Advanced pharmaceutical analysis

Using instruments

Instrumental analysis

- The use of spectrophotometer for the analysis of compounds which is divided into qualitative (what are they?) and quantitative (how much is there?)(Concentration or quantity)

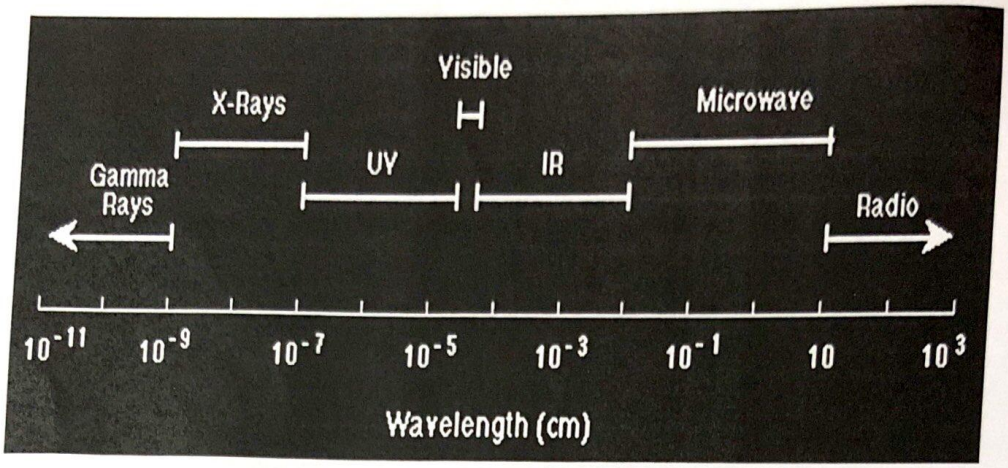
UV-Visible Spectroscopy

- It involves absorption within UV region 200-400 nm (for colorless compounds) and visible region 400-800 nm (for colored compounds).

The principle

- For absorption to occur within this region of spectrum the compounds should be **conjugated systems or aromatic compounds** while saturated hydrocarbons show no absorption in this region so they can be used as solvents e.g. cyclohexane, n- hexane.
- Some in organic species are colored and show absorption within the visible region e.g. chromic nitrate.
- Absorption spectra in the ultraviolet and visible regions are due to energy transitions of both **bonding and nonbonding *outer* electrons** of the molecule.

Electromagnetic spectrum



Electromagnetic spectrum

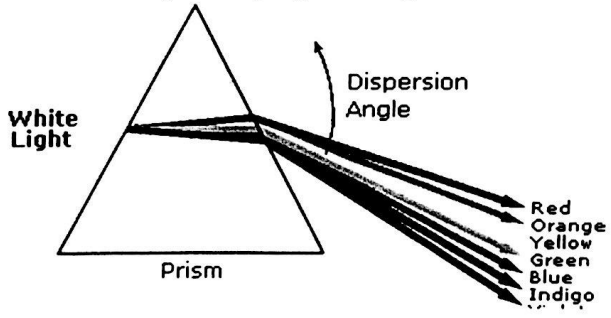
- Cosmic rays (highest energy, highest frequency, and shortest wave length)
- ϑ- rays
- X-rays
- UV-rays
- Visible rays
- IR-rays
- Micro waves
- Radio waves (lowest energy, lowest frequency, and longest wave length)

The principle

- Electrons in **σ bonds** (a single covalent bond) are tightly bound and radiation of high energy (short wave length) is needed to excite them.
- Certain atoms like N, O, and halogens have **non bonding electrons (lone pair)** that are less tightly bound than the previous and can be excited at a lower energy (longer wave length) radiation.
- Electrons in double or triple bonds **(π electrons)** are easy to be excited (loosely bound) and in compounds that contain series of alternating double bonds (**conjugated systems**), the π electrons are delocalized due to resonance and require less energy for excitation (longer wave length).

Visible light

- White light is a combination of lights of different wavelengths in the visible spectrum. Passing white light through a prism splits it up into the several colors of light observed in the visible spectrum between 400 nm and 780 nm.
- A common feature of colored compounds is a system of **extensively conjugated pi-electrons**.



Instrument parts

1. Light source: deuterium lamp (UV light), or tungsten lamp (visible).
2. Monochromator: allow the passage of light in certain selected wave length and neglecting the other unwanted wave lengths (using wave control knob).
3. Sample compartment: accommodate the sample (inside the cuvette) to be exposed to the monochromatic light.
4. Detector: responsible for converting light signals (transmitted) to electrical signals.
5. Microprocessor: translate the electrical signals to digital signals.
6. Displayer: display the digital signals on screen (A or %T).

Definitions

- ***Cuvette***: either quartz (UV) or glass (visible).
- The use of scratched or contaminated cuvettes should be avoided since they reflect and/or absorb radiation that will give you inaccurate measurements. Also, bubbles, turbidity, fingerprints, should be avoided since they will diminish the accuracy of readings. The cuvettes commonly used for accurate work have an optical path length of **1 cm** and require **2.5 to 3 ml of sample** for all accurate reading.
- ***Blank***: the medium in which the substance being measured is located may itself absorb light of certain wave length so in order to measure the absorbance due to only **a particular species** in solution, **zeroing** is needed, in which the blank is added in the light path and the light control knob is rotated until read 100%T (A=0).

Determination of the absorption spectrum of chromic nitrate solution $Cr(NO_3)_3$ (qualitative analysis)

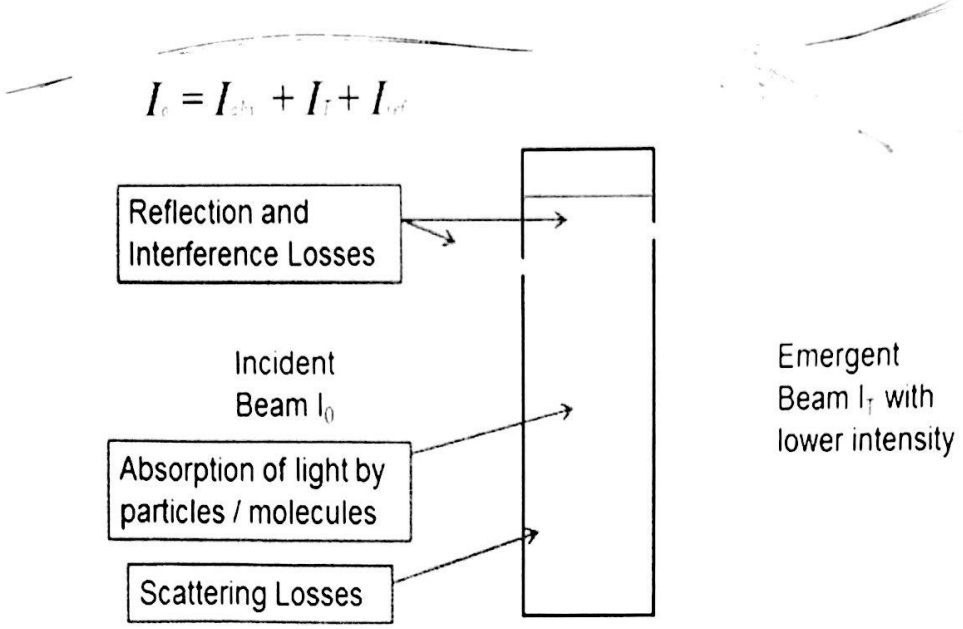
- By gradual scanning along wide range of wave lengths then reading A and %T
- Plot your data on graph paper (plot absorbance (A) on the y-axis as a function of wavelength (λ) on the x-axis).
- Plot %T on the y-axis as a function of wavelength (λ) on the x-axis.
- Establish the λ_{max} and the λ_{min} of the sample.

Wave length	Absorbance(A)	Transmittance %

Quantitative analysis

Introduction

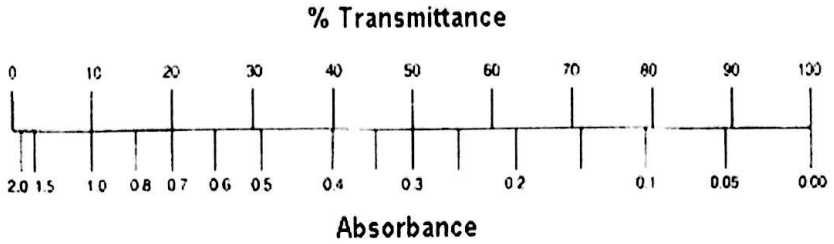
- Many compounds absorb ultraviolet or visible light. When a beam of monochromatic radiation I_o is directed at a solution, some will be absorbed, some transmitted I_T , and some reflected $I_{ref.}$
- I_{ref} is compensated by the blank



Relationship between absorbance and transmittance

- $T = I / I_0$ (the fraction of light that passes through the sample)
- where: I_0 = intensity of the incident radiation entering the medium.
- I = intensity of the transmitted radiation leaving the medium.
- T is usually expressed as percent transmittance, %T:
- **%T= T x 100**
- The relationship between percent transmittance (%T) and absorbance (A) is given by the following equation:
- **$A=\log I_0 / I$**
- **A= log 1/T**
- **A= -log T**
- **A= log 100/ %T**
- **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.

Beer-Lambert Law

- Two scientists had studied the factors that affect the extent of absorption of monochromatic radiation at certain wave length .
- **Lambert** studied the effect of the thickness of the medium on absorption , he stated that the intensity of transmitted light is inversely proportional to the thickness of the medium.

$$A=K\,t$$

- **Beer** studied the effect of concentration of solution on absorption, he stated that the intensity of transmitted light is inversely proportional to the concentration of solution.

$$A=K\,c$$

Where A =absorbance (unitless)

t= thickness of medium or sample path length (in centimeters usually 1cm)

c=concentration (in mole/L or %w/v)

K= constant

Beer-Lambert Law

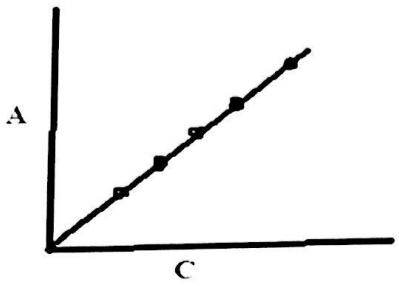
- Combining both equations
- A= K c t
- This means that the absorbance is directly proportional with the concentration of the sample absorbing the monochromatic radiation and the path length of sample (the path length of the cuvette).
- K is symbolized as ε and called the molar extinction coefficient or molar absorptivity (unit is L per mole per cm) if c is molar concentration.
- While if c is %w/v (g/100ml) then K is symbolized as $E^{\%}_{1cm}$ and called specific extinction coefficient.

$$\varepsilon = E^{\%}_{1cm} \times \text{molecular weight}/10$$

Molar absorptivity

- Molar absorbtivity is a constant for a particular substance, it is the probability of absorption of a sample at certain wave length. So if the concentration of the solution is halved the absorbance is halved also.
- A compound with a high molar absorbtivity is very effective at absorbing light (of the appropriate wavelength), and hence low concentrations of a compound with a high molar absorbtivity can be easily detected.

A1/C1=A2/C2=A3/C3



Beer-Lambert Law

- Plotting A against C we get a straight line passing through the origin this is called beer's law plot.

- Beer's law is obeyed for dilute solutions (the concentration should be low).
- Some solutions may not obey Beer's law as in sulfonamides (no straight line is obtained).

Beer-Lambert Law

- Purpose:

1. To demonstrate whether a solution obeys Beer's Law and at what λ max.
2. To find the concentration of an unknown solution from Beer's Law plot.

Calibration curve (standard curve)(Beer's plot)

- Prepare a series of standard solution with known concentration.
- Measure the absorbance of the standard solutions.
- Plot the graph Abs vs concentration of std.
- Find the "best' straight line.