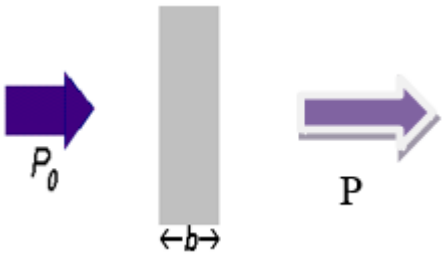
Beer Lambert law: It is the relationship between the absorbance of light is directly proportional to the thickness of the media through which the light is being transmitted multiplied by the concentration of absorbing medium.

Many compounds absorb ultraviolet (UV) or visible (Vis.) light. The diagram below shows a beam of monochromatic radiation of **radiant power P_0**, directed at a sample solution with molar concentration (c) and thickness of solution (b) Absorption takes place and the beam of radiation leaving the sample has **radiant power *P***.



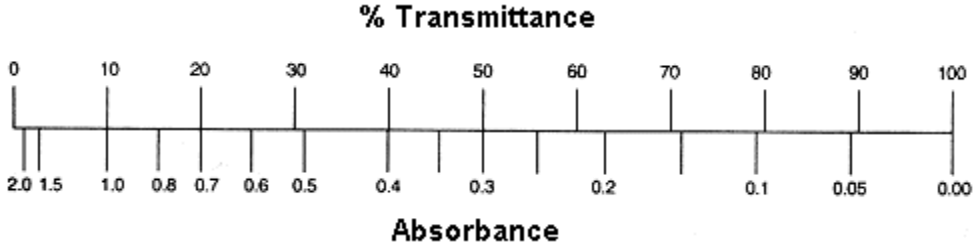
The amount of radiation absorbed may be measured in a number of ways:

Transmittance, $T = P / P_0$
% Transmittance, $\%T = 100\ T$

Absorbance= - log T

A= - log (%T /100)
$A = -(\log \%T - \log 100)$
$A = - \log \%T + \log 2$
$\boldsymbol{A = 2 - \log \%T}$

The relationship between **absorbance** and **transmittance** is illustrated in the following diagram:

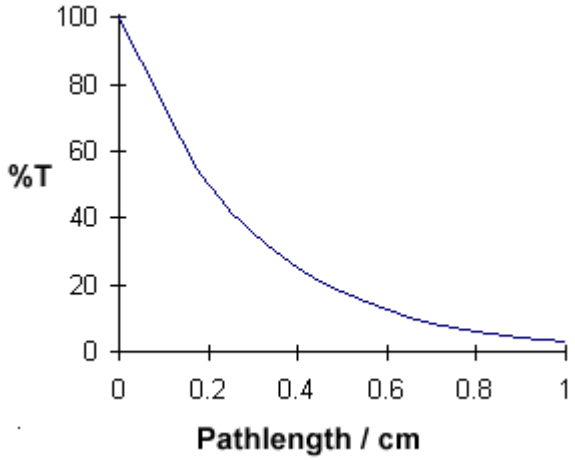


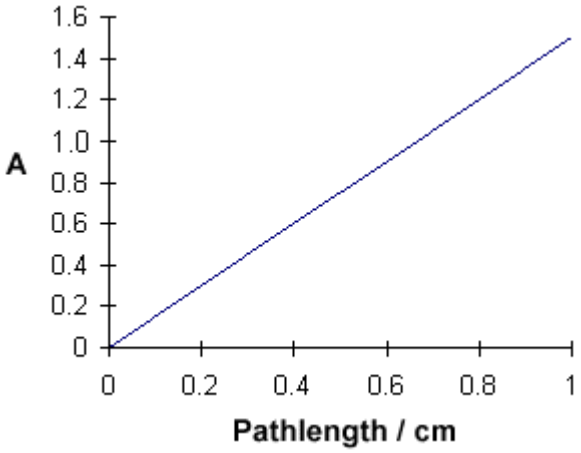
So, if all the light passes through a solution *without* any absorption, then absorbance is zero, and percent transmittance is 100%. If all the light is absorbed, then percent transmittance is zero, and absorption is infinite.

The Beer-Lambert Law

$$A=\varepsilon bc$$

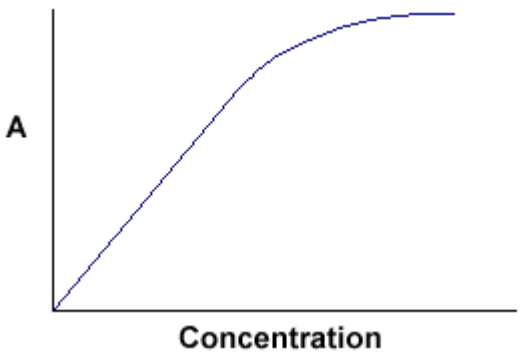
Where A is absorbance (no units, since $A= log_{10} P_0/P$) **Ɛ** is the molar absorptivity with units of L mol^{-1} cm^{-1} **b** is the path length of the sample - that is, the path length of the cuvette in which the sample is contained is (1). We will express this measurement in centimeters. **c** is the concentration of the compound in solution, expressed in mol L-1





The calibration in %T has the drawbacks of being non- linear and readings decreasing with increasing concentration.

A = a b c tells us that absorbance depends on the total quantity of the absorbing compound in the light path through the cuvette. If we plot absorbance against concentration, we get a straight



The linear relationship between concentration and absorbance is both simple and straightforward, which is why we prefer to express the Beer-Lambert law using absorbance as a measure of the absorption rather than %T.

QUANTITATIVE MEASUREMENTS

Before the analyst attempts to perform quantitative colorimetric analysis it is important to understand the theoretical aspects of the technique.

The relationship between concentration and the light absorbed is the basis of the following theoretical consideration; The seemingly obvious way of taking readings on a colorimeter is to measure % transmission and adjust the 'blank" to 100%. For example, consider a situation where a blank is measured followed by three standard solutions having concentrations of 1, 2 and 3 units respectively. Ideally, a colorimeter should be giving concentration readings directly, but consider the above solutions when analysed. The solution with a concentration of 1 unit reduces the light to 50% therefore, the solution with a concentration of 2 units will reduce the light to 25% and the solution with a concentration of 3 units will reduce the light to 12.5%.

In order to take measurements both directly and linearly in terms of concentration, %T readings must be converted into an inverse logarithmic form which are called optical density units (OD) or absorbance (A).

The formula is: = **$OD = \log_{10} 100/\%T$**

Therefore, for the given example, the relationship of OD to concentration is shown in the table below.

Concentration	%T	OD
0	100	0
1	50	0.3
2	25	0.6
3	12.5	0.9

A calibration curve or standard curve of OD against concentration linear and directly proportional at wave length constant.

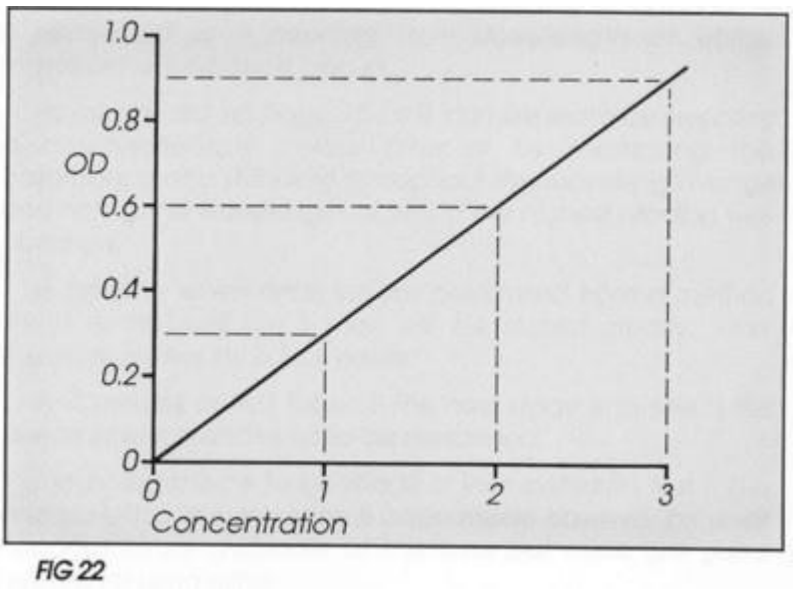


FIG 22

Optical density (absorbance) is used for colorimetric analysis so that readings relate directly to concentration.

Question: Why the absorbance does not exceed on 2? Hint:

depend on the relation **A= - log (%T /100**)

Spectrophotometer types

Spectrophotometers are instruments that measure and analyze the spectrum of samples. Which have different types depending on the various application requirements. Spectrophotometer can be divided into five subcategories according to the wavelength and application context:

1. VIS spectrophotometer
2. UV-VIS spectrophotometer
3. Infrared spectrophotometer
4. Fluorescence spectrophotometer
5. Atomic absorption spectrophotometer.

1. VIS spectrophotometer: An instrument used to measure absorbance and conduct quantitative analysis at the visible light (400 ~ 760nm), known as the visible spectrophotometer. Bacterial cell density can be determined at 600 nm.
2. UV-VIS spectrophotometer is used to measure the material of absorbance and quantitative analysis at the visible or ultraviolet light (200 ~ 760nm). Nucleic acid and protein concentrations can be measured and bacterial cell density can also be determined. UV spectrophotometer can be divided into single beam, split beam, double beam for different applications.

a) Single Beam:

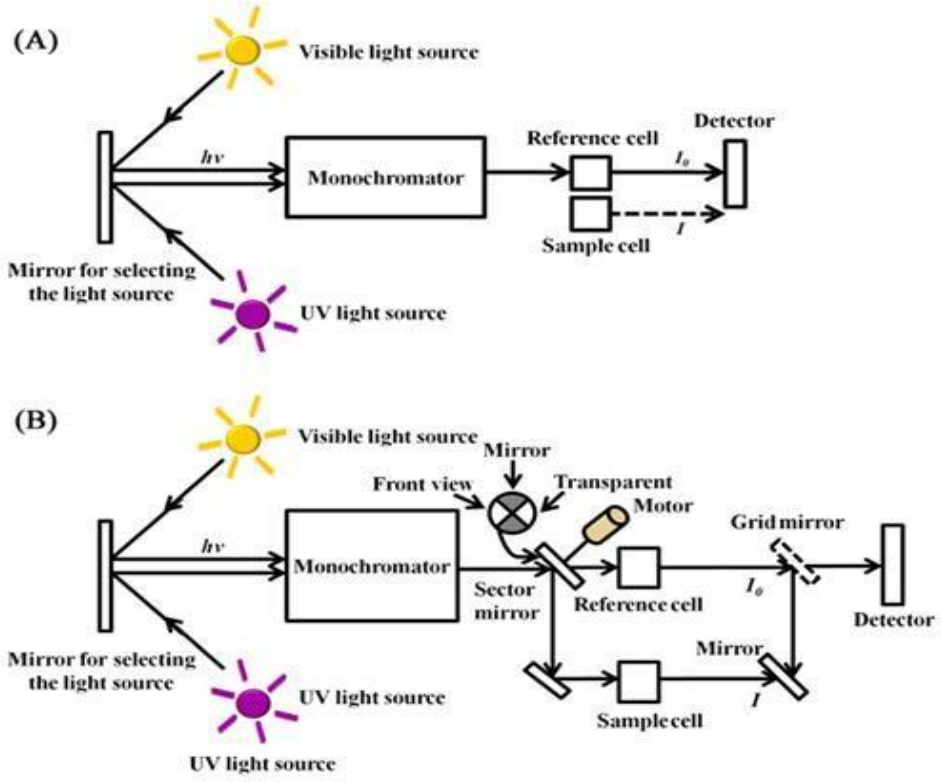
A single beam refers to the light emitted from a light source, passing through series of optical parts, absorption cell, and finally shining on the detector. It consists of a bunch of monochromatic light (the beam can only be alternately through the reference and sample solution), a cuvette, and a photoelectric converter. Work, a light path, first through the reference solution, and then through the sample solution for light intensity determination.

In general, single-beam UV-visible spectrophotometer is not suitable for high demanding pharmaceutical and quality inspection industries.

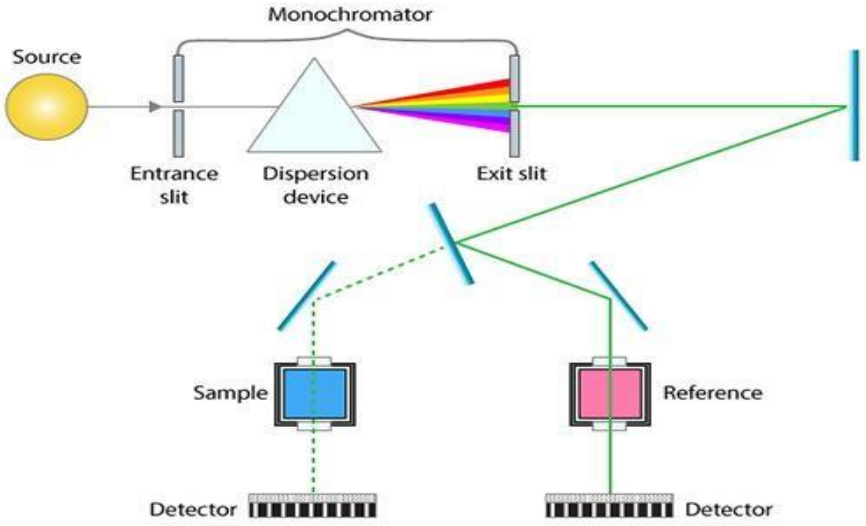
b) Double beam:

Double beam UV-visible spectrophotometer utilizes two monochromators, you can get two different wavelengths of monochromatic light. The two light beams alternately irradiate the same sample cell at regular intervals.

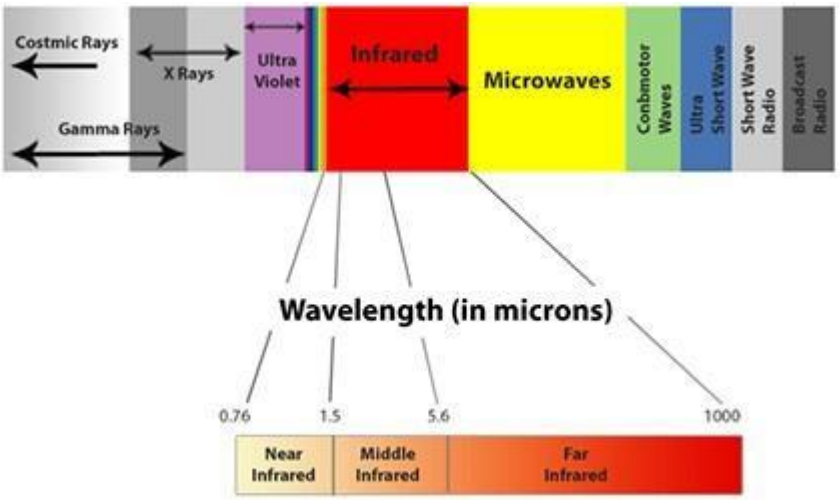
Dual-wavelength spectrophotometer can not only measure high-concentration samples, multi-component mixed samples, but also perform better in the turbid samples with more sensitivity than the single beam machine.



c) Split beam: The light emitted by the same monochromator is split into two beams, one of which reaches the detector directly and the other passes through the sample and reaches the other detector. The advantage of this instrument is that it monitors errors in the light source, but does not eliminate the effects of the reference.

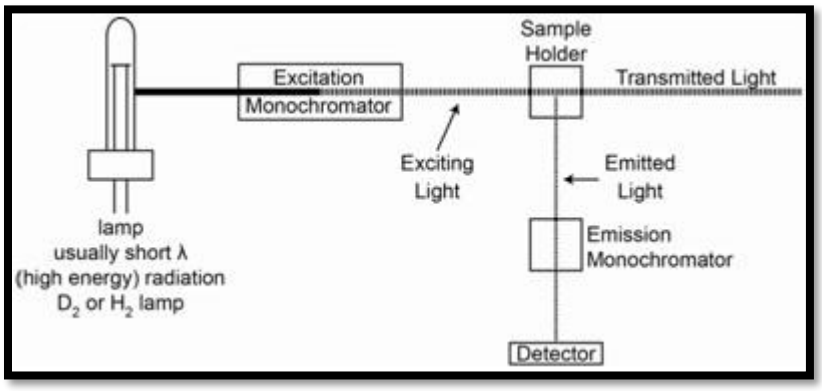


(3) Infrared spectrophotometer



The general infrared spectrum refers to the infrared spectrum greater than 760nm, which is the most commonly used spectral region of organic compounds, can analyze a variety of conditions (gas, liquid, solid) of the sample.
Infrared spectroscopy is characterized by fast, low sample volume (a few micrograms to a few milligrams), strong characterization (various substances have their own specific infrared spectrum),

4.Fluorescence spectrophotometer: is spectroscopy depend on the analysis of fluorescence light .It can be used in fields like chemistry ,medicine and pharmacy, biomedical research and environmental monitoring. In many ways the design of a fluorescence spectrophotometer is similar to an UV/VIS absorption spectrophotometer. Both are the same in that a sample is irradiated with monochromatic light from a light source after passing through a monochromator. The UV/Vis spectrometer detects light that has passed directly through the sample (absorption spectroscopy). In contrast, a spectrofluorometer detects fluorescent light emitted in the 90° direction and passed through an emission monochromator (emission spectroscopy).as shown in the following fig.



A diagram of the components of a fluorescence spectrophotometer

Ion measurement instrument (flame photometer)

Atomic absorption spectrophotometer(AAS) : is a device used in the field of Analytical Chemistry .Flame atomic absorption is applied measure the micro and trace metallic elements test, covering over 30 elements ,especially suitable for testing Cu, Zn, K, Na, Li, Ca, etc.. The instrument has simple structure, provide fast test with good repeat ability and less interference.

The principle depend on : The sample in solution is aspirated through an aspirator or nebulizer into the burner which the is flame is usually a propane / air fuel or, even, a purified natural gas/air mixture. The sample matrix evaporates followed by atomization of the sample. Atoms present in the high temperature zone of the flame are excited to higher energy levels by absorbing energy from the flame. As excited atoms return to the ground state they emit radiation in definite wavelength depending on the energy level from which each atom drop. This gives rise to a line spectrum. However, in flame photometry a pre-selected filter is used and it is the intensity of the emission line that is practically measured and is related to the original concentration of the sample in solution. The detector is usually a phototube or a photomultiphier tube depending on the quality of the instrument to convert it to digital reading. A schematic diagram of a simple flame photometer is shown is shown in Figure 1.

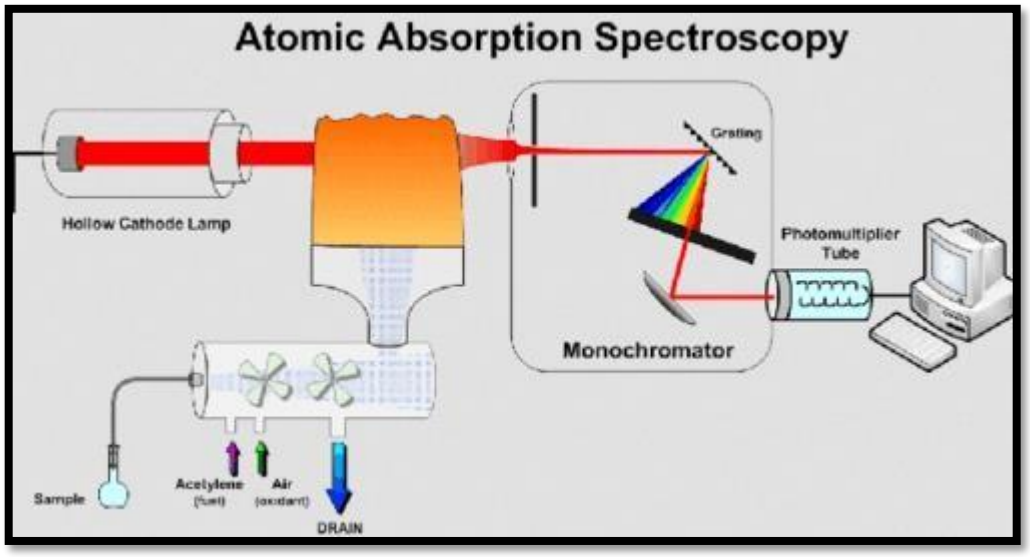
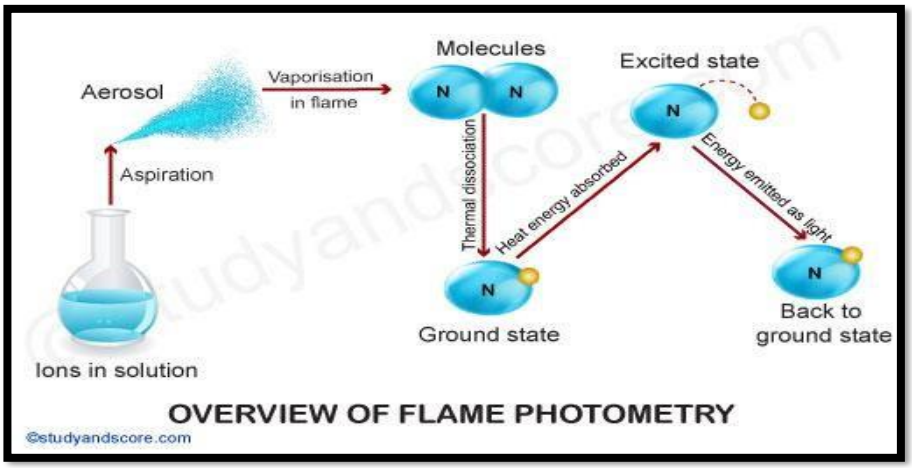


Fig. (1) schematic diagram of a simple flame photometer

A brief over view of the process:

1. First aerosol are formed from sample solution by a jet of compressed gas this process is called nebulization.
2. The liquid sample introduced into flame then the solution is evaporated leaving fine divided solid particles.

3. The solid particles move towards the flame where the gaseous atoms and ions are produced.
4. The ions absorb the energy from the flame and excited to high energy levels.
5. when the atoms return to the ground state, radiation of the characteristic element is emitted energy at characteristic wave length.
6. The measurement of the emitted photon from the element used photomultiplier tube detector.
7. The intensity of emitted light is related to the concentration of the element.



Wavelength of emitted light is specific for specific element

Element	Wavelength	*Observed Colour of flame*
Lithium	670	Red
Sodium	589	Yellow
Potassium	766	Violet
Calcium	622	Orange
Barium	554	Pale green

Calibration curve

In flame photometry emitted light intensity from the flame is directly proportional to the concentration of the species being aspirated. The graph below shows that the direct relationship between the emission and concentration is true only at relatively low concentrations of mg/L level (up to 50 mg/L).