
بسم الله الرحمن الرحيم

Spectroscopic Methods

- The spectroscopic methods of analysis are the most frequently employed technique in analysis . Many substances interact with electromagnetic radiation "Light" . Most of the spectroscopic methods are based on the fact that molecules are capable of absorbing radiant energy . The spectroscopic methods of analysis involve the measurement of the amount of light absorbed by the substance in solution .
- Spectrophotometry is used in clinical , industrial, educational and research areas for the analysis of drugs , food , beverages ,water and body fluids .

- **The importance of the spectrometric methods of analysis :**

1 - Qualitative and quantitative analysis of many compounds.

2 - Structure elucidation of organic compounds .

3 - Studying the stability of drugs .

4 - Studying the keinetic of drugs .

Nature of electromagnetic radiation

- Electromagnetic radiation is a form of radiant energy such as sun light , radio waves and x-rays . The white light can be split to produce different colors or wavelengths .
- The visible spectrum forms only a small part of the complete spectrum of electro-magnetic radiation which extents from the ultra short wave region rays at one end to that of the radio-waves at the other end .
- The **visible region of the spectrum extends from 380 nm to 800 nm** . The eye can normally detect only the colors within this wavelength range , that is why it is called visible . This colors include violet , indigo , blue , green , yellow , orange and red.

- Ultraviolet (UV) means “beyond the violet” . UV radiation has shorter wavelengths than violet light and cannot be seen by the eye .
- Infrared (IR) means “ below the red . IR radiation has longer wavelengths than red and also cannot be seen by the eye .

- When all the wavelengths or colors of the visible light are transmitted together, the light appears as white light , if all wavelengths or colors of the visible light are absorbed , it appears black .

- Colored substances appear colored because they selectively absorb some of the wavelengths of visible light and transmitted other wavelengths or colors (apparent color . For example , red substance absorb blue - green wavelengths from the visible region, so the transmitted light appears red , blue substance absorb the yellow wavelengths , so the transmitted light appears blue .

Properties of electromagnetic radiation

a - Wave properties :

such as reflection , refraction , scattering ,polarisation .

- Wavelength λ : is the distance between any two successive points on the wave (two successive maxima or minima of a wane) nm .
- Wavenumber ν : is the reciprocal of the wavelength ($1/\lambda$) in cm -1 , which is the number of waves per 1 cm .
- Frequenvy ν : is the number of waves emitted per second in Hz (cycle / s) .

$$1 / \lambda = \nu = \nu / c$$

- wavenumber = 1 / wavelelenth (cm) = frequency / speed of light (cm/second)

b - Particle properties :

- The interaction of EMR can be accounted for by the particle properties of light . This postulate can be used to illustrate absorption or emission of radiant energy . EMR behaves as it is a train of photons ; discrete wave packets of distinct particles .
- The energy of photon depends upon the frequency of the radiation

$$E = h\nu$$

- where h is plank,s constant (6.6 x 10 -34 j.s,)
- The relation between the frequency of light and its wavelength reveals that a photon of high frequency (short λ) has high energy content than one of lower frequency (longer λ) .

from $c = \lambda . \nu$

$$v = c / \lambda$$

then $E = h . c / \lambda$

- **Units used for the measurements of wavelengths in EMR Spectrum**

Unit	A°	nm	um	mm	cm	uses in spectroscopy
A°	1	10^{-1}	10^{-4}	10^{-7}	10^{-8}	gamma rays X - rays
nm	10	1	10^{-3}	10^{-6}	10^{-7}	UV-Visible
um	10^{4}	10^{3}	1	10^{-3}	10^{-4}	IR
mm	10^{7}	10^{6}	10^{3}	1	10^{-1}	
cm	10^{8}	10^{7}	10^{4}	10	1	

a) wave properties

wavelength ,

wave number ,

frequency ,

amplitude ,

intensity

b) particle properties

$E = h v$

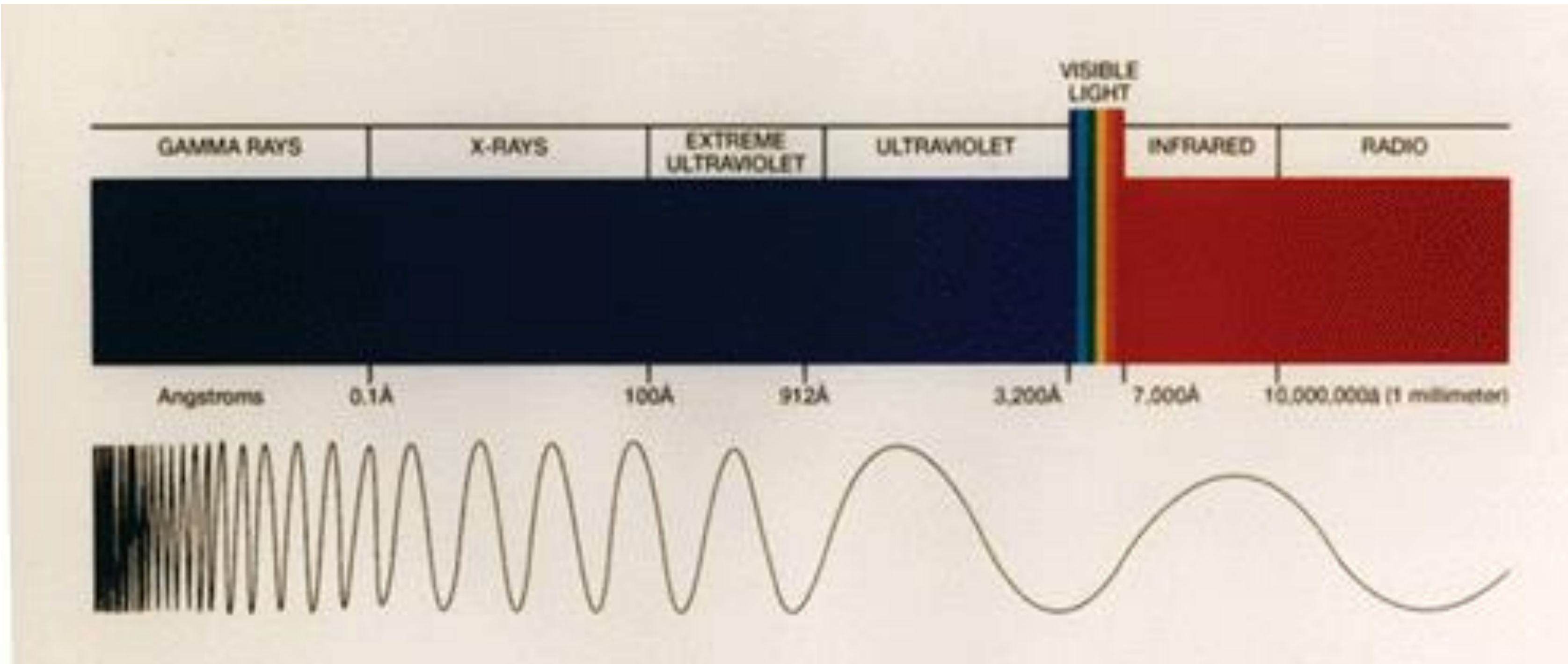
$E = hC/\lambda$

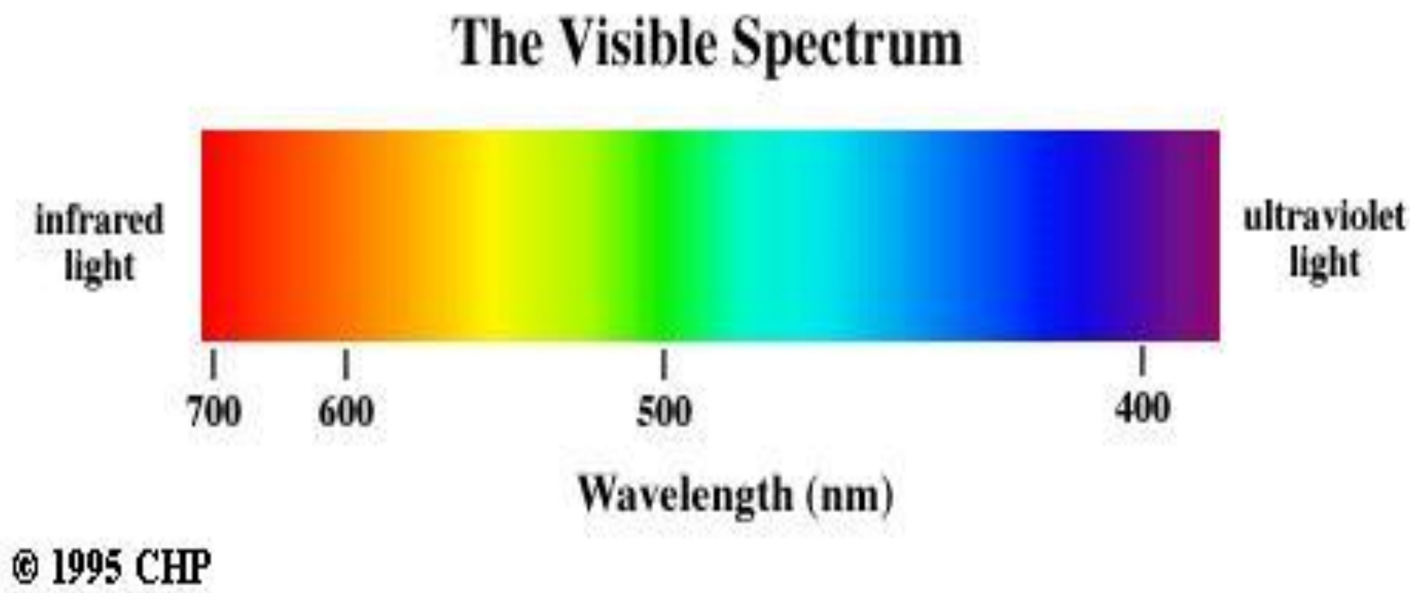
$E = hc\upsilon'$

Electromagnetic Spectrum

The following table lists the names of different spectral regions, the range of frequencies and wavelengths in those regions, and the type of transition that can occur when a photon in these spectral ranges interacts with matter.

Type of Radiation	Frequency Range(Hz)	Wavelength Range	Type of Transition
gamma-rays	1020-1024	<1 pm	nuclear
X-rays	1017-10201 nm-	1 pm	inner electron
Ultraviolet	1015-1017	400 nm-1 nm	outer electron
Visible	4-7.5x1014	750 nm-400 nm	outer electron
near-infrared vibrations	1x1014-4x1014	2.5µm-750 nm	outer electron molecular
Infrared	1013-1014	25 µm-2.5 µm	molecular vibrations
Microwaves	3x1011-1013	1mm-25 µm	molecular rotations,
			electron spin flips*
radio waves	<3x1011	>1 mm	nuclear spin flips*


VISIBLE LIGHT
GAMMA RAYS
X-RAYS
EXTREME ULTRAVIOLET
ULTRAVIOLET
INFRARED
RADIO
Angstroms
0.1Å
100Å
912Å
3,200Å
7,000Å
10,000,000Å (1 millimeter)

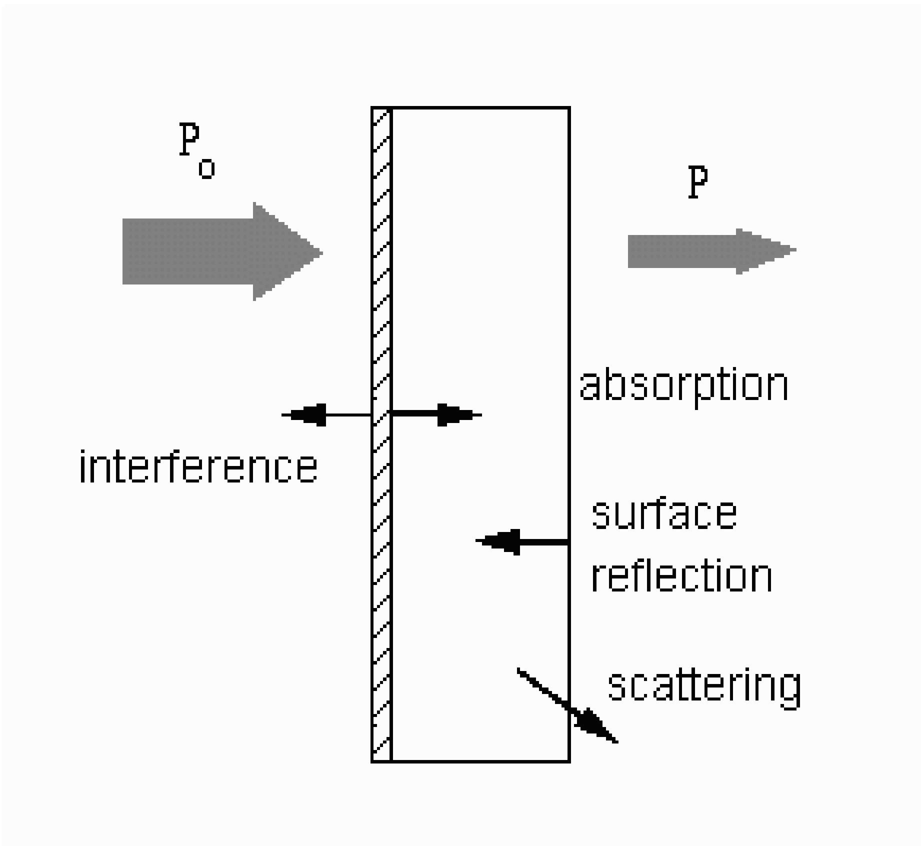

The Visible Spectrum
infrared light
ultraviolet light
700
600
500
400
Wavelength (nm)
© 1995 CHP

ABSORPTION OF RADIATION

Absorption
Transmission
EMR + matter → Emission
Reflection
Refraction
Scattering

M + hv → M* (excitation)

M* → M + heat (relaxation)
M + emission of radiation
(fluorescence)



P_o
P
absorption
interference
surface
reflection
scattering

- The interaction of radiation with matter can cause :
- Absorption:
- Transmission
- Emission:.
- Scattering:
- Reflection
- Refraction

Effect of Light (EMR) on Organic Molecules

- The effect of EMR on molecules depends upon the energy associated with EMR , i.e. according to the wavelength. These effects may be :

Effect	Wavelength	Spectral region
a - Ionization of molecules	< 0.1 Ao	gamma - rays X - rays
b - Excitation of the electronic system of the molecules	100 - 800 nm	UV-Visible region
c - Vibration of the atoms of the molecules	0.8 - 50 um	IR

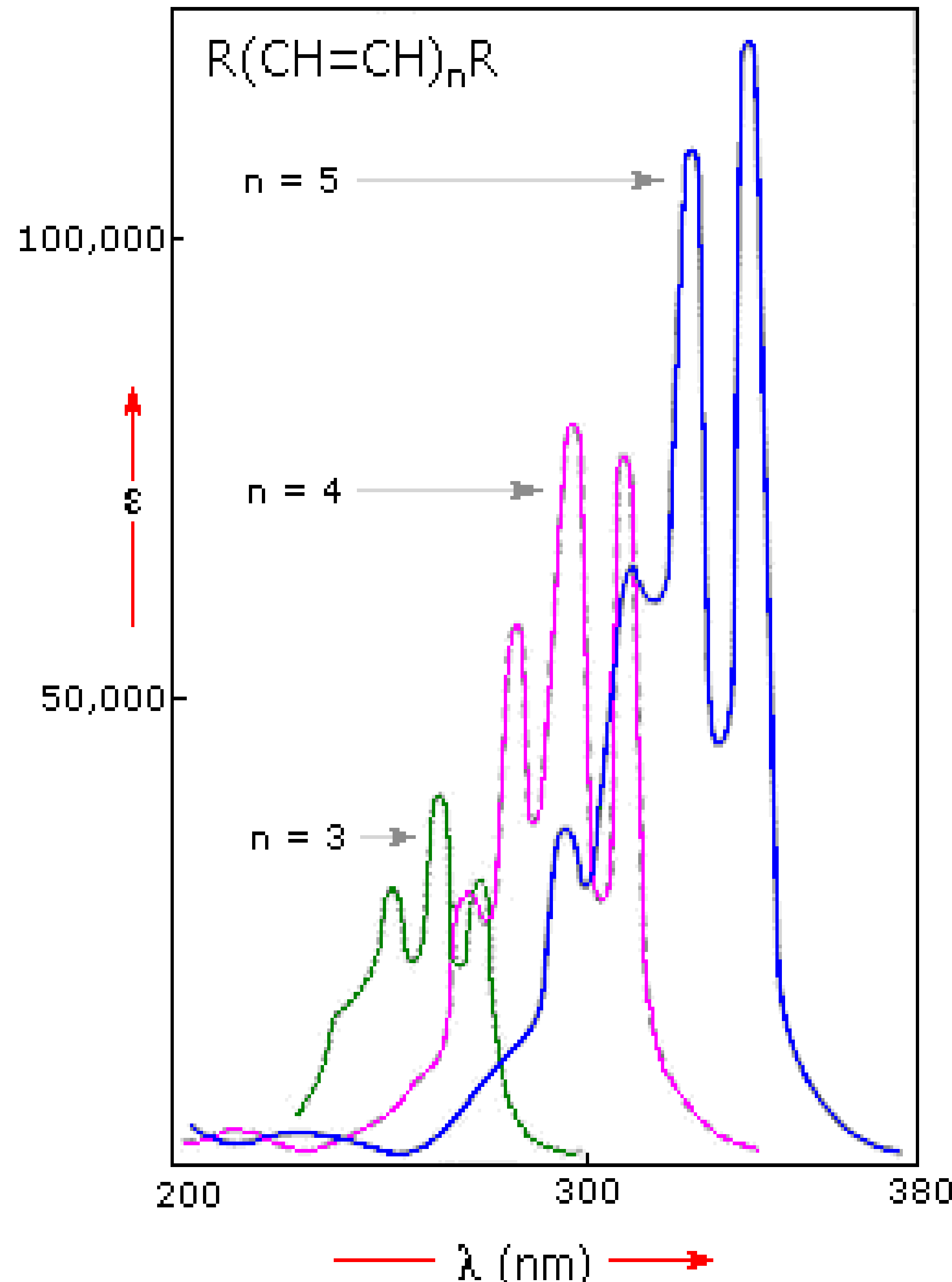
SPECTRA - STRUCTURE CORRELATION

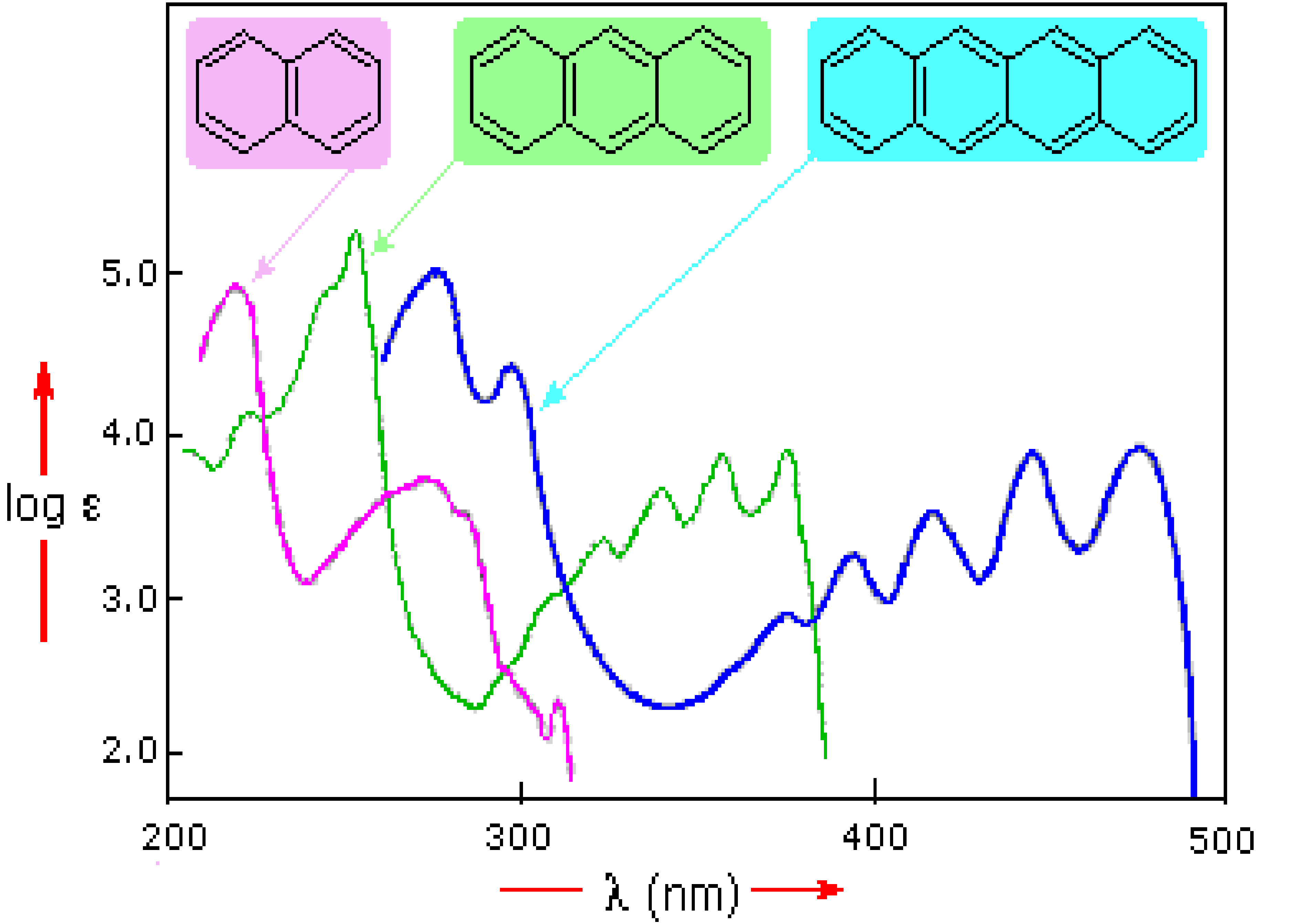
Chromophore: are functional groups which are responsible for absorbing light (radiation) eg .unsaturated bonds (double or triple) such as C=C,C=O , C-N , N=N , aromatic rings .

Auxochrome : are functional gps. which are by itself not able for absorbing light but have the ability to increase the effects of chromophors ie. When attached to a chromophore causes a shift to longer wavelength with increase in absorption intensity

A group which extends the conjugation of a chromophore by sharing of nonbonding electrons. eg. O-H , NH_2 .

- **Bathochromic shift** (red shift) : shift of absorption to longer wavelength due to substitution or solvent effect.
- **Hypthochromic shift** (blue shift) : shift of absorption to shorter wavelength due to substitution .
- **Hyperchromic effect** : increase in absorption intensity .
- **Hypochromic effect** : decrease in absorption intensity .
- **Monochromatic beam** : single beam of radiation .
- **Polychromatic beam** : multiple beam of radiation .


R(CH=CH)nR
n = 5
n = 4
n = 3
100,000
50,000
ε
200
300
380
λ (nm)



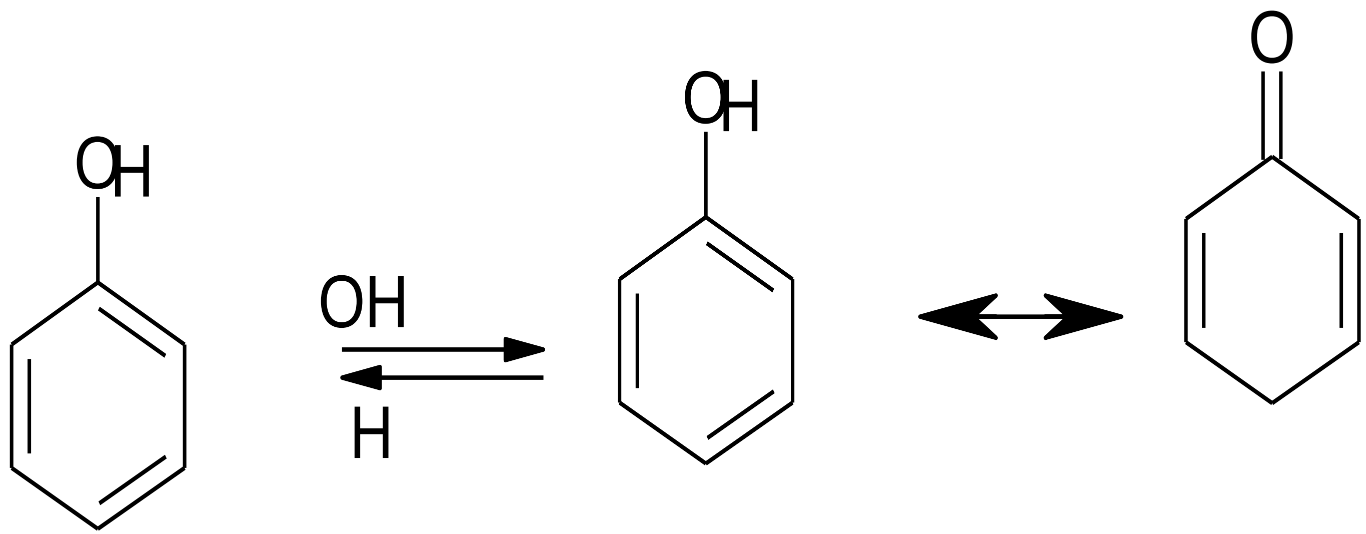
5.0
4.0
3.0
2.0
log ε
200
300
400
500
λ (nm)

- **Terminology for Absorption Shifts**

Nature of Shift	Descriptive Term
To Longer Wavelength	Bathochromic shift
To Shorter Wavelength	Hypsochromic shift
To Greater Absorbance	Hyperchromic effect
To Lower Absorbance	Hypochromic effect

Effect of pH on absorption spectra

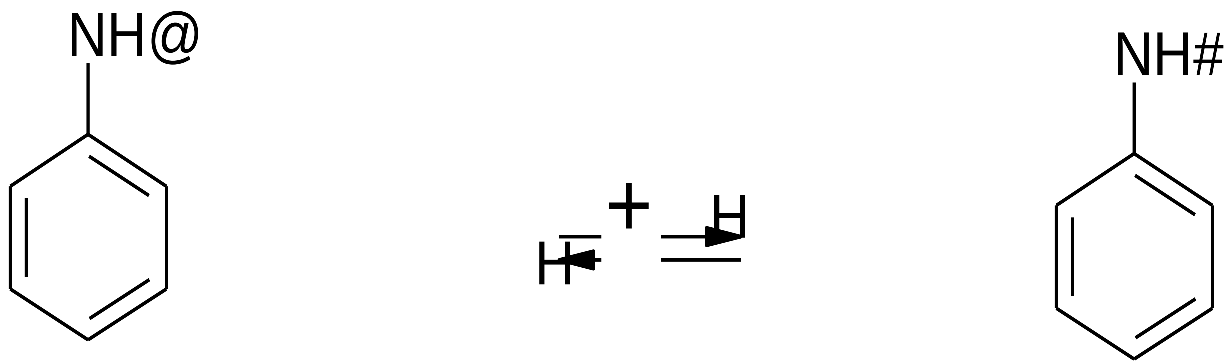
- The spectra of compounds containing acidic or basic gps. depends on the pH of the medium .
- The UV spectrum of phenol in acidic medium (molecular form) is completely different from its spectrum in alkaline medium (phenolate anion form) where bathochromis shift and hyperchromic effect occurs.
- The red shift is due to resonance effect and delocalization of the π electrons.



In acid mediom phenol λmax.=270 nm

in alkaline medium phenate λmax.=280 nm

- UV spectrum of aniline in acidic medium shows hypthochromic (blue) shift with hypochromic effect (decrease in absorption intensity). This blue shift is due to protonation of the amino gp , hence the pair electrons is no longer available available and the spectrum in this case is similar to that of benzene (benzenoid spectrum).



In alkaline medium — in acid medium

aniline ʎmax=280 nm — anilinium ionʎmax = 254 nm

Types of Electronic Transitions

- The outer electrons in an organic molecule may occupy one of three different energy levels :
- Sigma (σ) electrons : are bonding electrons which represent valence bonds and are formed from linear overlaps of electronic clouds of S or Sp orbitals . They possess the lowest energy level , i.e. most stable.
- Pi (π) electrons : bonding electrons constituting the pi bonds (double bonds) and result from lateral overlap of electronic clouds of P orbitals . They are of higher energy than sigma electrons .
- Non-bonding (n-electrons) : these are atomic orbitals of hetero-atoms (N, O, S, halogen) which do not participate in bonding , they usually occupy the highest energy level .

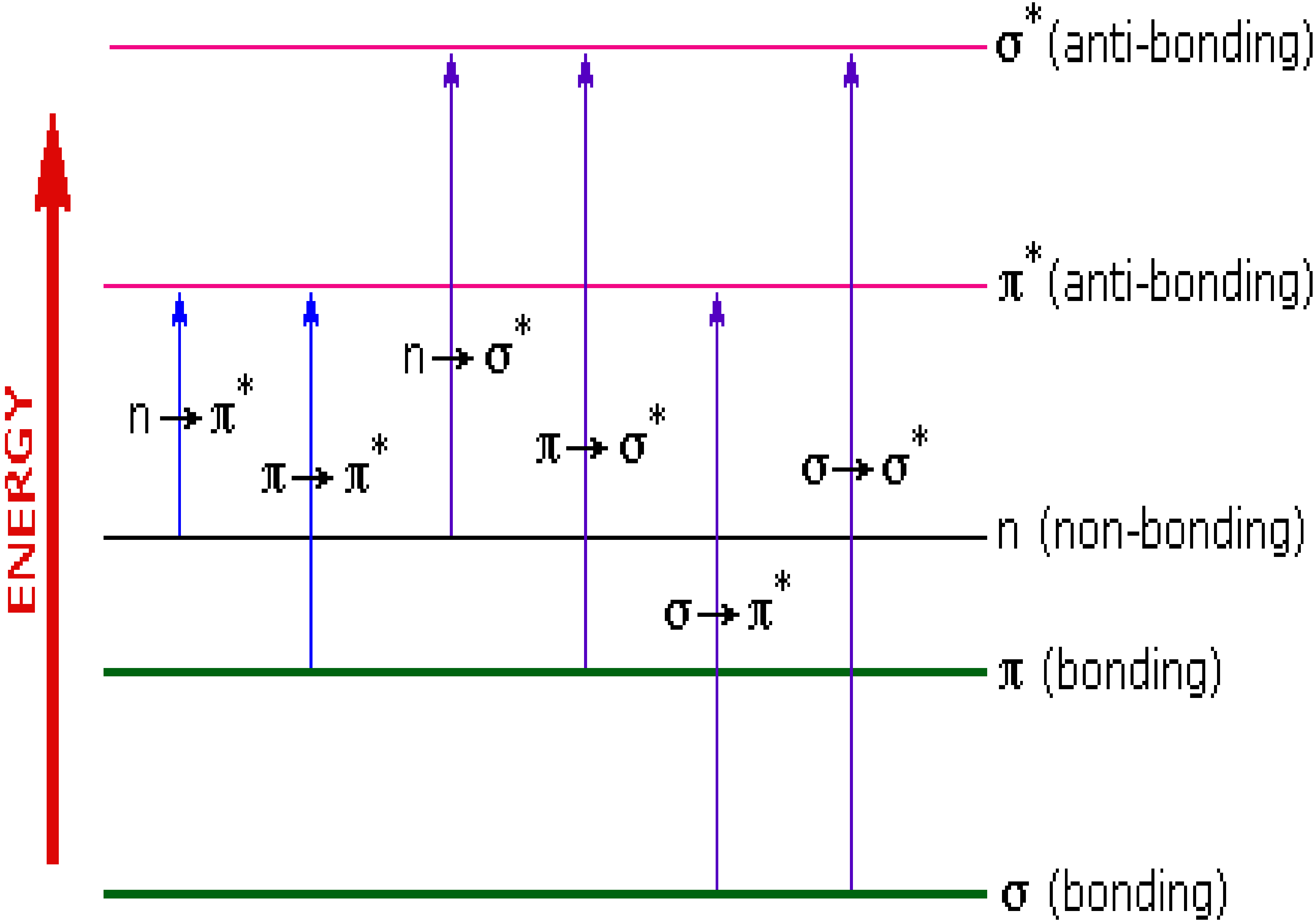
- **Types of Electronic Transitions**
- **σ - σ * transition : in vacuum uv e.g. saturated compounds (alkane)**

▪ σ to π* (carbonyl compounds)

- **π - π* transition : in 200 - 700 nm region e.g. unsaturated compounds.**
- (alkenes, carbonyl compounds, alkynes, azo compounds)

▪ η to σ * (oxygen, nitrogen, sulfur, and halogen compounds)

- **n - π* transition : in 150 - 250 nm region** (carbonyl compounds)


σ* (anti-bonding)
π* (anti-bonding)
n (non-bonding)
π (bonding)
σ (bonding)
ENERGY
n → π*
π → π*
n → σ*
π → σ*
σ → σ*
σ → π*

ABSORPTION SPECTRA

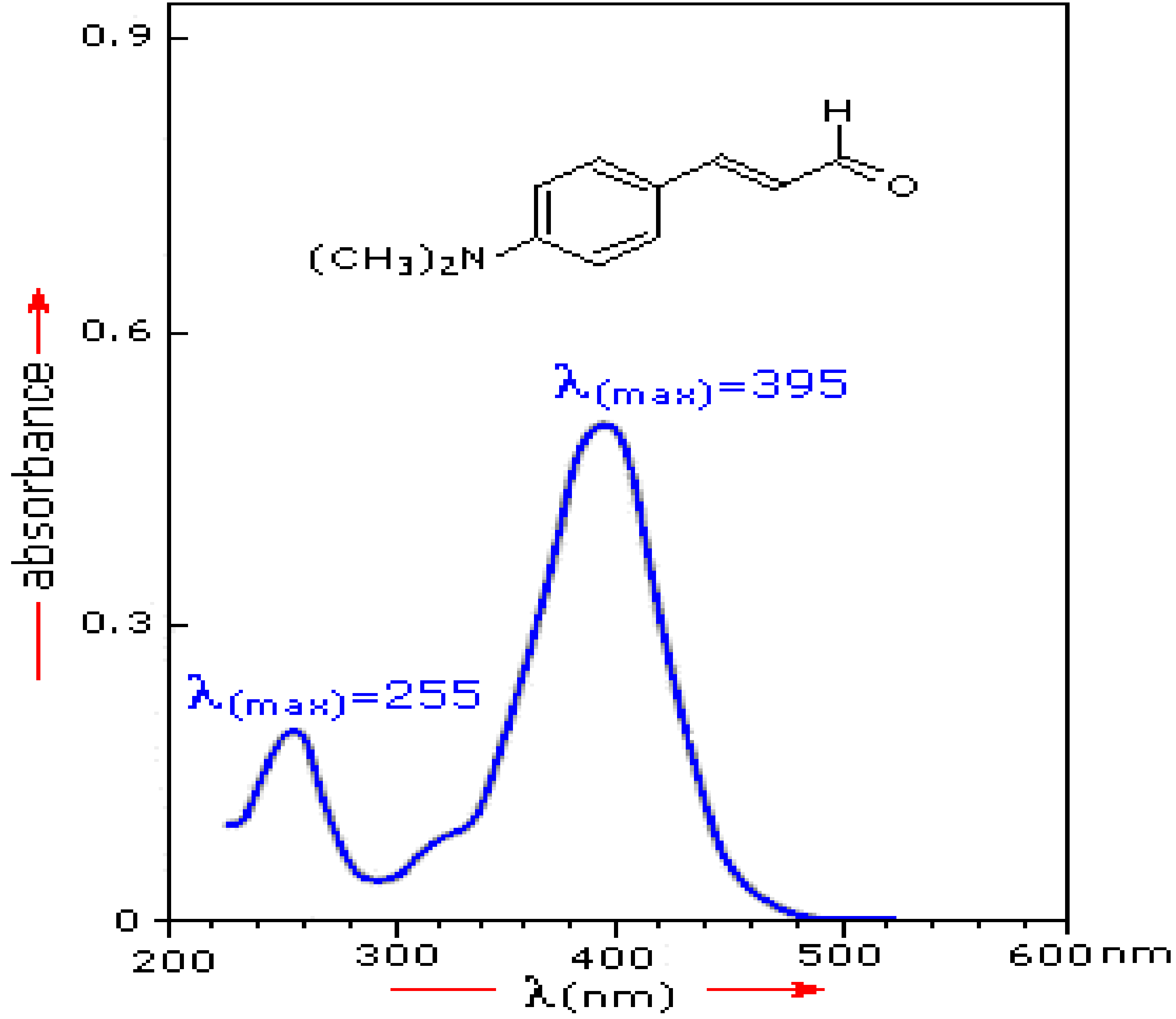
- Absorption spectrum is a plot of absorption of radiation vs wavelength . According to the absorbed radiation we can classified the absorption spectra into:

1 - UV - Spectrum

2 - Visible Spectrum

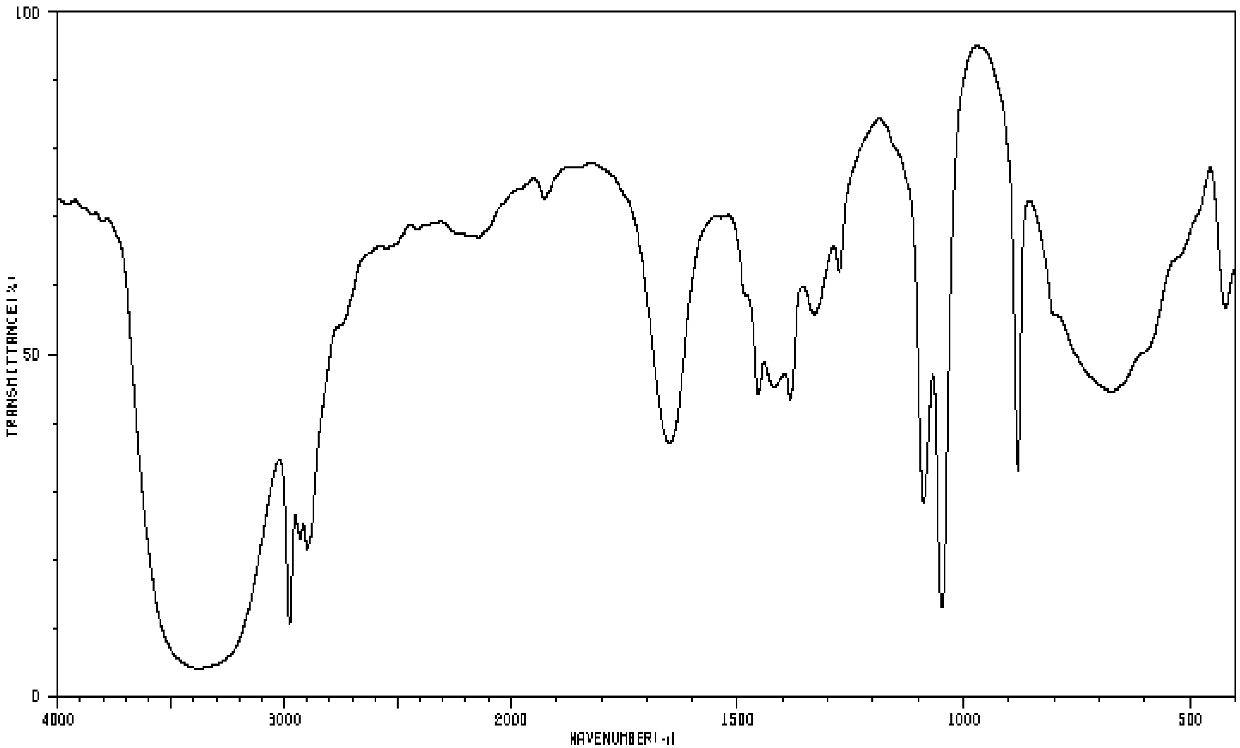
3 - IR - Spectrum

4 - NMR -Spectrum



0.9
0.6
0.3
0
absorbance
H
O
(CH3)2N
λ(max)=395
λ(max)=255
200
300
400
500
600 nm
λ(nm)

HIT-NO=1374	SCORE= ( )	SDBS-NO=1300	IR-NIDA-21941 : LIQUID FILM
ETHYL ALCOHOL			
C_2H_6O			



Theory

- Experimental measurements are usually made in terms of transmittance (T), which is defined as:

$$T = \frac{P}{P_0}$$

where P is the power of light after it passes through the sample and Po is the initial light power. **The relation between A and T is:**

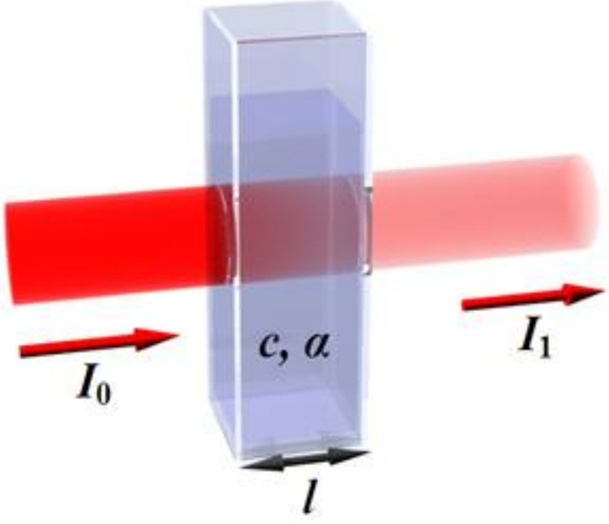
- $$A = -\log(T) = -\log\left(\frac{P}{P_0}\right)$$

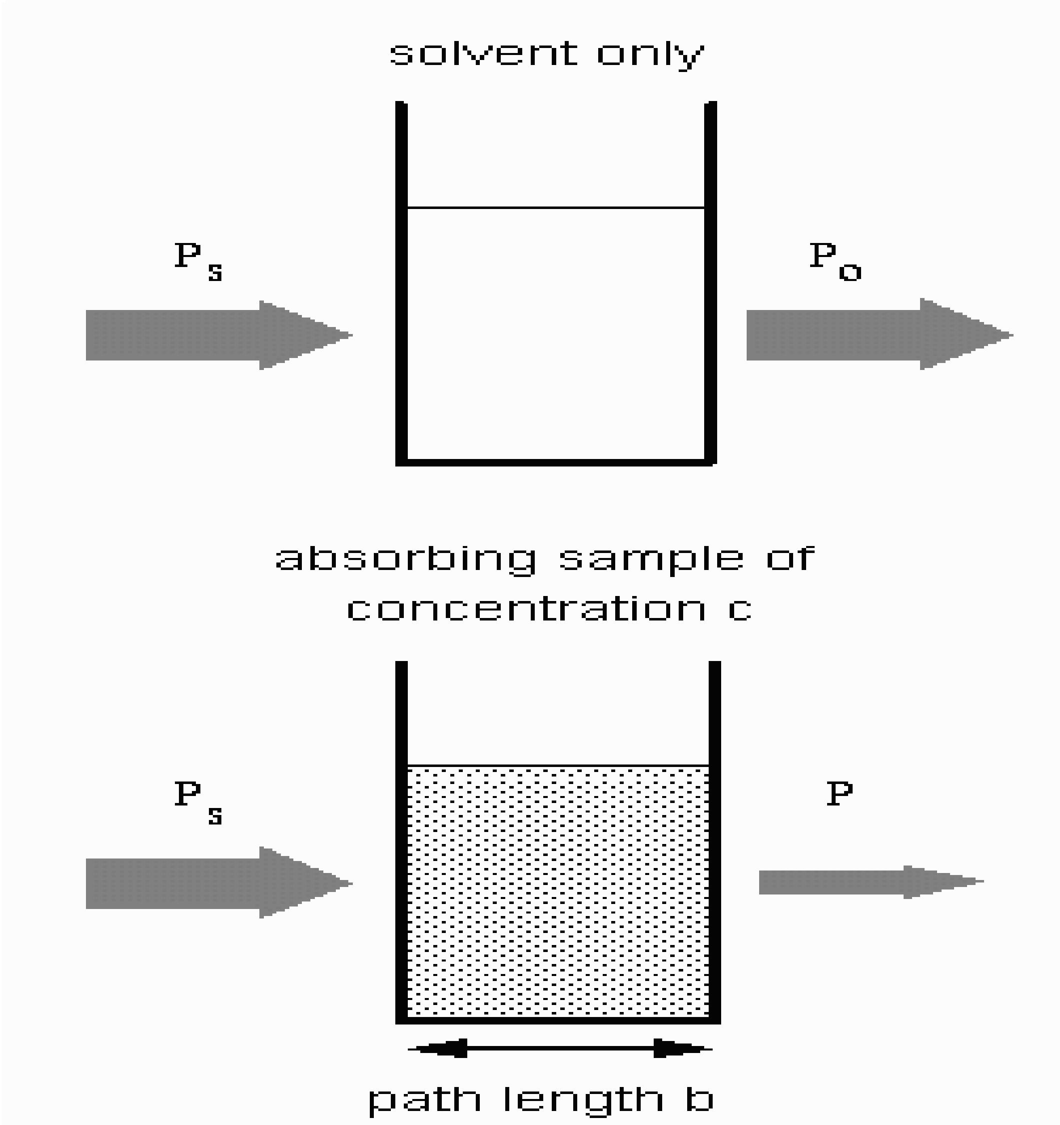
Laws of Light Absorption
Beer-Lambert Law

% Transmittance =

$(I / I_0) * 100\%$

- where:
- I = intensity of transmitted light
- I_0 = intensity of incident light




solvent only
Ps
Po
absorbing sample of
concentration c
Ps
P
path length b

- Modern absorption instruments can usually display the data as either transmittance, %-transmittance, or absorbance.
- An unknown concentration of an analyte can be determined by measuring the amount of light that a sample absorbs and applying Beer's law.
- If the absorptivity coefficient is not known, the unknown concentration can be determined using a working curve of absorbance versus concentration derived from standards.

a - Bouguert - Lambert,s Law

When abeam of a monochromatic radiation enters an absorbing medium , its intensity decreases exponentially with the increase of the thickness of the medium traversed(fig.)

$$\log \frac{Io}{I} = k\,b$$

(at constant concentration)

(k is a proportionality constant)

b - Beer,s Law

The intensity of a monochromatic radiation decreases exponentially as the concentration of the absorbing medium increases . (fig .   )

$$\log \frac{Io}{I} = k\,c \qquad (\text{ at constant } b\text{ })$$

(k is a proportionality constant)

c - Beer - Lambert,s Law

$$\log \frac{Io}{I} = abc \qquad (\text{ fig. } \quad)$$

where A : absorbnce ,

A = a b c

b : thickness cm

c : concentration g/L

- The value of "a" will depend upon the method of expression of the concentration :

If C is expressed in mole/liter , then "a" is given the symbol Ɛ ($1 mol^{-1} cm^{-1}$)and is called ***molar absorbitivity***

$$A = Ɛ\ b\ c$$

When c is in gm/100ml , b = 1 cm we get

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

$A^{1\%}$ can be converted easily to Ɛ by the equation

$$A^{1\%}_{1cm} = \frac{Ɛ \times 10}{mol.wt.}$$

DEVIATION FROM BEER,S LAW

- Real Deviation
- Instrumental Deviation
- Chemical Deviation

a - Real Deviation :

-dilution effect - molecular interaction

-complex formation

b - Instrumental Deviation (Errors)

<u>1 - Irregular   Deviation</u>

-unmatched cells

-unclean optics (cells - lenses - mirrors - lamps)

<u>2 - Regular Deviation</u>

- error in wavelength scale
- slit-width
- stray light

<u>3 - Non linear response of the phototubes</u>

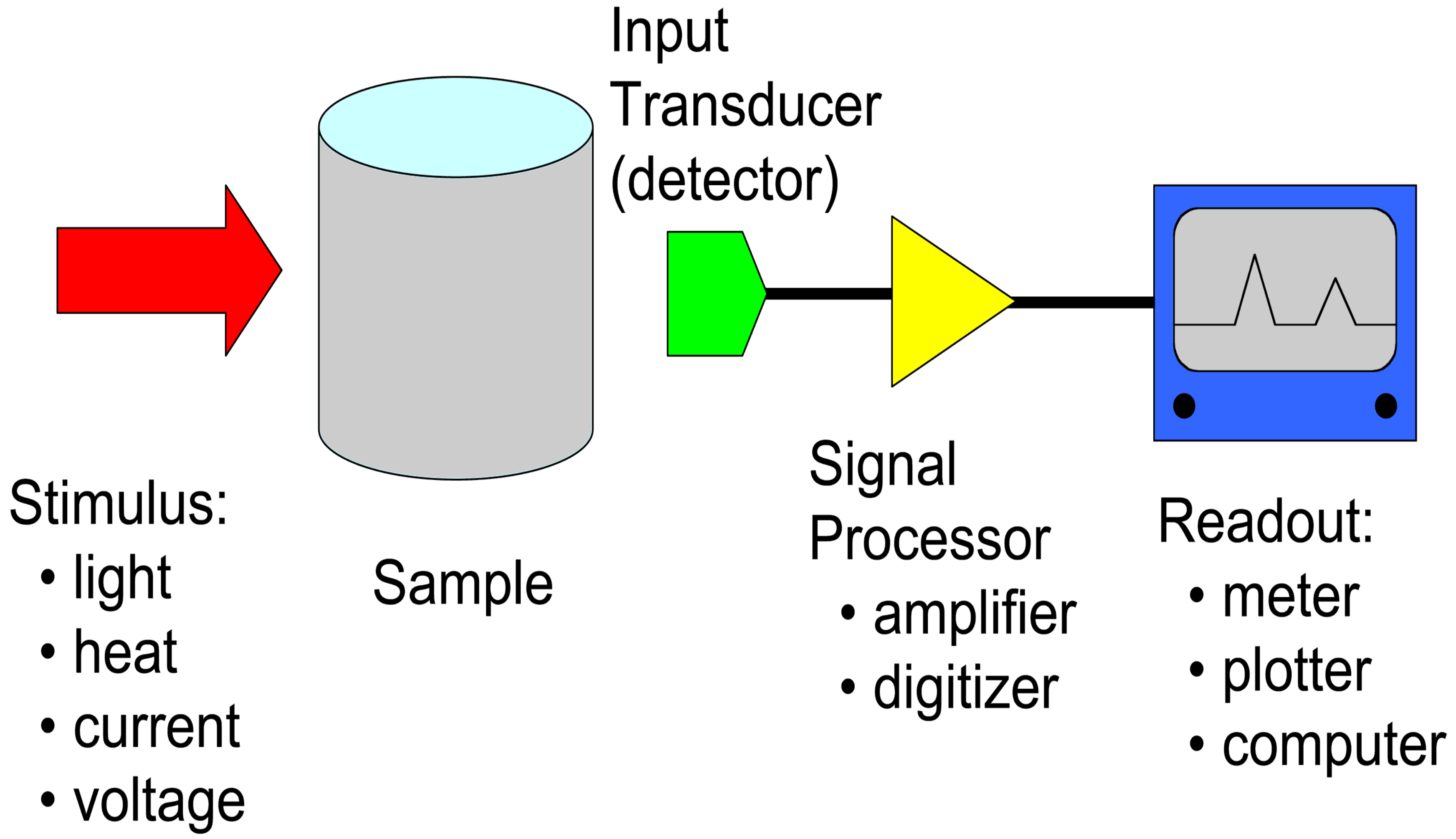
- Radio and TV interference's
- Voltage fluctuations

c - Chemical Deviations

- pH effect - solvent effect - temperature effect - photodegradation
- time factor (oxidation - reduction - hydrolysis - polymerization)

INSTRUMENTATION

- Spectrophotometry involves the use of a spectrophotometer.
- A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light.
- There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with(200 -900 nm,uv/vissible spectrophotometer), the measurement techniques they use(infra red spectrophotometer , spectrofluorometer, atomic absorption spectrophotometer) .


Input
Transducer
(detector)
Stimulus:
• light
• heat
• current
• voltage
Sample
Signal
Processor
• amplifier
• digitizer
Readout:
• meter
• plotter
• computer

- the most common application of spectrophotometers is the measurement of light absorption.
- The most common spectrophotometers are used in the UV (200 – 400nm) and visible (400 – 700nm) regions of the spectrum, and some of these instruments also operate into the near-infrared region as well.
- Visible region spectrophotometry is used extensively in colorimetry science .
- There are two major classes of spectrophotometers; single beam and double beam.

- The single beam spectrophotometer was the first invented, and all the light passes through the sample. In this case, to measure the intensity of the incident light, the sample must be removed so all the light can pass through (using blank solvent). This type is cheaper because there are less parts and the system is less complicated.

- Later, the double beam spectrophotometer was invented. In this type, the light source is split by a rotating mirror into two equal Intensity beams before it reaches the sample. One beam passes through the reference compartment containing only solvent and the other passes through the sample. This is advantageous because the reference reading and sample reading can be taken at the same time.
- In some double beam spectrophotometers, there are two detectors and the sample and reference beams can be measured simultaneously. Other double beam spectrophotometers that have only one detector use a beam chopper. This device inside blocks one beam at a time and the detector alternates between measuring the sample and reference beams

Spectrophotometers

A spectrophotometer is a device to measure light intensity at different wavelengths. It produces light with a light source, and after the light passes through a subject, the light is diffracted into a spectrum which is detected by a sensor and interpreted into results we can use.

<u>Components of Spectrophotometer</u>

1- source of light

2 – monochromator

3 – sample component

4 – detector

5 - meter

Application

A – Qualitative Analysis :

1 - Identification through comparison of

λ_{max}, Ɛ , $A^{1\%}$, $A_{\lambda 1}$ - $A_{\lambda 2}$ and $A_{\lambda 1}$ / $A_{\lambda 2}$ between sample and standard .

2 – identification of structure features through

1 - UV - Spectrum

2 - Visible Spectrum

3 - IR - Spectrum

4 - NMR -Spectrum

B - Quantitative Analysis

1 - Determinations of inorganic compounds

a – Cu forms blue colour with ammonia

b – Fe(III) forms red colour with thiocyanate

c – Fe(II) form red complex with 1,10-phenanthroline

2 - Determinatins of organic compounds

a – for aromatic primary amines: diazotisation and coupling forming red complex .

b – phenols:coupling with diazotised primary aromatic amines forming red complex .

c – carbonyl compounds: by reaction with 2,4-dinitro phenylhydrazine forming red complex .