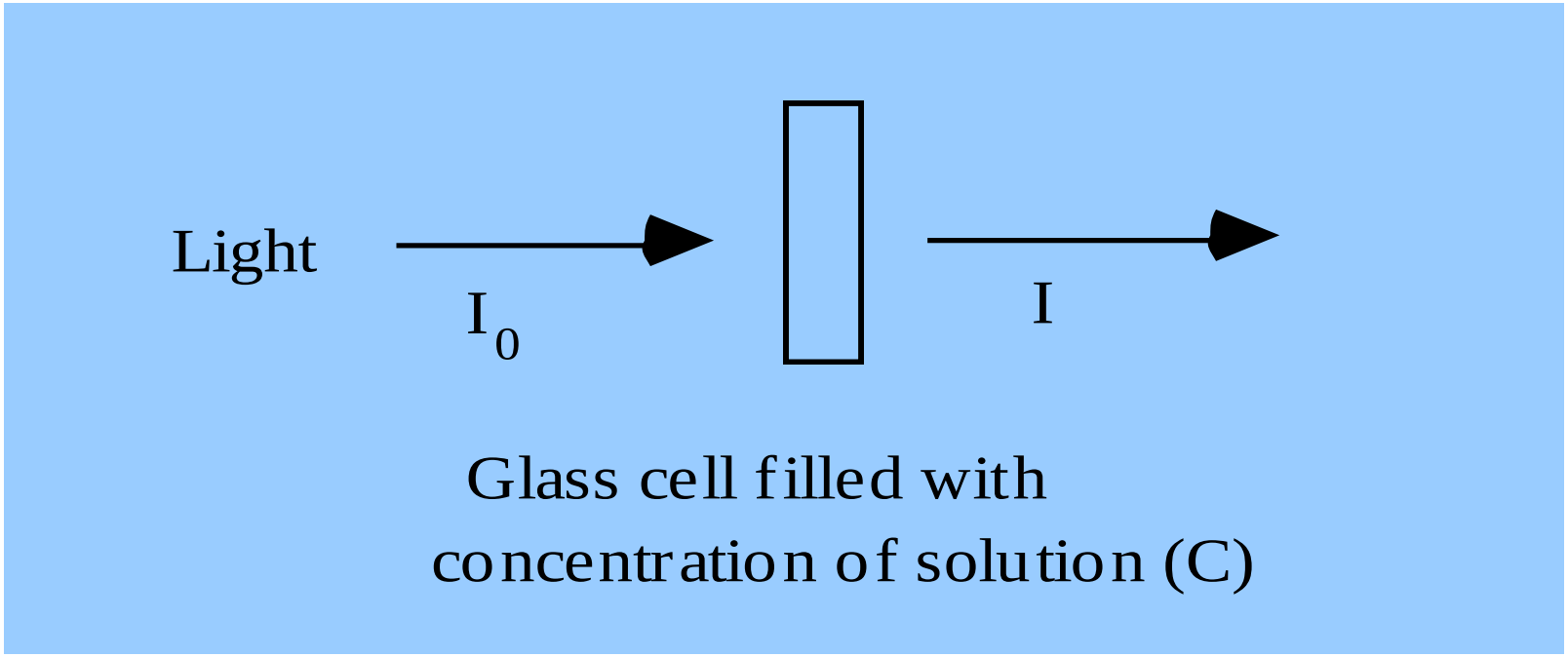
$$A = E. \, b. \, C$$

E = Koefisien ekstingsi spesifik (ml $g^{-1} cm^{-1}$)

b = tebal kuvet (cm)

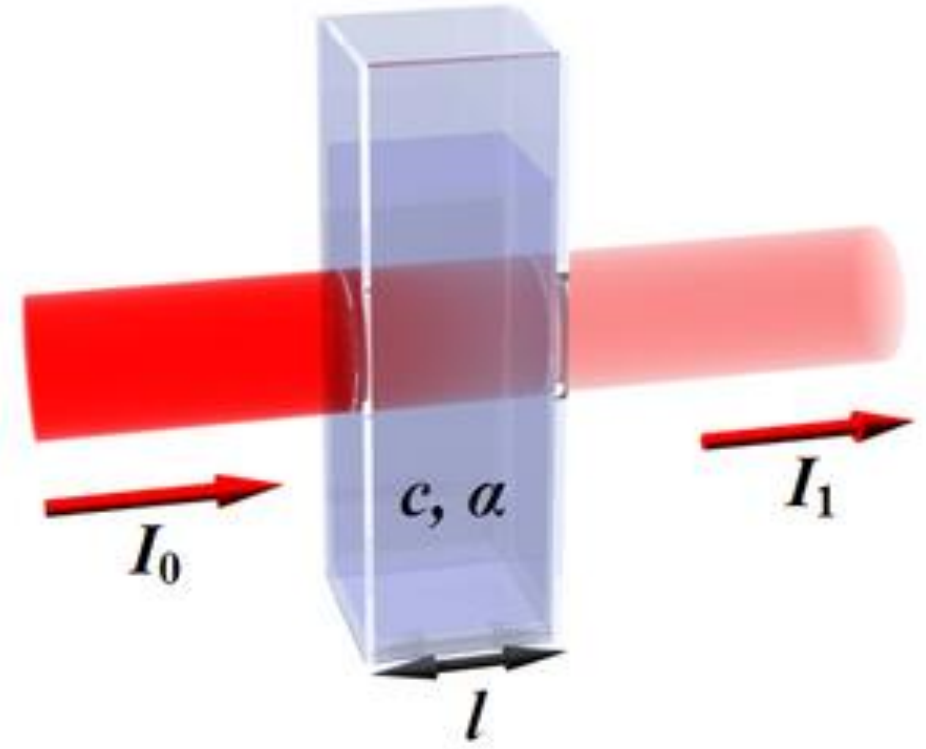
C = Konsentrasi (gram/100 ml).

BOURGER LAMBERT BEER LAW



As the cell thickness increases, the intensity of I (transmitted intensity of light) decreases.

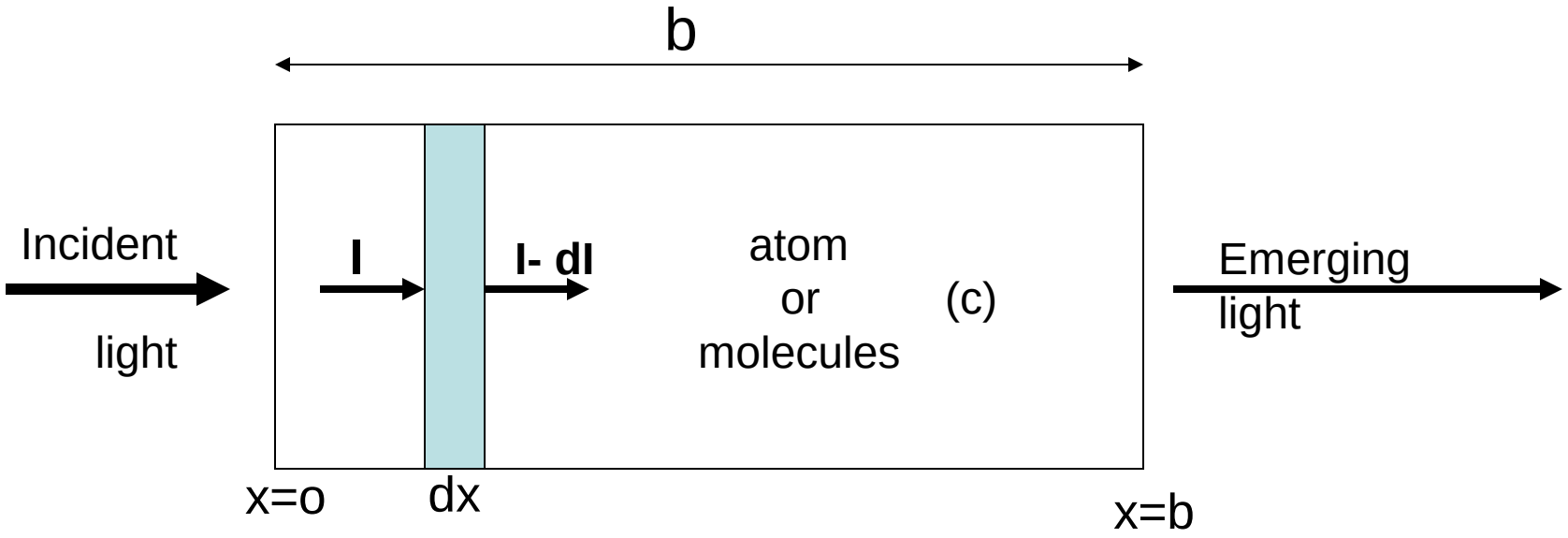
Quantitative



Pierre Bouguer (1729), Johann Heinrich Lambert (1760) and August Beer (1852) :

$$A = a.b.c$$

Transmittance : $T = I_1 : Io = It : Io$



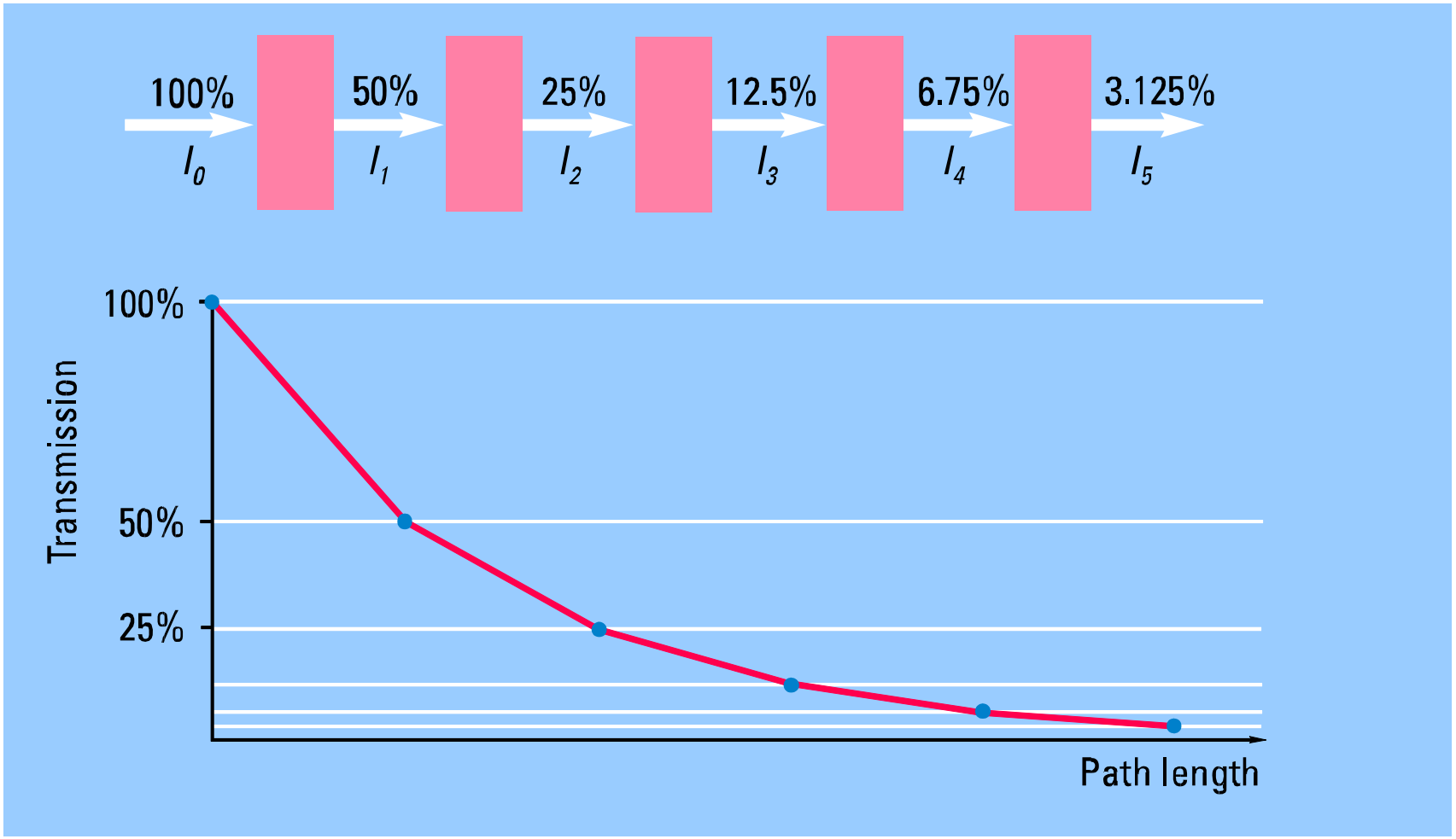
$dI = - ß\ I\ c\ dx$

$-dI/I = ßc\ dx$

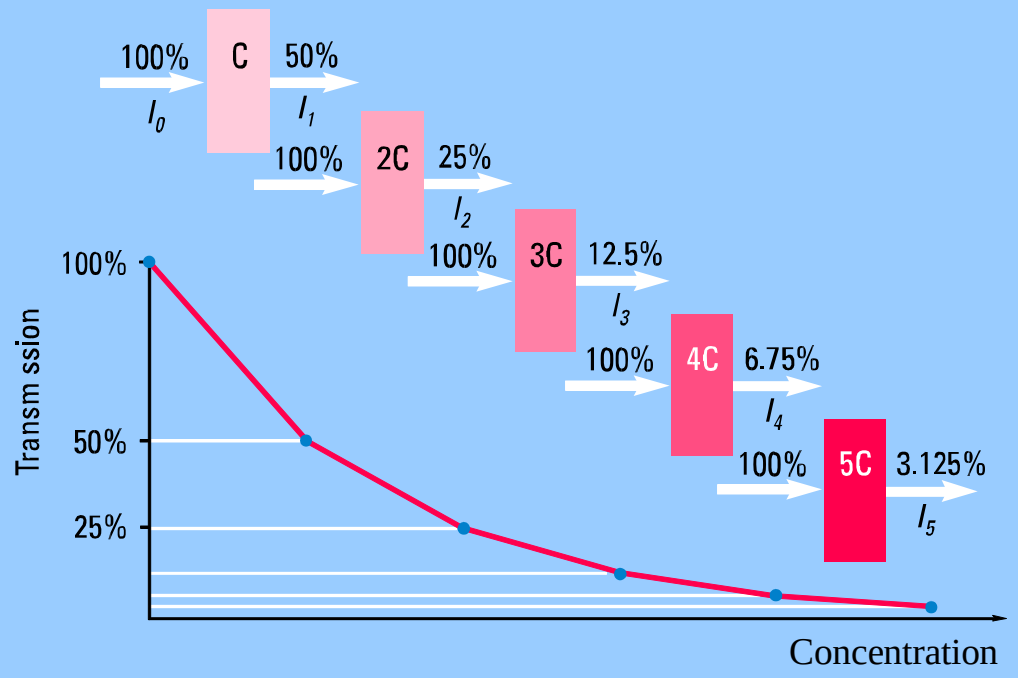
Integ $dI/I = ß$ c integ dx

⟶ $A = a\ b\ c$

Transmittance and Concentration
The Bouguer-Lambert Law

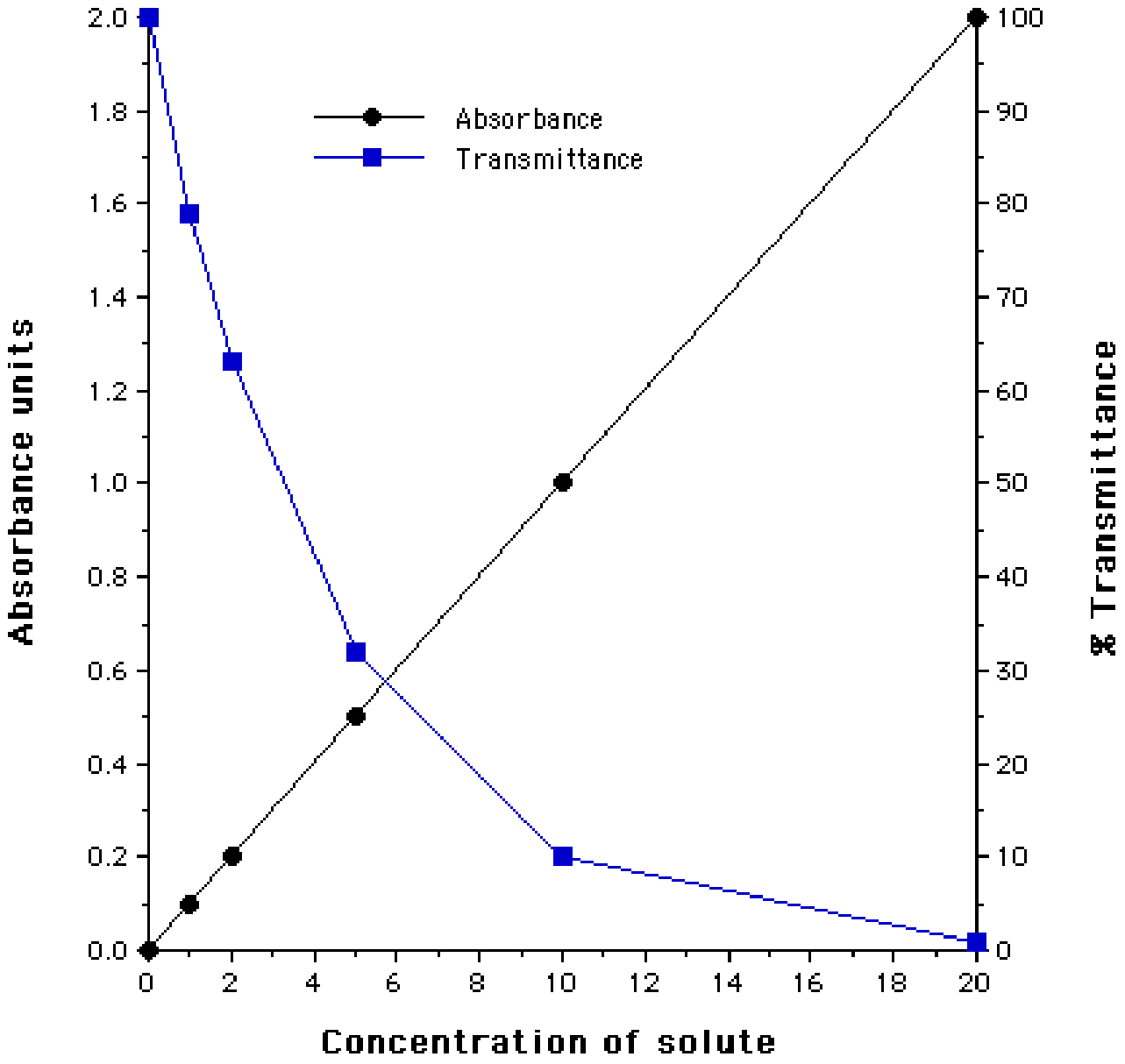


Transmittance and Path Length: Beer's Law

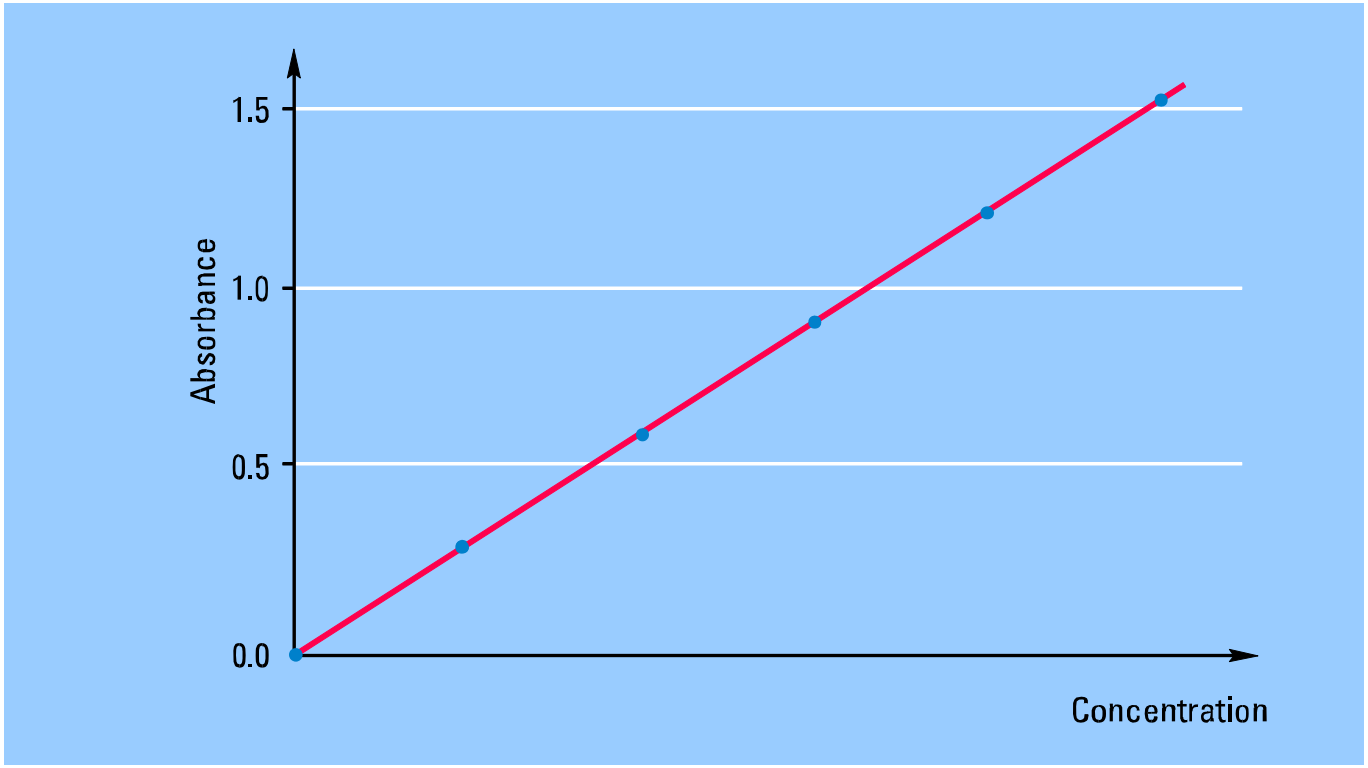


Relating Absorbance and Transmittance

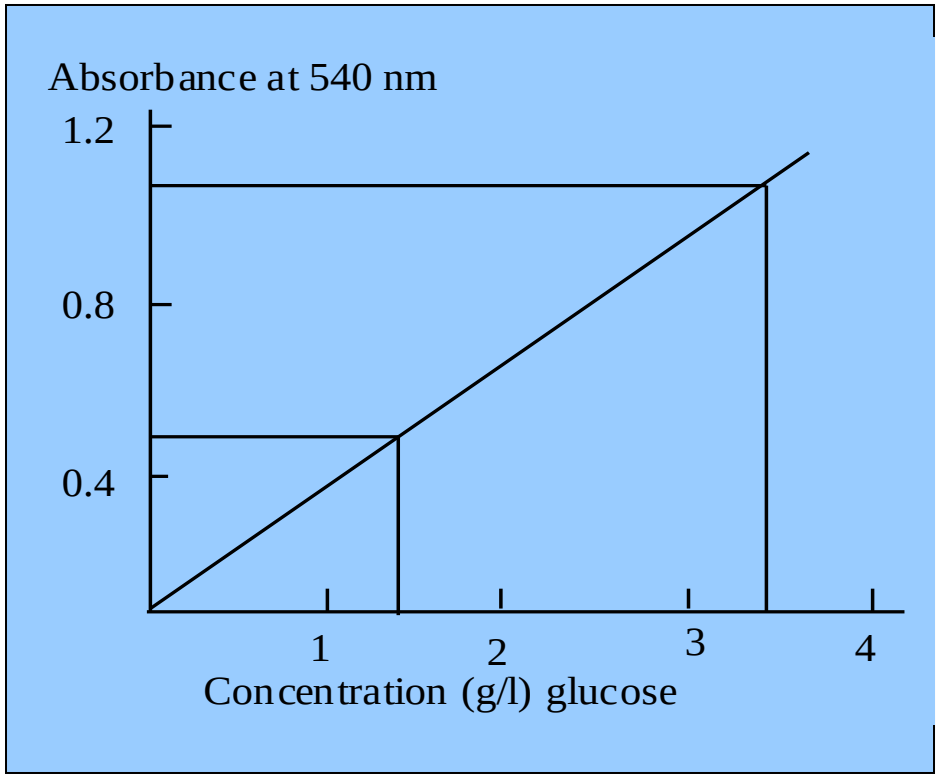
- **Absorbance rises linearly with concentration. Absorbance is measured in units.**
- **Transmittance decreases in a non-linear fashion.**
- **Transmittance is measured as a %.**
- **Absorbance = log10**
  - **(100/% transmittance)**


Absorbance
Transmittance
Absorbance units
% Transmittance
Concentration of solute
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
14
16
18
20

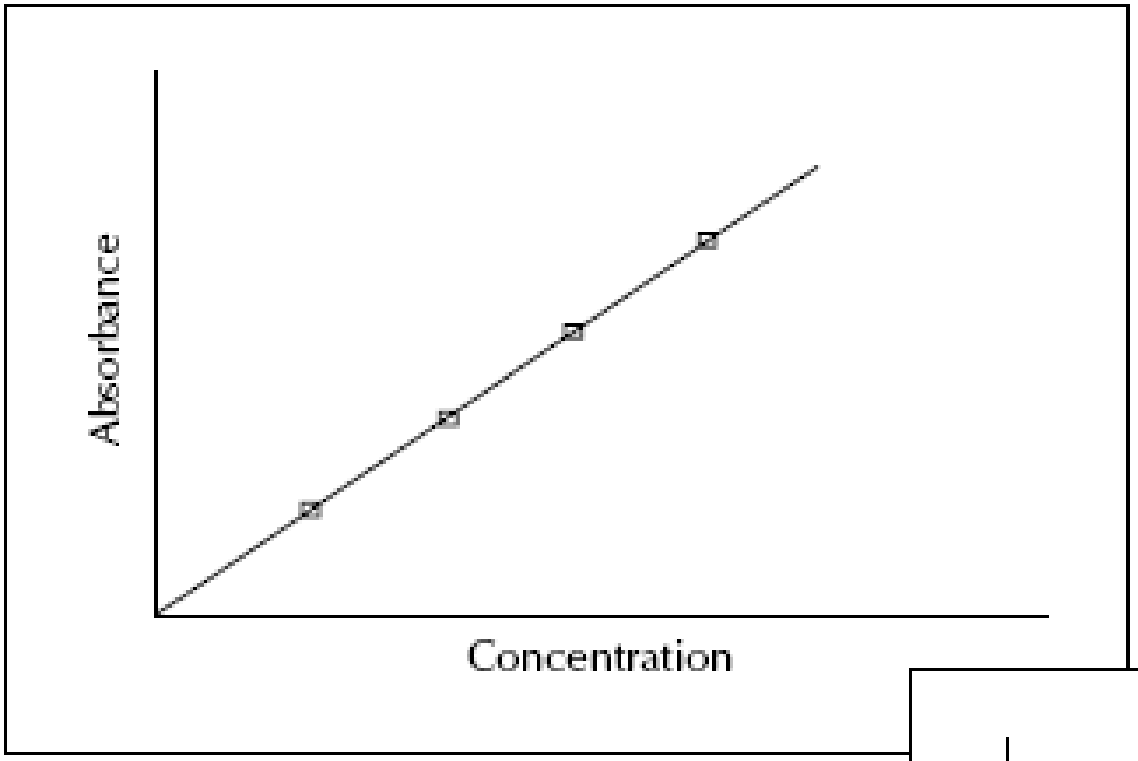
The Beer-Bouguer-Lambert Law

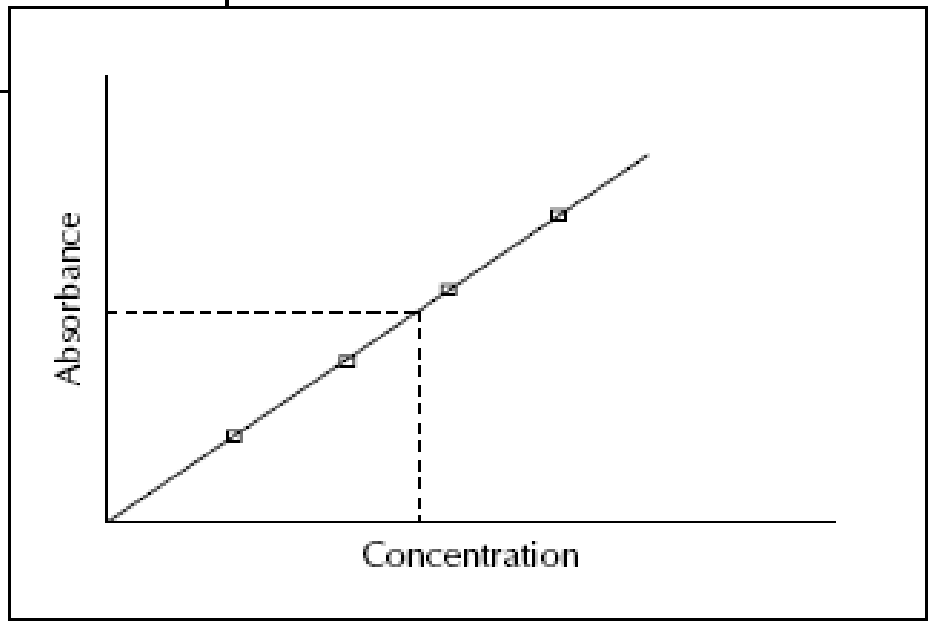


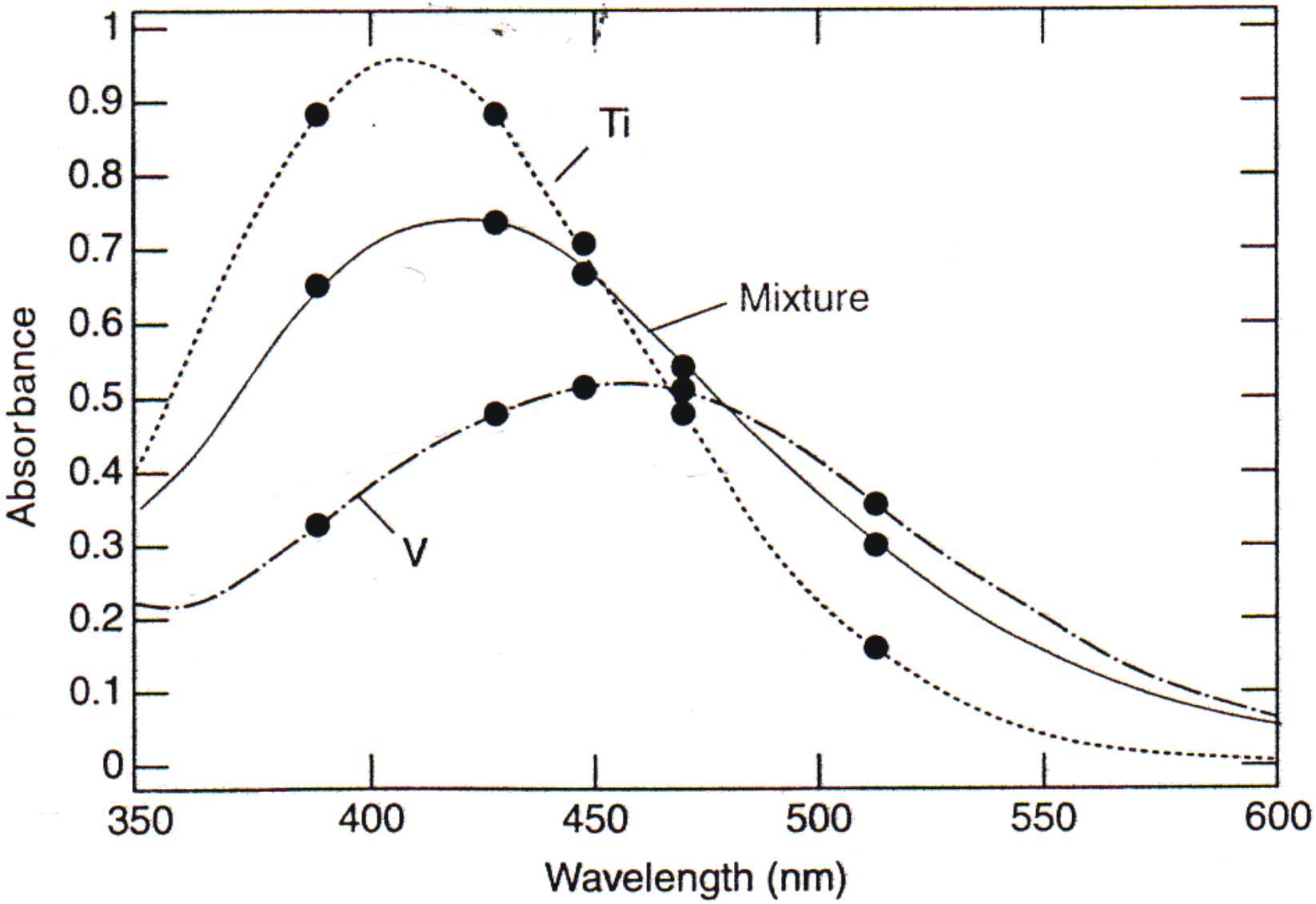
SPECTROMETRIC ANALYSIS USING STANDARD CURVE

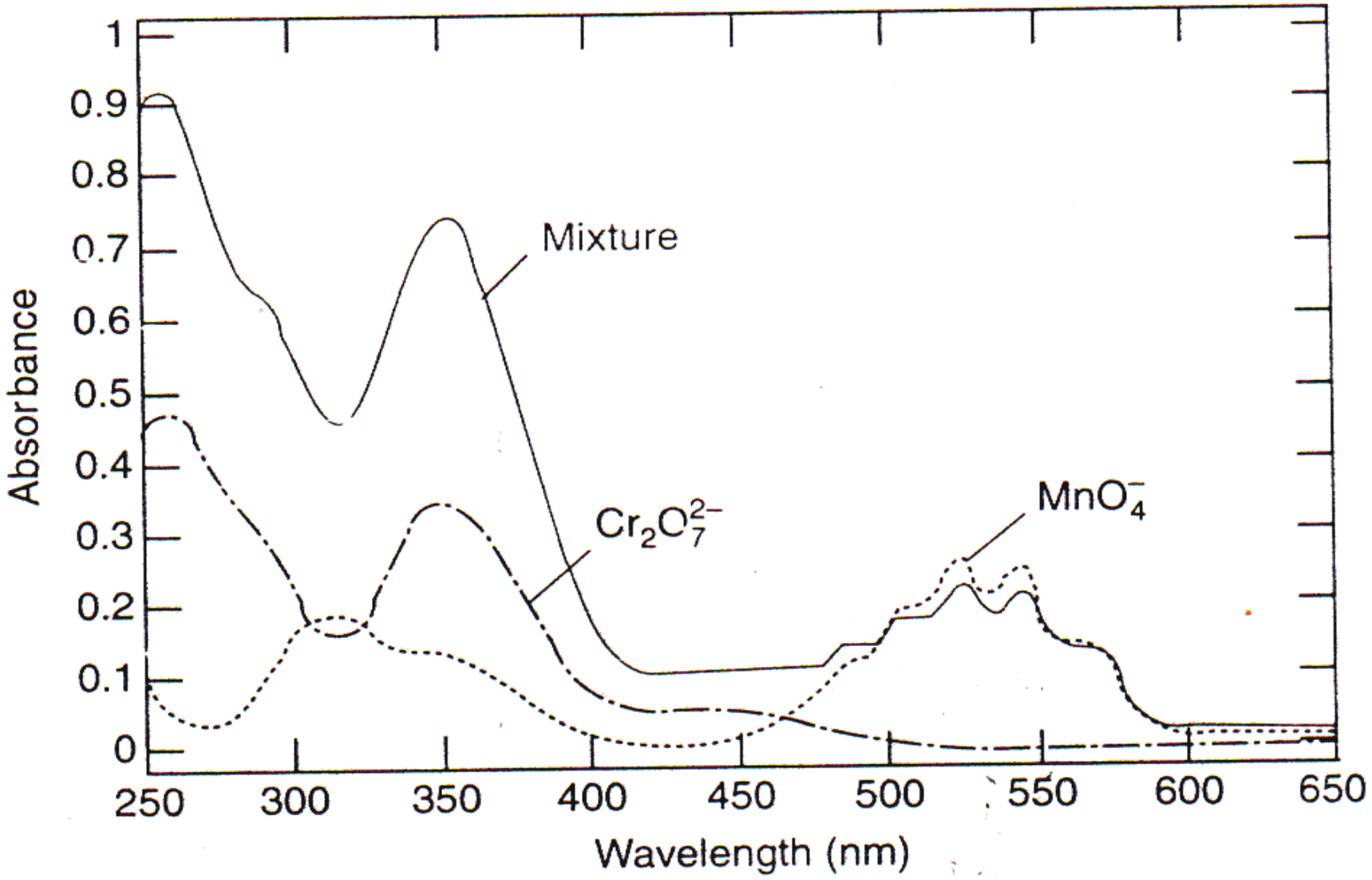


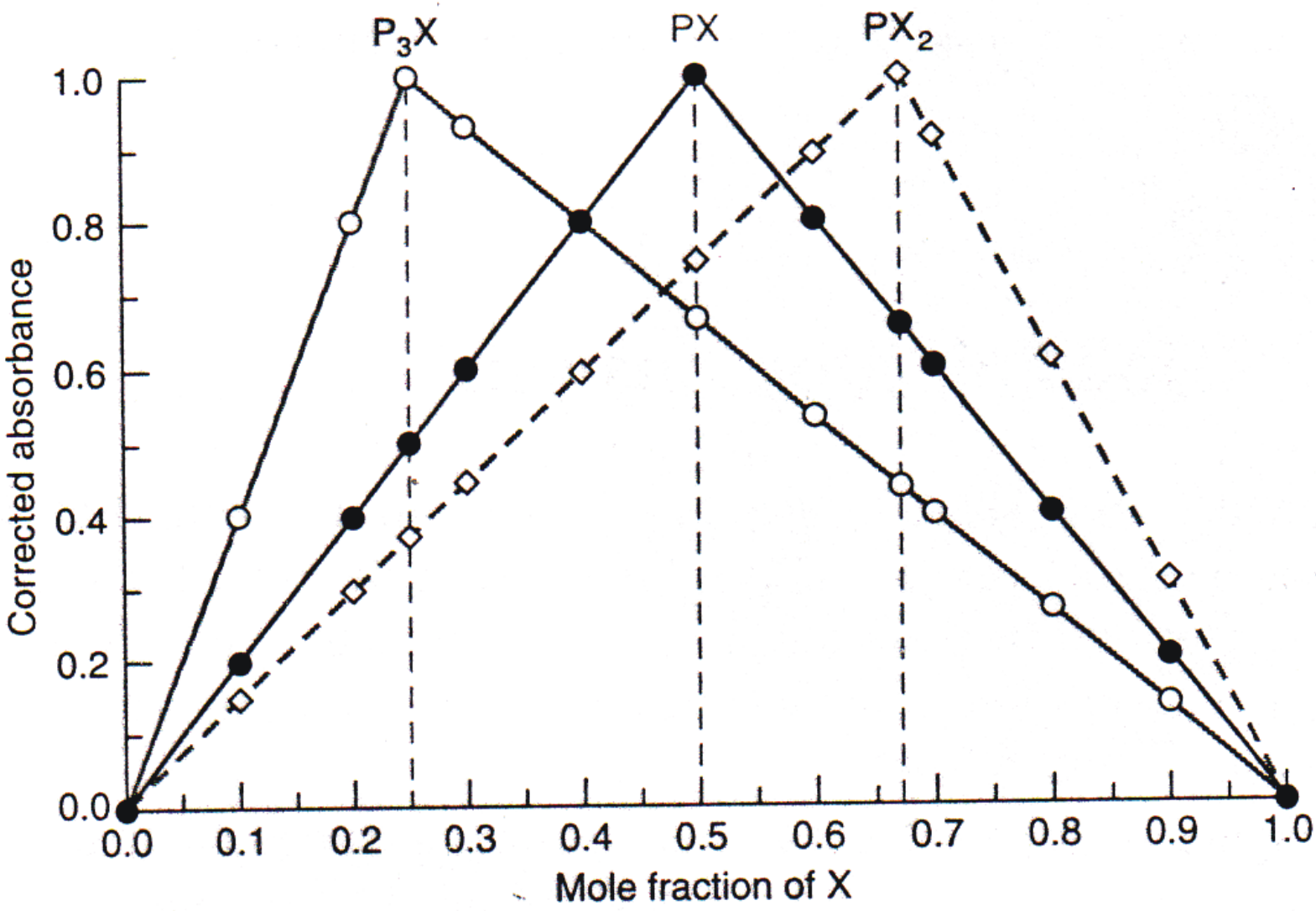
Avoid very high or low absorbencies when drawing a standard curve. The best results are obtained with $0.1 < A < 1$. Plot the Absorbance vs. Concentration to get a straight line

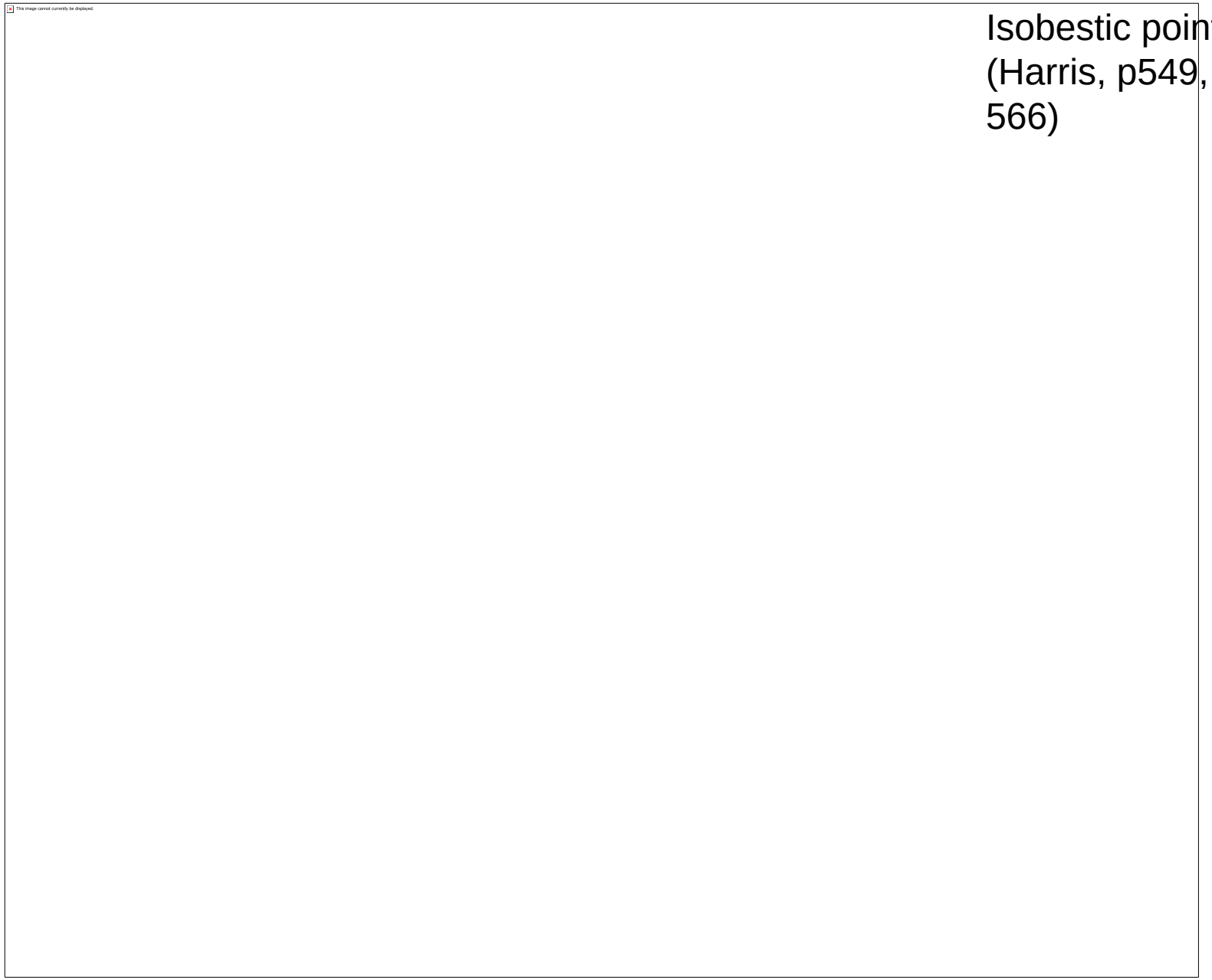

Absorbance
Concentration


Absorbance
Concentration


Absorbance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
350
400
450
500
550
600
Wavelength (nm)
Ti
Mixture
V

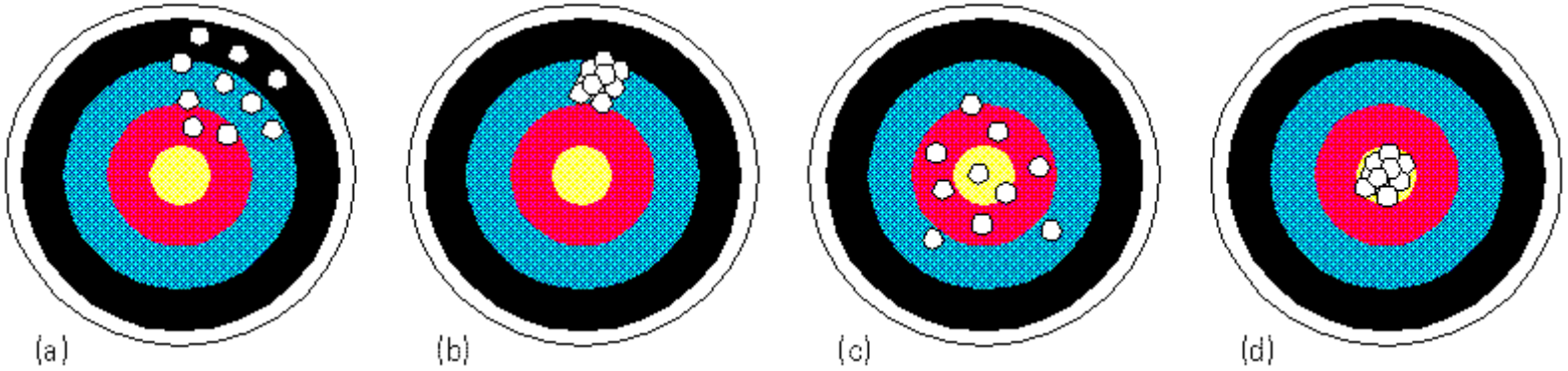

Absorbance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mixture
$Cr_2O_7^{2-}$
MnO_4^-
250
300
350
400
450
500
550
600
650
Wavelength (nm)


P_3X
PX
PX_2
1.0
0.8
0.6
0.4
0.2
0.0
Corrected absorbance
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Mole fraction of X



Isobestic point (Harris, p549, 566)

Precision and Accuracy

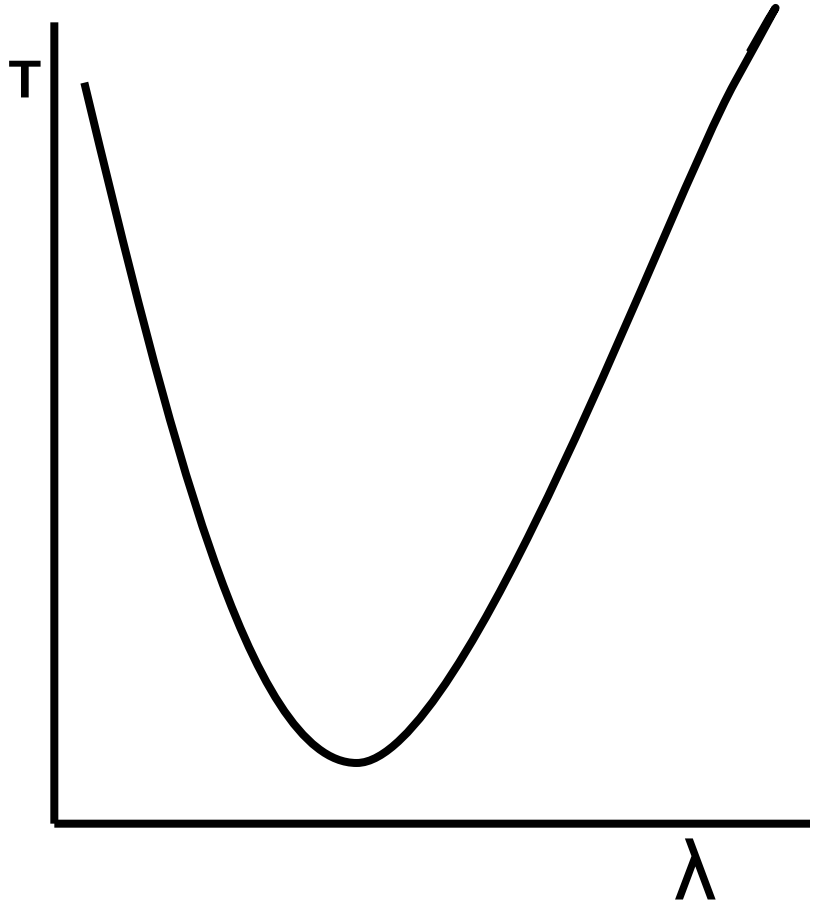


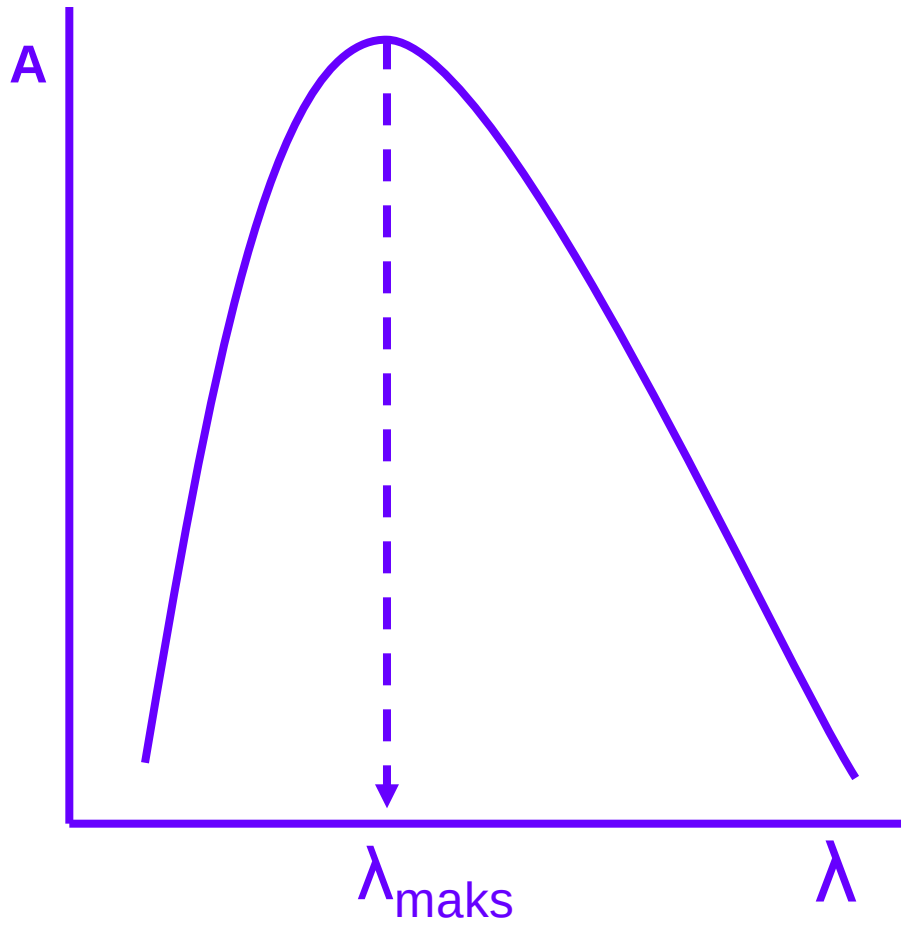
(a)	(b)	(c)	(d)
Precision –	**Precision +**	**Precision –**	**Precision +**
Accuracy –	**Accuracy –**	**Accuracy +**	**Accuracy +**

Photomultipliers are constructed from a glass vacuum tube which houses a dynode and an anode. Incident photons strike the photocathode material which is present as a thin deposit on the entry window of the device, with electrons being produced as a consequence of the photoelectric effect. These electrons are directed by the focusing electrode towards the electron multiplier, where electrons are multiplied by the process of secondary emission.

The electron multiplier consists of a number of electrodes, called dynodes. Each dynode is held at a more positive voltage than the previous one. The electrons leave the photocathode, having the energy of the incoming photon. As they move towards the first dynode they are accelerated by the electric field and arrive with much greater energy. On striking the first dynode, more low energy electrons are emitted and these, in turn, are accelerated toward the second dynode. The geometry of the dynode chain is such that a cascade occurs with an ever-increasing number of electrons being produced at each stage. Finally the anode is reached where the accumulation of charge results in a sharp current pulse indicating the arrival of a photon at the photocathode.

Read out system





An ultraviolet-visible spectrum is essentially a graph (or plot) of light absorbance vs. wavelength (represented by the symbol λ) in a range of ultraviolet and/or visible regions.

For the given substance, the wavelength at which maximum absorption in the spectrum occurs is called λmax, pronounced "Lambda-max".

To obtain absorption information, a sample is placed in the spectrophoto- meter, ultraviolet and/or visible light at a certain wavelength (or range of wavelengths) is transmitted through the sample.

The spectrophotometer measures how much of the light is absorbed by the sample. The intensity of light before going into a certain sample is symbolized by Io. The intensity of light remaining after it has gone through the sample is symbolized by It. The fraction of light transmittance is (It/Io), which is usually expressed as a percent Transmittance (%T).

From this information, the absorbance of the sample is determined for that wavelength or as a function for a range of wavelengths. Sophisticated UV/ Vis spectrophotometers often do this automatically.

Although the samples could be solid (or even gaseous), they are usually liquid. A transparent cell, often called a cuvette, is used to hold a liquid sample in the spectrophotometer. The pathlength **b** through the sample is then the width of the cell through which the light passes through.

Simple (economic) spectrophotometers may use cuvettes shaped like cylindrical test tubes, but more sophisticated ones use rectangular cuvettes, commonly 1 cm in width.

For just visible spectroscopy, ordinary glass cuvettes may be used, but ultraviolet spectroscopy requires special cuvettes made of a UV-transparent material such as quartz.

http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.htm


Ngadem yuk

Soal 1

- Jika absorbtivitas molar suatu kompleks bewarna pada 240 nm adalah 3,2 x10^{3}, hitung absorbansi suatu larutan dengan konsentrasi 5,0 x 10^{-5} M bila lebar selnya 5 cm dan ukur pada 240 nm!

Soal 2

- Hitung absorbtivitas suatu senyawa yang mempunyai berat molekul 144 jika 1 x 10-5 g/ml larutan senyawa tersebut mempunyai absorbansi 0,400 pada ssel 1 cm.

Soal 3

Ubahlah harga transmitan persen berikut menjadi harga adsorban.

a. 90 b.80 c. 50 d.10

Soal 4

Ubahlah harga adsorban berikut menjadi harga transmitan persen .

a. 0,10 b. 0,50

c. 1,00 d. 1,70

Soal 5

Transmitan persen sebuah larutan dalam 2,0 cm sel adalah 50. Hitung trasmitan persen larutan dalam sel-sel yang mempunyai ukuran panjang sebagai berikut.

a. 4,0 cm b. 1,0 cm c. 0,2 cm