Faculty of Pharmacy
Menoufia University

PHARMACEUTICAL ANALYSIS II

For

First Year Pharmacy Students

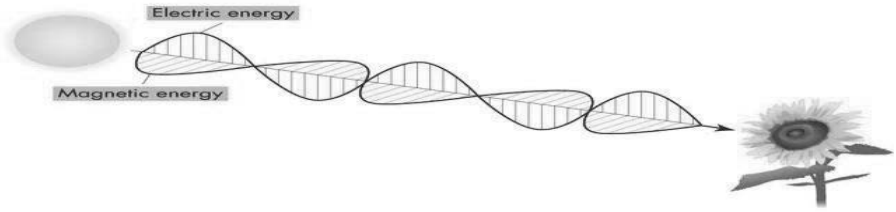
Second Semester

By Staff Members Of

Analytical Chemistry Department

Spectroscopy

What is spectrometry?
Why we see color of solution or matter?



From the earliest times, color has been used to identify chemical substances and spectrometry is an extensive of this primitive technique.

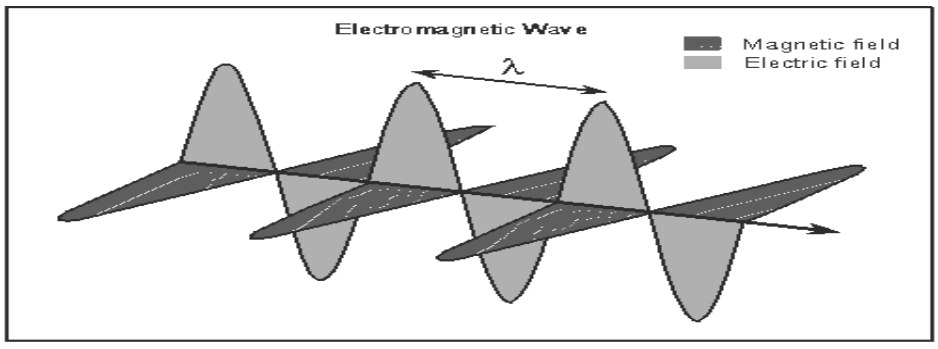
Historically, spectroscopy referred to a branch of science in which visible light was used for theoretical studies on the structure of matter and for qualitative and quantitative analyses.
Spectroscopy is based on the absorption of light by analyt. Also, defined as the study of the interaction between light and matter.

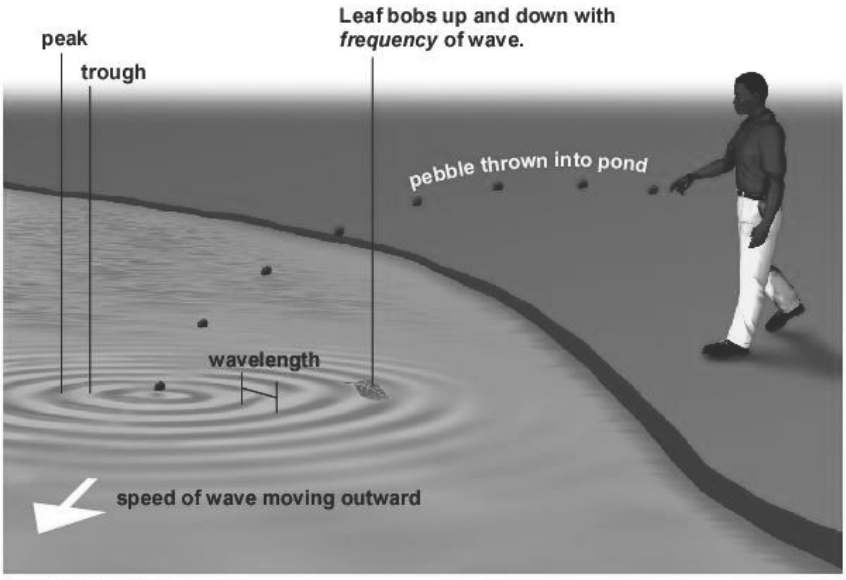
This lecture explains the meaning of the term spectrophotometry, the dual function of light as wave and energy. In addition to important terms usually used as chromophore, auxochrome and absorption spectrum.

What is Electromagnetic Radiation

Light is an electromagnetic radiation (*EMR*), composed of both electric and magnetic components. EMR is stream of discrete particles or wave packets of energy called photons or quanta.

It displays the property of continueous waves and can be described by the characteristics of wave motion

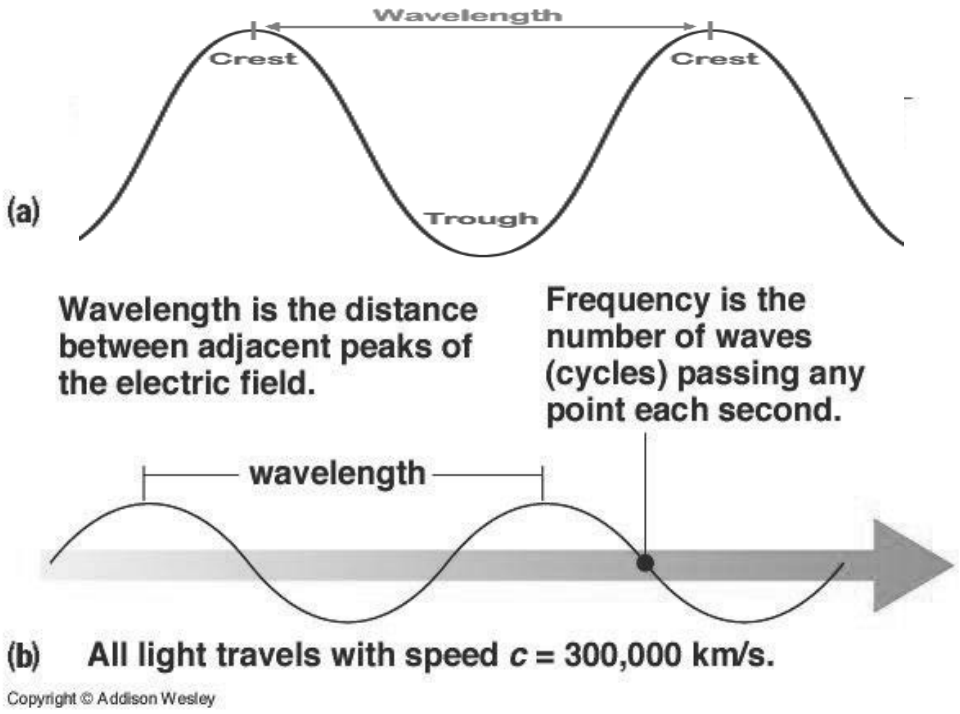




Wave Properties of Electromagnetic Radiation

Light exhibits wave property during its propagation and energy particle during its interaction with matter. Light waves, propagate at the highest known velocity of 3×10^{10}cm/sec.

Such wave motion may classified according to the wavelength (λ lambda) which is defined as: the linear distance measured along the line of propagation, between the crest of one wave to that of the next wave.



Different units of length are used to express wavelengths in different parts of the EM-spectrum.

The following units are in common use:

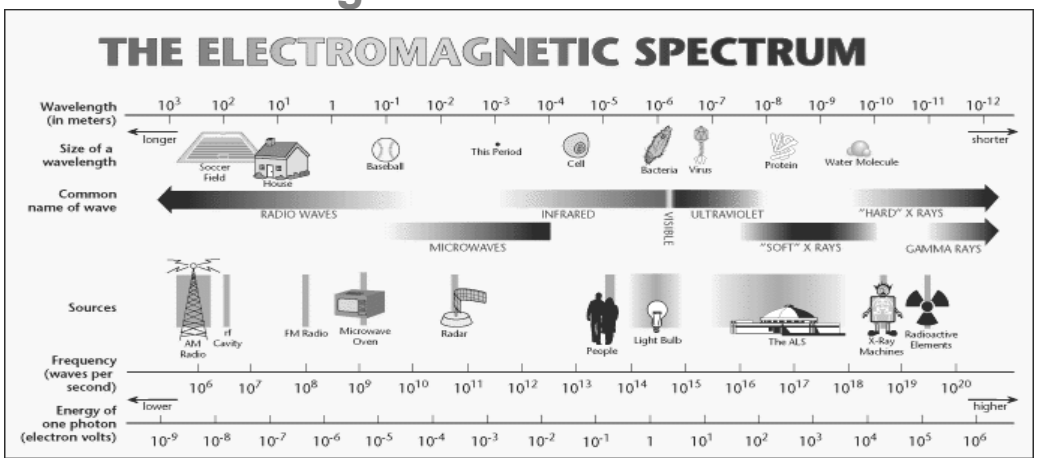
1 angstrom =1 A˚ =10^{-10}m = 10^{-8} cm

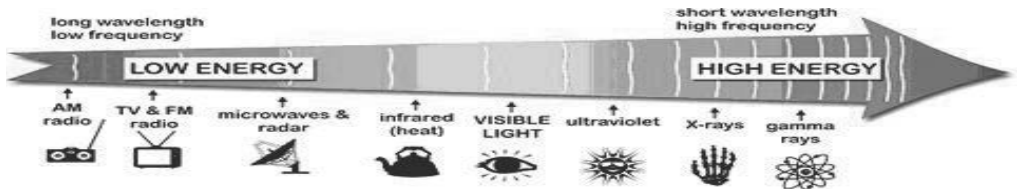
1 nanometre = 1 nm = 10A˚ =10^{-7} cm

1 micrometer = 1 um =10^{4} A˚ =10^{-4} cm

As indicated above, the electromagnetic spectrum is arbitrarely broken down into different regions according to wavelength. The various regions of spectrum are shown in next slide.
The behavior of EM radiation depends on its wavelength.

Light and radiation

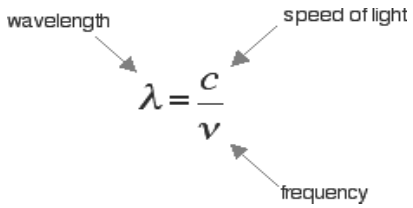




Some other terms are also used such as:

Frequency (v), which is the number of waves occurring per second and is expressed in cycles/sec(CPS) or Hertz(Hz)

Wavelength *inversely* proportional to the frequency,



and Wavenumber(ú) is number of waves per centimetre, which is expressed in cm^{-1}.

wavenumber (ú) =. 1/λ cm^{-1}

Relations between λ, v and ú are given by the following equations:

$$\frac{1}{\text{Wavelength } (\lambda)} = \text{wave number } (ú) = \frac{\text{Frequency } (\nu)}{\text{velocity of light(c)}}$$

Where **C** is the velocity of light in vacum = **3×10^{10}** cm/sec..

Note that, *the longer the wavelength, the lower the frequency and the smaller the wave number and vice versa.*

Example:

Particle properties of electromagnetic radiation

When a sample absorbs electromagnetic radiation it undergoes a change in energy,if we assume that electromagnetic radiation consist of a beam of energetic particles called photons.

Photon energy is directly proportional to the **frequency**

$$E = h\nu$$

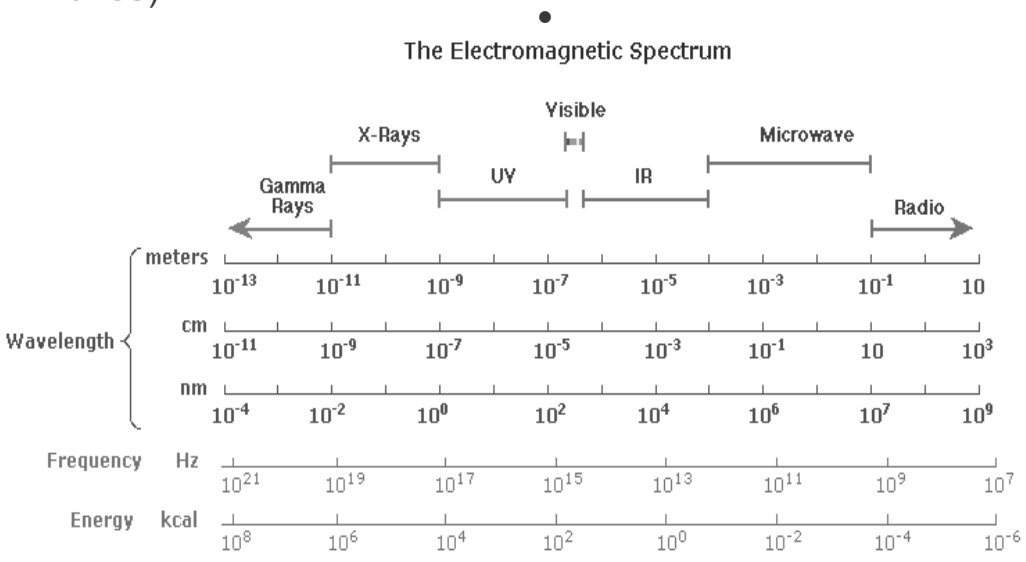
Where h is the Planck's constant (6.63×10^{-34} J.s)
The energy of a photon is [in joules]

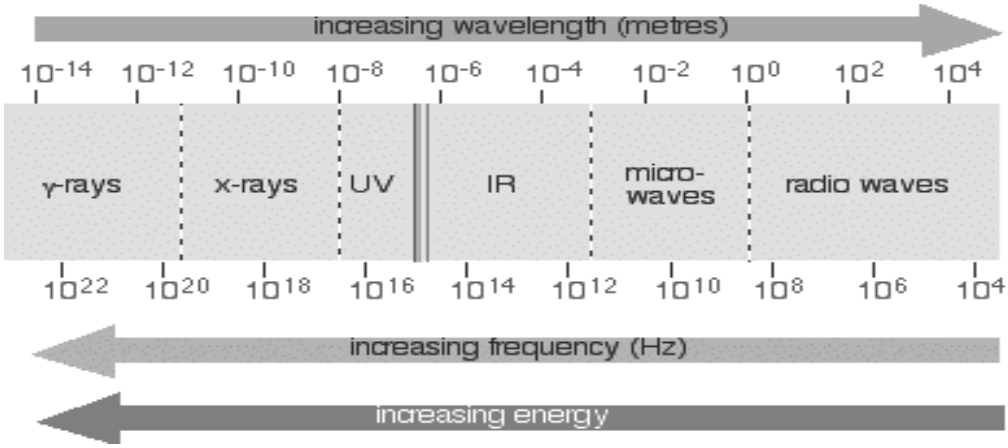
We can also relate the energy of radiation to wavelength and wave-number:

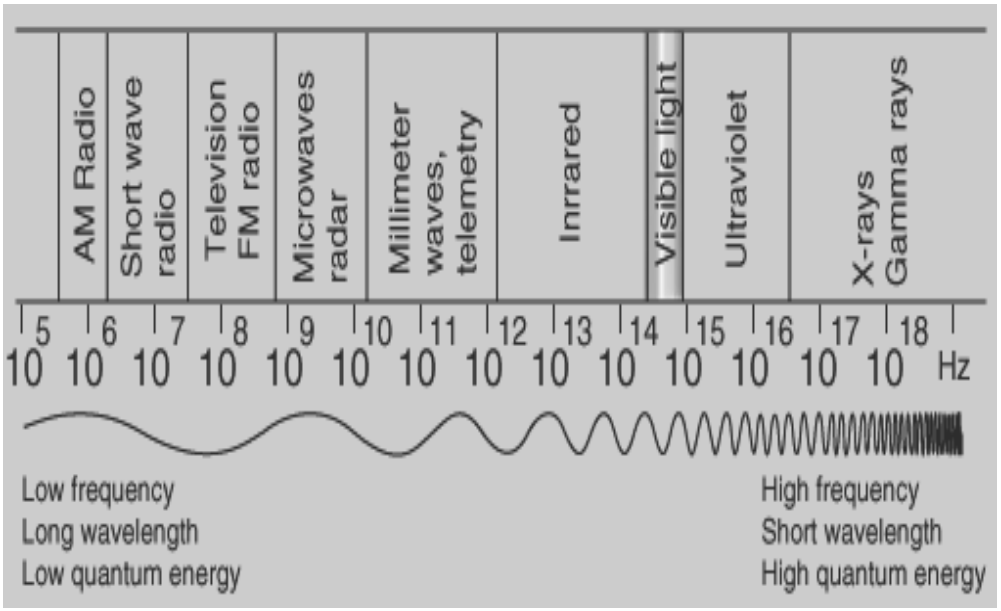
$$E = hc / \lambda = hc\,\acute{u}$$

Note that the *wave-number in common* with frequency is directly proportion to energy.

This **electromagnetic spectrum** ranges from very short wavelengths (including ***gamma and x-rays***) to very long wavelengths (including ***microwaves and broadcast radio waves***).







Why UV radiation burns? •

UV-Vis Luminescence Spectroscopy

- Visible spectrum:
- The human eye is only sensitive to a tiny proportion of the total electromagnetic spectrum between approximately 400 and 800 nm and within this area we perceive the colors of the rainbow from violet through to red[**It comes in colors as seen when white light is passed through a prism and broken into a rainbow**]

- UV-visible absorption spectra can be used to help identify compounds and to measure the concentrations of coloured solutions
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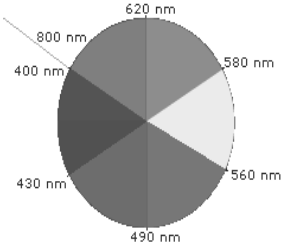
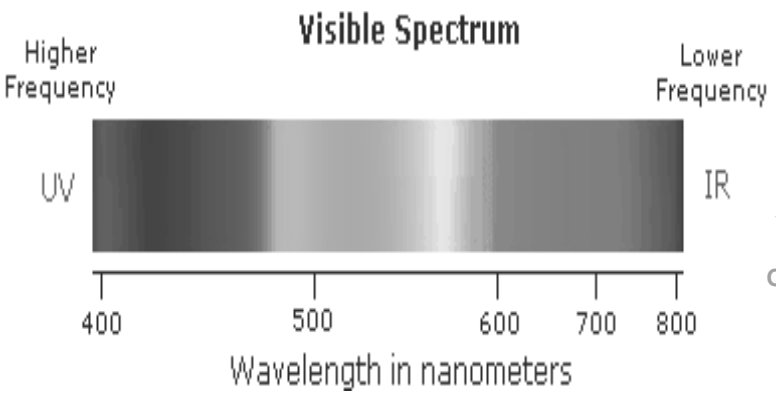
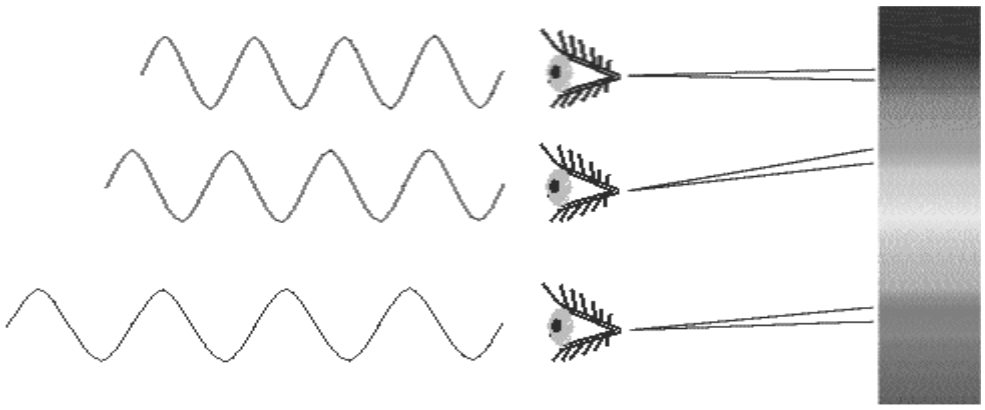
The visible spectrum forms only a small part [very tiny fraction] **of the complete *EMR* radiation spectrum.**
It extends from 400 to 800 nm and is further subdivided into colors according to their wavelengths.





- **Violet: 400 - 420 nm**
- **Indigo: 420 - 440 nm**
- **Blue: 440 - 490 nm**
- **Green: 490 - 570 nm**
- **Yellow: 570 - 585 nm**
- **Orange: 585 - 620 nm**
- **Red: 620 - 780 nm**

When polychromatic light (white light), which contains the whole spectrum of wavelength in the visible region is passed 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 color.



Interactions of photons with matter:

There are three basic process by which a molecule Can absorb radiation all involve raising the molecule to a higher internal energy level

1) By increasing the rotation of molecule about various axes so the molecule may absorb radiation and be raised to higher rotational energy level. (rotational transition

2) Atoms or groups of atoms within molecule vibrate relative to each other (vibrational transition).

3)By raising an electrons of molecule to a higher electron energy level (electronic transition).

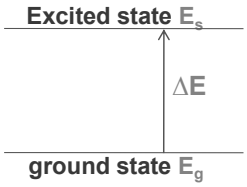
$$E_{total} = E_{electronic} + E_{vibrational} + E_{rotational}$$

The molecule at room temperature is usually in its lowest electronic energy state called ground state

When a molecule interacts with photons in the UV-VIS region, the absorption of the energy results in displacing an outer electron (valence electron) in the molecule. The molecule is said to undergo transition from the ground state of energy level (E_g) to an excited state of energy level (E_s).

Energy of transition is given by the equation:

$$\Delta E = E_s - E_g = h\nu = h\,C/\lambda$$



- **Electronic Spectra &Molecular Structure**
- **The electronic transitions that take place in the**
- **UV- Vis regions of the spectrum are due to the absorption of radiation by specific types of groups, bonds& functional groups within the molecule.** When light passes through a compound, some of the energy in the light kicks an electron from one of the bonding or non-bonding orbitals into one of the anti-bonding ones. The energy gaps between these levels determine the frequency (or wavelength) of the light absorbed, and those gaps will be different in different compounds.

- **Electrons in molecule can be classified into different types**

- Types of electrons:

(1)Closed shell – electrons that are not involved in bonding (very high excitation energies and don't contribute to absorption in Vis or UV regions) .

- (2)Covalent single –bond electrons (σ or sigma, electrons) These also posses too high excitation energy to contribute to absorption of Vis or UV radiation (eg.-CH_2-CH_2-).
- (3)Paired non –bonding outer shell electrons (n electrons) such as those on N,O,S and halo UV radiation.
- (4)electrons in π (pi) orbital for example in double or triple bonds.

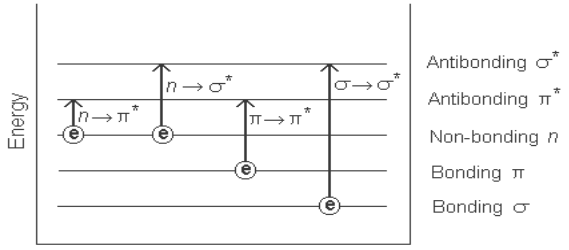
Accordingly, there are three types of Transitions:

a) **σ -electrons; They are bonding electrons, represent valence bonds and possess the lowest energy level (the most stable)**

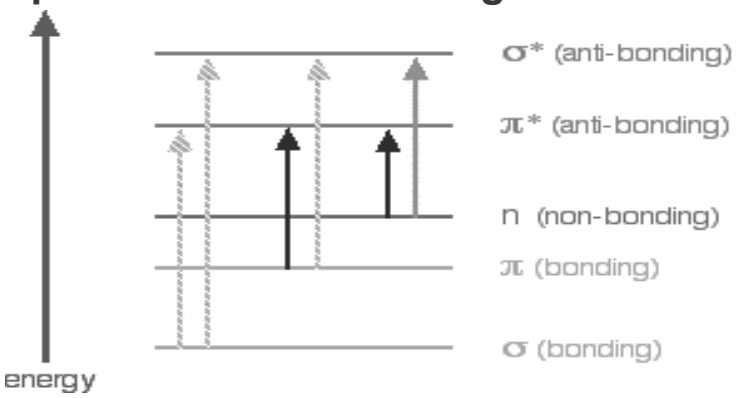
b) ***π*** **-electrons; They are bonding electrons, forming the π-bonds (double bounds), and possess higher energy than σ - electrons.**

c) **n -electrons; They are nonbonding electrons, present in atomic orbital of hetero atoms (N, 0, S or halogens). They usually occupy the highest energy level of the ground state.**

A molecule also possesses normally unoccupied orbitals called antibonding orbitals.



Look again at the possible jumps. This time, the important jumps are shown in black, and a less important one in grey. The grey dotted arrows show jumps which absorb light outside the region of the spectrum we are working in.



σ-Electrons:

Compounds containing only σ-electrons are the saturated hydrocarbons which absorb below 170nm (in the far UV region). They are transparent in the near UV region (200 - 400 nm) and this make them ideal solvents for other compounds studied in this range. They characterized by σ - σ * transition only.

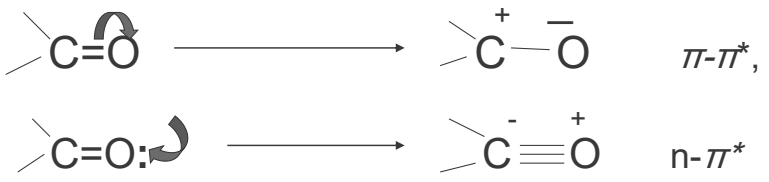
n-Electrons :

Saturated organic compounds containing hetero-atoms, possess n-electrons in addition to σ - electrons. So they characterized by the σ - σ * and n - σ * transitions. The majority of these compounds show no absorption in the near UV region. They are useful also as common solvents in the near UV .region.

However, their intense absorption usually extends to the edge of the near UV producing what is called end absorption (cut off wavelength) mostly in the 200 – 250 nm region.

π-Electrons

Unsaturated compounds containing no hetero atoms are characterized by the σ- σ * and *π- π** transitions. such as ethylene(CH_2=CH_2). When these compounds containing hetero atoms, they can undergo σ-σ*, *π-π**,n-σ* & n-*π** transitions, example acetone(CH_3-CO-CH_3).



Absorption spectrum:

The spectrum itself is a plot of absorbance vs wavelength and is characterized by the wavelength (λ_{max}) at which the absorbance is the greatest.

a) **Line spectrum:**

Occur with atomic spectra such as sodium metal which has a sharp line of λ at 589nm

b) **Band spectrum:**

Occurs with **molecules** due to the presence of different vibration and rotation sub-levels which the molecules may occupy on transition to excited state. That is, rotational and vibration modes will be found combined with electronic transition result in band rather than line spectra

According to the electronic transition that occur in each organic molecule, absorption spectrum is obtained by plotting **A** as a function of **λ.**

It has characteristic shape which show λ of maximum absorbance [λ_{max}] .

λ_{max} characteristic for each molecule according to its structure [Number & arrangement of electrons] and consequently types of transitions.

Therefore it is used for;

- identification of a chemical substance
- Used for quantitative measurement in order to increase sensitivity and to minimize error of analytical method.

There are two parameters which define an absorption band:

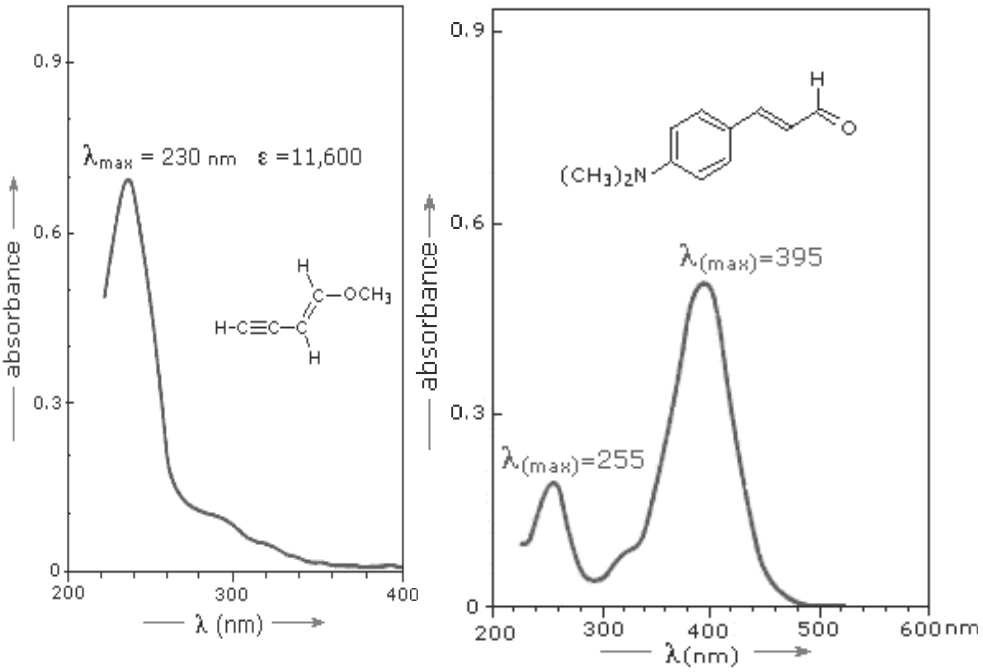
1) Its position (λ_{max}) on the wavelength scale.

2) Its intensity on the absorbance scale.
An excited molecule return to the ground state in about 10^{-8} sec. Energy must be released in the form of:

1- Heat: When excited electron returns directly to the ground state.

2- fluorescence or phosphorescence.

3- Molecular collisions.


λmax = 230 nm ε =11,600
absorbance
λ (nm)
0.9
0.6
0.3
0
200
300
400
H-C≡C-C
C-OCH3
H
(CH3)2N
λ(max)=395
λ(max)=255
500
600nm

Some important terms: spectra-structure correlation

THE ABSORBANCE OF EMR depends primarilly upon the number and arrangement of electrons in organic molecule of absorbing substance.

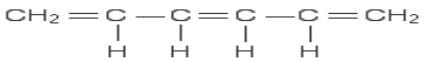
Chromophores: (Chrom = colour, phore = carrier).

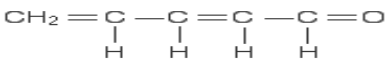
They are functional groups which confer colour on substances capable of absorbing UV and/or visible light (200 - -800 nm). They have unsaturated bonds (double or triple bonds) such as -C=C;-C=O,N=N , -C=N and etc...

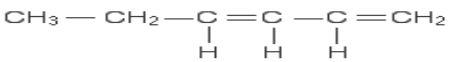
Chromophore	Example	Excitation	λ_{max}, nm	ε	Solvent
C=C	Ethene	π —> π*	171	15,000	hexane
C≡C	1-Hexyne	π —> π*	180	10,000	hexane
C=O	Ethanal	n —> π* π —> π*	290 180	15 10,000	hexane hexane
N=O	Nitromethane	n —> π* π —> π*	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	Methyl bromide Methyl Iodide	n —>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

The molecule containing a chromophore is called chromogen

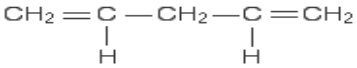
A chromophore (literally color-bearing) group is a functional group, not conjugated with another group, which exhibits a characteristic absorption spectrum in the ultraviolet or visible region. Some of the more important chromophoric groups are:





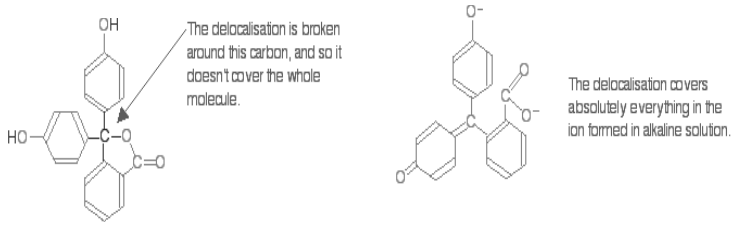


However, although the next molecule contains two double bonds, they aren't conjugated. They are separated by *two* single bonds.



If any of the simple chromophores is conjugated with another (of the same type or different type) a multiple chromophore is formed having a new absorption band which is more intense and at a longer wavelength that the strong bands of the chromophores .

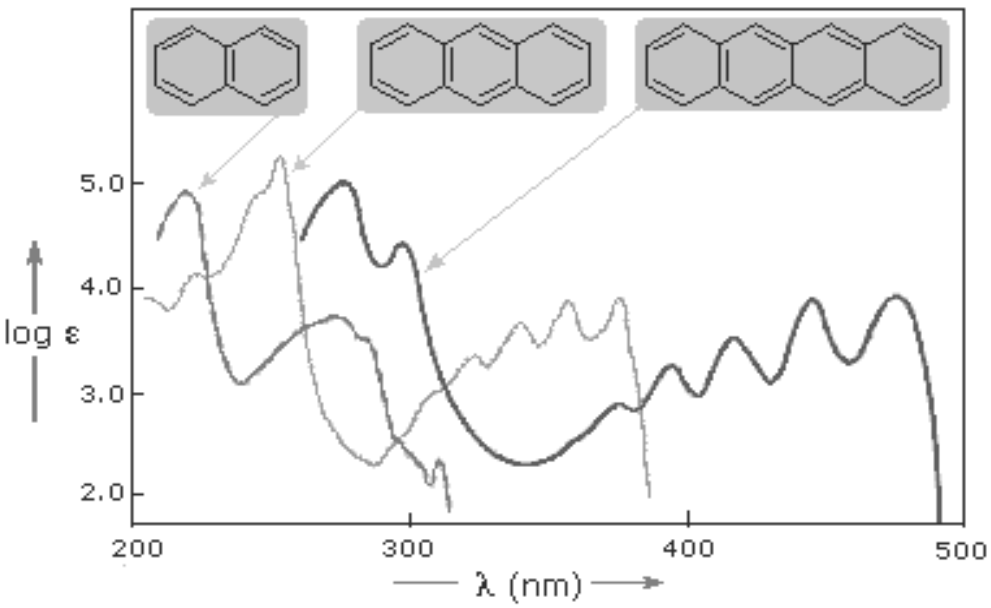
Another example about ph.ph . indicator



The rearrangement now lets the delocalisation extend over the entire ion. This greater delocalisation lowers the energy gap between the highest occupied molecular orbital and the lowest unoccupied pi anti-bonding orbital. It needs less energy to make the jump and so a longer wavelength of light is absorbed.

Remember: Increasing the amount of delocalisation shifts the absorption peak to a higher wavelength

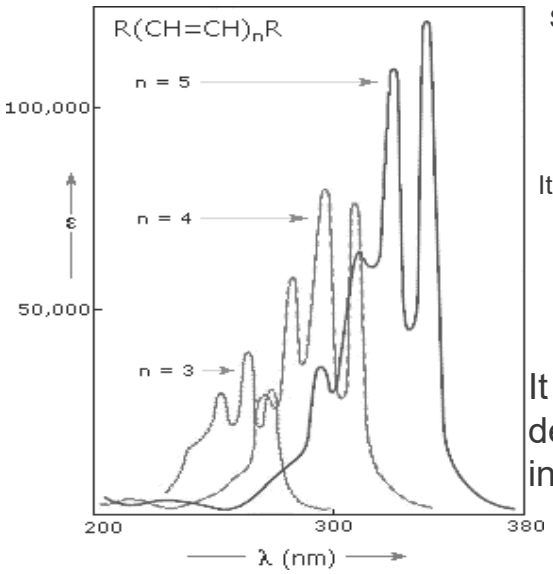
Relationship between the number of fused rings and wavelength



Auxochromes:

An auxochrome doesn't itself absorb radiation , but if present in amolecule , it can enhance the absorption by chromophore or shift the wave length of absorption when attached to the chromophore. Examples are hydroxyl groups [**OH**], Amino groups [NH_2]and halogens[Cl, Br, I].

Spectral changes



Bathochromic (Red) shift

Shift of absorption to longer wavelength due to substitution and solvent effects.

Hypsochromic (Blue) shift

It is shift of absorption to shorter wavelength

Hyperchromic & hypochromic effects:

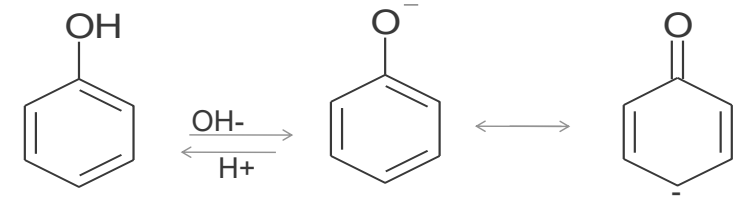
It is the increase and decrease in absorption intensity respectively.

Terminology for Absorption Shifts

Nature of Shift	Descriptive Term
To Longer Wavelength	Bathochromic
To ShorterWavelength	Hypsochromic
To Greater bsorbance	Hyperchromic
To Lower Absorbance	Hypochromic

Effect of pH on absorption spectra:

The spectra of compounds containing acidic or basic groups are dependent on the pH of the medium (e.g.) phenols and amines. The UV spectrum of phenol in acid medium (where the molecular form predominates) is completely different 'from its spectrum in alkaline medium (where the phenolate anion predominates). The spectrum in alkaline medium exhibits bathochromic shift with hyperchromic effect. The red shift is due to the participation of the pair of electrons in resonance with the π-electrons of the benzene ring, thus increasing the delocalization of the π-electrons.



in acid medium

On the other hand, UV spectrum of aniline in acid medium shows hypsochromic (blue) shift with hypochromic effect (decrease in absorption intensity). This blue shift is due to the protonation of the amino group, hence the pair of electrons is no longer available and the spectrum in this case is similar to that of benzene (thus called benzenoid spectrum)

Blue shift

Effect of Solvents on absorption spectra:

The solvents may have a strong effect on the position of λ max due to its effect on the energy of transition. Less polar solvents (e.g. hydrocarbons) interact less strongly with the solute than do polar solvents (e.g. water and alcohols)

*π-π**Transitions:

Two cases arise:

(a) *π-π**bands of dienes: Not shifted by any change of olvent polarity due to absence of charge separation in either ground or excited states.

(b) *π-π** bands of enones: are red shifted on increasing solvent polarity due to stabilization of excited state by dipole-dipole solvent interaction. This stabilization leads to lowering the energy of the excited state (i.e.) smaller transition energy and hence longer λ (↓E→↑λ)

n-π^* Transition:

Blue shift with increasing solvent polarity due to stabilization of the ground state by hydrogen bonding: ,R-C=O…….H-OR Hydrogen bonding lowers the energy of the ground state (i.e.)increase energy of transition and hence decrease λ (Fig. 7, b).

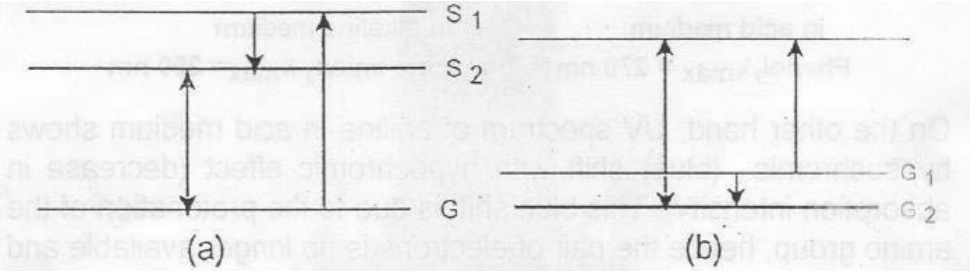
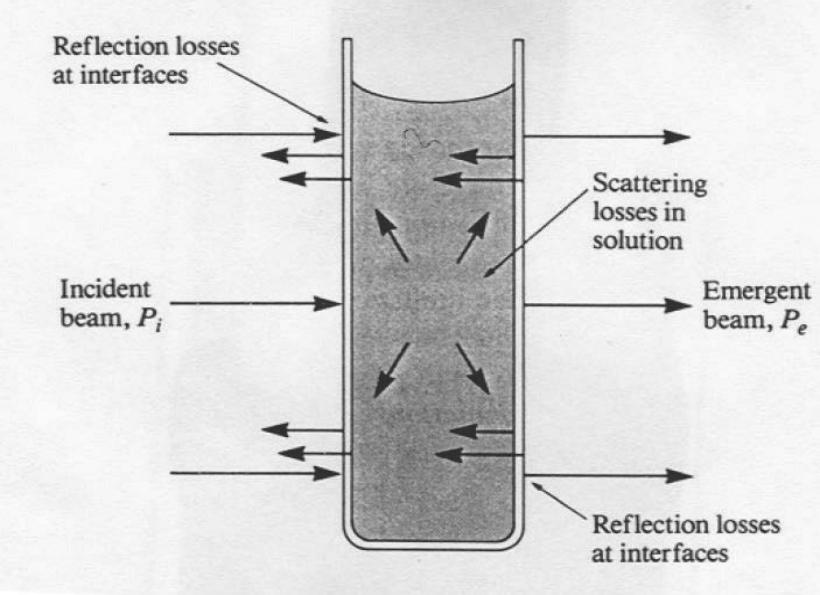


Fig.7, Effect of solvents on π – π* transitions of enones & n – π* transitions.

The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species. Peaks resulting from n-π^* transitions are shifted to shorter wavelengths (*blue shift*) with increasing solvent polarity. This arises from increased solvation of the lone pair, which lowers the energy of the *n* orbital. Often (but *not* always), the reverse (i.e. *red shift*) is seen for π-π^*transitions. This is caused by attractive polarisation forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states. This effect is greater for the excited state, and so the energy difference between the excited and unexcited states is slightly reduced - resulting in a small red shift. This effect also influences n-π^* transitions but is overshadowed by the blue shift resulting from solvation of lone pairs.

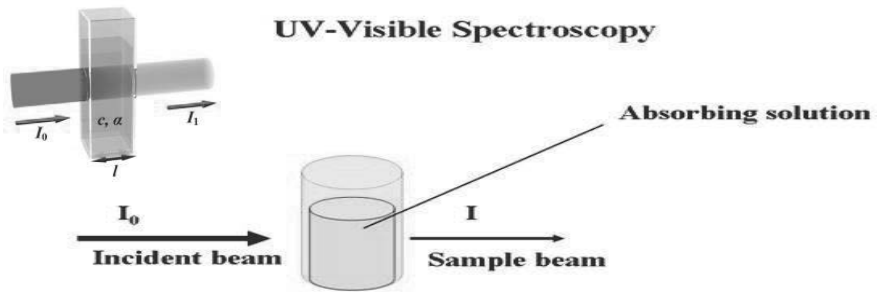
Terms employed in absorption spectroscopy :



$$I^{\circ} = I_t + I_a + I_r + I_{f+} I_s$$

- I_t= transmitted light
- I_a=absorbed light
- I_r=reflected light
- I_f= refrected light
- I_s= scatterd light
- For clear solutions,I_s=0
- I_r can be cancelled by using blank & I_f solution

Many compounds absorb ultraviolet (UV: ~190 nm - ~370 nm) or visible (Vis.: ~370 nm - ~800 nm) light.
The diagram below shows a beam of monochromatic radiation of radiant power I_0, directed at a sample solution. Absorption takes place and the beam of radiation leaving the sample has radiant power I.



•The maximum wavelength is the wavelength at which the sample absorbs the most light.
•At this wavelength, most light is absorbed so the sample beam is weakest.

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

Transmittance

Transmittance : $T = I / I_0$

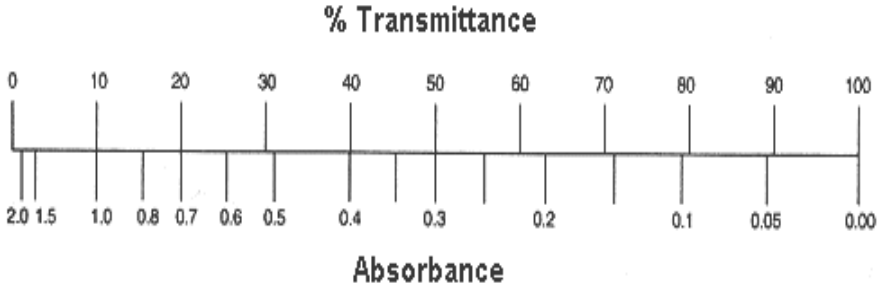
% Transmittance: $\%T = 100\ T$

Absorbance:

$$A = \log_{10} (1 / T) \quad = -\log_{10} (T)$$
$$A = \log_{10} (100 / \%T)$$
$$A = 2 - \log_{10} (\%T)$$

The absorption of a solution increases as the transmittance decreases

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.

Lambert's Law:

When a beam of monochromatic radiation enters an absorbing medium its intensity decreases exponentially with the increase of thickness [b] of the medium traversed mathematically

$$\log I_0/I_t = Kb$$

concentration c held constant

K is the proportionality constant , I_0 and I_t are intensity of incident and transmitted radiations.

Beer's Law:

It relates absorption capacity .to the concentration of an absorbing solute. It stated that absorption is proportional to the number of absorbant molecules in the light path

$$\log I_o/I = K\,C$$

where K is proportionality constant and C is the concentration while b is held constant.

Beer's-Lambert's law:

This is a combination of both laws;

$$\text{Log } I_o/I = abc$$

where (a) is a constant called absorptivity, (b) is the pathlength in cm and **(c)** is the concentration in *grams/Liter.* Log I_o/I is usually substituted by A (Absorbance), then the equation become;

$$A = abc$$

The value of (a) will clearly depend upon the method of expression of the concentration.

If **(c)** is expressed in ***moles/liter,*** and b in centimetres then a is given the symbol ε Epsilon ($l.mol^{-1}cm^{-1}$) and is called the molar absorption coefficient or molar absoptivity

$$A=\varepsilon bc$$

when ε is measured at λ_{max} it is called ε_{max}
when (**c**) is in ***g/100 ml*** and b in centimetres. We get

$$A=A^{(1\%,1cm)}cb$$

The $A^{1\%}_{1cm}$ is valuable for natural products identification when their constituent is unknown (molecular weight is not definitely known). $A^{1\%}_{1cm}$ can be converted easily to ε by the equation;

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

Deviations from Beer-Lambert Law:

When the results obey Beer-Lambert law, the concentration versus absorbance gives a straight line passing through the origin (calibration curve) as indicated by the solid line in figure 9.

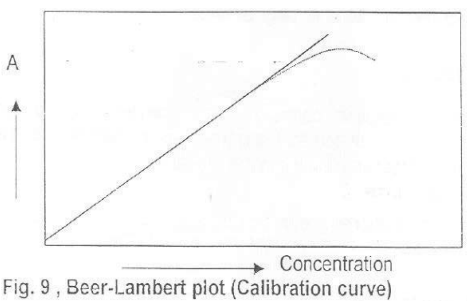
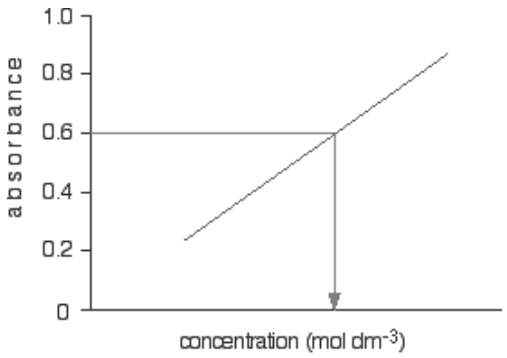


Fig. 9 , Beer-Lambert plot (Calibration curve)



In some cases, deviation from Beer-Lambert law occur (dotted line in the figure) which may be:

(1) Real deviation:

In high concentrations, due to crowding, molecular interaction and association as well as charge distribution.

(2) Instrumental deviation (errors):

a) **Irregular deviation due to: Unmatched cells, unclean handling and unclean optics.**

b) **Regular deviation: due to:**

- Errors in wavelength scale.

- Slit width control.

- Stray light is any radiation of wavelength other than those which are absorbed. Also includes any light reaches the detector without passing through the sample..

c) Other errors:

- Non-linear response of photo cells.
- Radio and TV interferences.
- Unstabilized power supply.

(3) Chemical deviations:

-pH effects.

-Solvents interaction due to high concentrations.

-Temperature effects.

-Dipole interactions

-Time factor affect oxidation, reduction, or hydrolysis reactions which may be occur.

Colorimetry

The energy of transition determines λ of absorption and if the λ_{max} is in the visible range (400-800 nm), a colour is obtained and its measurement can be made visually by using simple apparatus.
Thus colorimetry is the technique of measuring visible radiation.

colorimetry is the method for quantitative analysis which depends on measuring the absorption of VIS radiation

It must be noted that; the absorbed colour is different from the actually observed color. The observed colour is complementary color which remained after the wave length absorbed from white light (see the following table and colour wheel).

chromogen(reagent):

is substance capable of conversion into a pigment or dye.(lacks definite color but may be transformed into a pigment)

General Requirement for an Ideal chromogen:

- **It should be Colorless**

- **its excess easily separated from the color product.**

- **Has no absorbance at λ_{max} of the coloured product**

- **Selective (react with analyte only)**

- **Reacts quantitatively with the analyzed substance (stiochiometry)**

- **Give one single colored product with specified λ_{max}.**

- **Sensitive & gives highly color product.**

- **Color development must be rapid**

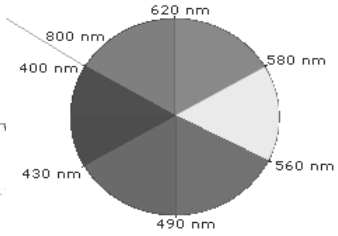
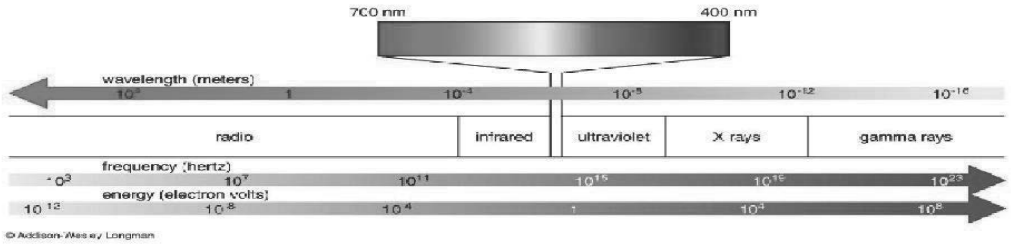


Fig. 11, Colour wheel

λ of absorption	Absorbed color	Observed color
400	Violet	Yellow
450	Blue	Orange
500	Green	Red
570	Yellow	Violet
590	Orange	Blue
620	Red	Green



Note **The range from 200 - 400 nm can not be detected by the eye and must be measured spectrophotometrically (using spectrophotometers).**

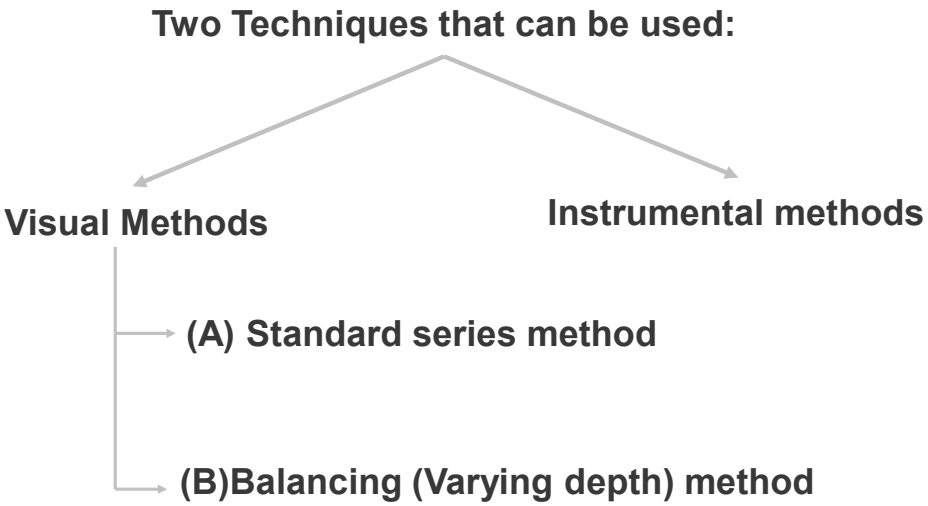
Advantages of colorimetry:

1- Give more accurate results at low concentrations than the corresponding titrimetric and gravimetric procedures.

2- It is frequently applied under conditions where no satisfactory gravimetric or titrimetric procedure exists, e.g. for biological substances.

3- It is considered a simple and rapid techniqe if compared with titrimetry and gravimetry.

Quantitative Measurements of Elctromagnetic Radiation Absorption



[I] Visual Methods

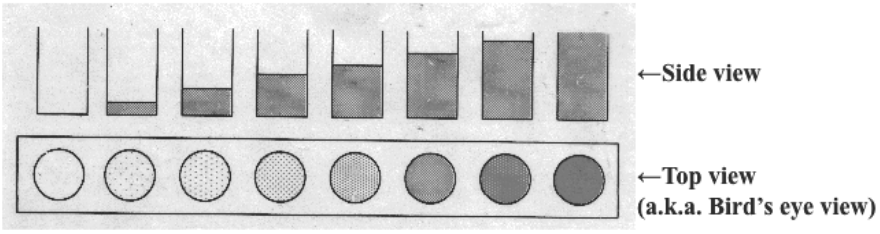
Are used for measuring the coloured solutions only

(A) Standard series method

The test solution contained in a test tube is matched with a series of standards similarly prepared, then the concentration of unknown solution is equal to that of the standard solution whose colour is matched with it exactly. Example, is the determination of copper with ammonia by forming an azur blue colour or determination of iron (III) by forming blood red colour with thiocyanate anion.

Visual Colorimetry

Intensity: For light shining through a colored solution,the observed intensity of the color is found to be **dependent on both the thickness of the absorbing layer (pathlength) and the concentration of the colored species.**



For One Color: A series of solutions of a single color demonstrates the **effect of either concentration or pathlength**, depending on how it is viewed.

(B) Balancing (Varying depth) method

Involve the use of more accurate devise (Dubosq Colorimeter), which uses a dual matched optical system made of glass plungers. The depth of their immersion in the coloured solution brings about-change in colour thickness of the sample and the standard. Equality of optical density of the test and standard solutions is obtained by varying the thickness of the layer through which a light beam travels and according to

the relation:
Ax = $\varepsilon b_x C_x$ (for unknown) $A_s = \varepsilon b_s C_s$ (for standard), Where Ax & As are the optical densities of the test and standard solutions respectively. bx & bs are the layers thickness or the test and standard solutions respectively. Cx &Cs are the concentrations of the test and standard solutions respectively, and ε is the molar absorptivity of the coloured compound. At the moment of matching: $A_x = A_s$
then: Cx/Cs= bs/bx thus:

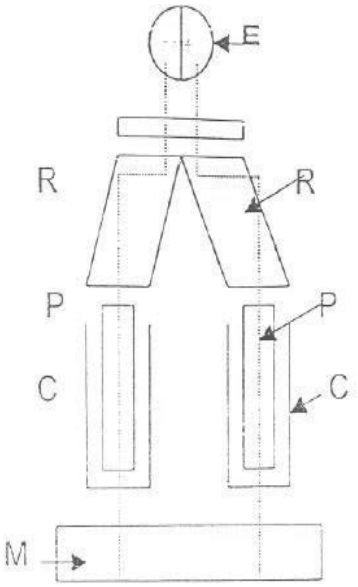
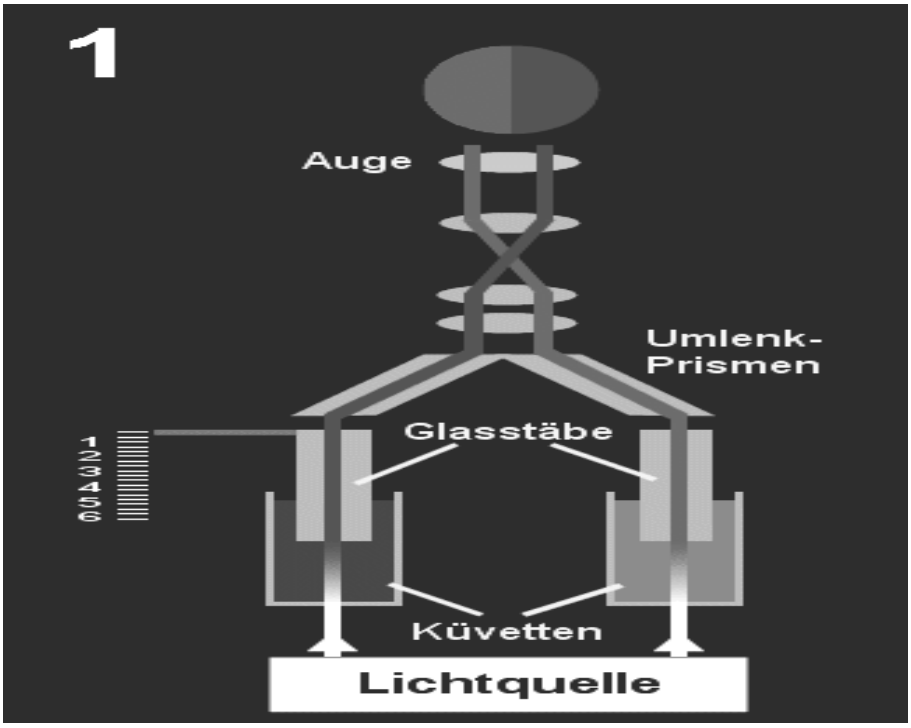


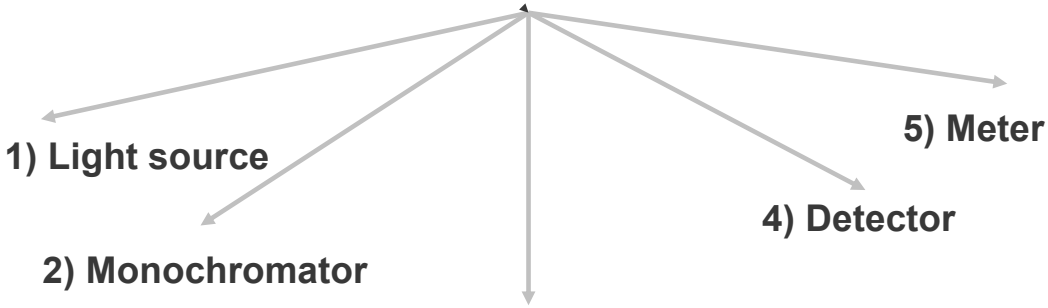
Fig. 12 , Duboscq Colorimeter
E = Field of view
R = Prism
P = Plunger
M = Mirror
C = Cell



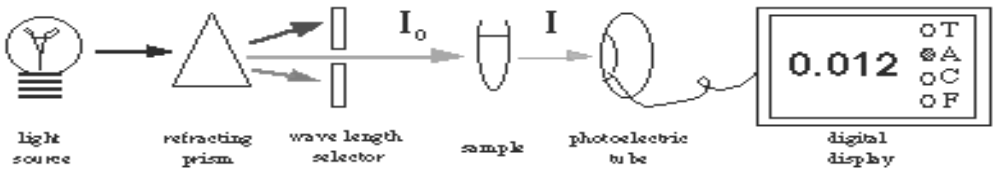
photoelectric instrument

In photoelectric colorimeters and spectrophotometers, intensity of light absorbed by the sample is measured electrically. In addition, monochromatic light is used instead of polychromatic light frequently used in visual methods.

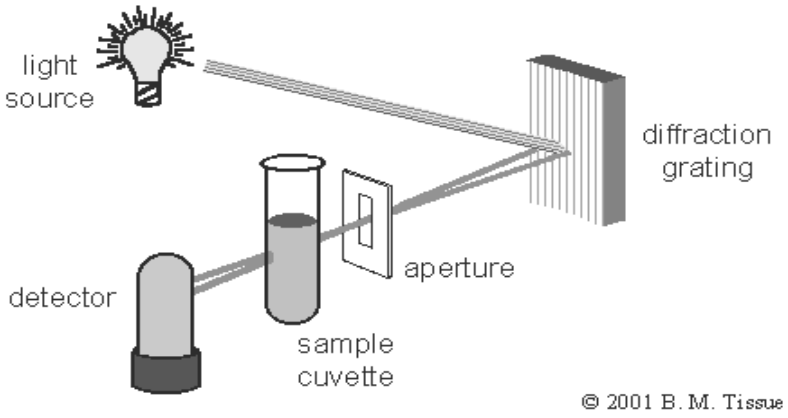
Components of photoelectric instrument:



Essential components of a photoelectric instrument are shown in Fig. which include:



- Schematic of a wavelength-selectable, single-beam UV-Vis spectrophotometer



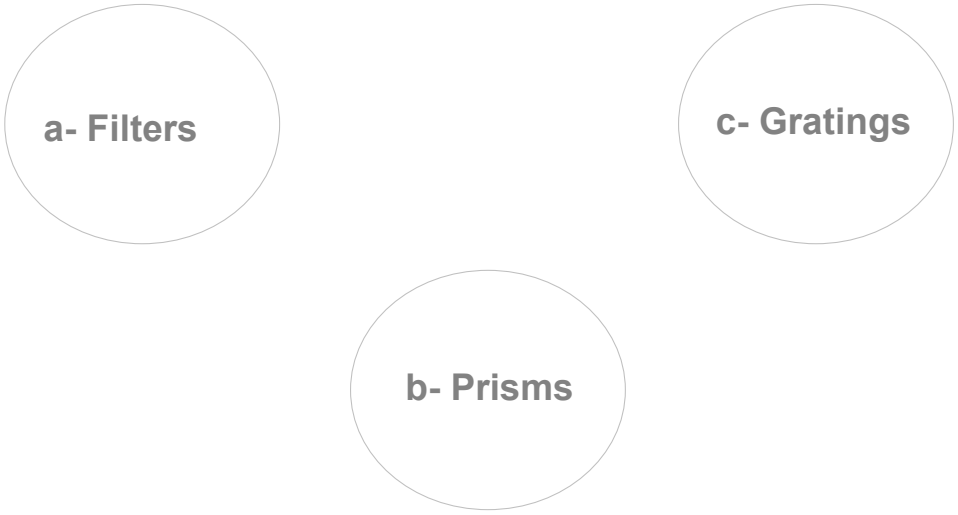
1) Light Source:

a-For UV measurements: Hydrogen or Deuterium discharge lamp (give radiations from 190 - 375 nm) is used.

b- For visible measurements: Tungesten lamp is used (give radiations from 350 - 1000 nm)

2) Monochromators:

Monochromatic light may be obtained by one of the following methods:



a- Filters

Functioning via selective absorption of unwanted wavelengths and transmitting the complementary color which is needed to be absorbed by the sample to be analysed. Filters used to isolate certain wavelengths of light. Filter made of glass and contain chemicals (dyes)that absorb all radiation except that desired to be passed.

b- Prisms

Functioning via refraction of light [Fig. 14 (a)].

When Electromagnitic radiation passes through a prism,it is refracted,because the index of refraction of the prism material is diffrent form that in air.

Shorter wavelenghts are refracted more than longer wavelenghts

By rotation of prism ,different wavelenght of the spectrum can be made to pass through An exit slit and through the sample

c- Gratings

Consists of a large number of parallel lines ruled very close to each other on a highly polished surface, e.g. aluminium or aluminized glass (600 groove/mm).Each filled groove functions as a scattering center for light falling on its edge and through diffraction and interference the grating disperses the light beam into almost single λ [Fig. 14 (b)].

b- Prisms **c- Gratings**

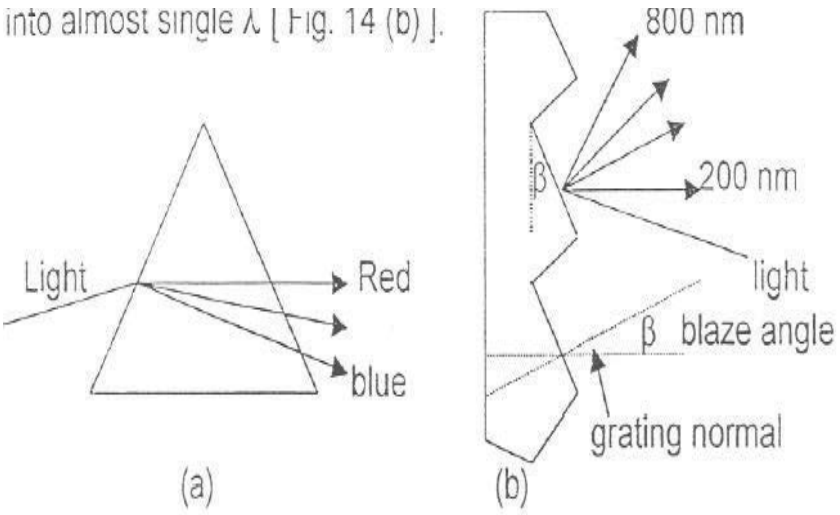


Fig. 14,(a) Dispersion of light by a prism, (b) diffraction of radiation from a grating.

3) Sample cells:

Usually either quartz cells for UV measurements and glass for visible measurements, of 1 or 1/2 cm pathlength.

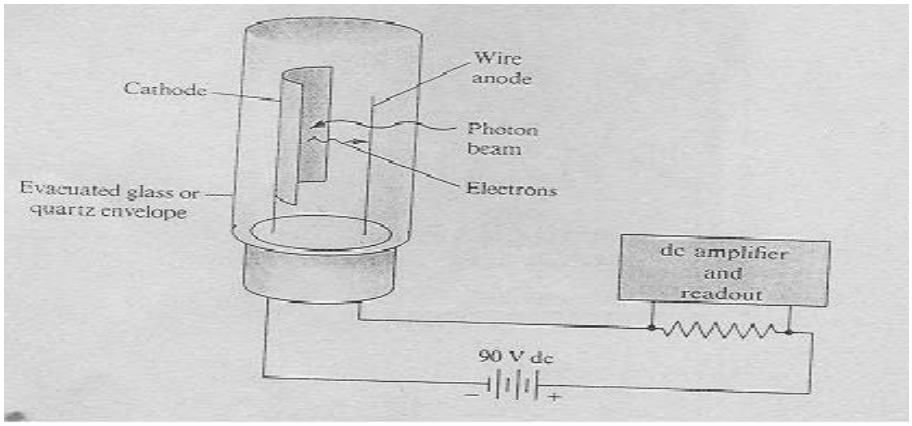
4) Light Detectors:

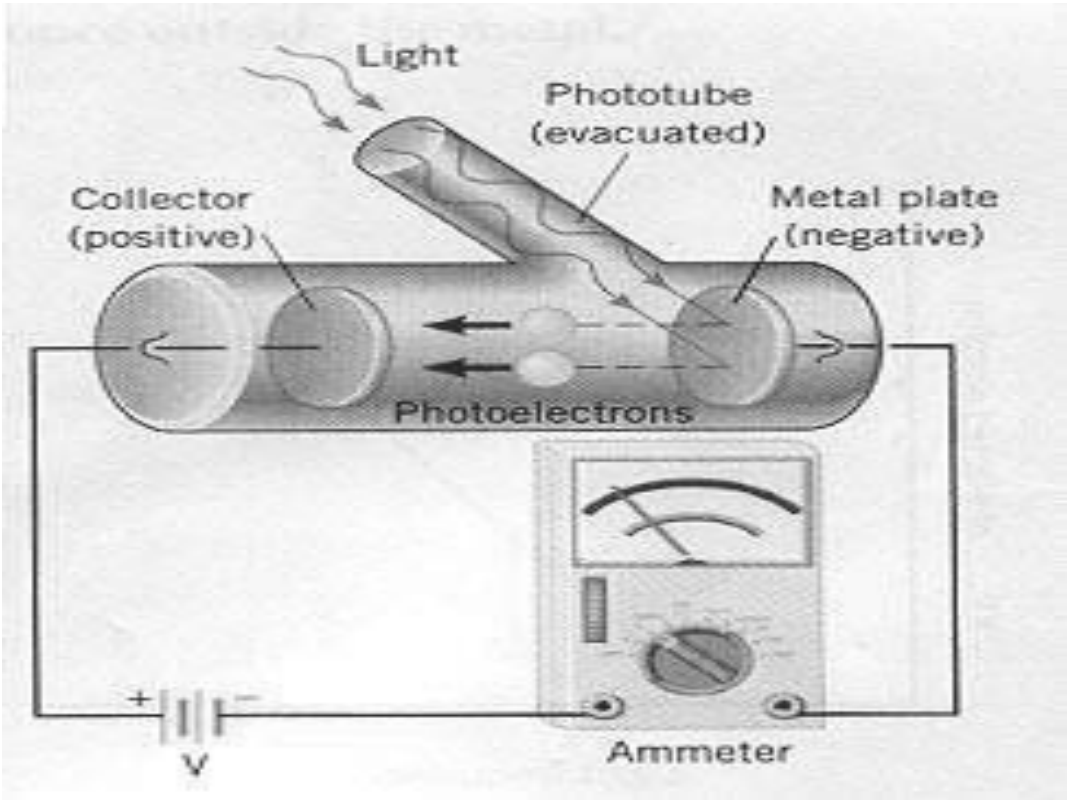
a- Photocells

b- Phototubes

a- Photocells:

The light (radiant energy) falls on the cathode surface which excites electrons and generates an electric current which is proportional to light intensity.





Phototubes consists of a semi-cylindrical cathode and wire anode sealed inside an evacuated transparent envelope the concave surface of the cathode supports alayer of photo-emissive material such as an alkali metal or metal oxide that tends to emit electrons when a potential is applied across the electrodes the emitted photoelectrons flow to the wire anode producing a current (photo-current) that's readily amplified and displayed or recorded.

The number of electron ejected from a photo-emissive surface is directly proportional to the radiant power of the beam striking that surface

b-Photomultiplier

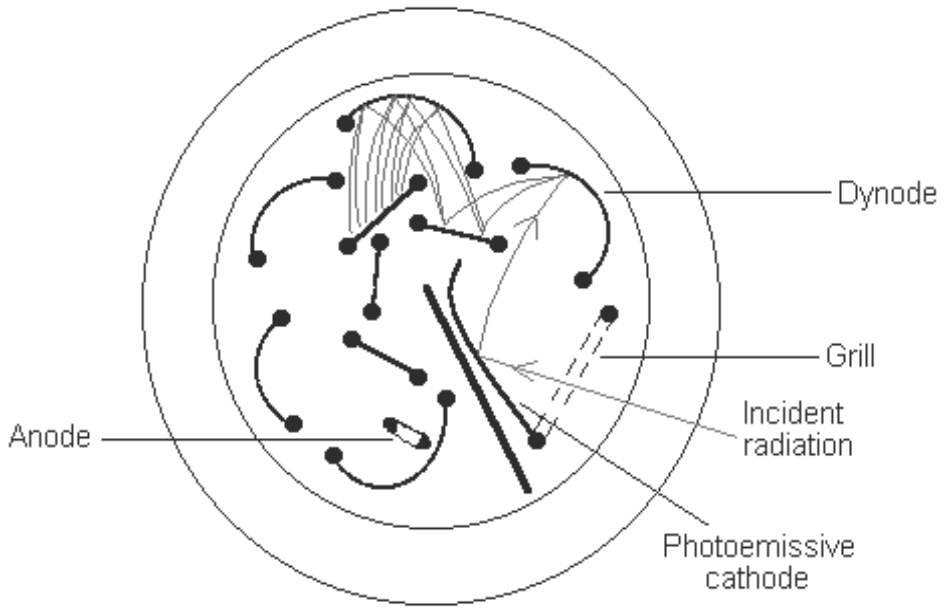
Is similar in construction to the phototube but is significantly more sensitive

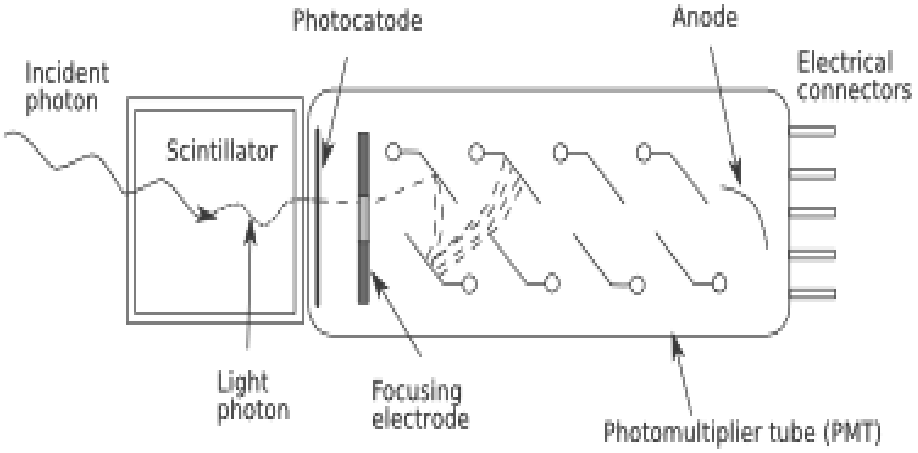
Electrons being emitted upon exposure to radiation .the emitted electrons are accelerated towards a dynode more positive than cathode.

The cascade of electrons is finally collected at the anode the resulting current is then further amplified electronically and measured.

Give greater sensitivity. Used when light signal is weak. composed of a photocathode and several anodes of gradually increased potential. Electrons from the photocathode are attracted to dynode 1[more positive] and liberates more electrons which travel to dynode 2 due to its higher potential and so on to the last dynode. The final photocurrent is many times greater than the primary current.

Signals from photocells and phototubes are then passed through amplifier to enhance the response, then passed to the recorder.



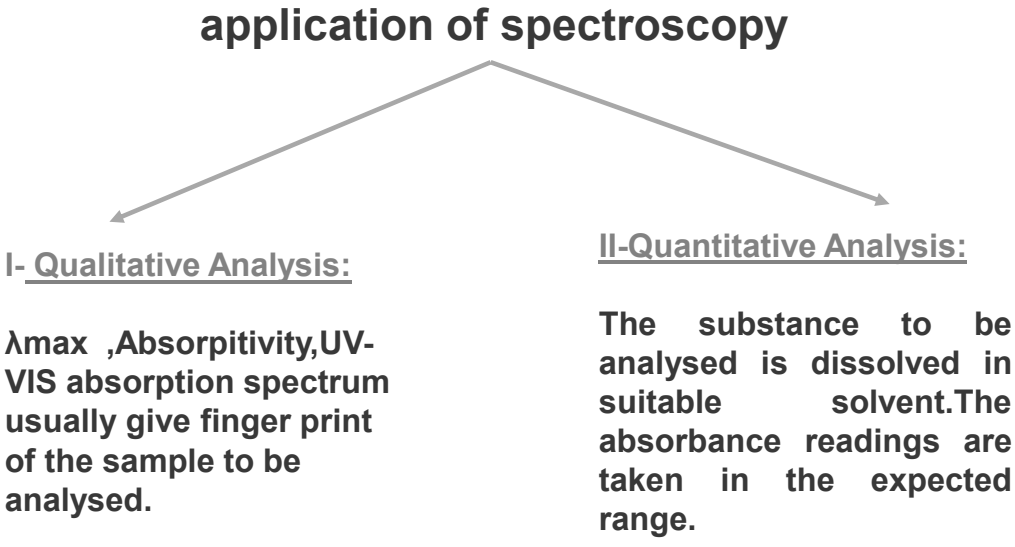


5) Meters (Recorders):

Many types are used such as galvanometers, electric recorders or electric cells (to give digits).

Application of spectrophotometry

It includes application of spectroscopy in Qualitative & Quantitative analysis including both organic and inorganic pharmaceutical compounds and determination of some physical constants.



a) Analysis of inorganic:

Copper can be determined by forming an azur blue colour with ammonia. Iron (III), is determined by forming blood red colour with thiocyanate anion and iron (II) by forming red complex with 1,10 - phenanthroline.

b)Analysis of organic compounds:

Examples:

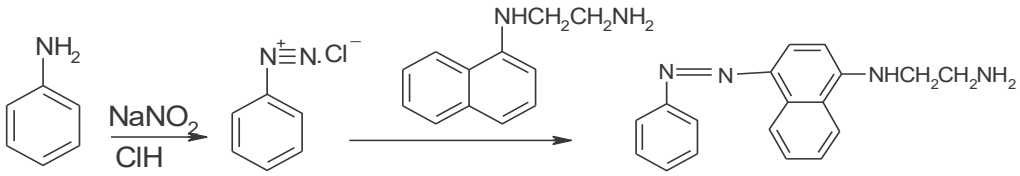
Amines

Carbonyl compounds

Phenols

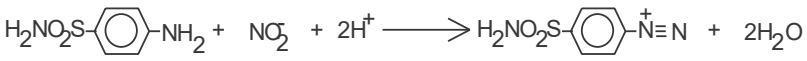
1-Amines

For small quantities of amines, diazotisation and coupling method is valuable for aromatic primary amines.

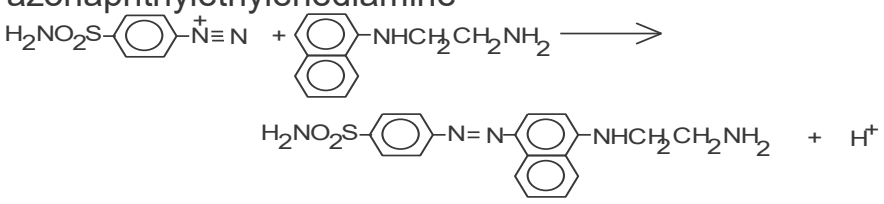


Aniline **red colour λ_{max} about 450-520 nm**

Nitrite can be measured easily and with good sensitivity by a coupling reaction known as diazotization. Under acidic conditions, nitrite ions and aromatic amines can form reactive diazonium salts. In the test commonly employed for nitrite, the aromatic amine used is sulfanilamide. This forms p-diazobenzenesulfonamide :

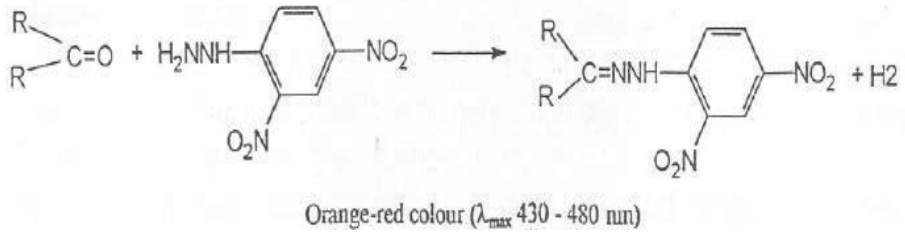


To improve the intensity of the absorbance band, this is reacted with N-(1-naphthyl)ethylenediamine to form a reddish purple azo dye, p-benzenesulfonamide azonaphthylethylenediamine



2-Carbonyl compounds

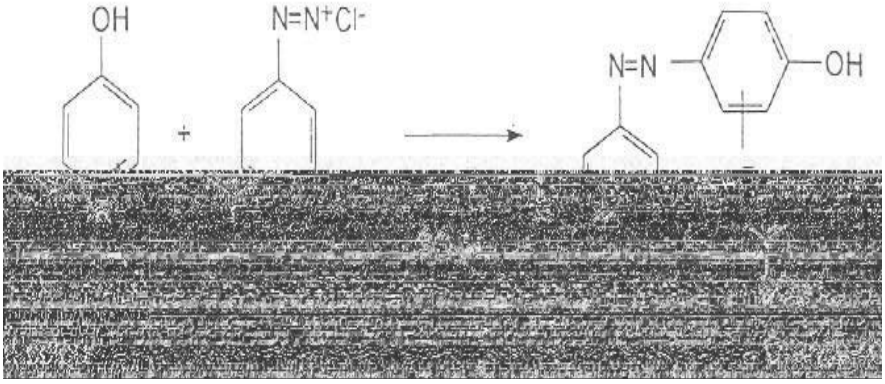
Carbonyl compounds can be determined spectrophotometry after their conversion to phenyl hydrazones by reaction. with .2,4-dinitrophenylhydrazine



Orange-red colour (λ_{max} 430 - 480 nm)

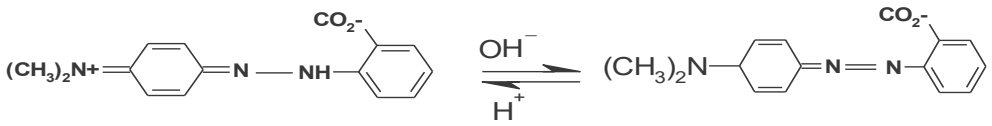
3- Phenols

Phenols can be determined after formation of coloured compounds by couoling with diazotised primary aromatic amines.



c) Spectrophotometric determination of pKa of an indicator

Example:Determination of pK_a of methyl red indicator: Acidic (HMR) and basic (MR-)forms of methyl red are shown as:



The pK_a of methyl red indicator is given by the equation:

$$pK_a = pH - \log [MR-]/[HMR] ,$$

Both HMR and MR- have strong absorption peaks in the visible portion of the spectrum (Fig. 15). The colour change interval from pH 4 to pH 6 can be obtained with acetate buffer system. At pH = 4, the acid is completely unionized (HB). At pH = 6,the acid is completely ionized(B-). At intermediate pH values, the two species are present. Plotting absorbance (A) against pH values at λ_1 and λ_2 gives two curves. The pH at the point of intersection represents, the. pKa of the indicator.

Molecular Photoluminescence Spectroscopy

Photoluminescence is the process of emission of radiation from the excited electronic state.
It is divided into two categories:

1-Fluorescence

2-phosphorescence

Molecular Fluorescence and Phosphorescence [Photoluminescence]

General Interactions of Light and Matter

When a beam of radiation strikes any object it can be absorbed, transmitted, scattered, reflected or it can excite fluorescence.
With fluorescence ,a photon is first absorbed and excited the molecule to a higher energy state. The molecule then drops back to an intermediate energy level by re-emitting a photon. Since some of the energy of the incident photon is retained in the molecule or is lost by a non-radiative process such as collision with another molecule.
The emitted photon has less energy and hence a longer wavelength than the absorbed photon.
Like scatter, fluorescent radiation is also emitted uniformly in all directions.

An analyte in an excited state possesses an energy,E_2,that is greater than that when it is in a lowerenergy-state,E_1,

$$\Delta E=E_2-E_1$$

ΔE is excess energy which must be released

There are several ways an excited atom or molecule can give up its excess energy and relaxe to its ground state

Relaxation occurs through :

1-N*onradiative*

a-release of heat

A* → A + heat

b-decomposition reaction

A* → X + Y

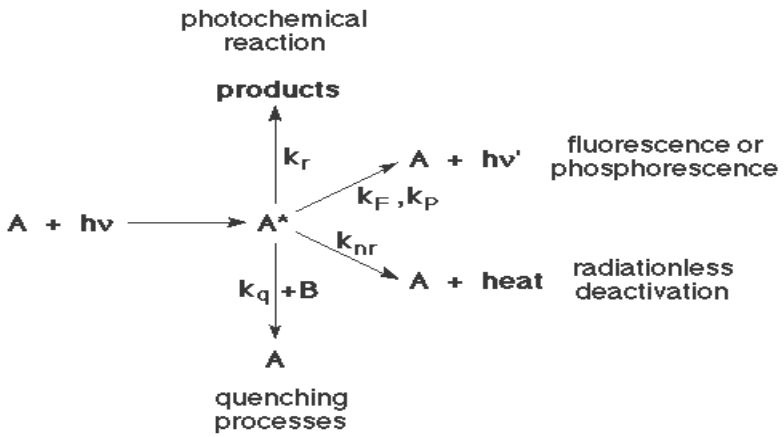
excess energy is used up in the chemical reaction

c-reaction between A* and another species

A* + Z → X+Y

2-Emission of photons {with lower energy (longer wavelength) than was absorbed}

A* → A + *hu*



- **Lifetime :**

The length of time that an analyte stays in an excited state before returning to a lower-energy state.

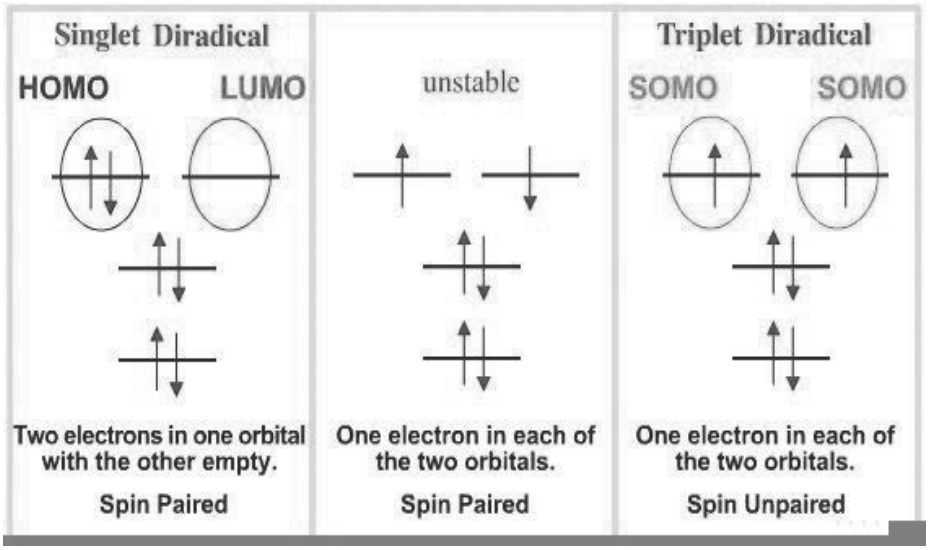
Relaxation:

Any process by which an analyte returns to a lower-energy state from a higher-energy state.

A molecule at room temperature normally resides in ground state. The ground state is usually a **singlet state** $S_0$with all electrons paired. Electrons that occupy the same molecular orbital must be "paired"that is ,have opposite spins.

If electrons have the same spin they are unpaired and the molecule is in a **triplet state** T_1.

Singlet State	Triplet State
Electrons are paired and spin in opposite direction S_0	**Electrons are unpaired by flipping and spin in the same direction T_1**
Higher energy	**Lower energy**



Molecular fluorescence and phosphorescence

To appreciate the origin of molecular fluorescence and phosphorescence, we must consider what happens to a molecule following the absorption of a photon..Let's assume that the molecule initially occupies the lowest vibrational energy level of its electronic ground state. The ground state is a singlet state labeled S◦..Absorption of photon of correct energy excites the molecule to one of several vibrational energy levels in the first excited electronic state,S_1, or the second excited electronic d state,S_2,

Both of which are singlet states.Relaxation to the ground state from these excited states occurs by a number of mechanisms that are either

(a) radiationless,in that no photons are emitted, or involve

(b) **the emission of a photon.**

(a) Radiationless Deactivation:

It involves the following forms:

1- Vibrational relaxation
2-Internal conversion
3-External conversion
4-Intersystem crossing

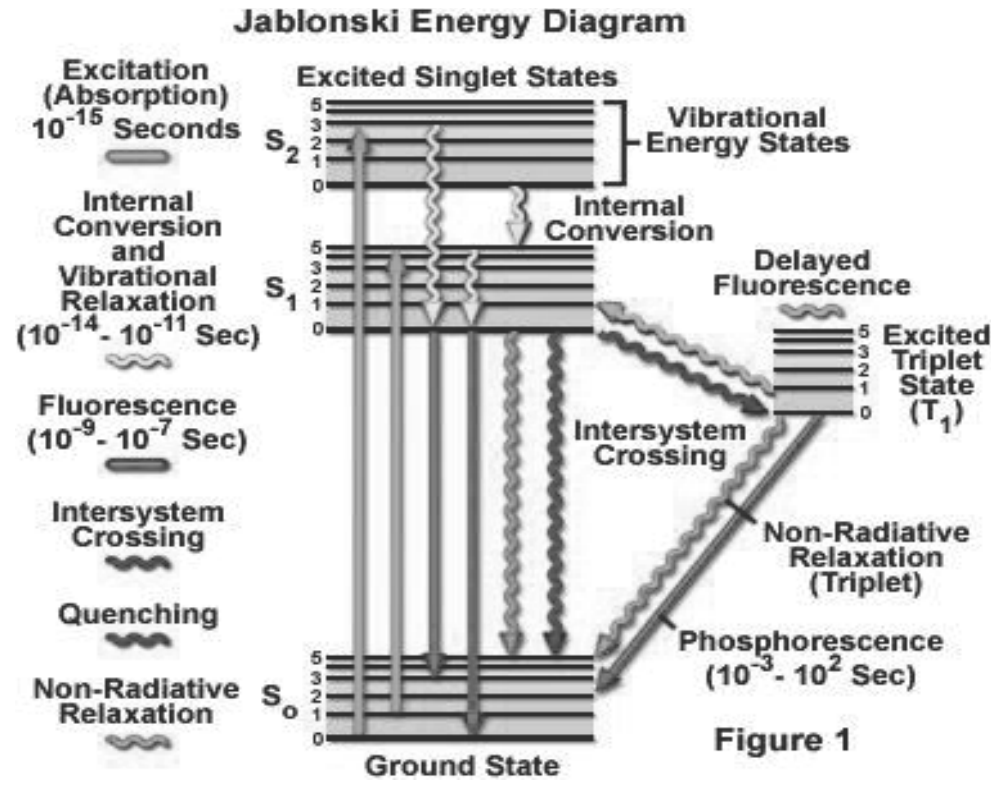
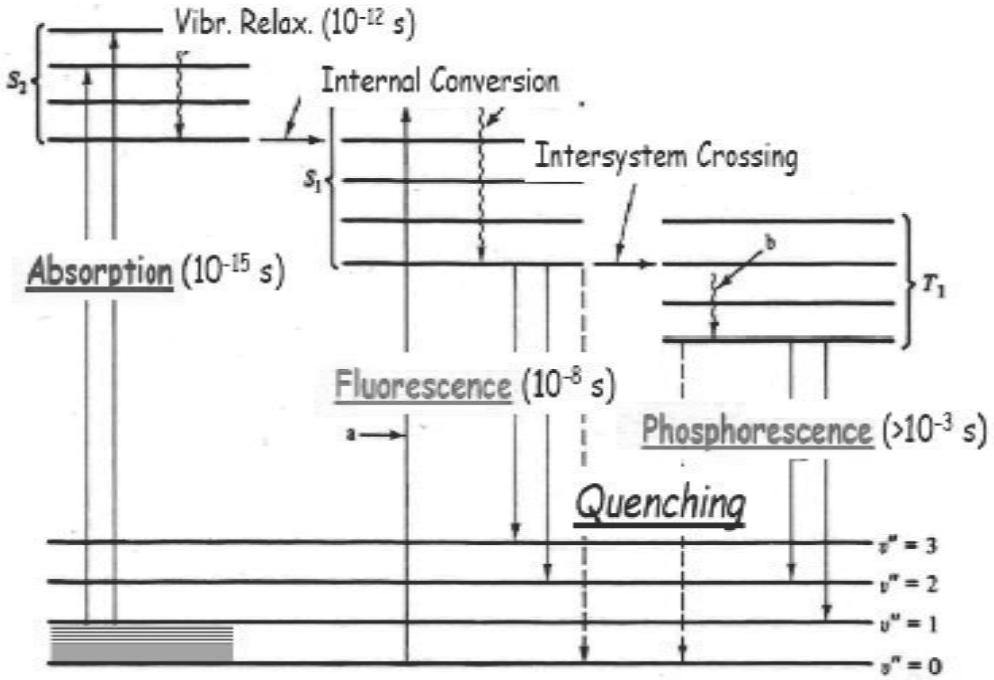

Jablonski Energy Diagram
Excitation (Absorption) 10^{-15} Seconds
Internal Conversion and Vibrational Relaxation (10^{-14} - 10^{-11} Sec)
Fluorescence (10^{-9} - 10^{-7} Sec)
Intersystem Crossing
Quenching
Non-Radiative Relaxation
Excited Singlet States
S_2
S_1
S_o
Vibrational Energy States
Internal Conversion
Delayed Fluorescence
Excited Triplet State (T_1)
Intersystem Crossing
Non-Radiative Relaxation (Triplet)
Phosphorescence (10^{-3} - 10^{2} Sec)
Ground State

Figure 1


Vibr. Relax. (10^{-12} s)
Internal Conversion
Intersystem Crossing
Absorption (10^{-15} s)
Fluorescence (10^{-8} s)
Phosphorescence (>10^{-3} s)
Quenching
T_1
S_1
S_0
v" = 3
v" = 2
v" = 1
v" = 0

- 1-Vibrational relaxation:
- . It is a form of radiationless relaxation in which an analyte moves from a higher vibrational energy level to a lower vibrational energy level in the same electronic level. It takes place during collision between excited molecules & molecules of solvet. Also collision with solvent molecules at this point rapidly removes the excess energy from the higher vibrational level of S_1
- 2-Internal conversion:
- A form of radiationless relaxation in which the analyte moves from a higher electronic energy level to a lower electronic energy level.It occurs between lowest vibrational level of an excited electronic state & the upper vibrational level of another electronic state.

- 3-External conversion:
- A form of radiationless relaxation in which energy is transferred to the solvent or sample matrix

4-Intersystem crossing:

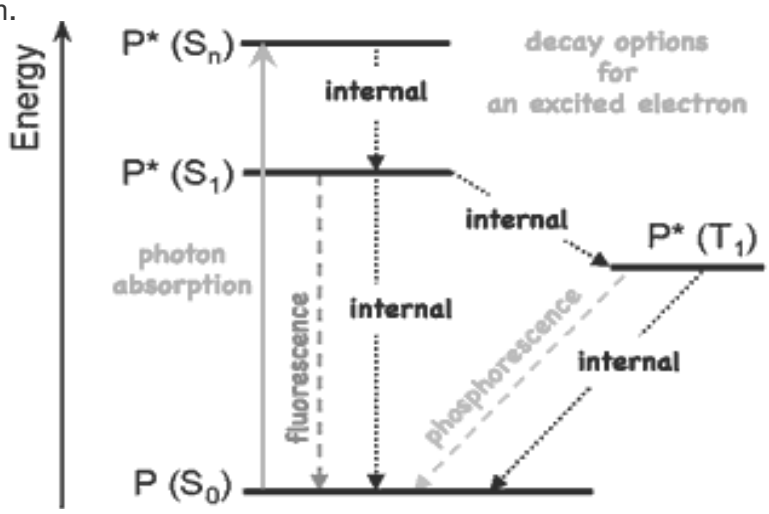
A form of radiationless relaxation in which the analyze moves from a higher electronic energy level to a lower electronic energy level with a different spin state[one electron to reverse its spin]

- , and the molecule transfer to a lower-energy triplet state . From here the molecule can return to the ground state by emission of photon (phosphorescence).

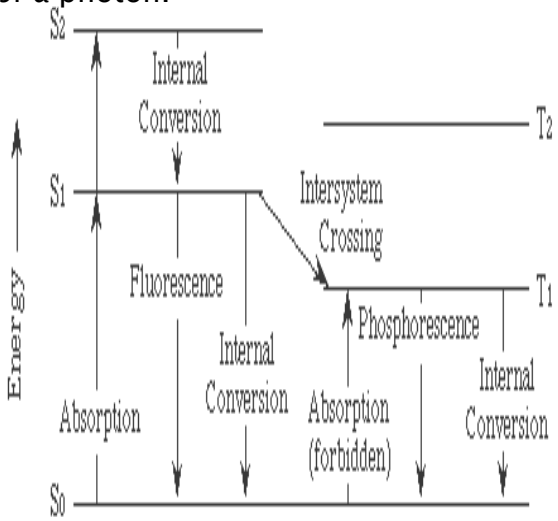
(b)-the emission of a photon:

1-Fluorescence:

Fluorescence occurs when a molecule in the lowest vibrational energy level of an excited electronic state returns to a lower energy electronic state by emitting a photon.Since molecules return to their ground state by the fastest mechanism.



- 2-Phosphorescence:
- A molecule in the lowest vibrational energy level of an excited triplet electronic state normally relaxes to the ground state by an intersystem crossing to a triplet state or by external conversion. Phosphorescence is observed when relaxation occurs by the emission of a photon.



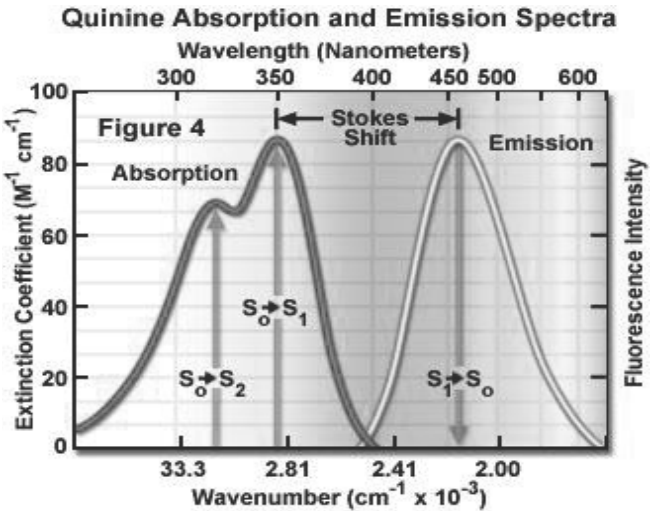
Main difference between fluorescence & phosphorescence

Fluorescence	Phosphorescence
1-The spin state of the excited electron doesn't change during transfer from excited state to the ground state (G) **2- Relaxation takes place from lowest singlet state (electron paired) to singlet ground state $S_1 \rightarrow S_0$** **$\_\_\downarrow\_\_S_1$** **$__\uparrow\downarrow__$G paired $\_\_\uparrow\_\_S_0$** **3-it occurs very soon i.e lifetime 10^{-6} --10^{-9}sec. It decays rapidly after the excitation source is removed.** **4- Measurement take place during excitation**	**1-Change in spin state of electron during transition from X^* to G** **2-Relaxation from lowest triplet state to G (electron unpaired)** **$T_1 \rightarrow G$** **$\_\uparrow\uparrow\_\_T_1$ unpaired** **$\_\uparrow\uparrow\_\_G$** **3-Lifetime longer 10^{-4}- 10^4 sec** **4-Measurement takes place even after excitation ceases.**

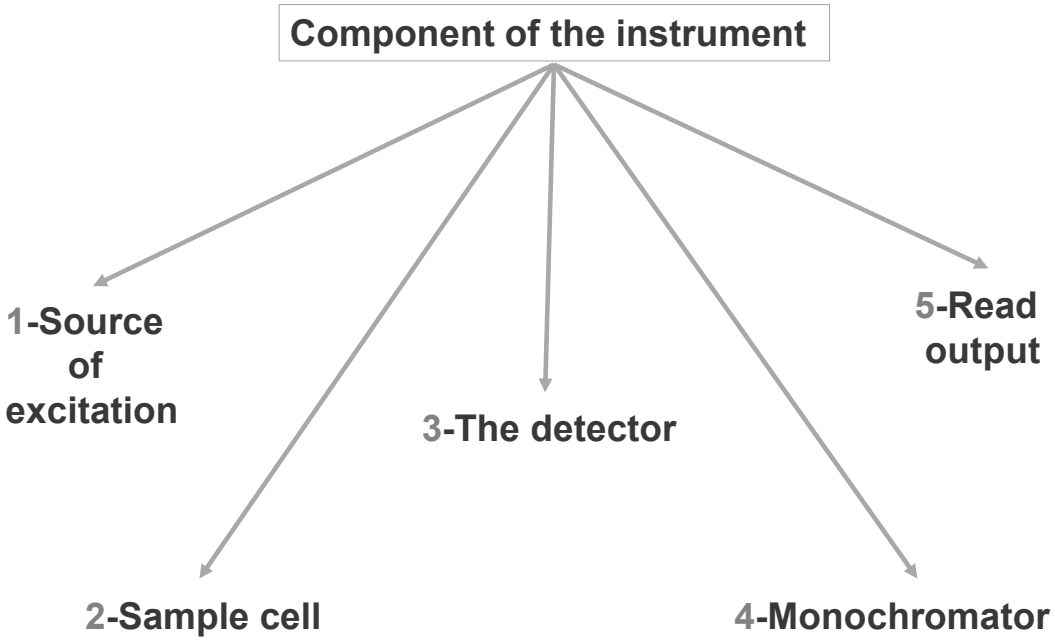
Excitation and Emission Spectra

If we plot the intensity of fluorescence obtained when the sample is irradiated with monochromatic radiation (λ excitation) versus wavelength an emission spectrum is obtained.

If we plot the excitation and emission spectra of a compound on the same chart the displacement of emission band to a longer wavelength (λ) (is known as stock's effect).


Quinine Absorption and Emission Spectra
Wavelength (Nanometers)
300
350
400
450
500
600
Figure 4
Stokes Shift
Emission
Absorption
$S_o \rightarrow S_1$
$S_o \rightarrow S_2$
$S_1 \rightarrow S_o$
Extinction Coefficient (M^{-1} cm^{-1})
Fluorescence Intensity
100
80
60
40
20
0
33.3
2.81
2.41
2.00
Wavenumber (cm^{-1} x 10^{-3})

Instrumentation of Fluorescence



1-Source of excitation.

Either xenon arc lamp (ultra violet) or mercury arc lamp (visible).

2-Sample cell

Transparent in all sides either glass (visible) or quartz (ultra violet) usually present at right angle with respect to the source and detector.

3-The detector

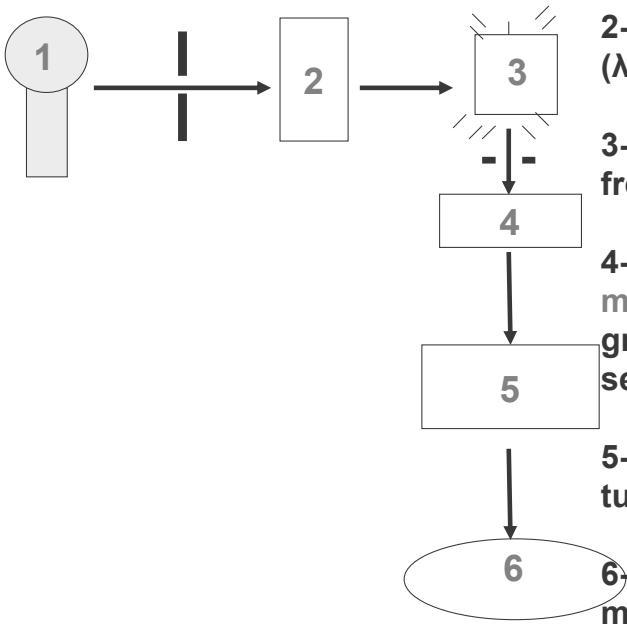
Phototube or photomultiplier tube.

4-Monochromator

Filters or grating.
Two monochromators are used .
One is used for wavelength selection of excitation
and
the second is used for emission.

5-Read output

Recorder or meter scale.



1- Source of radiation Hg-arc xenon lamp

2- Primary monochromater (λ excitation selector)

3- Sample cell transparent from all sides

4- Secondary monochromator (filter or grating) (λ emission selector)

5- Detector (photomultiplier tube)

6- Read output recorder or meter scale

Relation between concentration and fluorescence intensity

- A quantitative expression of the efficiency of fluorescence is the fluorecent quantum yield , Φ, which is the fraction of excited molecules returning to the ground state by fluorescence. Quantum yields range from 1, when every molecule in an excited state undergoes fluorescence, to 0 when flyorescence does not occur.
- $$\Phi = \frac{\text{number of molecules that fluorescence}}{\text{total number of excited molecules}}$$

The intensity if fluorescence, I_f, is proportional to the amount of radiation from the excitation source that is absorbed and the quantum yield for fluorescence where k is a constant accounting for the efficiency of collecting and detecting the fluorescent emission. From Beer's law

Florescence Intensity (I_f) is directly proportional to the concentration of the emitter at low concentration (< 10^{-5} Molar solution).

$$I_f = 2.3\ K\ \Phi_f\ P_0\ (\varepsilon\ b\ c)$$

K = photon measured / photon emitted

P_0 = Radiation power.

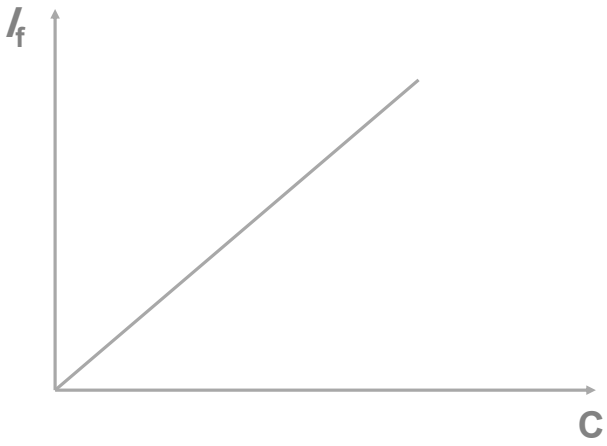
Φ_f = quantum yield or quantum efficiency

Qf = no. of molecule fluoresce : no. of molecule excited

The intensity of fuorescence increase with an increase in quantum efficiency,incident power of the excitation source, and the molar absorpitivity and concentration of the fluorescing species.

For quantitative analysis

Plot of F versus C , constitutes fluorescence calibration curve



Variables affecting fluorescence intensity

1- Quantum yield or quantum efficiency

2- Transition type in fluorescence

3- Fluorescence and Structure

4- Temperature and solvent effect

5- Effect of PH

1-Quantum yield or quantum efficiency

$$Q_f = \frac{\text{No. of fluoresced molecules}}{\text{No. of excited molecules}}$$

It reaches unity in highly fluorescent substances and reaches 0 in non- fluorescent substances.

2- Transition type in fluorescence

Fluorescence is seldom seen in σ* - σ transition using ultraviolet wavelength less than 250 (highly energetic so cause rupture of the bonds.)
Fluorescence emission due to less energetic transition Π*- Π and Π*- n transition.

3-Fluorescence and Structure

Fluorophore:

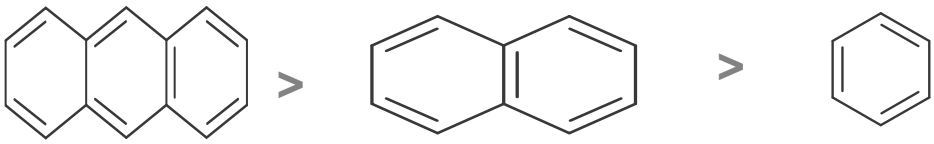
Molecule that absorbs light but then returns to the ground state by emitting some of the light as a photon rather than losing all the energy as heat.

Any molecule that absorbs ultraviolet radiation could fluorescence. The greater the absorption by a molecule, the greater its fluorescence intensty. Many aromatic and heterocyclic compounds fluorescence. Compounds with multiple congugated double bonds are favorable to fluorescence. One or more electron –donating groups such as –OH,-NH_2&-OCH_3 *enhances* the fluorescence.

Groups such as –NO_2,-COOH, -CH_2COOH, -Br, -I,& azo gps tend to *inhibit* fluorescence.

Effect of structure rigidity

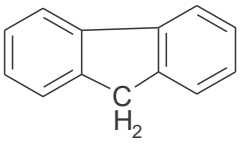
Quantum efficiency or quantum yield increase with increasing the number of fused rings.



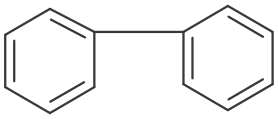
Anthracene **naphthalene** **benzene**

Effect of structure rigidity

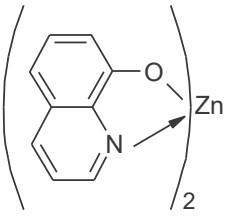
Fluorescence is favored by rigidity of structure therefore:
Eg.
Fluorene (more rigid) where Q_f = 1 , Biphenyl (less rigid) where Q_f = 0.2



Fluorene

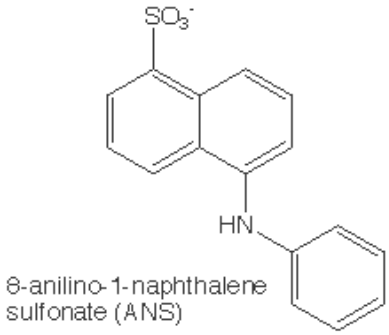


Biphenyl



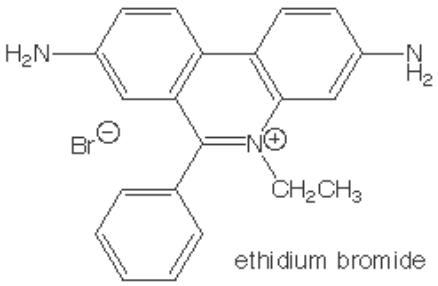
The Zinc complex

Lack of rigidity enhance internal conversion and decrease Q_f



8-anilino-1-naphthalene sulfonate (ANS)

Absorption λ_{max} = 374 nm
Emission λ_{max} = 454 nm
Emission τ_F = 16 ns
Sensitivity 67×10^{-2}



ethidium bromide

Absorption λ_{max} = 515 nm
Emission λ_{max} = 600 nm
Emission τ_F = 26.5 ns
Sensitivity 38×10^{-2}

4- Temperature and solvent effect

Φ decreases by increasing temperature due to collision

5-Effect of PH

Fluorescence of aromatic compounds with acidic or basic PH-dependent groups (phenol , aniline)

Aniline PH-NH_2 with lone pair of electrons in alkaline medium fluorescence at about 400 nm while in acid medium it does not fluoresce.

Phenol in acid medium differ in its fluorescence from phenate form in alkaline medium [not fluoresce .

Application of Fluorimetry

Compounds which are intrinsically fluorescent (has native fluorescence) are easily determined at very low concentration by simple fluorimetric method for example , phenobarbitone, quinine , emetine , adrenaline, cinchonine, reserpine, vitamin A, riboflavine , thalium I(while thalium III does not fluoresce), cerium III (while cerium IV does not fluoresce), UO_2^+ and many other pharmaceuticals and natural products.

Also non-fluorescent substances can be determined after chemical reaction.

Inorganic ions can be determined either by formation of fluorescent chelates upon reaction with fluorimetric reagents

e.g.

8-hydroxyquinoline (for Al), benzoin(for Zn) or flavonol (for Zr) or by measuring the quenching of fluorescence of a fluorescent substance in presence of some ions

- Fluorescence Quenching
- One difficulty frequently encountered in fluorescence is that of fluorescence quenching by many substances. These are substances that, in effect, compete for the electronic excitation energy and decrease the quantum yield(the efficiency of conversion of absorbed radiation to fluorescent radiation see below). *Iodide ion* is an extremely effective quencher. Iodide and bromide substituent groups decrease the quantum yield. Substances such as this maybe determined indirectly by measuring the extent of fluorescence quenching. Some molecules do not fluoresce because they may have a bond whose dissociation energy is less than that of the radiation. In other words, a molecular bond maybe broken, preventing fluorescence.

Advantages of fluorimetry

1- Highly sensitive to very low concentrations(10-5 M).

2- highly selective two λ_{ex} and λ_{em}

3- Only aromatic compounds fluoresce while alicyclic or acyclic do not fluoresce therefore useful in their mixture analysis.

Disadvantages of fluorimetry

1-Quenching by presence of non-fluorescent compounds.

2-Many organic compounds undergo photochemical reaction by u.v radiation.

3-It does not exhibits high precision or accuracy +/- 2 to 10 % however its sensitivity and selectivity makes it a method of choice

Atomic Spectroscopy

This section deals with the spectroscopy of atoms. Since atoms cannot rotate or vibrate as a molecule does ,only electronic transitions can take place when energy is absorbed.

Advantages of atomic spectroscopy:

1-sensitivity (ppm)

2-speed

3-convenience

4-high selectivity

5-moderate instrument costs

Spectroscopic determination of atomic species can only be performed on a gaseous medium in which the individual atoms are well separated from one another. There are various ways to obtain free atoms and to measure the absorption or emission of radiation by these.

The principal techniques are emission spectroscopy, in which atoms are excited in an electron arc or spark.

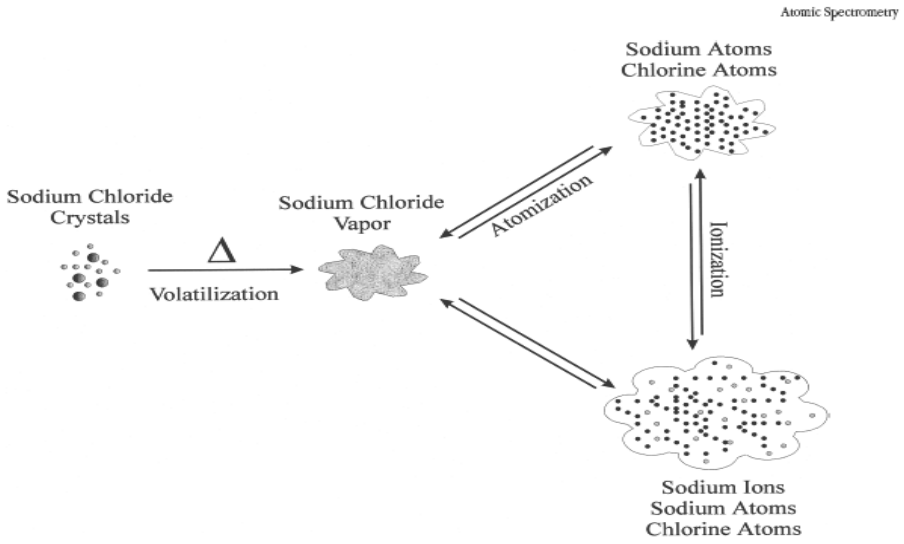
Flame emission spectroscopy in which atoms are excited by flame.

Atomic absorption spectrophotometry, in which the amount of radiation absorbed by ground-state atoms in a flame is measured.

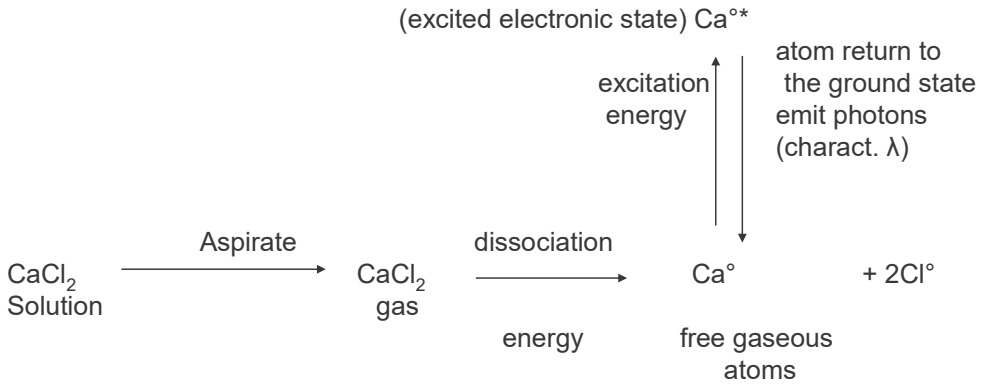
The first step in all atomic spectroscopic procedures is atomization.

Atomization:

It is a process in which a sample is converted into gaseous atoms



Example



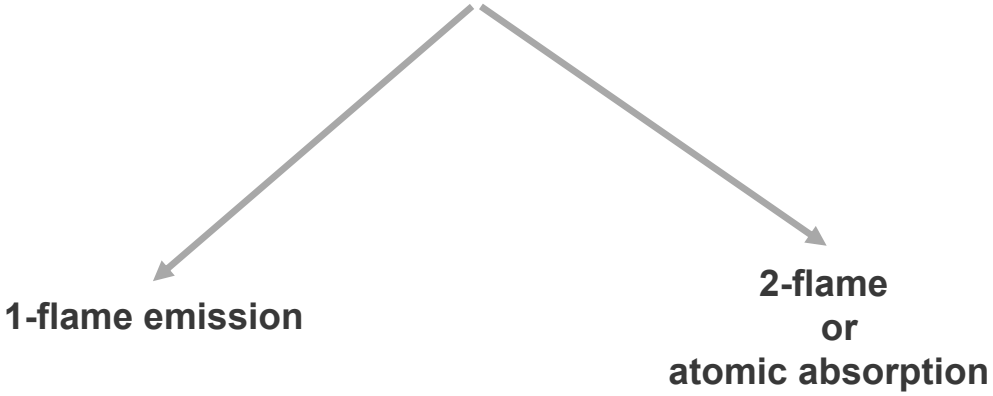
λ Detected with a conventional monochromator detector setup

When a solution of inorganic salt is aspirated (introduce in very fine droplets) in flame or heated on electrically heated surface,the solvent will be evaporated. A certain fraction of these atoms can absorb energy from the flam and be raised to an excited electronic state.These excited atoms, on retuning to the ground state, emit photons of characteristic wavelength. These can be detected with a conventional monochromator-detector setup.

The intensity of emission is directly proportional to the concentration of the analyte in the solution being aspirated.
So a calibration curve of emission intensity as a function of concentration is prepared.

Classification of flame spectroscopy and flame spectroscopic methods:

■**they are classified into two main classes:**



1-flame emission

atoms in gaseous state in the flame absorb thermal energy from the flame itself. Some of the atoms get excited and the outer most electrons are promoted to higher energy levels ;as they return back to the ground state the emit radiation having energy equal to that absorbed.

the emission is proportional to the total number of atoms in the flame i.e. the sample concentration .the method is known by flame emission .

if the atoms in the gaseous state are excited by an outer highly illuminated source of radiation, the method is known by flame fluorescence.

2-Flame absorption or atomic absorption (AAS).

In this method we study and\ or use the absorption of electromagnetic radiation (EM) by gaseous atomic particles in the flame, for their determination .the atoms in the flame are subjected to an external source of radiation producing EM of definite wavelength which will be absorbed by the atoms. The absorbance A and the sample concentration ,within certain limits, obeys Beer's law.

However in any of the above methods the thermal energy supplied must be only that will cause atomization and not ionization i.e. less than the ionization potential of the atoms.

Flame spectra

The spectra of gaseous ,atomic particles consist of well defined narrow discrete lines arising from electronic transition of outermost electrons.

each line represents one of the electronic transitions of an excited electron, to one of the permissible higher energy levels (the energy absorbed and\ or emitted is exactly the difference between the ground and excited energy levels).

Notice that ,since there is no bonds, atoms, undergo electronic transition only,no vibrational or rotational transitions. Thus when atoms in the flame are subjected to the thermal energy,UV,or VIS Radiation not all the energy or radiation are absorbed or emitted, but only few quantized energy levels which correspond to radiation of definite wavelength ,e.g. in case of sodium atom 20 lines appear of these only two which are intense and can be used for its analysis.

E absorbed or emitted = Eexcited state –Eground state =h c\λ

Theory of flame emission:

The number of atoms of an element excited by the flame depends on:

1-flame temperature

2- the energy difference between the excited and ground states.

3-the quantum states of the electrons in the ground and excited energy levels.

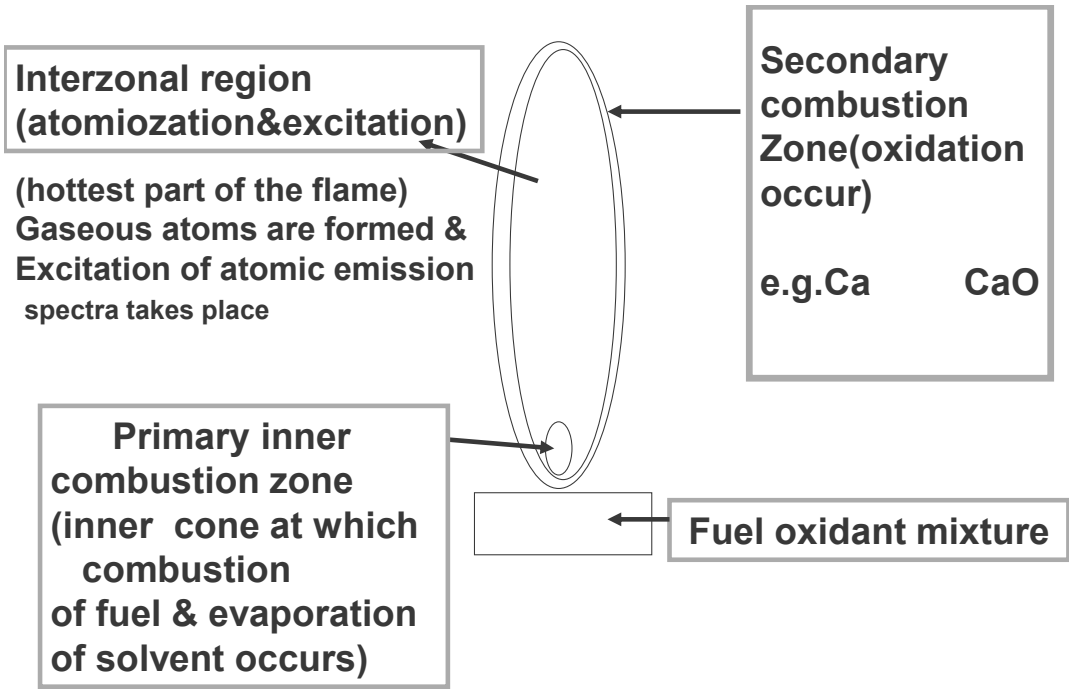
flame temperature

flame temperature must be

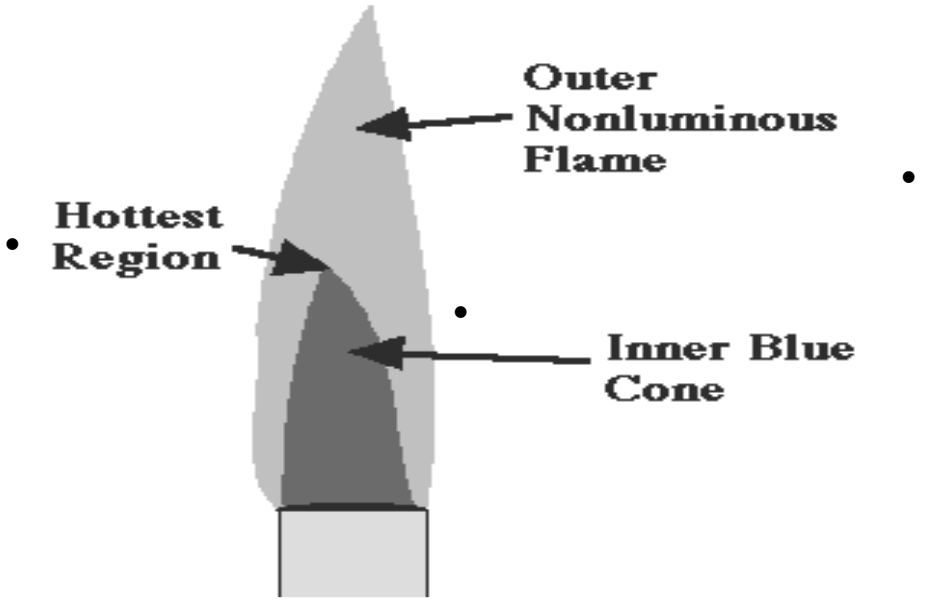
a- Regular
b- It must be sufficient to cause atomization only and not ionization

flame temperature must be

a- Regular
b- It must be sufficient to cause atomization only and not ionization



Region in a flame



Flame mixture	Maximum temperature ◦C
Propane-air	1725
Hydrogen-air	2045
Acetylene-air	2250
Propane-oxygen	2900
Acetylene-nitrous oxide	2955

The sensitivity of the flame spectroscopic method depends on:

The conditions under which flame atomizer operates.

Dimensions of the burner (they are usually standard)and its position nebulization, temperature and the rate of introduction of the sample to the flame.

•As the oxidant flows it withdraws the sample from the capillary in very fine droplets which with oxidant are mixed in the premixing chamber with the fuel in laminar burner or the three are mixed at the top of the burner in the turbulent type. The nebulization depends on the rate at which the oxidant is introduced . the premixing chamber contains baffles which retain large droplets to pass to waste drain. The fuel-oxidant-sample aerosol mixture passes to the burner

In the burner the combustion of fuel occurs producing the necessary heat for atomization and excitation. It is either circular or rectangular with standard dimensions and position. The temperature of the flame produced depends on fuel-oxidant ratio and kind. The mixture of natural gas or propane or butane with air has a temperature below 2000°k, while mixture of acetylene with air or nitrous oxide or oxygen has a temperature above 2000° k. In case of potassium, sodium, lithium and calcium, they are atomized and excited below 2000° k above 2500° k ionization occurs.

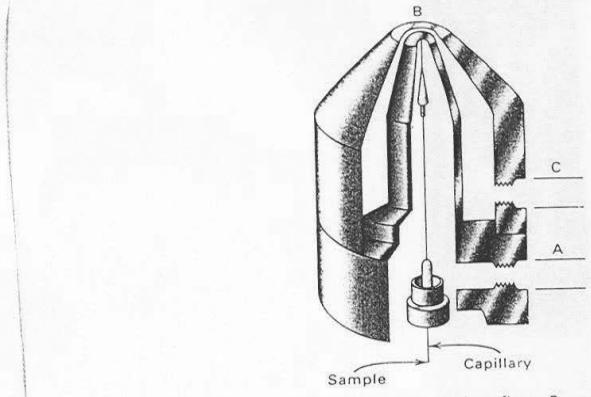
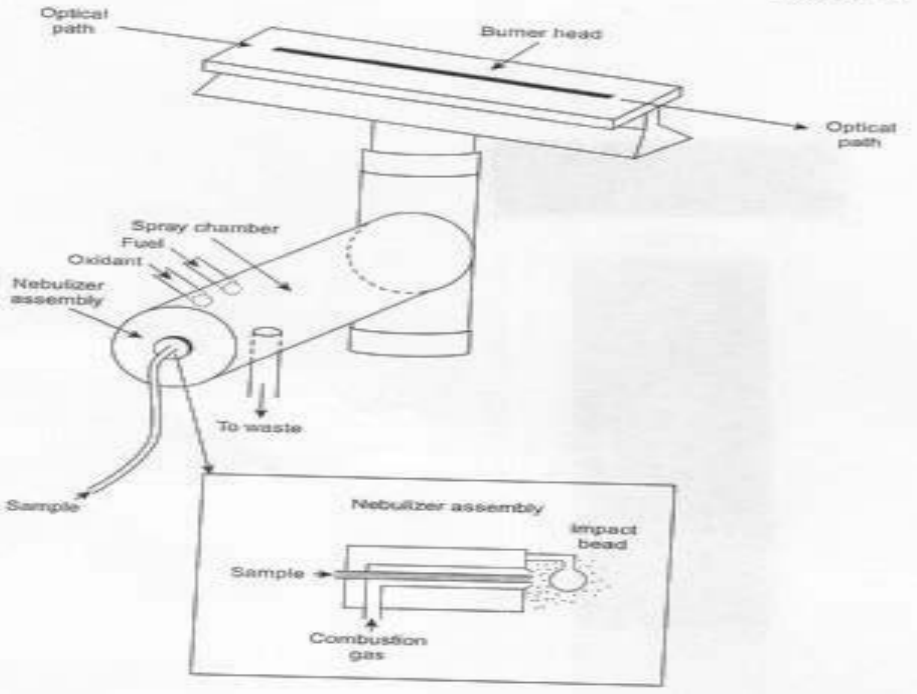


FIGURE 15.6 Total-consumption burner: *A*, oxidant flow; *B*, surface mixing; *C*, fuel flow. (From G. D. Christian and F. J. Feldman. *Atomic Absorption Spectroscopy. Applications in Agriculture, Biology, and Medicine.* New York: Interscience, 1970. Reproduced by permission of John Wiley & Sons, Inc.)



Theory of flame emission

The effect of the three factors is given by Boltzmann equation:
Nj \Ng = Pj \ Pg exp –(Ej –Eg) \KT
Nj and Ng are the number of atoms in the excited and ground states

Ej-Eg =∆E =hc \λ=difference in energy levels
λ is the resonance wavelength.
K= Boltzmann constant =1.38 ×10 -16
T= the flame temperature in Kelvin degree
Pj and Pg are the statistical weights of the respective states and are related to the total quantum number that an electron may have e.g. in case of sodium the promotion will be from 3s to 3p in the s orbital two probable total quantum number ,while in the 3p there are six probable total quantum number.
Pj \Pg = 6\2 = 3

According to the above equation ,it is clear that:
1-the number of excited atoms in the flame is considerably small, even in the case of alkaline metals which are easily excited. For example, sodium at 2500 K 0 ,0.017% of the atoms are excited ,other metals ; the number of excited atoms is extremely small e.g. in case of Zinc only 10^{-9} are excited.
2- Any increase of the flame temperature is accompanied by great increase in the number of excited atoms.

Limitation of flame emission photometry:

1-the number of excited atoms in the flame is very small. It is the alkaline and alkaline earth metals that can be practically determined.
2- Heavy and transition metals have large number of closely spaced energy levels electrons; thus the number of absorption and emission lines is enormous and difficult to be resoluted, the spectra are complex.
3- Interference by other elements is not easy to be eliminated.
4-It needs perfect control of flame temperature.

Instrument for flame emission

The instrument used is formed of:

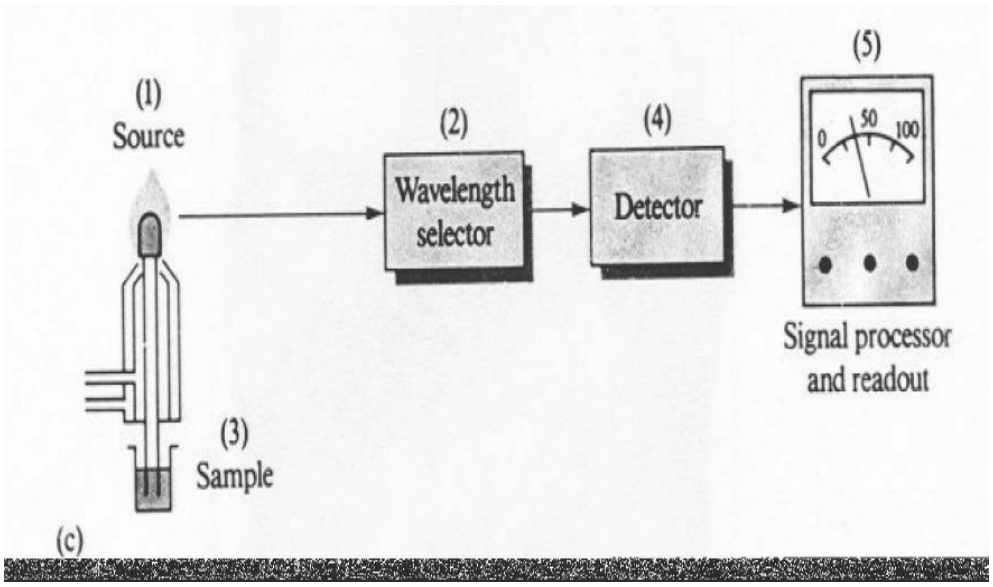
1-flame atomizer.

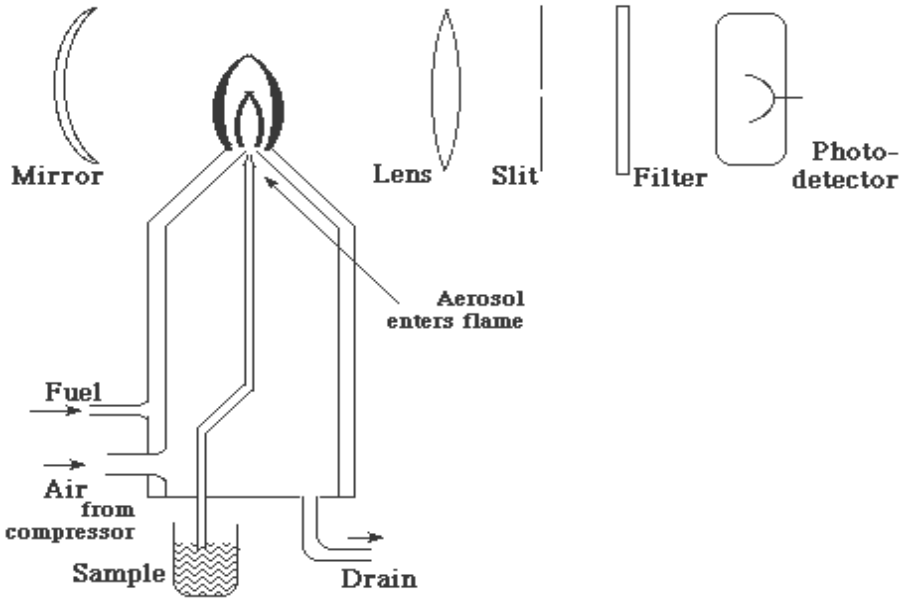
2-Monochromator

3-Detector.

4-readout meter.

The flame atomic emission instrument





1-flame atomizer

it serves two functions

a)Atomization of the sample.

b)Source of the energy to excited the atoms .

The atomizer is composed of :

Nebulizer ,and burner (both total consumption (turbulent flow) and premixed (laminar flow) are used.)

Nebulizer

It is a device by which sample solution is divided into very fine droplets which are aspirated into fine spray or aerosol. It is formed of fine capillary , its lower end is bend and dipped in the sample solution, the order end has a fine tip which is surrounded by the exit of tube through which the oxidant flows at high pressure, both open in the premixing chamber in case of laminar type burner or the nebulizer and burner are one unit in case of turbulent type burner

2- Mononochromator

Either grating *or* interference filters which allow the resonance
wavelength to pass. Notice that radiation passes through monochromator to the detector will that emitted from the sample and that from the flame itself (flame noise), however the later is the same in the blank and experiment.

3- Detector

Photomultiplier tube.

4-Read out meter

Measure transmittance percent.

APPLICATION OF FLAME EMISSION

a-Calibration Curve Method:

1- prepare a series of standard solution of A.R.grade of the analyte (KCl, NaCl, CaCl2) containing 2,4,6,8 and 10 PPM.
2- Choose the suitable filter (K ,Na ,Ca)
3-Adjust the zero of transmittance scale upon spraying deionized water.
4-Adjust the 100% trans1nittance scale upon spraying the highest
5- Determine the transmittance % of each of the prepared solutions.
6- Plot a calibration curve representing the % transmittance against the concentration in PPM.
7 Spray the unknown solution and determine % transmittance then the concentration can be obtained from the calibration curve

-Standard Addition method:

1- prepare standard solution of suitable concentration e.g. 6 PPM.

2- Choose the suitable filter.

3- Adjust zero as above.

4- Adjust 50% transmittance while spraying the standard.

5- Add the same amount of standard to the sample and spray, then by difference you can calculate the sample concentration.

ATOMIC ABSORPTION SPECTROSCOPY

In atomic absorption spectroscopic method, atoms in the vapor state are subjected to external source of radiation which produces beam of monochromatic light with single wavelength. This wavelength is a resonance one for the atoms and that will be absorbed by them. Since all elements can be determined by this technique.

Atomic absorption spectra	Molecular absorption spectra
1- The outer most electrons occupy one of the atomic energy level (K,L,M,N,…. s, s,p s,p,d s,p,d,f)	**1-The outer most electrons occupy σ ,π or n electronic energy levels in the ground state**
2- Upon excitation ,electrons are promoted to any permissible higher atomic energy levels.	**2-Upon excitation, electrons are raised to π* or σ* energy levels.**
3- Since there are no bonds there are no vibrational or rotational energy levels in either the ground or excited state.	**3-Since there are bonds,there are vibrational and rotational energy levels in both the ground and excited states.**
4- The analytical wavelength is the resonance wavelength of the analyte.	**4-The analytical wavelength is the λ_{max}**
5- The spectra are line form.	**5-The spectra are in the form of bands due to the presence of very close, superimposed and unresolved vibrational and rotational energy levels in the excited state.**

Theory of Atomic Absorption:

Atoms - in the vapor state - absorb radiation with specific wavelengths which is necessary to promote the outer most electrons to definite higher energy levels. Thus upon subjecting the atoms to a source of radiation producing one of these specific radiation a decrease III intensity of the radiation occurs due to absorption by atoms.
The absorbance takes place follows **Beer's law:**

$A = \log I0/I = 0.434\ A\ k\ c\ b$
k = is a coefficient depends on the nature of atoms and the radiation.
c = is concentration of atoms which depends on and represented by the sample concentration.
b = is the light path-length which depends on the dimensions of the flame which are standard and constant for each instruments.

INSTRUMENT FOR ATOMIC ABSORPTION

The main parts of atomic absorption spectrophotometer (AAS) are:

1- Source of radiation

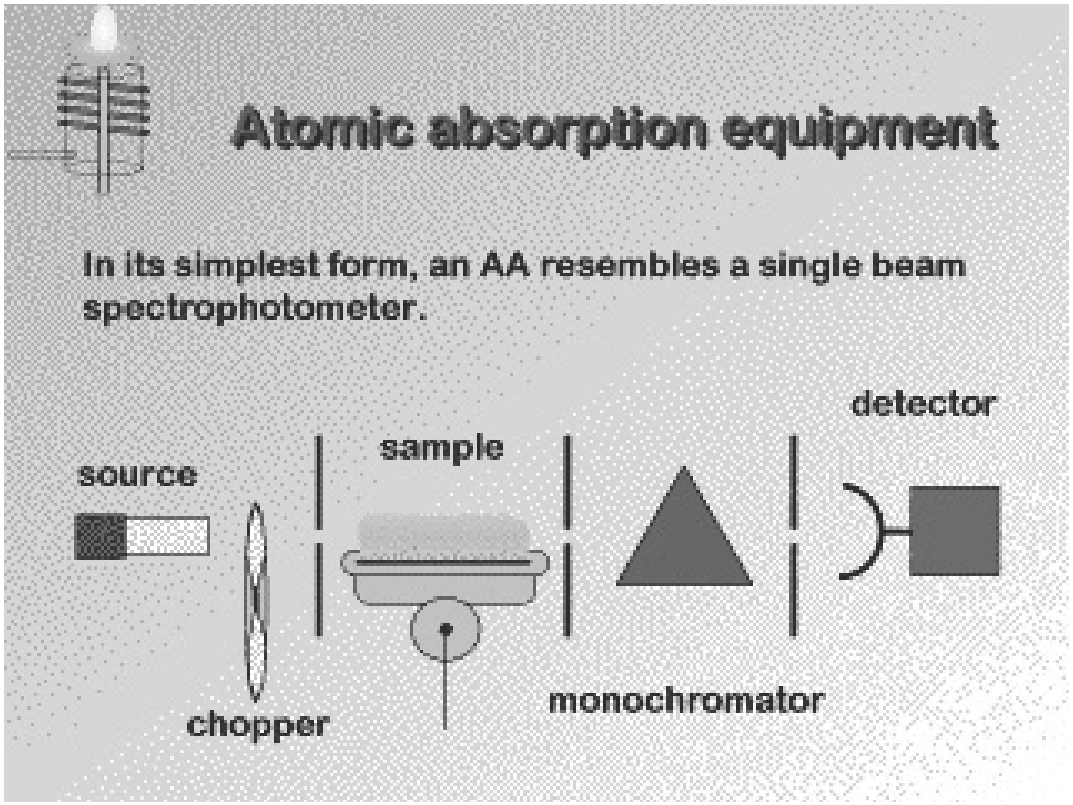
2- Chopper

3- Atomizer.

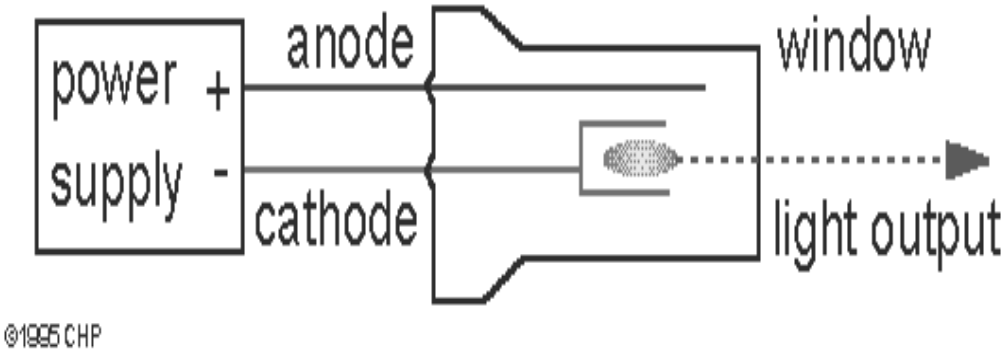
4-Monochromator

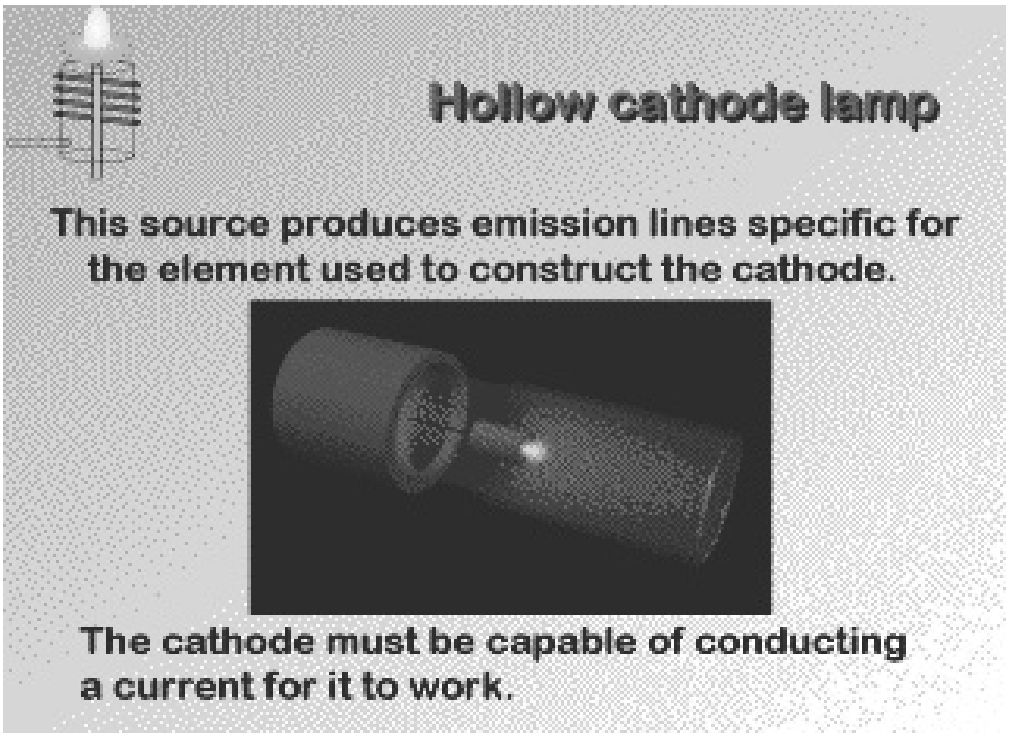
5- Detector.

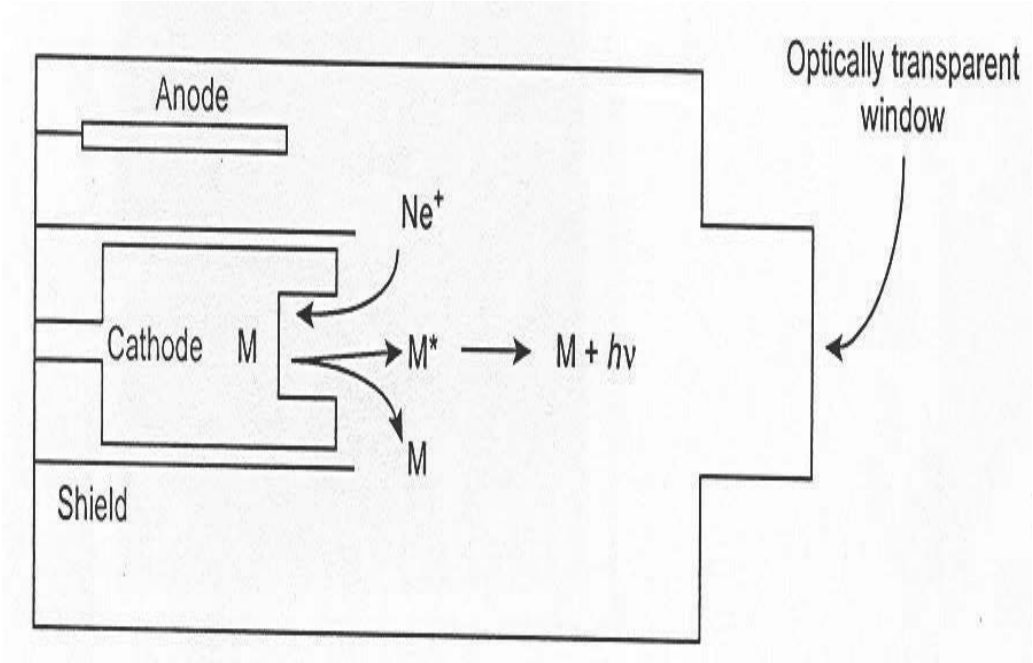
6- Read out Meter.

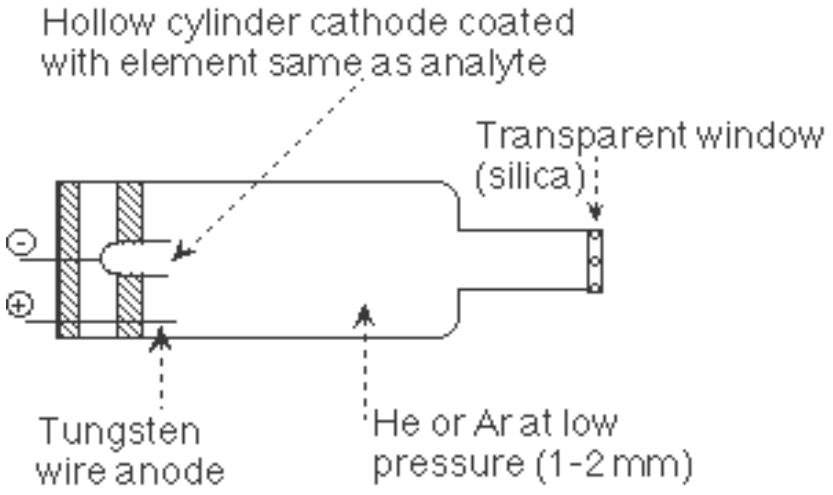


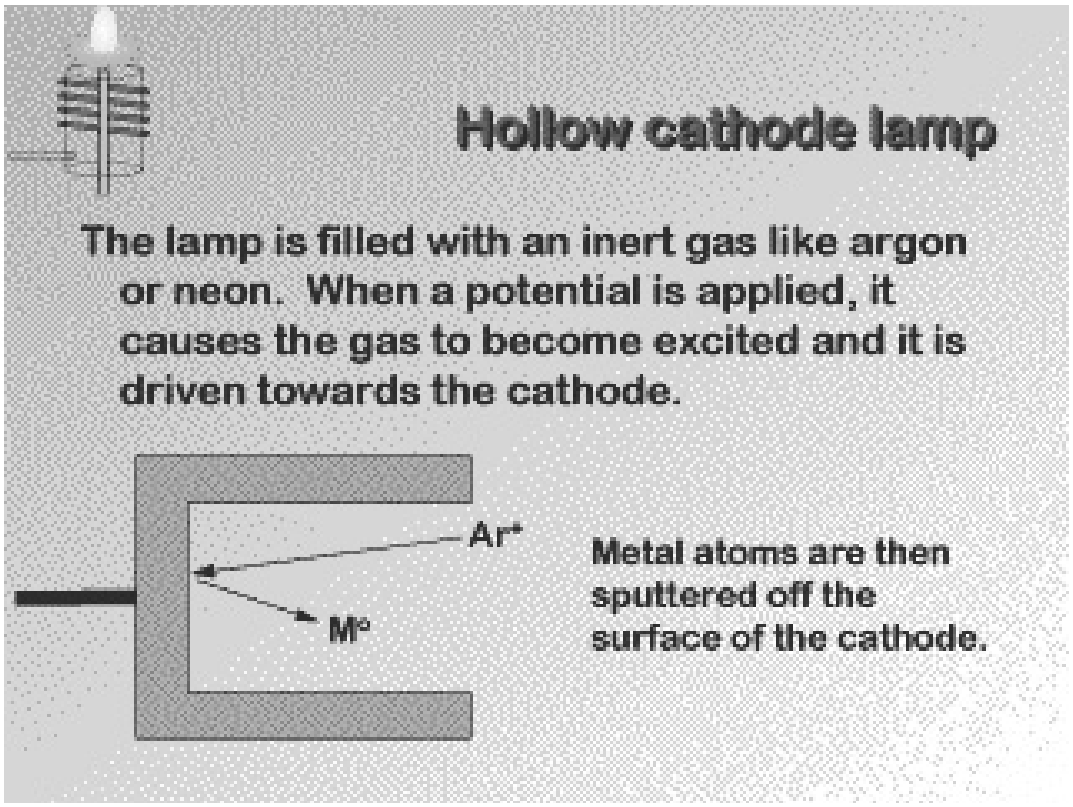
<u>1-source of radiation</u>: ***hollow-cathode lamp***

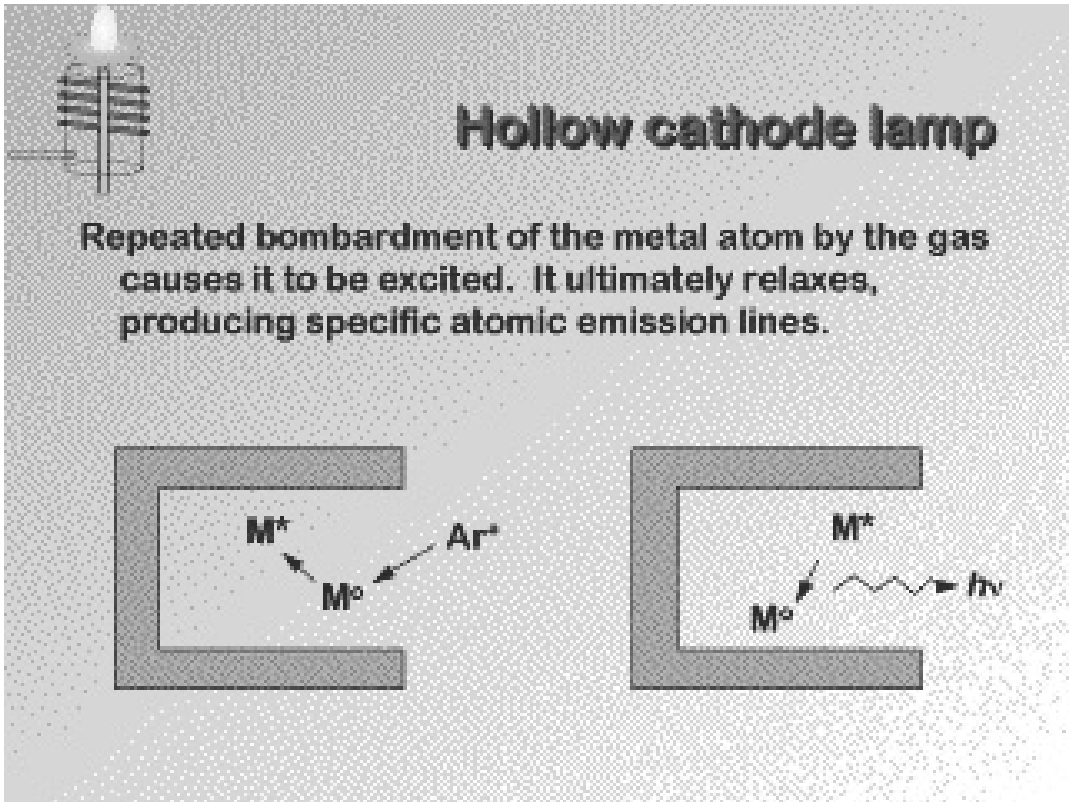



Hollow cathode lamp
This source produces emission lines specific for the element used to construct the cathode.
The cathode must be capable of conducting a current for it to work.


Optically transparent window
Anode
Ne+
Cathode
M
M*
M + hν
M
Shield


Hollow cylinder cathode coated with element same as analyte
Transparent window (silica)
Tungsten wire anode
He or Ar at low pressure (1-2 mm)

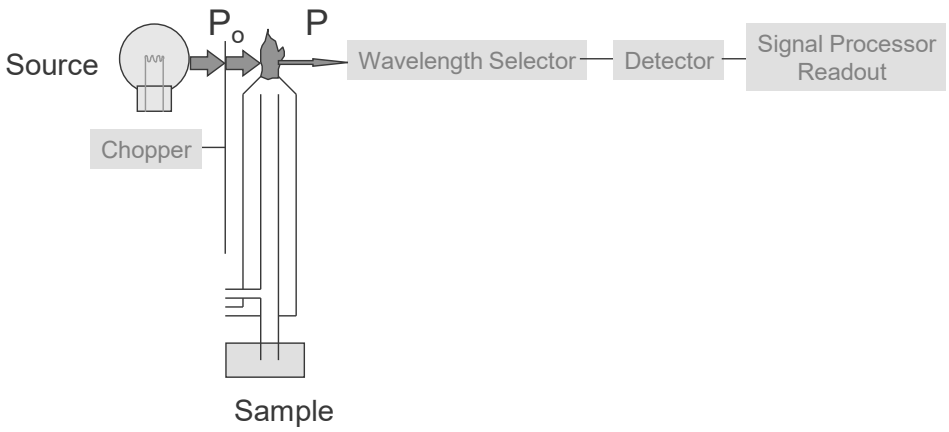

Hollow cathode lamp
The lamp is filled with an inert gas like argon or neon. When a potential is applied, it causes the gas to become excited and it is driven towards the cathode.
Ar+
M°
Metal atoms are then sputtered off the surface of the cathode.

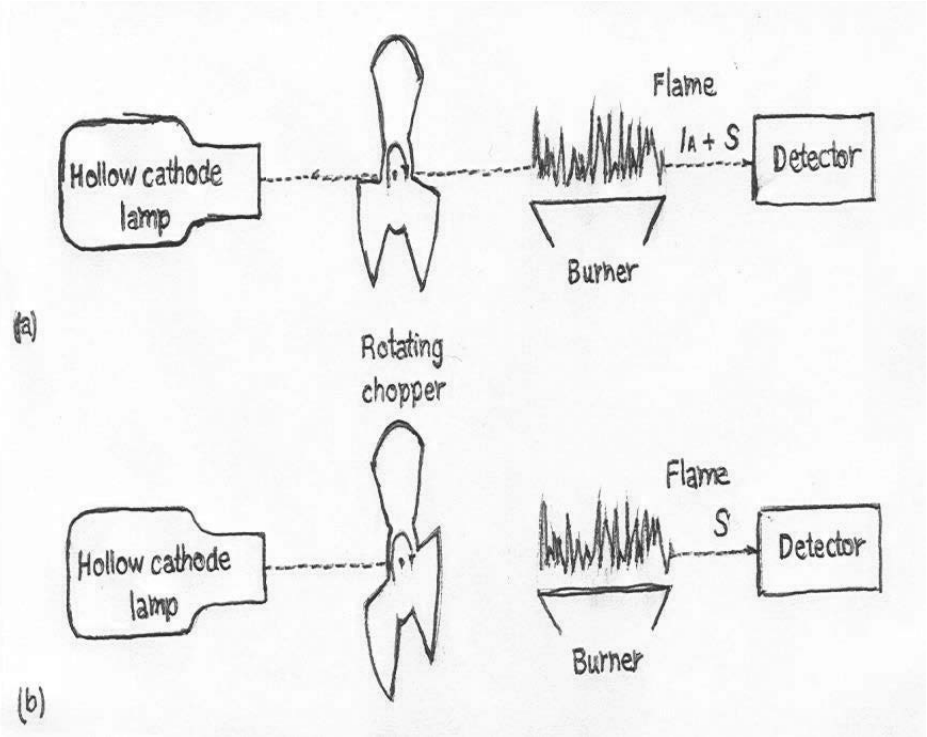


A deuterium *or* hydrogen discharge lamp cannot be used for atomic absorption because the band width obtained even by the use of very efficient monochromator is much broader than the absorption line to be absorbed by the atoms in the flame. Thus a hollow cathode lamp is used. The above fig. shows the hollow cathode lamp which is a tube with a front quartz 'window contains an anode of tungsten and a **cylindrical cathode, the material of which is the same element of the sample to be determined**. The glass tube is filled with neon or argon at a pressure of 1 to 5 tore. When a low potential is applied between the two electrodes ionization of the gas occurs; ions move rapidly to the cathode and hit it causing dislodge of atoms and produce an atomic cloud (sputtering). Some *of* the free atoms are in the excited states and emit their characteristic radiation, then diffuse back to the cathode surface. The cylindrical configuration of the cathode tends to concentrate the radiation in a limited region of the tube with production of line beam of exactly the same wavelength to be absorbed by the sample.

2-The chopper

In front of the lamp there is the chopper its function is to fluctuate the source output. It is a circular disc divided into four quarters two are mirrored and two are opened. The disc rotates at high constant speed, when the mirrored quarter in front of the lamp, it reflects the radiation, the second moment the open in front of the lamp and the radiation passes to the sample being absorbed it and reaches the by detector in pulses. The detector converts radiation to alternating current signal and amplified it . The radiation coming the flame itself and from atoms excited by the flame will reach the detector continuously and converted to direct current signal which can be suppressed and eliminated . So the detected signal will be that coming from the source only. This process is known by modulation of the source output.





3-Atommizers

There are two types of atomizers:

a- Flame atomizer: it is the same as in flame emission technique but the burner must be rectangular.

b- Flamless or non flame atomizer: its a graphite furnace heated

electrically up to 6000°C and contains a ribbon or boat in which one can inject the sample. Upon heating the furnace, the sample is ashen ,then atomized by action of heat.

Advantages of non-flame atomizer:

- The sample volume is small few micro-liter.
- Unusual high sensitivity 10-12ppm.
-Solid sample can be used directly.
-Heat distribution is uniform and temperature is steady.
- No need for fuel -oxidant mixture.
- No flame noise.

4- Monochromator:

Grating to eliminate other resonance lines from the source or
other radiation from the flame or sample.

5- Detector:

Photomultiplier.

6- Readout meter:

Absorbance or transmittance output

APPLICATIONS OF ATOMIC ABSORPTION

Calibration curve method:

1- Choose the suitable Lamp.
2- Prepare a series of standard solutions of A.R. grade of a salt of the Metal to be detenI1ined, in the solvent to be used in the
sample. The concentration range in PPM
3-Adjust zero absorbance while spraying a solvent blank.
4- Measure the absorbance while spraying each of the standard solutions.
5- Plot a graph-representing the concentration against the absorbance.
6- Spray the sample and measure the absorbance and from the graph the concentration can be obtained.

Standard Addition Method:

1- Prepare a standard solution of exact known concentration e.g. 100 ppm

2- A volume of the solution is added to the sample solution so that the final dilution an exactly known amount of the standard added e.g. 5 ppm

3- Measure the absorbance while spraying the sample A_1 and the solution containing the standard and the sample A_2.

$A_1 = KC_x$ $A_2 = K(C_x + C_s)$

where C_x is the sample concentration and Cs is the standard concentration.

$$A_1 = \frac{A_2.C_x}{C_x + C_s} \qquad A_1C_x+A_1.C_s = A_2C_x$$

$$C_x = \frac{A_1.\ C_s}{(A_2 - A_1)}$$

PRACTICAL PHARMACEUTICAL ANALYSIS "II"

FIRST YEAR PHARMACY

SPECTROSCOPY

Definition: it is the study of interaction of spectrum of light with a substance to be analysed, for its identification (qualitative analysis) as well as determination of its amount (quantitative analysis).

Wavelength (λ): is the linear distance measured along the line of propagation between crest of one wave to the next.

λmax: is the wavelength of maximum absorbance

Isosbestic point: is the wavelength at which absorbance is not pH dependant but concentration dependant.

Units of wavelength:

Micron (μ) = 1 x 10-6m = 1 x 10-4cm = 1 x 10-3mm

Millimicron (m(μ) = nanometer (nm) = 1 x 10-9m = 1 x 10-7cm.

Angstrom (A°) = 1 x 10-10m = 1 x 10-8 cm = 1 x 10-7.

Frequency (ν): number of waves per seconds, unit: Hz

Velocity of light (C): 3 x 108m/sec

Where C= λ x ν

Wave number (δ): is the number of waves per cm

Where δ = 1/λ (λ in cm)

Energy of photon (E): E = hν where h is max plank constant.

Colorimetry: is the technique in which we are measuring the absorbance (A) of visible radiation by colored sample.

Spectrophotometry: the technique in which we are measuring the absorbance (A) of UV or visible radiation either by colorless or colored sample, respectively.

LAWS OF LIGHT ABSORBANCE

Beer-Lambert's law:

$A = \log I°/It = \log 1/T = \log 100/ \%T = abc$

Where:

A = Absorbance

I° = Intensity of incident light

It = intensity of transmitted light

T = Transmittance

% T= Percentage transmittance

b= Path length of light in sample

C= Concentration

a= is a constant, known as absorptivity, which is the absorbance when thickness of solution is unity (1cm) and concentration is unity.

Molar absorptivity or epsilon (ε)

If the unit of concentration is 1M, (a) is known as molar absorptivity or epsilon (ε) or molar extinction coefficient (unit of (ε) is L mol-1 cm-1).

A(1%-1cm): if unity of concentration is 1%, (a) is known as A(1%-1cm).

PROBLEMS

1. (a) A 6.40 x 10-5 M solution of a drug had an absorbance of 0.847 in a 1 cm cell at 255 nm. Calculate the molar absorptivity of the compound.

(b) Are the data of part (a) sufficient to tell if Beer's law is obeyed by this compound?

(c) Exactly 10.0 mg of a sample of this drug (MW 200.0) was dissolved in water to make 1 liter. At 255 nm the absorbance, in a 1 cm, was 0.556. Calculate the purity of the sample.

(d) A sample of Poldine methylsulphate weighing 500.0 mg was dissolved in enough methanol to make 1 liter. The absorbance of this solution in a 2 cm cell at 257 nm was 0.946. Assume that any impurities present do not absorb light of this wavelength, calculate the percent purity of the sample.

2.Convert each of these absorbance values into the corresponding transmittance.

(a) A= 0.015

(b) A= 0.500

(c) A= 1.000

(d) A= 1.300

3.Convert each of these transmittance values into the corresponding absorbance.

(a) T= 0.001

(b) T= 0.25

(c) T= 0.75

(d) T= 0.99

4. Tolbutamide, MW 270.4 has molar absorptivity 703 at 262 nm. If a single tablets of tolbutamide is dissolved in water and the solution is diluted to 2500 ml, its absorbance will be 0.520 at 262 nm in a 1 cm cell. What weight of tolbutamide is contained in the tablet?

Tutorial (1)

Spectrophotometry

Definition:

Study of interaction between light and substance for the purpose of identification and quantification.

Dual nature of light (dualism):

1- Light as a Wave

2- Light as energy particle (photon)

1- Light as a Wave:

A- Wavelength (λ): is distance between successive maxima (crest) or minima (trough) of a wave.

Different units of length are used to express wavelengths:

The following units are in common use:

$1\ m = 100\ cm = 1000\ mm = 1 \times 10^{6}\ \mu m = 1 \times 10^{9}\ nm = 1 \times 10^{10}\ A^{o}$

OR

$A^{o} = 1 \times 10^{-1}\ nm = 1 \times 10^{-4}\ \mu m = 1 \times 10^{-7}\ mm = 1 \times 10^{-8}\ cm = 1 \times 10^{-10}\ m.$

B- Frequency (ν): which is the number of waves occurring per second and is expressed in cycles/sec (CPS) or Hertz (Hz).

C- Wavenumber (ν`): which is number of waves per centimeter, which is expressed in cm^{-1}.

wavenumber (ν`) = 1/λ cm^{-1}

D- Velocity (c):

Constant measured in Km/sec or m/sec or cm/sec = **300,000 km/sec**

= 3 x 10^8 m/sec = 3 x 10^{10} cm/sec

Relations between λ, ν and ν` are given by the following equations:

$$\frac{1}{\text{Wave number } (\nu`)} = \text{Wavelength } (\lambda) = \frac{\text{Velocity of light (c)}}{\text{Frequency } (\nu)}$$

Note that, *the longer the wavelength, the lower the frequency and the smaller the wave number and vice versa. (↑ λ, ↓ν, ↓ν`).*

2- Light as an energy particle:

$$E = h\nu$$

where h is Plank`s constant (6.63 x 10^{-34} j.s or 6.63 x 10^{-27} erg.sec)

$$\nu = c/\lambda \qquad E = hc/\lambda$$

Therefore, the **shorter** the wavelength, the **greater** the energy of the photons and the **more powerful** the radiation.

Example (1)

Convert 4000 A^o into frequency (Hz) and into wave number (cm^{-1}).

Solution

Wavelength λ 4000 A^o = 4000 x 10^{-8} cm

= 4 x 10^{-5} cm

Wave number ν′ = 1/4 x 10^{-5} = 25000 cm^{-1}

Frequency ν = c/λ

= 3 x 10^{10} / 4 x 10^{-5}

= 0.75 x 10^{15} Hz

Example (2)

Calculate the frequency in Hz and energy in joules of x-ray photon with wavelength of 2.7 A^o.

Solution

Wavelength λ 2.7 A^o = 2.7 x 10^{-8} cm

Frequency ν = c/λ

= 3 x 10^{10} / 2.7x 10^{-8}

= 1.11x 10^{18} Hz

Energy $E = h\nu$

$= 6.63 \times 10^{-34} \times 1.11 \times 10^{18}$

$= 7.359 \times 10^{-16}$ joules.

Problems

1) Find wave number of wave has $\lambda = 480$ nm.

2) find frequency of wave has $\lambda = 490$ nm , $c = 3 \times 10^{8}$ m/s.

3) Find the wave number of a yellow light beam has ν = 589 Hz, knowing that c =3 x10^8 m.

4) Express the wavelength 2500 A^o in micrometer (μm) and nanometer (nm).

5) Convert wavelength 4000 A^o into wave number and frequency.
(c = 3 x 10^{10} cm/sec).

6) Convert the following into meters (m) and centimeters (cm).

a- 1 um

b- 1 nm

c- 1 A^o

7) Express the following in terms of angstrom (A^o) and nanometers (nm).

a- 150 cm b- 55 cm c- 15 cm

d- 1.2 m e- 8500 mm f- 75 cm

8) Calculate the wavelength in cm and energy in joules associated with a signal at 220 Hz.

9) What is the energy content of one quantum of blue light with wavelength of 420 nm.

10) A certain quantum of light has an energy content of 2.9×10^{-9} joules. What is its frequency and wavelength?

Tutorial (2)

Beer's Lambert's law

Lambert's law

The intensity of transmitted light is decreased exponentially with the increase of thickness (b) of the absorbing medium at constant concentration.

OR

Absorbance of light by sample is directly proportional to the path length of light in sample provided that concentration is constant.

- Thickness (b) increases → Absorption increases
- Abs α b (at constant conc)
- $A = \log I_0 / It = k\ b$
- k constant
- I_0 and It are intensity of incident and transmitted radiations.

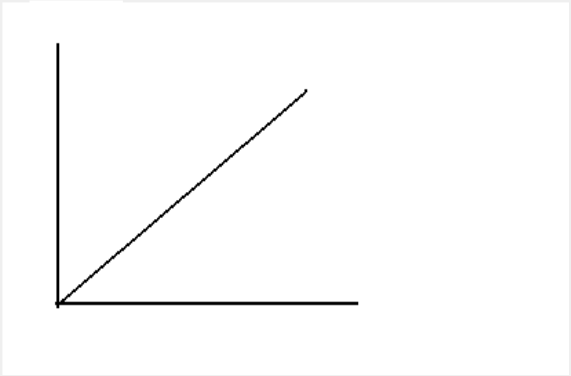
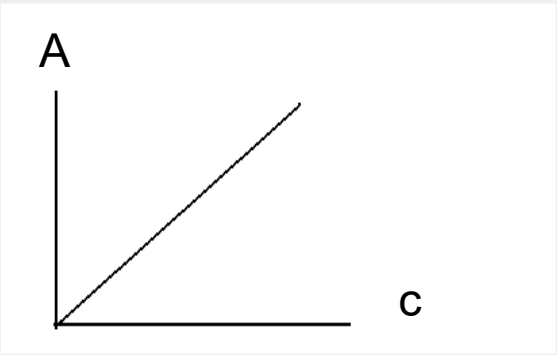
Beer's law

The intensity of transmitted radiation is exponentially decreased with the concentration (c) of the solution at constant thickness.

OR

Absorbance of light by sample is directly proportional to the concentration of sample provided that the path length is constant.

- Concentration (c) increases → Absorption increases
- Abs α c (at constant b)
- $A = \log I_0 / It = k'\ c$
- k' constant
- I_0 and It are intensity of incident and transmitted radiations

Lambert's law	Beer's law
Abs α b **(c is constant)** **A = log I_0/ It = k b (k constant)**	**Abs α c** **(b is constant)** **A = log I_0/ It = k' c (k' constant)**
	

It/I_0 called transmittance (T) so

A = log 1/ T or A = - log T

Beer's - Lambert's Law:

This is a combination of both laws;

$$A = a\ b\ c$$

Absorbance Absorptivity Thickness Concentration

Absorptivity constant

- It is a constant for each compound at certain λ.
- It has 3 forms according to unit of Concentration:
  - When concentration in **g\L** it is called **absorptivity (a).**
  - When concentration in **mole\L** it is called **molar absorbativity (ε).**
  - When concentration in **g%** it is **($A^{1cm}{}_{1\%}$).**

$$A^{1cm}{}_{1\%} = \varepsilon \times 10 \setminus Mwt$$

$$A^{1cm}{}_{1\%} = a \times 10$$

$$a = \varepsilon \setminus Mwt$$

Example (1)

A colored sample has maximum absorbance at λ 385nm. A solution of 20 mg/L has Abs of 0.84 using 2 cm cell. (Mwt = 50).
Calculate: 1- $A^{1cm}{}_{1\%}$
2- Molar absorptivity
3- How many mg of sample present in 25ml if It has Abs of 0.65 at 385 nm when measured in 1 cm cell.

1) $A^{1cm}{}_{1\%}$

$A^{1cm}{}_{1\%} = A / b\ c$

C = 20 mg/L → ? g% (g / 100ml)

$= 2 \times 10^{-3}$ g%

$A^{1cm}{}_{1\%} = 0.84 / (2 \times 2 \times 10^{-3}) = 210$

2) Molar absorptivity

$€ = A^{1cm}{}_{1\%} \times Mwt \setminus 10$

$= 210 \times 50 / 10 = 1050$ $L.mol^{-1}cm^{-1}$

3) How many mg of sample present in 25ml if it has Abs of 0.65 at 385 nm when measured in 1 cm cell.

$A = A^{1cm}{}_{1\%} \times b \times c$

$0.65 = 210 \times 1 \times c$

$c = 0.65 / 210$

= 0.0031 g% → mg/ 25ml

= 0.0031 x 1000\4 = 0.7738 mg/ 25ml

Problems

1) If compound X has % T = 30%, calculate its absorbance at λ =270nm.

2) Compound Y has T = 0.7, find %T.

3)

a) If drug Z has absorbance 0.324 calculate %T

b) Calculate the concentration of a solution of drug Z in water which exhibits an absorbance of 0.613 at λ_{max} 262 nm in 1 cm cell if ε = 703 $L.mol^{-1}cm^{-1}$.

4) A drug has maximum absorbance at 412nm, when 10 mg were dissolved in H_2O and the total volume is completed to 500 mL, its absorbance was 0.324.

a) Calculate $A^{1\%}_{1cm}$

b) Calculate the concentration of unknown solution of the same drug if its absorbance at the same λ but in 2 cm cell is 0.486 assuming that Beer's Lambert law is obeyed.

c) Calculate the corresponding ε if M.wt =400

5) Calculate the concentration in mole/L of a solution of tolbutamide in water which exhibits an absorbance of 0.613at λ_{max} 262 nm in 1 cm cell if ε=703 L.mol^{-1} cm^{-1}.
If you know that M.wt of the drug is 270 calculate its $A^{1\%}_{1cm}$ and (a).

6) A solution of concentration 0.0008 % of a compound (M.wt 308.4) has an absorbance of 1.1 in 1cm. cell.
Calculate its ($A^{1\%}_{1cm}$), (ε) & (a).

7) Calculate the concentration in Mole/Liter of a solution of Tryptophan in 0.1N HCl giving an absorbance 0.613 at λ_{max} 277 nm in cell 1cm its ε at 277nm = 5432 $L.mol^{-1}$ cm^{-1}.

8) A colored substance (x) has a maximum absorbance at 385 nm, A solution contains 20 mg/L has absorbance of 0.840 using 2 cm cell the M.wt = 50

a) Calculate $A1^{\%}_{1cm}$ at 385 nm.

b) Calculate ε at 385 nm.

9) A compound of formula weight 280 transmits 35% of radiation at certain wavelength in 2 cm cell at concentration of 15 ug\mL.

Calculate its molar absorptivity at this wavelength.

Tutorial (3)

Absorbance curve

Definition:

Relation between absorbance and wave length at constant concentration

- Constructed by:

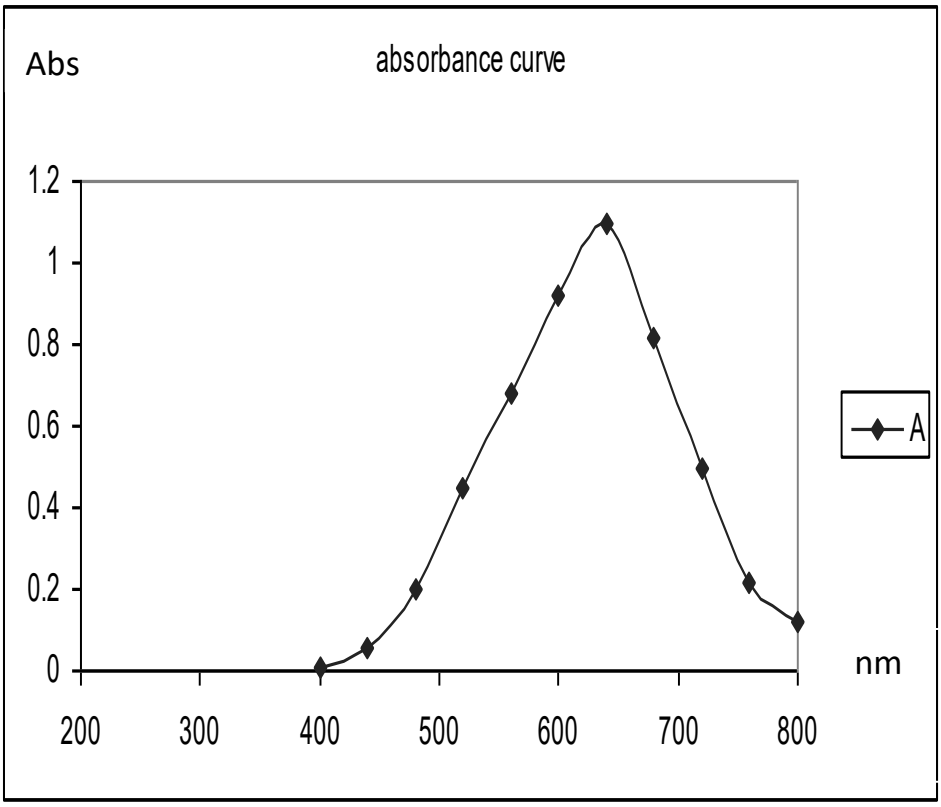
1- Changing the incident wavelength

2- And get the absorbance at each

3- To get a table like this

Wavelength	absorbance
400	0.011
440	0.06
480	0.2
520	0.45
560	0.68
600	0.92
640	1.1
680	0.82
720	0.5
760	0.22
800	0.12

Then draw absorbance (A) at y axis And Wavelength (λ) at x axis



Then we use this curve to determine λmax

- **What is λmax?**

λ at which the sample has the highest absorbance.

- **What is the importance of λmax?**

  1- Qualitative analysis

  2- Quantitative analysis to: ↑ Sensitivity

  ↓ Error

Determination of λ_{max} of $KMnO_4$

You are provided with table contains the wavelengths and corresponding absorbance of a sample of $KMnO_4$

λ	absorbance
515	**0.584**
520	**0.627**
525	**0.658**
530	**0.632**
535	**0.61**

Draw the absorbance curve and find its λ_{max}

Tutorial (4)

Calibration curve

Relation between absorbance and concentration

Its characters:

1- It should obey Beer's Lambert law.

2- Straight line passes through the origin.

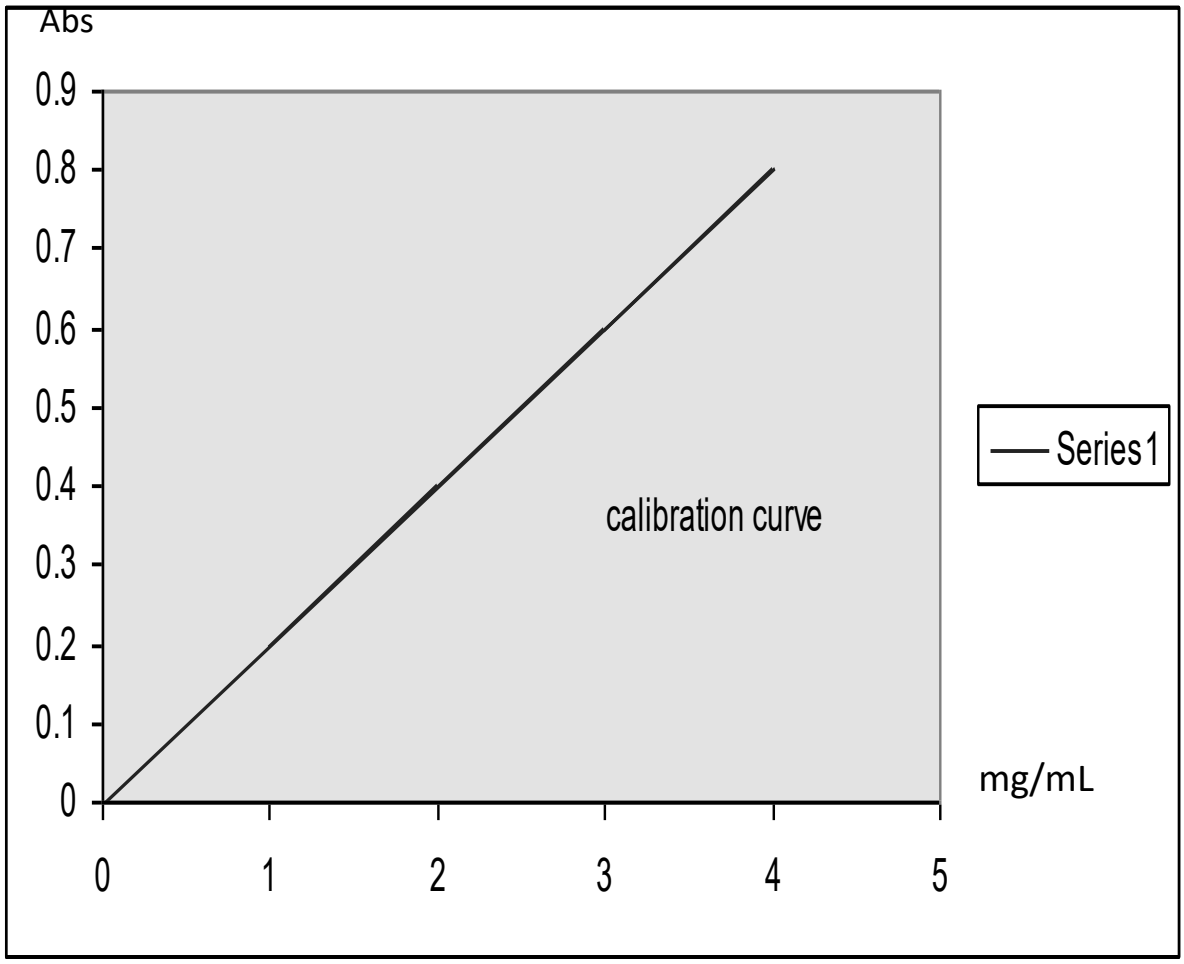
3- To get concentration of unknown sample.

How to construct the calibration curve?

- ❑ Prepare standard series of your substance.
- ❑ Adjust spectrophotometer at λmax.
- ❑ Get the absorbance of each standard.
- ❑ Arrange the results in a table like that.

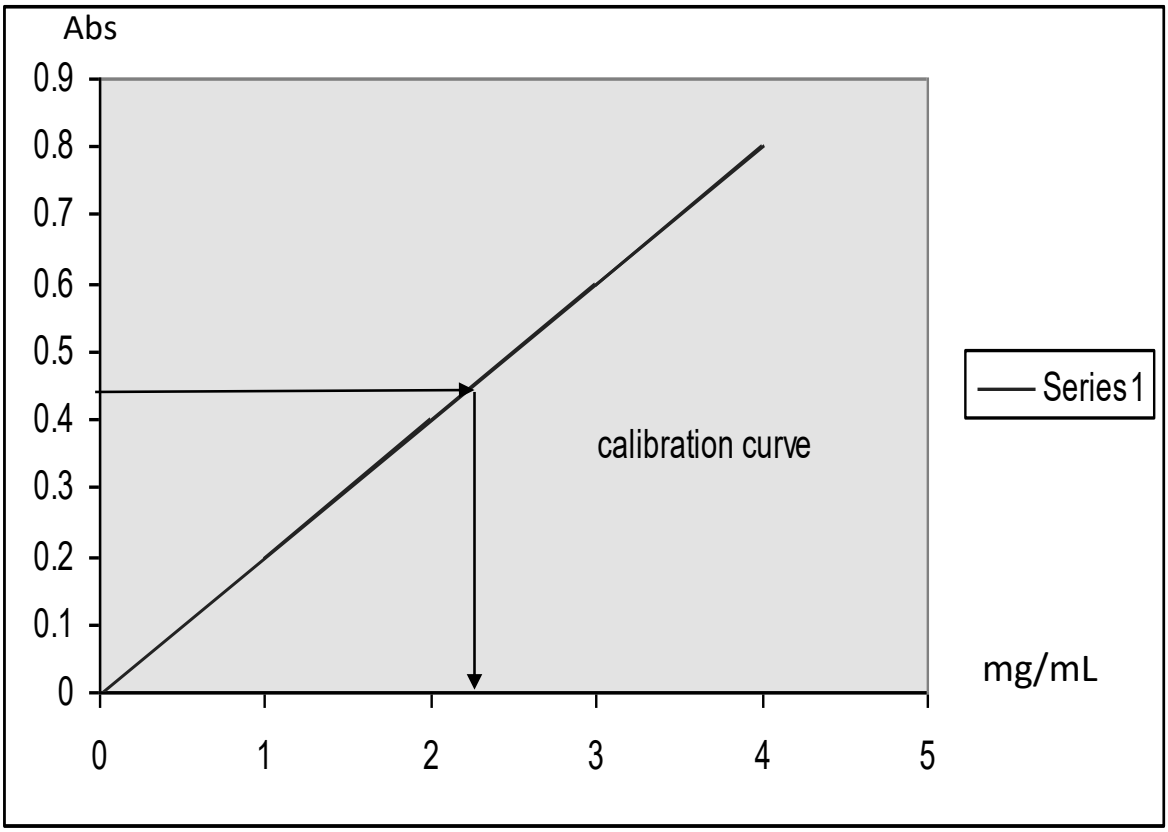
Concentration	absorbance
1	0.2
2	0.4
3	0.6
4	0.8

Draw absorbance at y axis against concentration at x axis to get the calibration curve.



To get the concentration of unknown sample

1- Get the absorbance of the sample from the spectrophotometer.
2- Point the absorbance of the sample on the curve draw a line parallel to x axis.
3- It will cut the straight line at a certain point.
4- At this point draw a perpendicular line to cut x axis in a point
5- This point is the unknown concentration.



Now draw your calibration curve and get the unknown concentration from the applied data.

Conc (mole / L)	absorbance
0.026	0.285
0.052	0.57
0.157	1.59
0.210	1.92
0.105	unknown

VISUAL METHODS

A. Serial dilution method

Determination of Fe3+ sample

Principle:

Ferric ions react with thiocyanate ions (added in excess) in acid medium to form blood red colored complex. The intensity of the red color depends on the ferric ions concentration, so the concentration of the unknown iron sample can be determined by the comparison with the red color of standard series.

Procedure:

1. Pipette 1, 2, 3, 4, 5 ml of standard iron solution into five similar test tubes.

2. Add 1 ml of HNO3 to each tube, boil for 2 minutes and cool.

3. Add 1ml 10% KSCN and complete the volume to 10 ml with distilled water.

4. Transfer 5 ml of the unknown sample solution to a similar test tube and proceed as under standard solutions.

5. Compare the color of the unknown with equivalent color of standard.

Results:

B. Variable depth method

Determination of copper sample

Principle:

In this method, the colored test and standard solutions are placed in two similar vessels (Nessler tubes). The layer thickness (depth) of one of the solutions (more intense) is varied until the colors are matched. Since the

concentration of the standard solution is known, that of the test solution can be computed from the formula:

Ct = Cs x Ls/Lt

Where Cs and Ct are the concentrations of the standard and unknown solutions, respectively. While, Ls and Lt are the thickness of standard and unknown solutions layers.

Procedure:

In two identical Nessler tubes:

1. In tube 1, add 5ml of standard copper solution (1mglml), 5ml conc. NH3 and complete to the mark (50ml) with distilled water.

2. In tube 2, add 5ml of unknown copper solution, 5ml conc. NH3 and complete to the mark (50ml) with distilled water.

3. Compare the formed colors and decrease the volume of the more intense colored solution until the colors are matched.

4. Measure the length of solutions layers by ruler.

PHTOELECTRIC METHODS

1. Absorption spectrum and calibration curve of potassium permanganate:

Theory: Transition elements of the lanthanide group contain partially filled ″d″ orbital. Manganese is one of the transition elements. Electronic transitions between the ″d″ orbitals are possible leading to highly colored solutions and high intensity of absorption. Potassium permanganate has two absorption maxima in wavelength range 500-600 nm.

Experiment 1:

Single component analysis of KMnO4

You are provided with:

- Stock KMnO4 solution 100mg%

- Sample of KMnO4 of unknown concentration

You are required to calculate the concentration of KMnO4 in the sample

Practical steps

Solution number	mls taken	Conc (mg%)	Abs at 525nm
1	2		
2	3		
3	4		
4	5		
5	6		
6	7		
	Sample		

1-Prepare serial standard solutions of $KMnO_4$

(Deliver from a biurette 2, 3, 4, 5, 6 and 7 ml of stock $KMnO_4$ 100mg% into a set of 100 ml volumetric flasks, complete to mark with tap water)

2- Measure the Absorbance of each prepared solution and the sample and record it in the provided table.

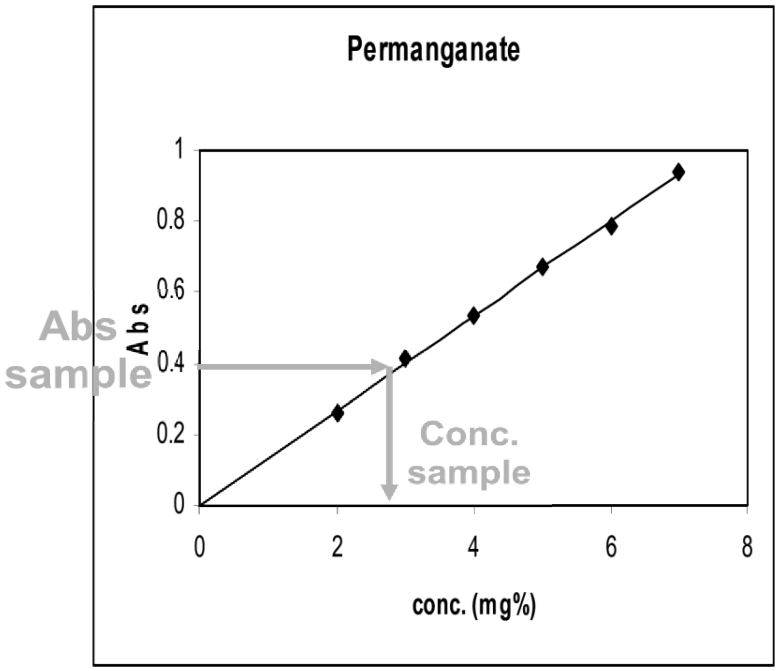
3- Calculate the concentration of each solution and record them in the table

C*V (before dilution) = C*V (after dilution)

100 mg% * (2,3,4,5,6,7 ml) = ? mg% * 100

4- Draw the calibration graph between the measured Abs against concentration.

5- Calculate the concentration of the sample graphically



Results:

Experiment 2:

Effect of pH on the absorption of sulphanilamide:

Theory:

In alkaline solution the primary amino group is retained as the auxochrome. In acid solution quaterniaztion occur to a co-ordinatively saturated auxochrome. (Hypsochromic shift and hypochromic effect resulting from the removal of the non bonded electrons of the –NH2 auxochrome from the conjugated system).

N H 2 SO2NH2 H+ H3N SO2NH2 OH- +

A. Determination of absorption spectrum:

Procedure:

1. Pipette 10 ml of sulphanilamide solution into 100 ml volumetric flask, dilute the mark with 1N HCl and mix.

2. Determine the absorbance of this solution in 1 cm cell at 10 nm interval from 210-230 nm, using 1 N HCl as blank (use 5 nm interval around □max).

3. Repeat step(1) but using instead of 1 n HCl, 1 N NaOH.

4. Plot the absorption curves of the acid and alkaline solutions on the same graph paper.

5. Determine λmax and calculate molar absorptivity (ε) for each solution.

Results:

B. Determination of calibration curve (standard curve):

Procedure:

1. Into five 100-ml volumetric flask, transfer by pipette 2, 5, 10, 15 and 20 ml of sulphanilamide solution. Complete to the mark with 1 N NaOH solution and mix.

2. Measure the absorbance of each solution at the λmax of sulphanilamide in 1 N NaOH. Plot the standard curve (A vs. C).

3. Dilute 10 ml of the given unknown to the mark with 1N NaOH and read its absorbance at □max. Locate its position on the standard line, and read the concentration from the other axis.

4. The concentration of unknown solution in other way can be calculated by the following equation:

Cu= Cs x Au/ As

Where :-
Cu = concentration of the unknown in μg/ml in the final solution.
Cs = concentration of the standard solution in μg/ml in the final solution.
Au = absorbance of the unknown solution.

As = absorbance of the standard solution

Results:

Experiment 3:

Effect of pH on the absorption spectrum of thymol blue:

Theory:
Thymol blue is an acid-base indicator, it has phenolic –OH group in its structure. In alkaline solution it changes into phenolate anion and the interaction of the negative charge on the oxygen atom with the ring leads to different resonating structures. As a result a bathochromic shift and a hyperchromic effect occur and the color changes from red (acid medium) into blue (alkaline medium).

Solutions:
- 0.02 g % w/v Thymol aqueous solution.
- 1N HCl.
- 1N NaOH

Procedure:
1. Pipette 10 ml of thymol blue solution into 25 ml volumetric flask, dilute to the mark with 1 N HCl and mix. Determine the absorbance of this solution in 1 cm cell at 10 nm interval from 450-650 nm, using 1N HCl as a blank (use 5 nm interval around the λ_{max}).

2. Repeat step 1 using 1N NaOH instead of 1N HCl.

3. Plot the absorption curves of the acid and alkaline solutions on the same graph paper.

4. Determine λ_{max} and calculate molar absorptivity (ε) for each solution.

Results:

Experiment 4:

Determination of the molar ratio of Iron-Thiocyanate complex

(job's method for continuous variation)

Theory:

- Neither ferric nor thiocyanate ions in nitric acid solution have absorption maximum at 500 nm.
- When they react with each other, a blood red color is formed that has a high absorption value at 500 nm.
  $m\ Fe^{3+} + n\ SCN^{-} \longrightarrow [Fem(SCN)n]^{3m-n}$
- Prepare equimolar solutions of Fe3+ (A) and SCN-(B) and series of mixtures of the two solutions in different ratios in which the sum of the number of moles of Fe3+ and SCN-, is kept constant (total number of moles is constant).
- The absorbance of each mixture is measured against water and the absorbance is plotted versus the corresponding mole fraction of Fe3+.

  $$\text{Mole fraction of Fe3+} = \frac{\text{No. of moles of Fe3+}}{\text{Total no. of moles (Fe3+ +SCN-)}}$$
- The resultant plot is round shaped curve. When the straight lines of the curve are extrapolated, the point on the abscissa corresponding to their intersection represents the molar ratio of the complex.

Solutions:

Solution (A): Ferric solution: 3x103- M ferric ammonium sulphate.

Solution (B): Thiocyanate solution : 3x103- M potassium thiocyanate.

Procedure:

1. Prepare a series of solutions by mixing the indicated volumes of solution (A) and solution (B).

1 ml solution A +9 ml solution B

9 ml solution A +1 ml solution B ……(etc.)……

2. Measure the absorbance of each mixture against water as blank at 500 nm.

3. Plot the absorbance versus the corresponding mole fraction of ferric.

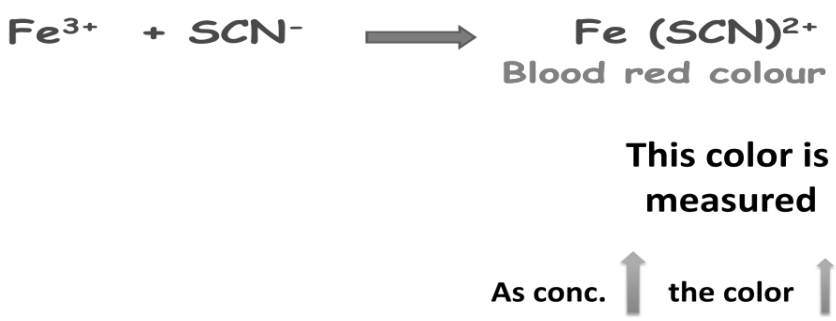
4. The resultant plot is round shaped curve, the straight lines of the curve are extrapolated.

5. The point on the abscissa corresponding to their intersection represents the molar ratio of the complex.

Results:

Experiment 5:

Colorimetric determination of FeCl3 by Complexation with SCN^-



You are provided with:

- Stock FeCl3 solution 20mg% in dil nitric acid

- Sample of FeCl3 of unknown concentration

You are required to calculate the concentration of Fe Cl3 in the sample

Solution number	mls taken	Conc (mg%)	Abs at 471nm
1	8		
2	10		
3	12		
4	14		
5	16		
	Sample		

1-Prepare serial standard solutions of Fe Cl_3 (Deliver from a burette 8, 10, 12, 14 and 16 ml of stock Fe Cl_3 20mg% into a set of 100 ml volumetric flasks.

2- Add 2ml 10% NH4 SCN (from a burette) and complete to mark with dist. water) and mix well.

3- Measure the Absorbance of each prepared solution and the sample against blank solution (blank is prepared by adding 10ml dil. nitric acid in 100 ml volumetric flask + 2ml 10% NH4 SCN then complete to volume with dist water) and record them in the provided table.

5- Calculate the concentration of each solution and record them in the table

C*V (before dilution) = C*V (after dilution)

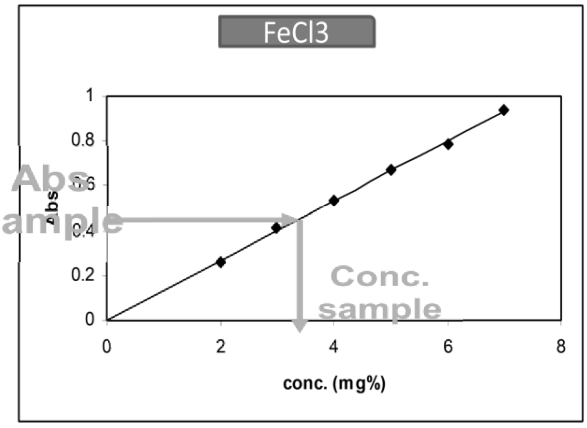
20 mg% * (8, 10, 12, 14, 16 ml) = ? mg% * 100

6- Draw the calibration graph between

the measured Abs against concentration

7- Calculate the concentration of the sample

graphically

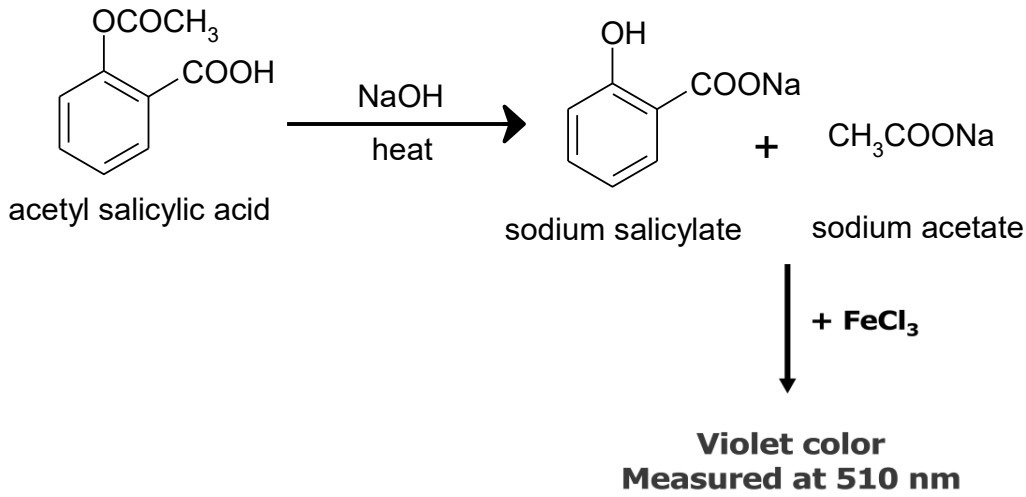


Results:

Experiment 6:

Colorimetric determination of Aspirin

Theory



When aspirin is heated with NaOH, it gives sod.salicylate which gives with $FeCl_3$ violet color measured at 510 nm

N.B. Excess NaOH must be removed before addition of FeCl3 to prevent its pptn as Fe(OH)3

Practical steps

1- Put one Aspirin tablet in 100 ml beaker.

2- Add 20 ml distilled water (cylinder).

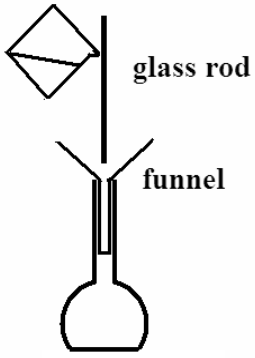
3- Add 5 ml 0.5 N NaOH (cylinder).

4- Heat 5 min. on hot plate.

5- Neutralize xss NaOH .

6- Add 2 drops ph.ph indicator to the beaker where pink color is

7- Add C. HCl dropwise till disappearance of the pink color.



8- Transfer the contents of the beaker quantitatively into 250 ml volumetric flask using funnel and glass rod.

9- Wash the beaker and the glass rod with water and transfer the wash into the volumetric flask using the funnel.

10- Complete to the mark with distilled water and mix well.

11- Pipette 5 ml from the 250 ml volumetric into a 100 ml volumetric flask.

12- Add 5 ml 5% $FeCl_3$ into the 100 ml volumetric flask where violet color is developed.

13-Complete to the mark with distilled water and mix well.

14-Measure the Abs. of this solution at 510 nm.

Calculate the concentration of Aspirin in the measured solution

1- Using Beer's law

A= a b c

a= 0.067

b= 1 cm

c= ?? mg%

2- Using regression equation

Abs= a + b*Conc.

a (intercept) = 0.005

b (slope) =0.455

Abs.= measured Abs.

Conc.= ?? mg%

Calculate the % recovery of Aspirin tablet

% recovery = Found conc. / labeled conc. * 100

Found conc. (calculated using Beer' law or regression equation)

Labeled conc. ???

Each Aspirin tablet is labeled to contain 320 mg aspirin. We put amount equivalent to one tablet into 250 ml vol. flask from which we took 5 ml into 100 ml volumetric flask

320 mg (found in) 250 ml vol. falsk

??? (found in) 5 ml (taken) ??= 6.4 mg

6.4 mg (found in) 100 ml vol. flask (measured solution)

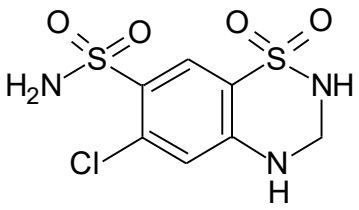
So the conc. in the measured solution is

Conc. mg% = wt/vol*100 = 6.4/100*100= 6.4 mg%

Results:

Experiment 7:

Spectrophotometric determination of Hydrochlorothiazide (Tablets)



Theory

Spectrophotometric determination of hydrochlorothiazide in alkaline solution

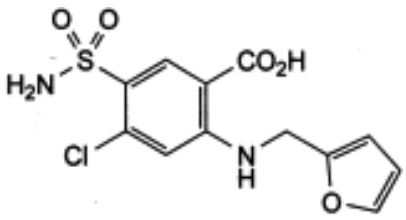
Procedure:

1. Weigh and powder 20 tablets.
2. To a quantity of the powder containing 30 mg of Hydrochlorothiazide add 50 ml of 0.1M *sodium hydroxide*.
3. Shake for 20 minutes and dilute to 100 ml with 0.1M *sodium hydroxide*.
4. Mix, filter, dilute 5 ml of the filtrate to 100 ml with *water*.
5. Measure the *absorbance* of the resulting solution at the maximum at 273 nm taking 520 as the value of A(1%, 1 cm) .

Results:

Experiment 8:

Spectrophotometric determination of Furosemide (injections)



Theory

Spectrophotometric Determination of furosemide in alkaline solution

Procedure:

1. To a volume containing the equivalent of 20 mg of Furosemide ,
2. Add sufficient water to produce 100 ml.
3. Dilute 5 ml to 100 ml with 0.1M sodium hydroxide.
4. Measure the absorbance of the resulting solution at the maximum at 271 nm taking 580 as the value of A(1%, 1 cm).

Results:

CALCULATION OF λ_{MAX}

1. THE WOODWARD-FIESER RULES FOR DIENES

EMPIRICAL RULES FOR DIENES

parent	λ= 214 nm
Increments for:	
Double bond extending conjugation	30
Homocyclic diene component	39
Alkyl substituent or ring residue	5
Exocyclic double bond	5
Polar groupings:	
-$OCOCH_3$	0
-OR	6
-Cl, -Br	5
NR_2	60

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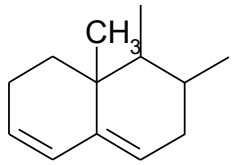
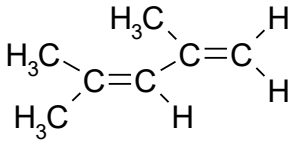
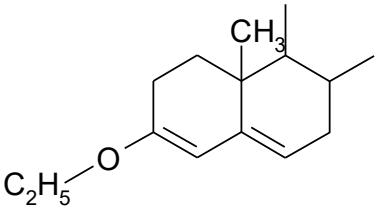
SOLVED EXAMPLES ON DIENE SYSTEM:



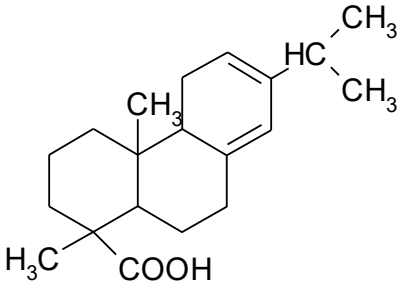
Parent	214
Ring residue: 3x5=	15
Exocyclic double bond:	5
	234



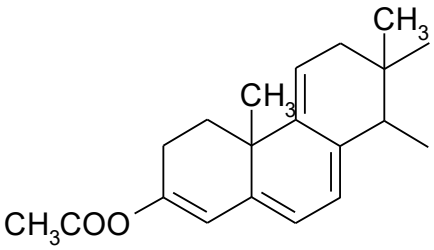
Parent:	214
Alkyl groups: 3 x5=	15
	229



Parent	214
Ring residue: 3x5=	15
Exocyclic double bond:	5
-OR:	6
	240



Parent:	214
Alkyl group: =	5
Ring residue: 3x5=	15
Exocyclic double bond:	5
Homocyclic diene:	39
	278



Parent	214

Homocyclic diene: 39

Ring residue: 5 x5= 25

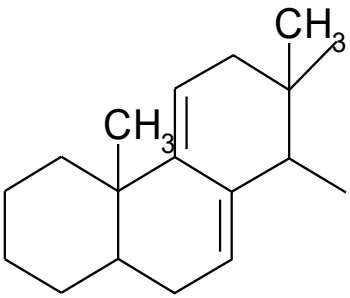
353

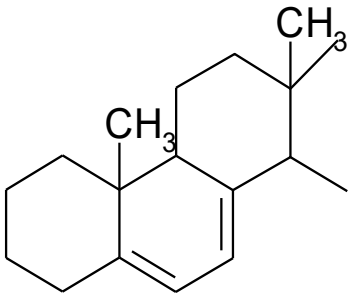
Double bond extension= 2 x 30= 60

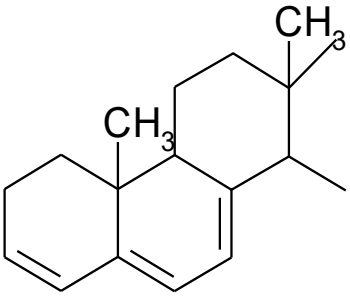
Exocyclic double bond: 3 x 5= 15

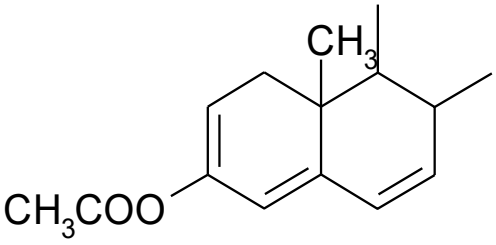
CH3COO-: 0

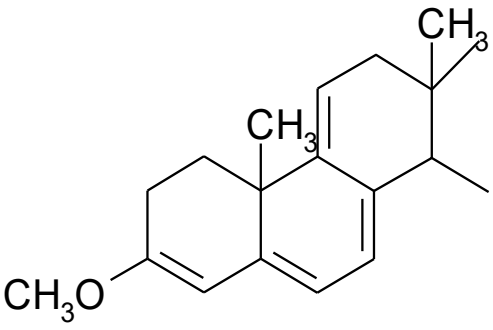
calculate λ_{max} for the following compounds:







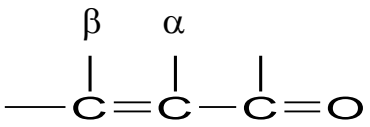




ANSWERS:

2. THE WOODWARD-FIESER RULES FOR ENONES

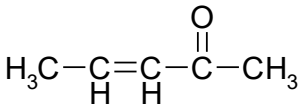
EMPIRICAL RULES FOR ENONES

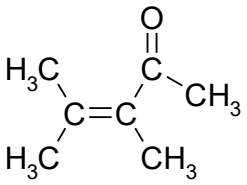


Parent		
6-membered ring or acyclic parent enone (ketone)		λ= 215
5-membered ring parent enone (ketone)		λ= 202
Aldehyde parent enone		λ= 208
Increments for:		
Double bond extending conjugation		30
Homocyclic diene component		39
Alkyl substituent or ring residue	α	10
	β	12
	γ and higher	18
Exocyclic double bond		5
Polar groupings:		
-OCOCH$_3$	α, β, δ	6
-OR	α	35
	β	30
	γ	17
	δ	31
-OH	α	35
	β	30
	δ	50
-Cl	α	15
	β	12
-Br	α	25
	β	30

-NR_2	β	95

SOLVED EXAMPLES ON ENONE SYSTEM:





Acyclic enone: 215

β-CH3: 12

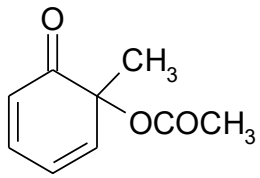
227

Acyclic enone: 215

α-CH3: 10

β-CH3: 2 x 12= 24

249



6-membered enone: 215

double bond extension: 30

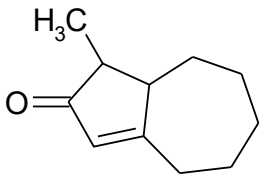
Homocyclic diene: 39

5

Alkyl substituent 18

231

302



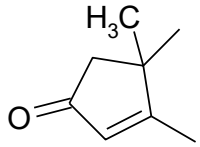
5-membered enone:

202

β-ring residue:2 x 12=

24

exocyclic double bond:



5-membered enone: 202

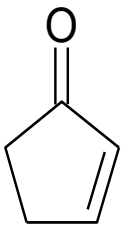
α-Br: 25

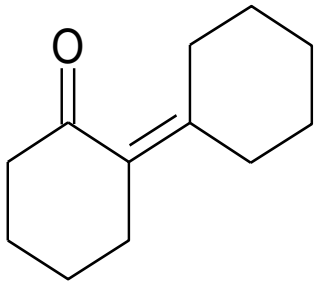
β-ring residue:2 x 12= 24

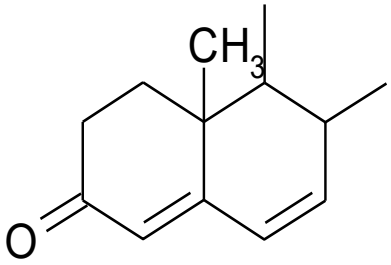
exocyclic double bond: 5

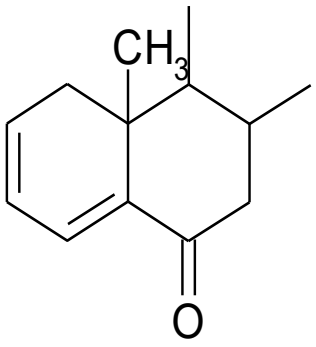
256

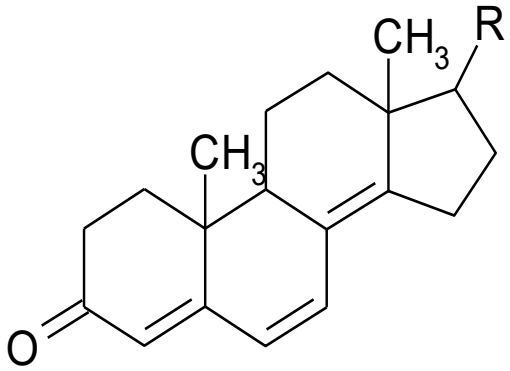
calculate λ_{max} for the following compounds:











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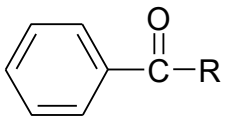
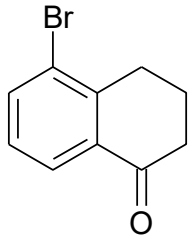
3. Empirical rules for Benzoyl derivative

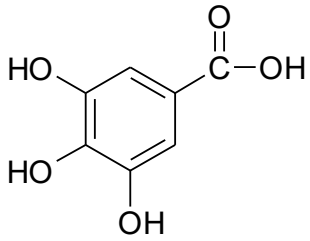
Parent Chromophore		
		
R= Alkyl or ring residue		246
R= H		250
R= OH or O-Alkyl		230
Increment for each substitution		
-Alkyl or ring residue	*o-, m-*	3
	p-	10
-OH, -OCH_3, -O-Alkyl	*o-, m-*	7
	p-	25
-O^-	*o-*	11
	m-	20
	p-	78
-Cl	*o-, m-*	6
	p-	10
-Br	*o-, m-*	2
	p-	15
-NH_2	*o-, m-*	13
	p-	58
-$NHCOCH_3$	*o-, m-*	20
	p-	45
-NHCH3	*p-*	73
-$N(CH_3)_2$	*o-, m-*	20
	p-	85

SOLVED EXAMPLES ON BENZOYL DERIVATIVE SYSTEM:

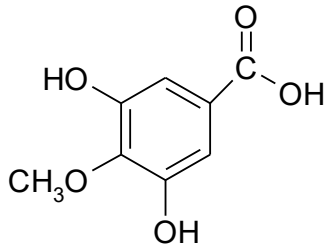


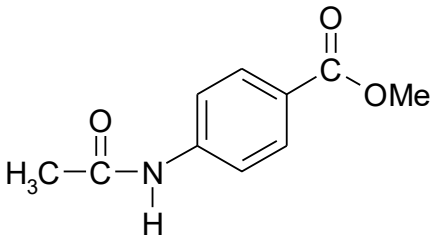
Parent: :	246
o-ring residue:	3
m-Br:	2
	251

269



Parent: :	230
m-OH: 2 X 7=	14
p-OH:	25





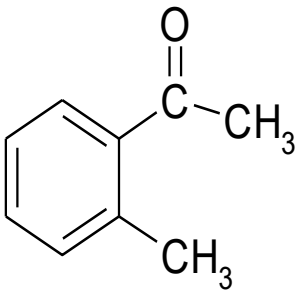
Parent: 230 Parent: 230

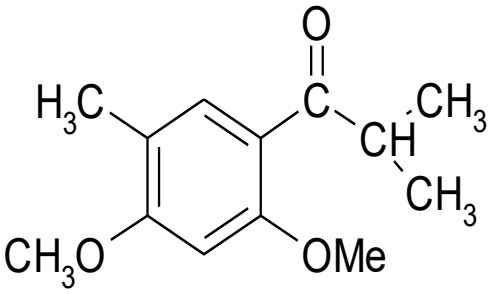
m-OH: 2 X 7= 14 *p*-NHCOCH3: 45

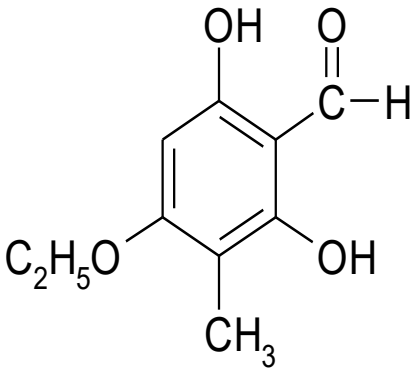
p-O-Alkyl: _____ 25 275

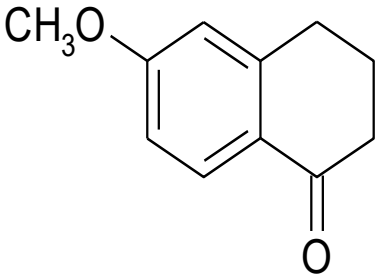
269

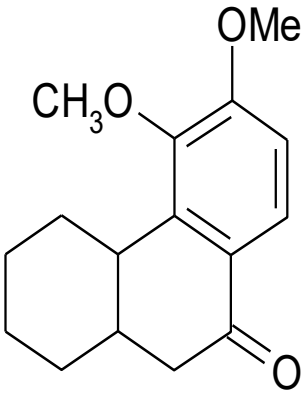
<u>calculate λ_{max} for the following compounds:</u>


O
C
CH3
CH3


O
H3C
C
CH3
CH
CH3
CH3O
OMe


OH
O
C
H
C2H5O
OH
CH3


CH3O
O


OMe
CH3O
O