Pharmaceutical Instrumental Analysis

الأستاذ الدكتور جمعه الزهوري (دكتوراه صيدلة-ألمانيا 1991)

Prof.Dr.Joumaa Al- Zehouri(Ph. D Germany 1991)

Damascus university

Faculty of Pharmacy

كيف تحصل على المحاضرات ؟

رابط المحاضرات على موقع جامعة دمشق :

http://new.damascusuniversity.edu.sy/faculties/pharm/2013-07-18-11-11-13/2013-07-18-11-22-15/174-2014-02-04-13-03-09

رابط المحاضرات على موقع جامعة الرشيد :

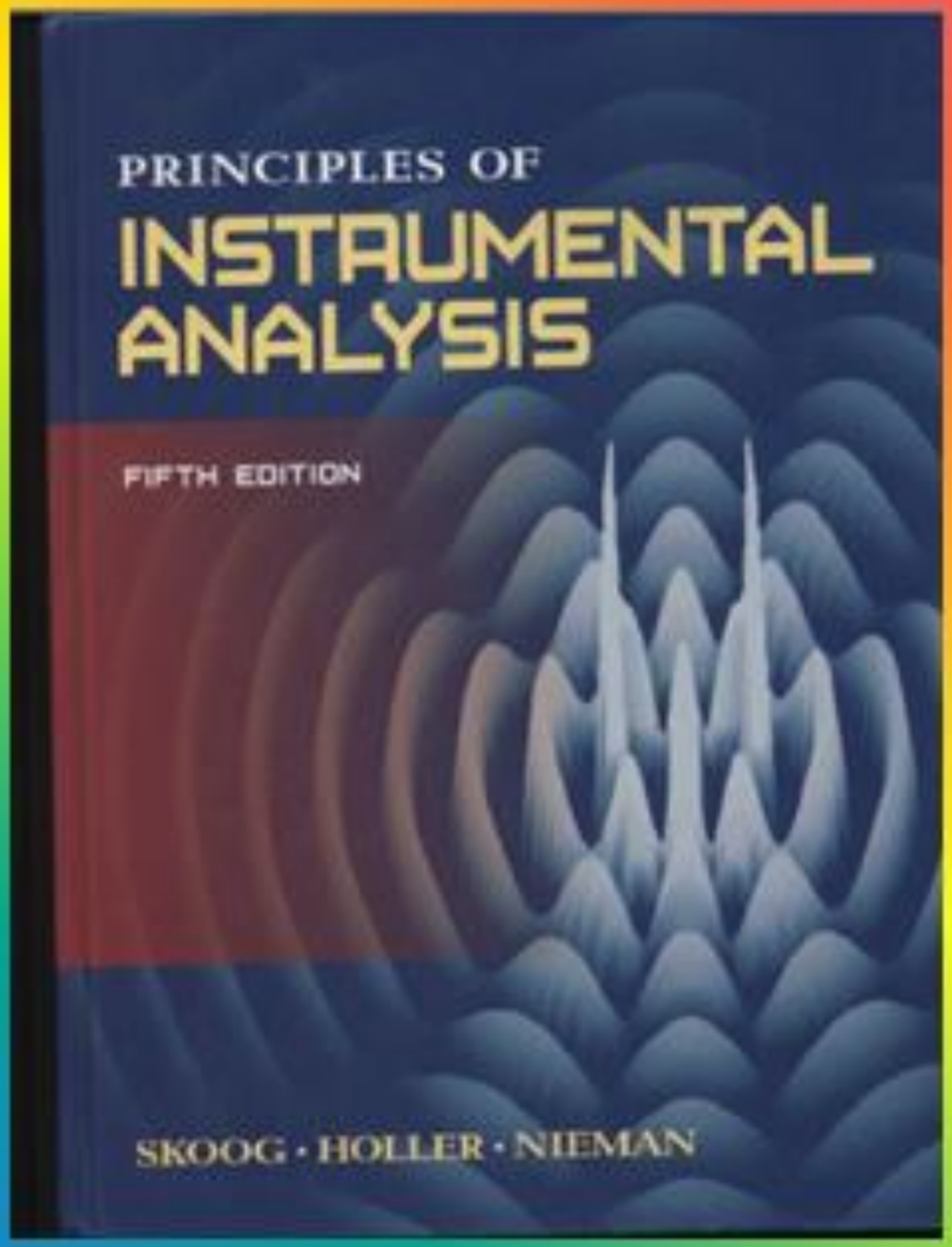
https://onedrive.live.com/?authkey=%21AHTRlDW3auHIvlc&id=6E6E9FDA4EA6B639%212048&cid=6E6E9FDA4EA6B639

الكتاب متوفر
ب 200 ل.س

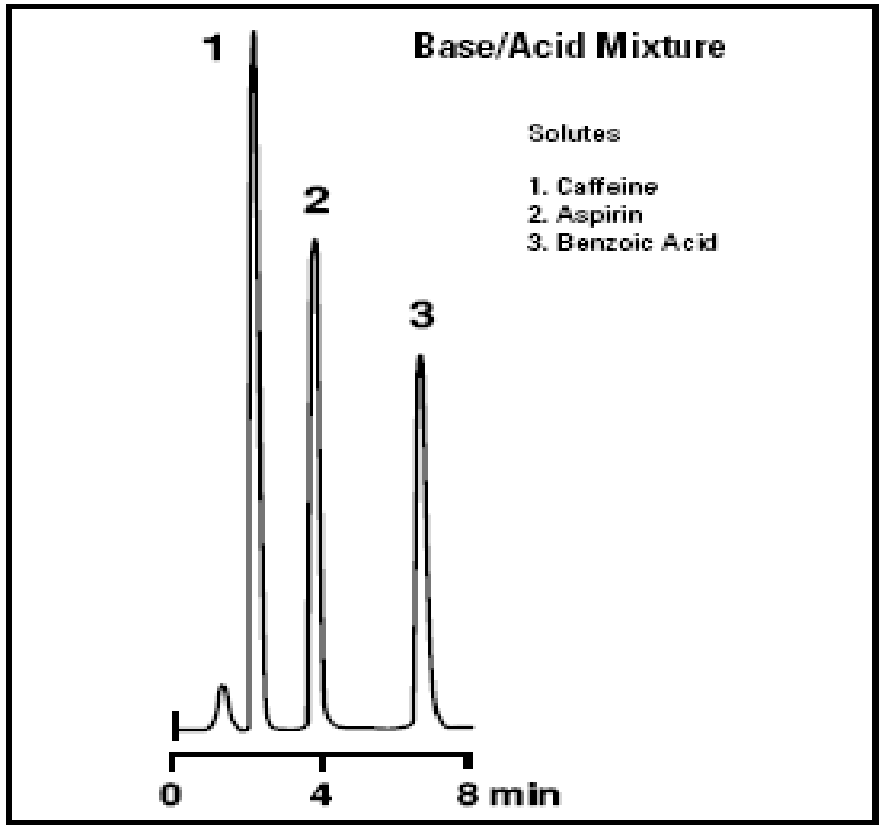
11-66

127-168

Principles of
INSTRUMENTAL ANALYSIS
for SKOOG.HOLLER.NIEMAN



Prof. J .Al-Zehouri



Column	Hypersil BDS C18, 5µ,150x4.6mm
Buent	Methanol:0.05M Phosphate Buffer, pH3.5 (40:60)
Flow Rate	1.0 ml/min
Detection	UV 254nm

	Quantity/1000 Tablets (g)
	500.00
	65.00
	15.00
	10.00
l)	5.00
	33.00
	8.00
	1.00
M PH102)	10.00
P)	7.00
l)	5.00
	2.00
	4.00
	155.00

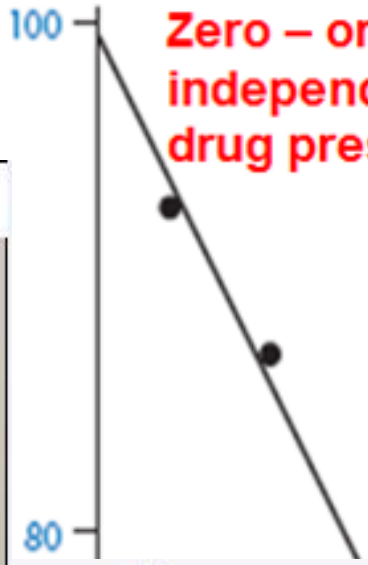
أ.د.جمعه الزهوري

Rate $= -\frac{dc}{dt} = K$

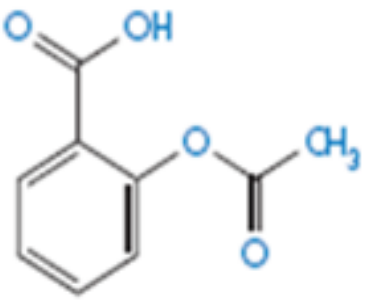
Integrated rate $x = K t$

$t_{1/2} = \frac{a}{2k}$

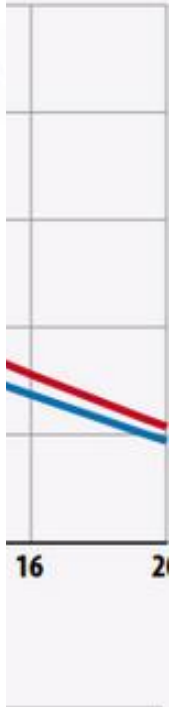
t (shelf) or $t_{90}\% = \frac{a}{10k}$



Zero – order rate constan
independent on the amo
drug present

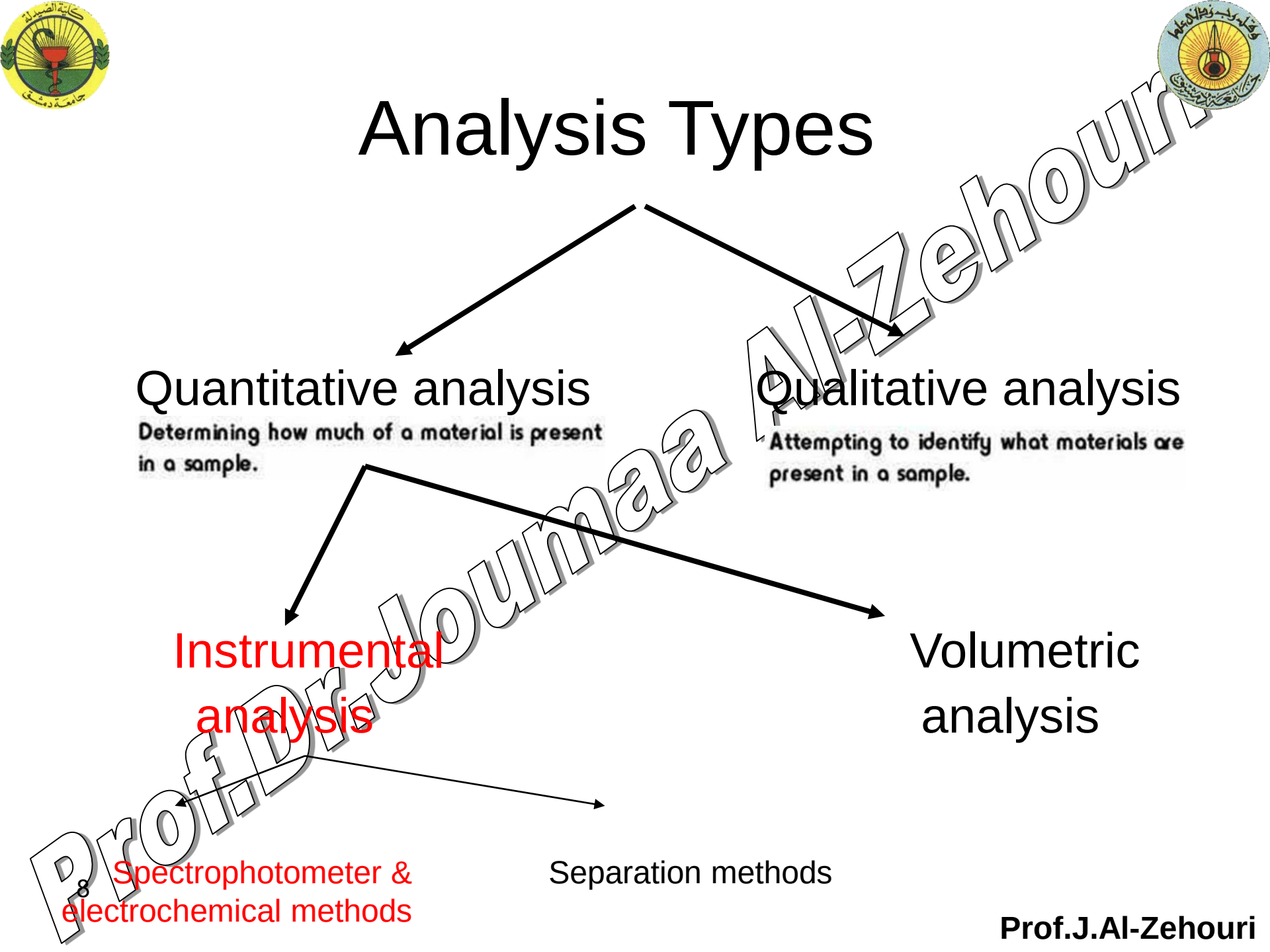


RAPEUTIC DRUG MONITORING



Prof. J. Al-Zehouri

Analysis Types

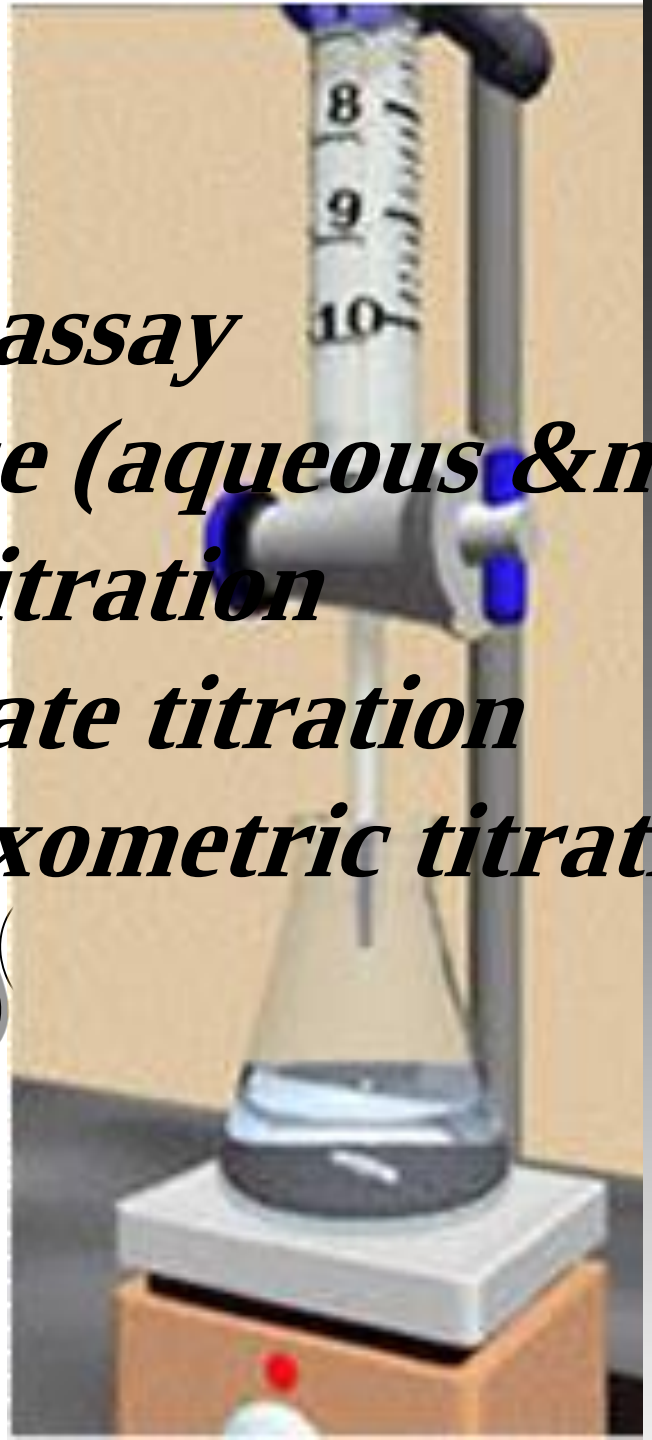


Acid base (aqueous) titration
Substance must have basic or acidic characters

(calculate the sample in case of finished product)

Weigh and powder a 20 tablets. Add a quantity of the powder containing 1 g of API ???

$$C\% = \frac{12}{12.\square} \times \frac{\text{weight of sample taken}}{}$$



Volumetric assay

- *acid base (aqueous &non) titration*
- *Redox titration*
- *precipitate titration*
- *Complexometric titration*
- *others*

Why ? Instrumental analysis

- Impossibility of assay using volumetric and Gravimetric analysis.
- Very low sensitivity (ng,pg….) .
- Simultaneous assay
- Huge Number of analysis
- Identification analysis
- Structure analysis

Instrumental Analysis

Spectrophotometric methods

* UV-Vis
*Fluorescence Spectroscopy
*IR
* MS
* NMR (H,C)
*AAS
* AES = Flame Photometry
* X-ray Spectrometry

Chromatographic methods

* TLC , PC
* HPTLC
* GC (GSC , GLC)
* HPLC ,LSC,LLC
* Ion-ExchangeChromato.
* Gel Chromatography

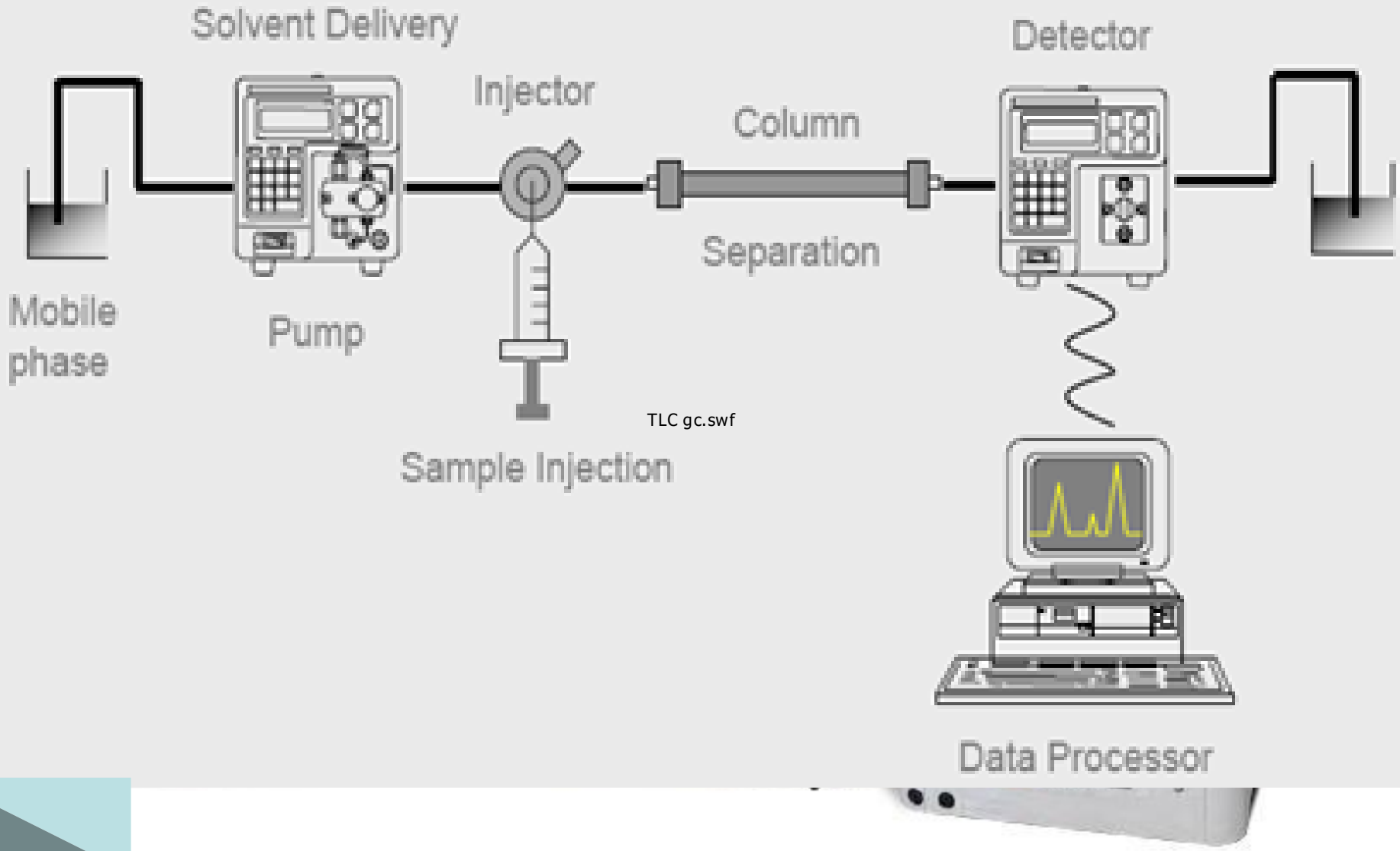
Electrochemical methods

* Voltametry (Polarography)
* Amperometry
* Conductometry
* Coulonmetry
* Electrogravimetry

Immunoassay methods

* RIA
* EIA
* FluorescenceImmuno assay
* PCR

TLC& GC &HPLC



Prof. J. Al-Zehouri

Spectrochemical Analysis

Outline

- Spectroscopic Methods of Analysis : Making Measurements with light.
- Instruments for Measuring Absorption.
- Applying Molecular Spectroscopic Methods

- ***Spectrochemical methods are among the most popular of the instrumental analytical techniques.***
- ***Ultraviolet and visible absorption spectroscopy are applied for quantitative determinations in many areas of life sciences.***
- ***Likewise infrared absorption methods are frequently used to identify molecules and to supply structural information.***
- ***Atomic spectrometric methods use for quantitative elemental analysis.***

Spectroscopic Methods

Of Analysis

Colorimetric and Spectrophotometric methods

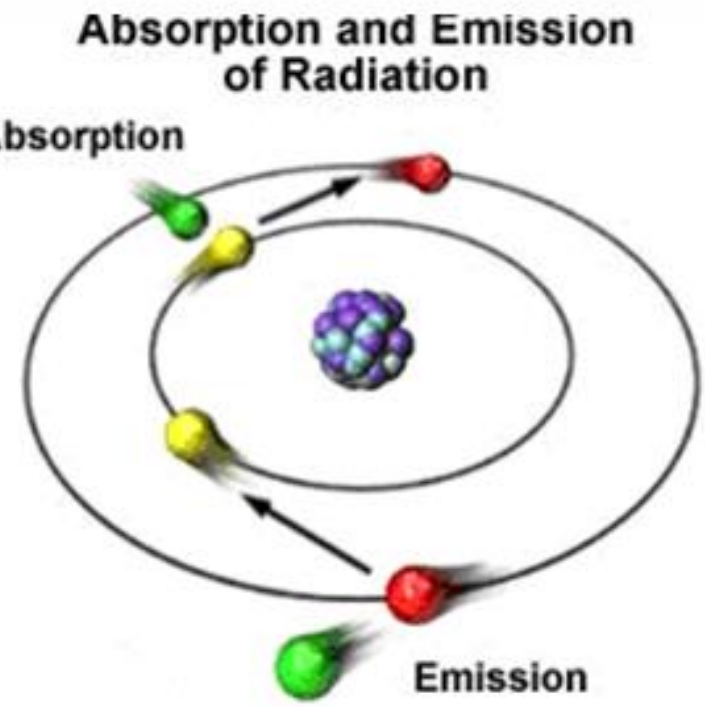
A group of techniques that relies on the interaction of electromagnetic radiation and matter.

There are many types of methods based on either molecular or atomic interactions.

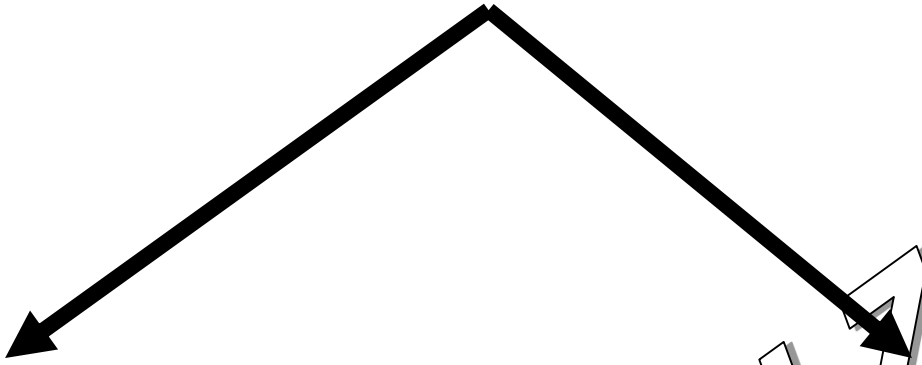
Absorption

Emission

Fluorescence



Spectrometric Methods of Analysis



Absorption Spectrometry

- **Molecular UV Absorption Spectrometry**
- **Molecular Visible Absorption Spectrometry**
- **Infrared Spectrometry**
- **Nuclear Magnetic Resonance (NMR)**
- **Atomic Absorption Spectrometry (AAS)**

Mass spectrometry

Emission Spectrometry

- **Fluorimetry**
- **Atomic emission spectrometry (Flame Photometry)**

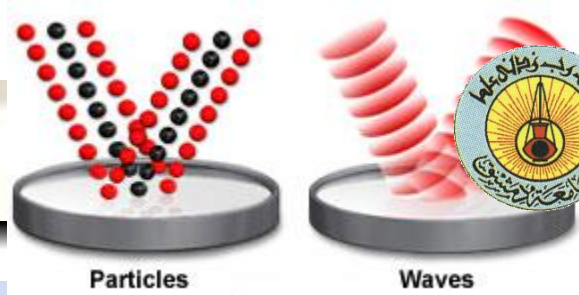
Chlorpropamide Tablets

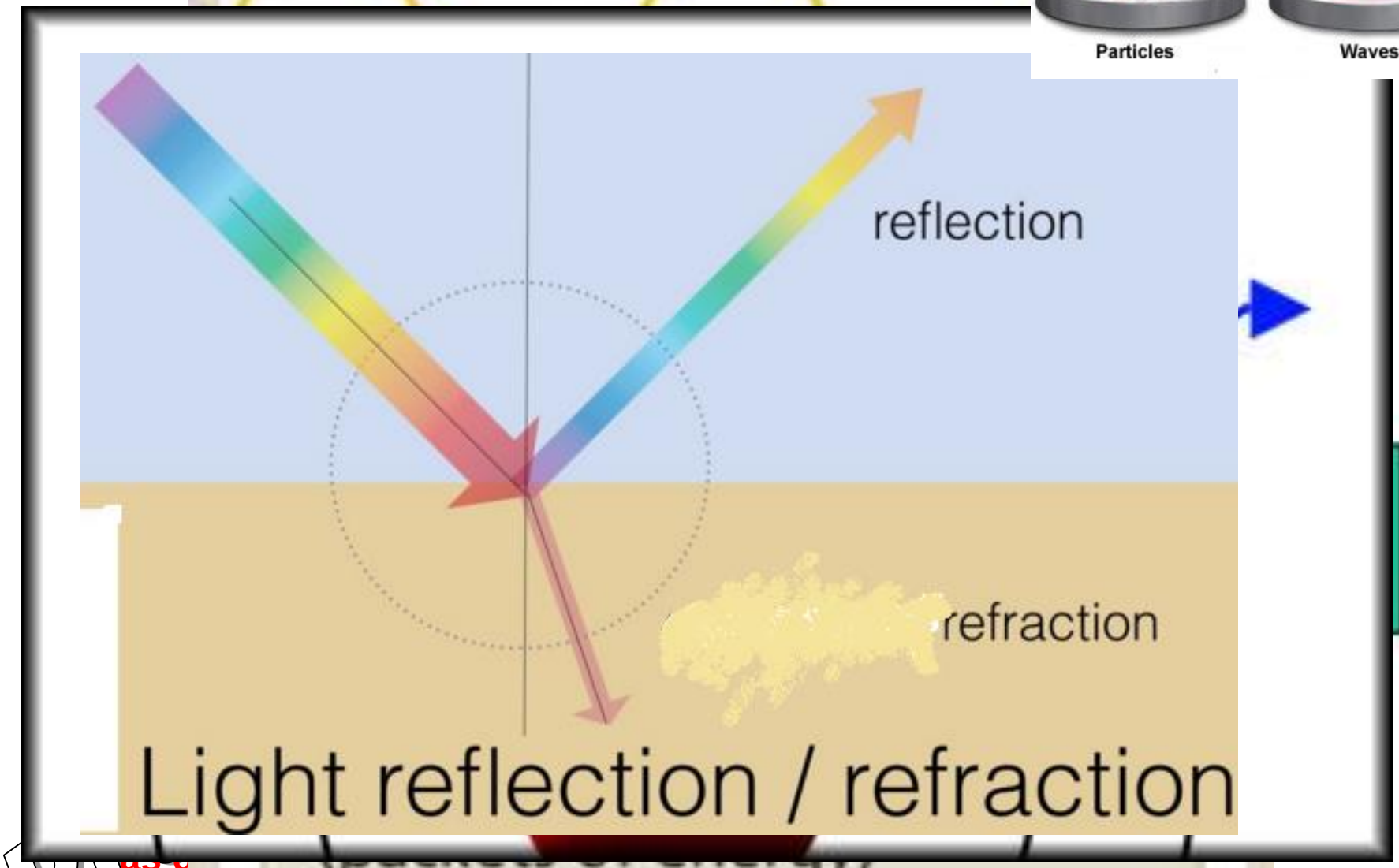
Action and use

Hypoglycaemic.

Assay : Weigh and poweder 20 tablets, Shake a quantity of the powder containing 0.25 g of Chlorpropamide with 40 ml of methanol for 20 minutes, add sufficient methanol to produce 50 ml, mix ,filter and dilute 5 ml of the filtrate to 100 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at 232 nm, appendix II B .Calculate the content of Chloropropamide taking 598 as the value of A(1%,1cm) at the maximum at 232 nm.

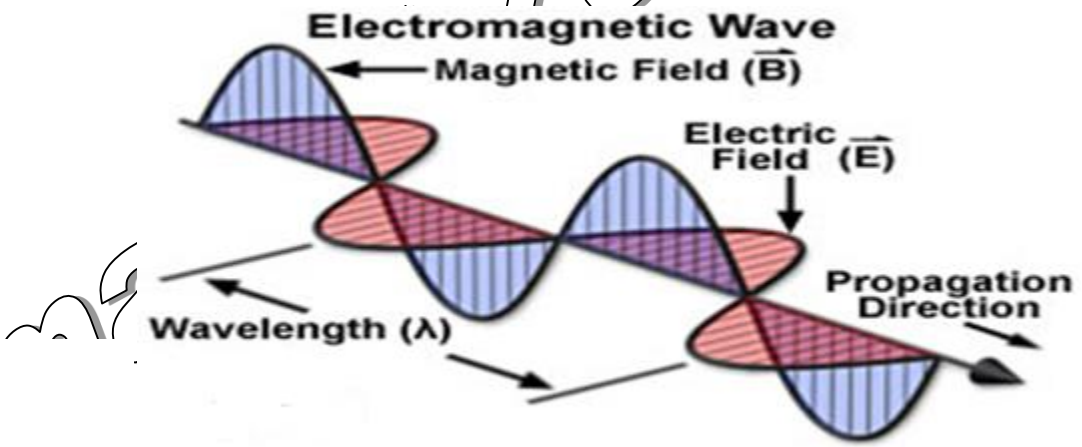
Wh

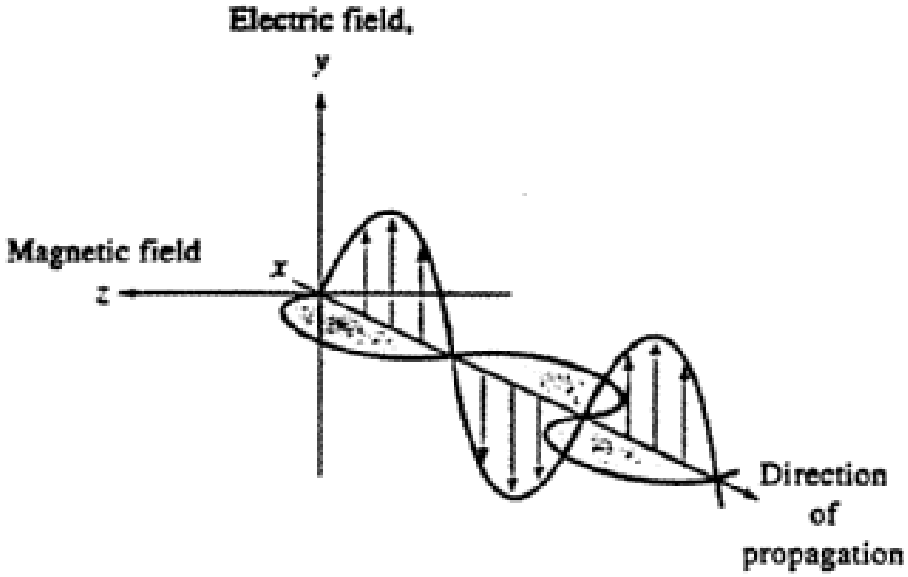

Particles
Waves

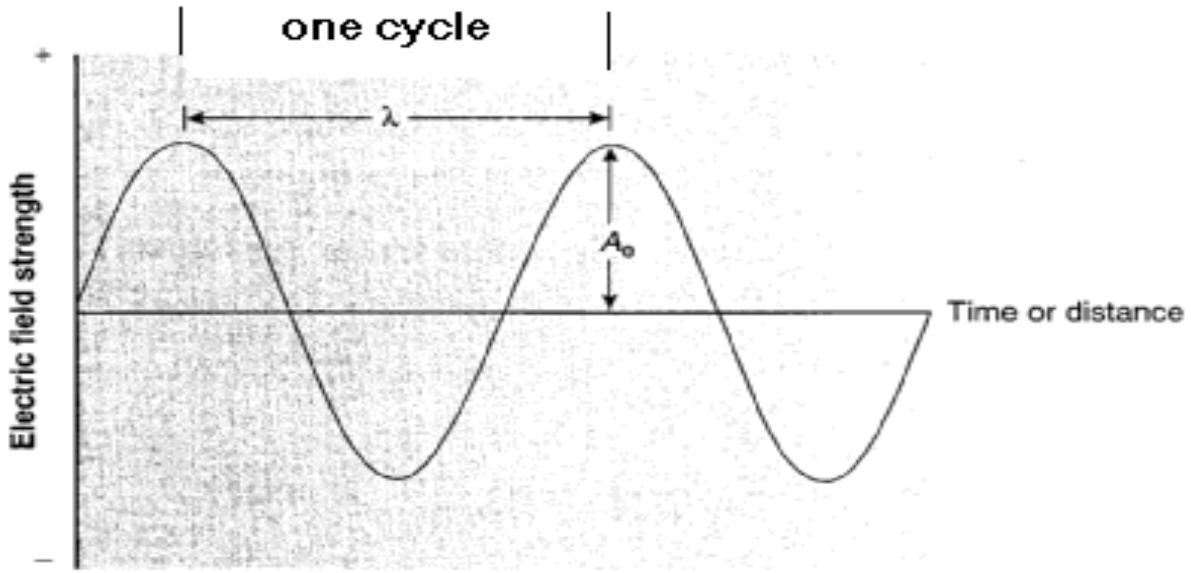

reflection
refraction
Light reflection / refraction

Nature of Electromagnetic Radiation

1. Wave Properties







- **λ**= wavelength : The distance between any two consecutive maxima or minima of an electromagnetic wave (distance that the wave moves during one cycle)
- **ν**= frequency : The No. of oscillation of electromagnetic wave per second (No.of cycles/s) **;has units of hertz (Hz), which is one oscillation /s**
- $\bar{\nu}$= wave number : The reciprocal of wavelength **,has units cm^{-1}**
- **A**= wave amplitude : the maximum disturbance from the horizontal axis.
- t= period : the time for one complete cycle

or, The time required for successive peaks of an electromagnetic wave to pass a fixed point in space.

Wavelength, frequency, and wave number are interrelated

The relationship between the wavelength and frequency is :

$$\lambda = \frac{C}{V}$$

C (Velocity of light = $3X10^{10}$ cm/s)

V (frequency)

$$V = \frac{1}{\lambda} = \frac{V}{C}$$

Nature of electromagnetic Radiation

Particle Properties of Electromagnetic Radiation When a sample absorbs electromagnetic radiation it undergoes a change in energy. The interaction between the sample and the electromagnetic radiation is easiest to understand if we assume that electromagnetic radiation consists of a beam of energetic particles called **photons.** When a photon is absorbed by a sample, it is "destroyed," and its energy acquired by the sample.[5] The energy of a photon, in joules, its frequency, wavelength, or wavenumber by the following equations

$$\lambda = \frac{c}{\nu}$$

$$E = h\nu$$

$$= \frac{hc}{\lambda}$$

$$= hc\bar{\nu}$$

$$\bar{\nu} = \frac{1}{\lambda} = \frac{\nu}{c}$$

where h is Planck's constant, which has a value of 6.626×10^{-34} J • s.

Angstrom unit (Å) : A unit of length equal to 1 x 10^{-10} meter.

Nanometer unit (nm) : A unit of length equal to 1x10^{-9} meter

Å = angstrom = 10^{-10} meter = 10^{-8} centimeter = 10^{-4} micrometer

nm = nanometer = 10^{-9} meter = 10 angstroms = 10^{-3} micrometer

μm = micrometer = 10^{-6} meter = 10^{4} angstroms

Example

What is the energy per photon of the sodium D line (λ = 589 nm)?

SOLUTION

The energy of the sodium D line is

$$E = \frac{hc}{\lambda} = \frac{(6.626 \times 10^{-34}\ \text{J} \cdot \text{s})(3.00 \times 10^{8}\ \text{m/s})}{589 \times 10^{-9}\ \text{m}} = 3.37 \times 10^{-19}\ \text{J}$$

From the equation $E = h\nu$ and $\lambda \nu = c$, derive an equation which relates energy to wavelength.

$$E = h\nu \quad \nu = c/\lambda$$

$$E = hc/\lambda$$

What is the energy of photons with a wave-length equal to 0.05 nm?

$$E = h\nu = hc/\lambda$$

$$E = \frac{6.63 \times 10^{-34}\ J\,s \mid 3.0 \times 10^{10}\ cm\,s^{-1}}{5 \times 10^{-9}\ cm \mid s}$$

$$E = 4.0 \times 10^{-15}\ J$$

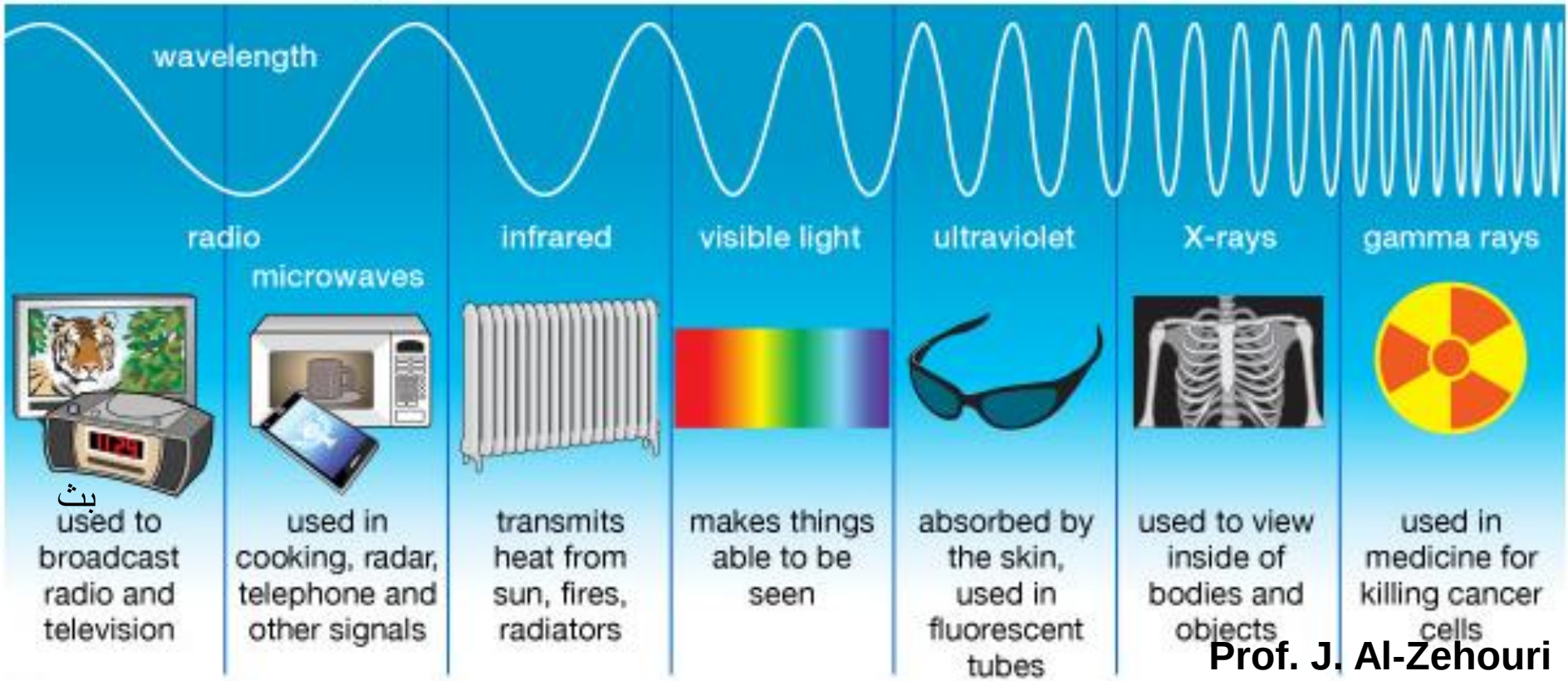
Electromagnetic radiation is divided into several regions called γ-ray , x-ray , ultraviolet, visible, infrared, mi[illegible], and radio wave.

γ-rays	X-rays	UV	IR	Microwave	Radio

λ (nm) 0.01 10 380 780

Visible

Types of Electromagnetic Radiation



بث

Wavelength Units for Various Spectral Regions

Region	Unit	Definition
X-ray	Angstrom unit, Å	10^{-10} m
Ultraviolet/visible	Nanometer, nm	10^{-9} m
Infrared	Micrometer, μm	10^{-6} m

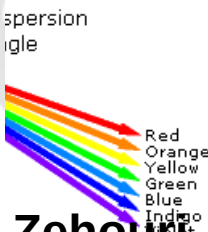
The different wavelength regions in electromagnetic radiation and the type of transition :

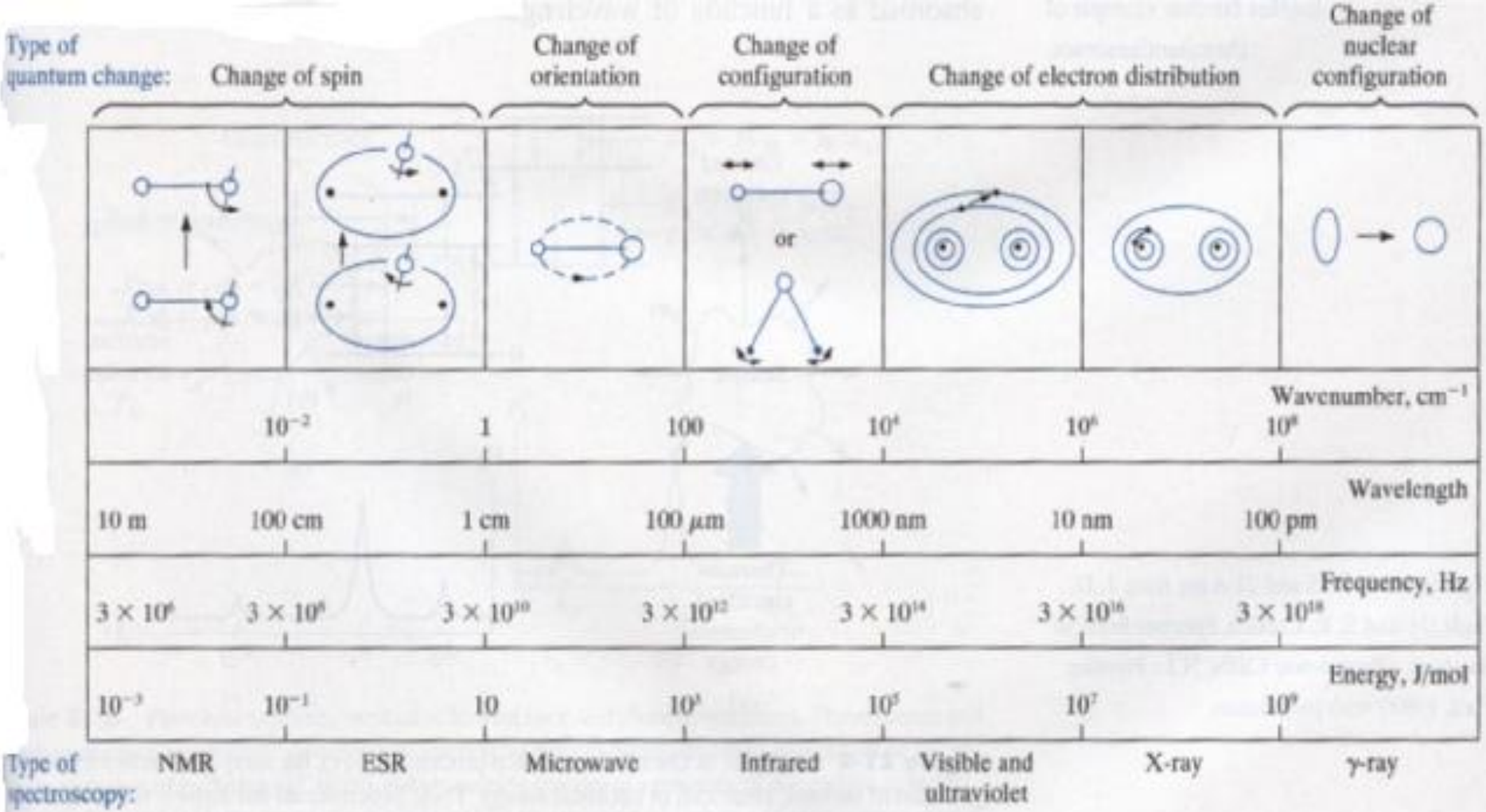
Spectral region (Type of radiation)	Wavelength	Type of transition
γ-ray	< 0.01 nm	Nuclear
x-ray	0.01 – 10 nm	Core – level electrons
Far- UV	10- 200 nm	Valence electron
Near –UV	200-380 nm	Valence electron
Visible	380-780 nm	Valence electron
IR	0.780 – 400 μm	Molecular rotation
Microwave	0.04- 3 cm	Molecular rotation; Electron spin
Radio wave	> 3 cm	Nuclear spin

UV-VIS SPECTROSCOPY

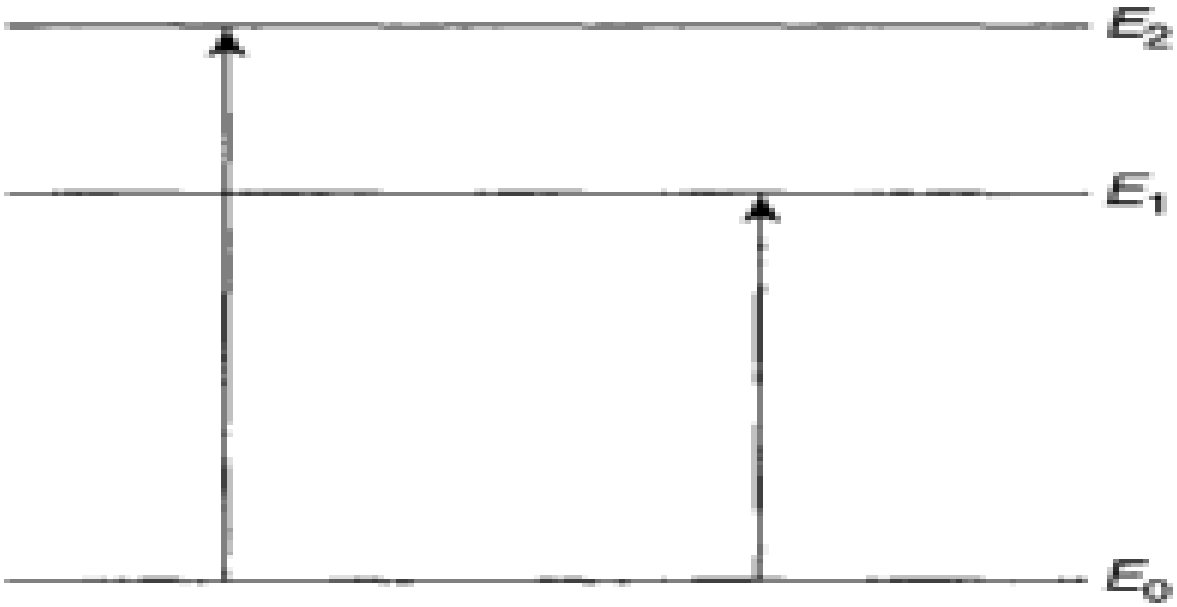
Complementary Colors

λ_{max}	Color Observed	Color Absorbed
380-420	Green-yellow	Violet
420-440	Yellow	Violet-blue
440-470	Orange	Blue
470-500	Red	Blue-green
500-520	Purple-red	Green
520-550	Violet	Yellow-green
550-580	Violet-blue	Yellow
580-620	Blue	Orange
620-680	Blue-green	Red
680-780	Green	Red





The regions of the electromagnetic spectrum. Interaction of an analyte with electromagnetic radiation can result in the types of changes shown.



Simplified energy level diagram showing absorption of a photon.

Q Below is an energy diagram which represents the four lowest energy levels of an atom:

If an atom is in its lowest energy state, which level represents the energy of the atom?

A E_1

Q What happens to the energy of the atom as it is excited from E_1 to E_2 to E_3, etc.?

A The energy increases.

Q What must happen before the atom can be excited from E_1 to E_2?

A The atom must absorb energy.

Q Write an expression for the amount of energy necessary to excite the atom from the first to the second energy level.

A

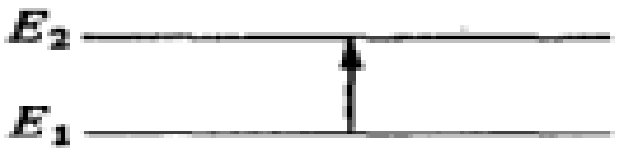
First level E_1

Second level E_2

The amount of energy necessary to excite the atom would be the energy difference between the two levels.

$$E_2 - E_1 = \Delta E_{2,1}$$

Q Give an expression which relates the energy of absorption to the wavelength of light absorbed in the process



A

$$\Delta E_{2,1} = E_2 - E_1$$

$$E = hc/\lambda$$

Therefore,

$$\Delta E_{2,1} = hc/\lambda$$

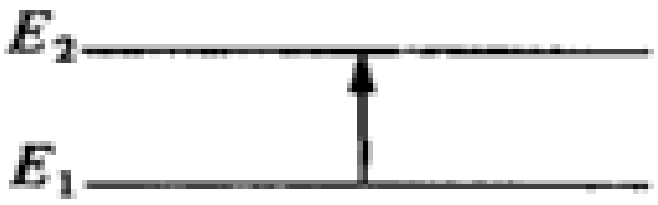
Q- What factor determines the wavelength of light absorbed in a spectrum?

A The differences between the various energy levels in the atom or molecule.

Q What explanation would account for the fact that the absorption spectrum for each kind of atom and molecule is unique?

A Each atom or molecule has unique differences between its energy levels.

R The energy of atoms and molecules is quantized (only certain allowed energy levels). An absorption spectrum is the result of an atom or molecule being excited to a higher energy level by absorption of a quantum of energy.



$$E_2 - E_1 = \Delta E_{2,1} = hc/\lambda = hc\bar{\nu}$$

$E_2 - E_1$ = transition energy.

Each different kind of atom or molecule gives its own characteristic absorption spectrum.

Spectroscopy Based on Absorption
ULTRAVIOLET AND VISIBLE SPECTRA

Ultraviolet and visible spectroscopy is useful in determining structures of organic molecules and it quantitative analysis. This chapter will deal mainly with the theory of electronic transitions

Electronic Transitions

After completing this section you should :

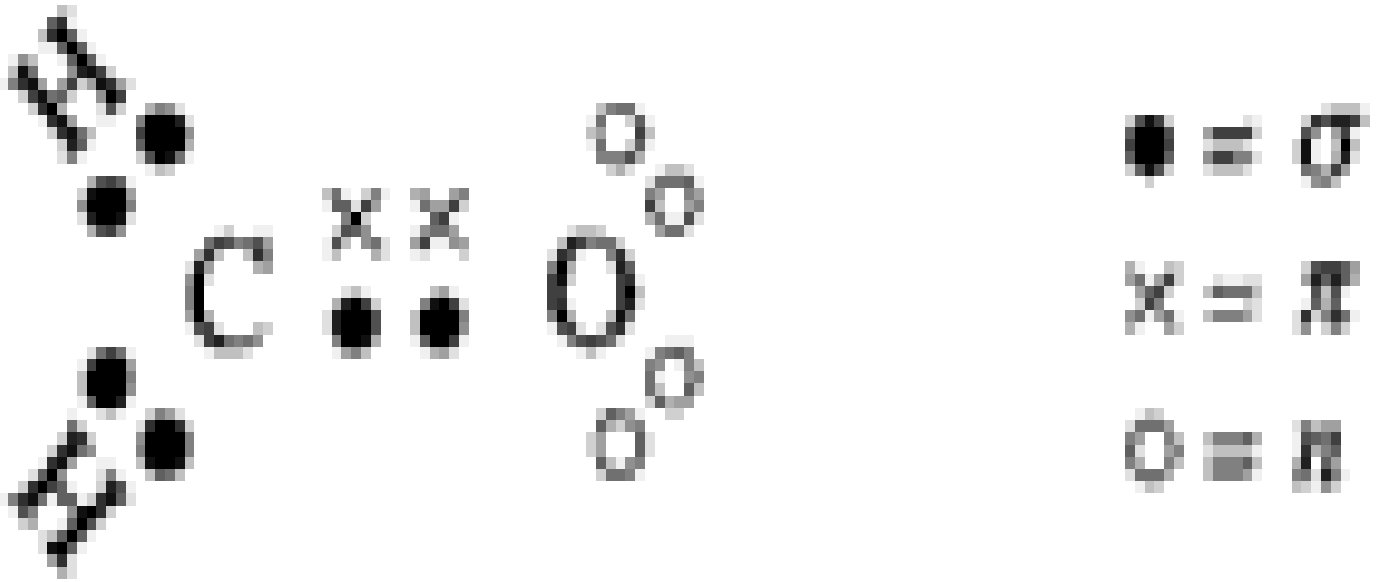
a) be able to determine the types of chromophores present in a molecule and determine which chromophores gives to the lowest energy transition

b) be able to predict approximate wavelength regions for different types of transitions.

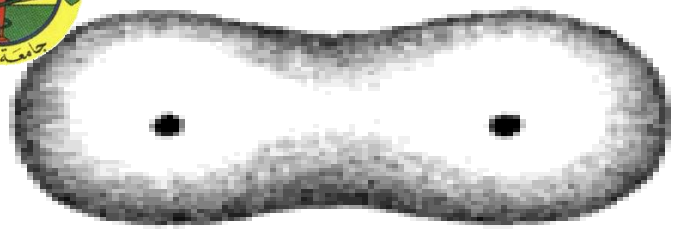
c) Understand why conjugated system absorb at longer wavelength.

d) be able to predict solvent effects on ππ* and nπ* transition

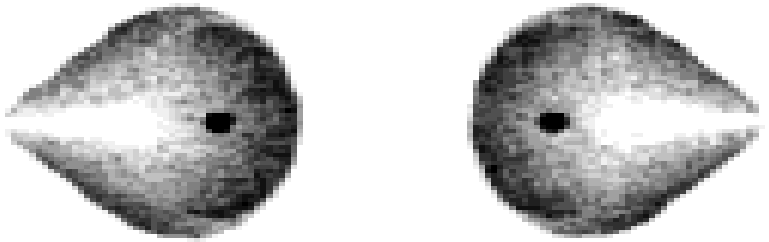
e) be able to calculate ε from the Lambert-Beer



Types of molecular orbitals in formaldehyde



(a) σ orbital



(c) σ* orbital

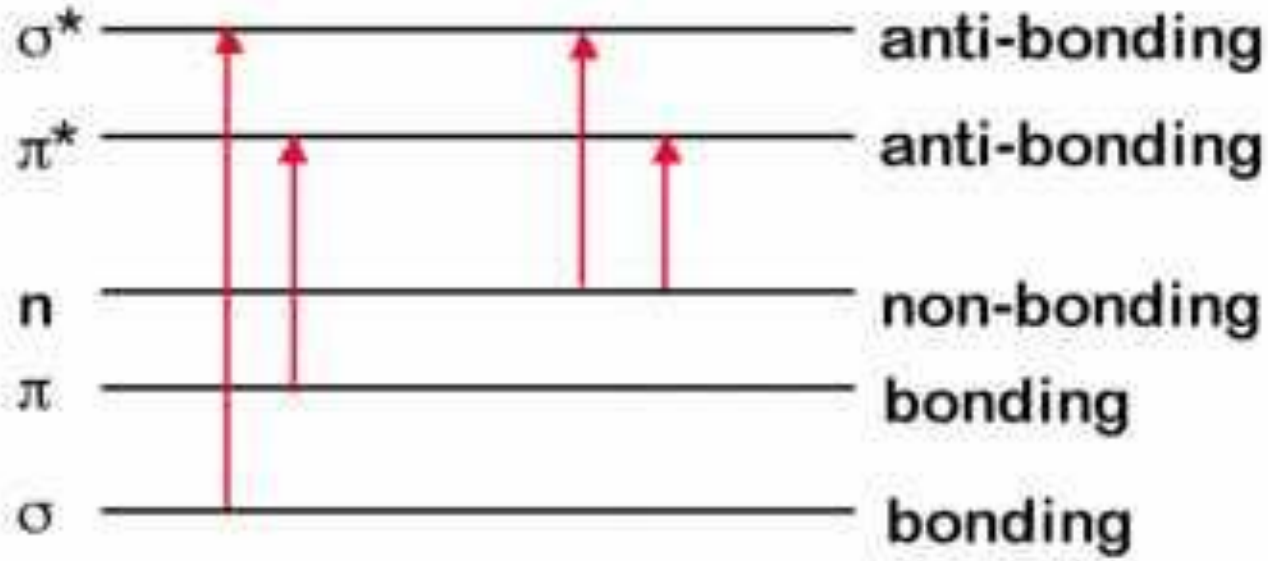
(b) π orbital

(d) π * orbital

Electron distribution in sigma and pi molecular orbitals

UV/Vis absorption

UV/Vis absorption by organic compounds requires that the energy absorbed corresponds to a jump from a populated orbital to an unpopulated one.

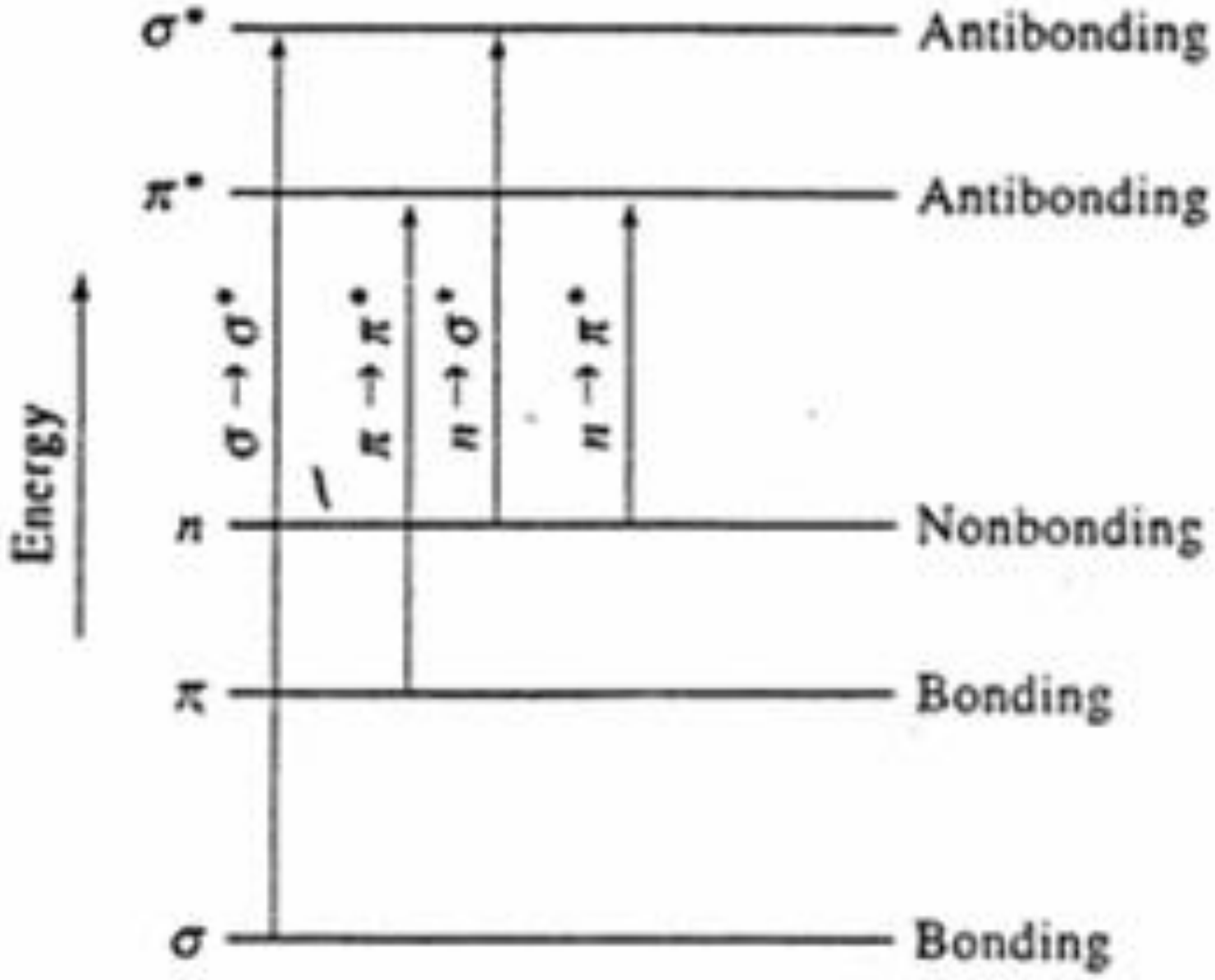


CH_3COCH_3

CH2CH2

The electronic spectrum of a molecule results from a transition between two different molecular electronic energy levels. A transition between two states will be denoted by using the notation illustrated below:

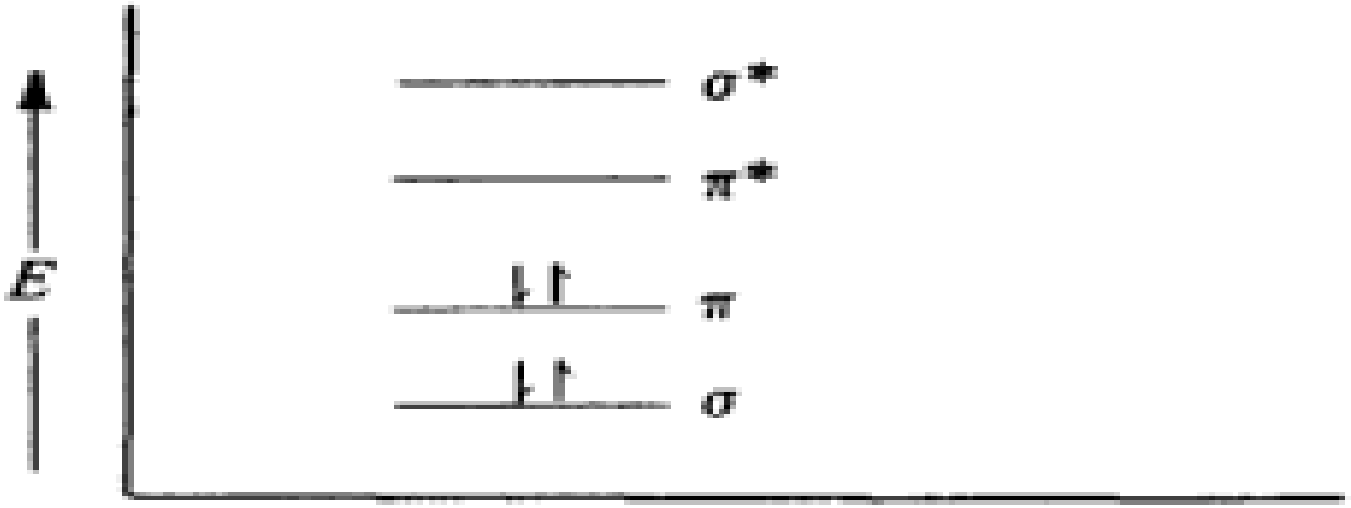
denoted as $\sigma \rightarrow \sigma^*$



Electronic molecular energy levels.

Q

The energy level diagram for ethylene is given below. What kind of electronic transitions can the highest energy electrons undergo?



A л л*

Systems responsible for the absorption of light are called chromophores or chromophoric group, Chromophores which give rise to σσ* transitions are system which contain electrons in σ molecular orbital's, Compounds containing only σ molecular orbital's are saturated organic molecules which do not contain atoms with Ione pair electrons, Examples of σ σ* type

Ch

$$\geqslant C - C \leqslant \text{ and } \geqslant C - H$$

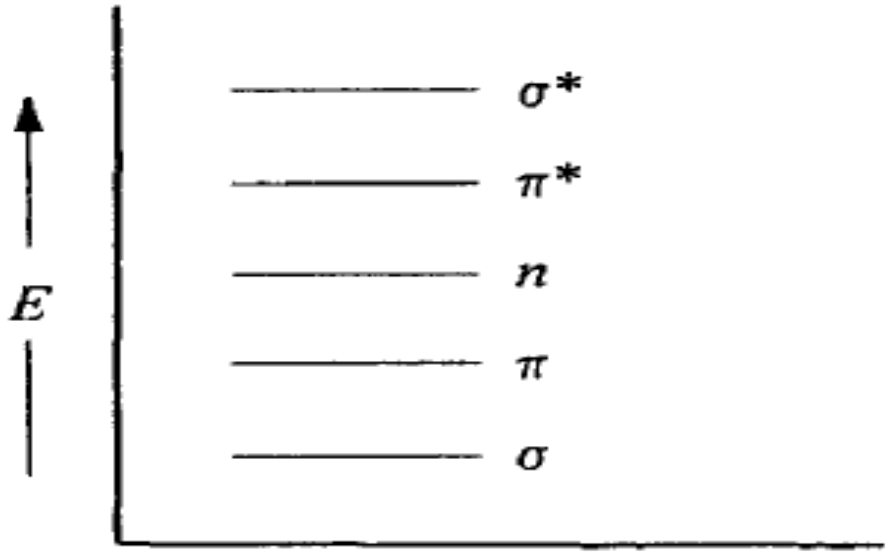
Chromophores which give rise to n σ* transitions are systems which contain electrons is non-bonding and σ molecular orbital's. Compounds containing only n and σ molecular orbital's are saturated organic molecules which contain one or more atoms with Ione pair electrons . Examples of n σ* type chromophores are

Chromophores which give rise to $\pi\pi^*$ type transitions are systems which contain electrons in π molecular orbitals, unsaturated organic compounds have $\pi\pi^*$ type chromophores are

$$>C=C< \quad \text{and} \quad -C\equiv C-$$

Q Which of the following transitions requires the most energy?

$\sigma \rightarrow \sigma^*, n \rightarrow \sigma^*, \pi \rightarrow \pi^*$



A $\sigma \rightarrow \sigma^*$ requires the most energy.

Q What types of transitions are possible in cyclopentene (C_5H_8)?

A $\sigma \rightarrow \sigma^*$

$\pi \rightarrow \pi^*$

Q What *chromophore* in cyclopentene is responsible for the lowest energy transition?

A 

The $\pi \rightarrow \pi^*$ is the lowest energy transition.

UV/Vis absorption

$\sigma \rightarrow \sigma^*$ transitions

Not observed in in normal UV/Vis work.

The absorption maxima are < 150 nm.

The energy is too great.

This type of absorption corresponds to breaking of C-C, C-H, C-O, C-X, ... bonds

UV/Vis absorption

n -> σ* transitions

The compound must contain atoms with unshared electron pairs.

Compounds containing O, S, N and halogens can absorb via this type of transition.

Absorptions are typically in the 150-260 nm region and are not very intense.

180-500 nm 225-600

$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ transitions : UV and visible

Q What is the chromophore in each of the following molecules which gives rise to the lowest energy transition?

a. 

b. CH_3OH

A

a. Chromophore $>C=C<$.
$\pi \rightarrow \pi^*$

b. Chromophore $-\ddot{O}-$.
$n \rightarrow \sigma^*$

Electronic Transitions Involving *n*, σ, and π Molecular Orbitals

Transition	Wavelength Range (nm)	Examples
$\sigma \rightarrow \sigma^*$	< 150	C—C, C—H
$n \rightarrow \sigma^*$	150–260	H_2O, CH_3OH, CH_3Cl
$\pi \rightarrow \pi^*$	180–500	C=C, C=O, C=N, C≡C
$n \rightarrow \pi^*$	225–600	C=O, C=N, N=N, N=O

Q For the three compounds given below, can the observed absorption bands be due to the same type of transition? Explain.

$CH_3 - Cl$	172 nm
$CH_3 - I$	258 nm
$CH_3 - Br$	204 nm

A Yes. All the absorptions are due to $n \rightarrow \sigma^*$ transitions. Because the electronegativity of each halogen is different, the electronic environment in each molecule will be different; this results in a different degree of interaction of molecular orbitals and, consequently, a difference in energy between the n and σ^* states.

Cl 17 2-8-7

Br 35 2-8-18-7

I 53 2-8-18-18-7

كثافة اليكترونية

Molecular absorption

Absorbing species

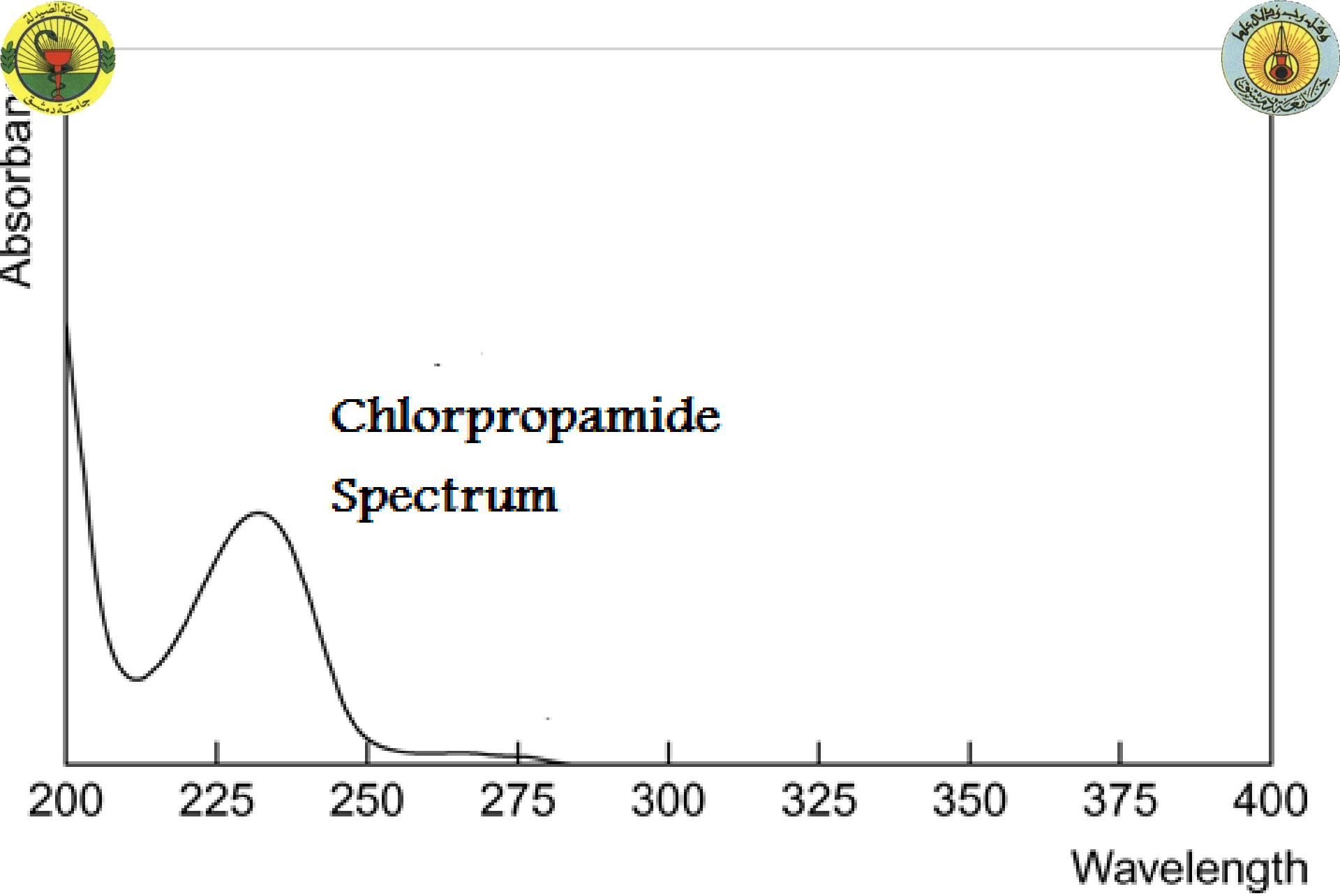
UV/Vis

We are dealing with electronic transitions.

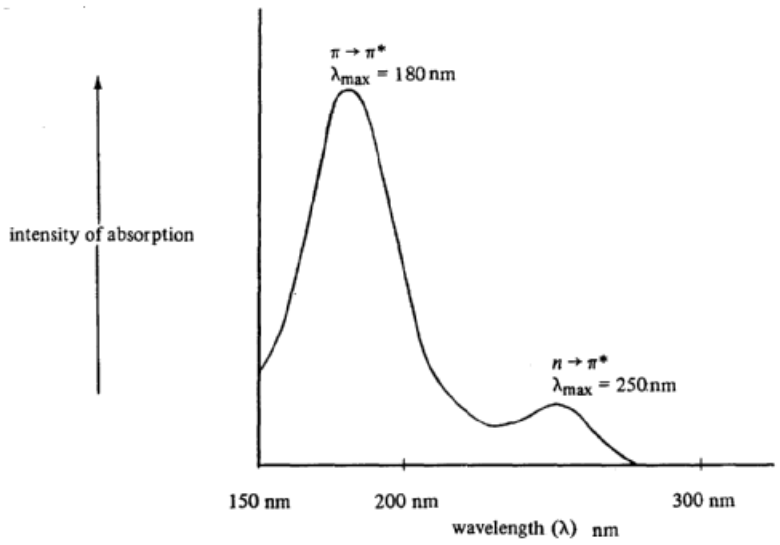
Due to the large number of vibrational and rotational states, the spectra appear as bands.

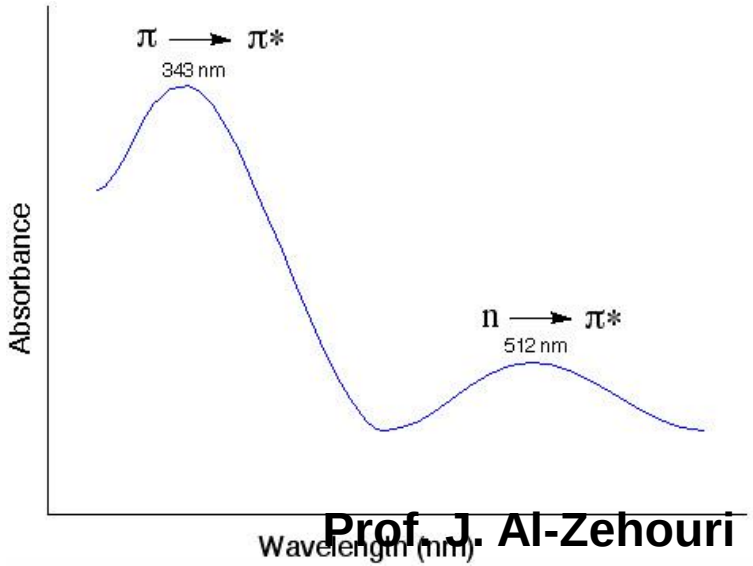
Absorbance spectrum

A graph of a sample ُs absorbance of electromagnetic radiation versus wavelength.


Absorbance
Chlorpropamide
Spectrum
200
225
250
275
300
325
350
375
400
Wavelength

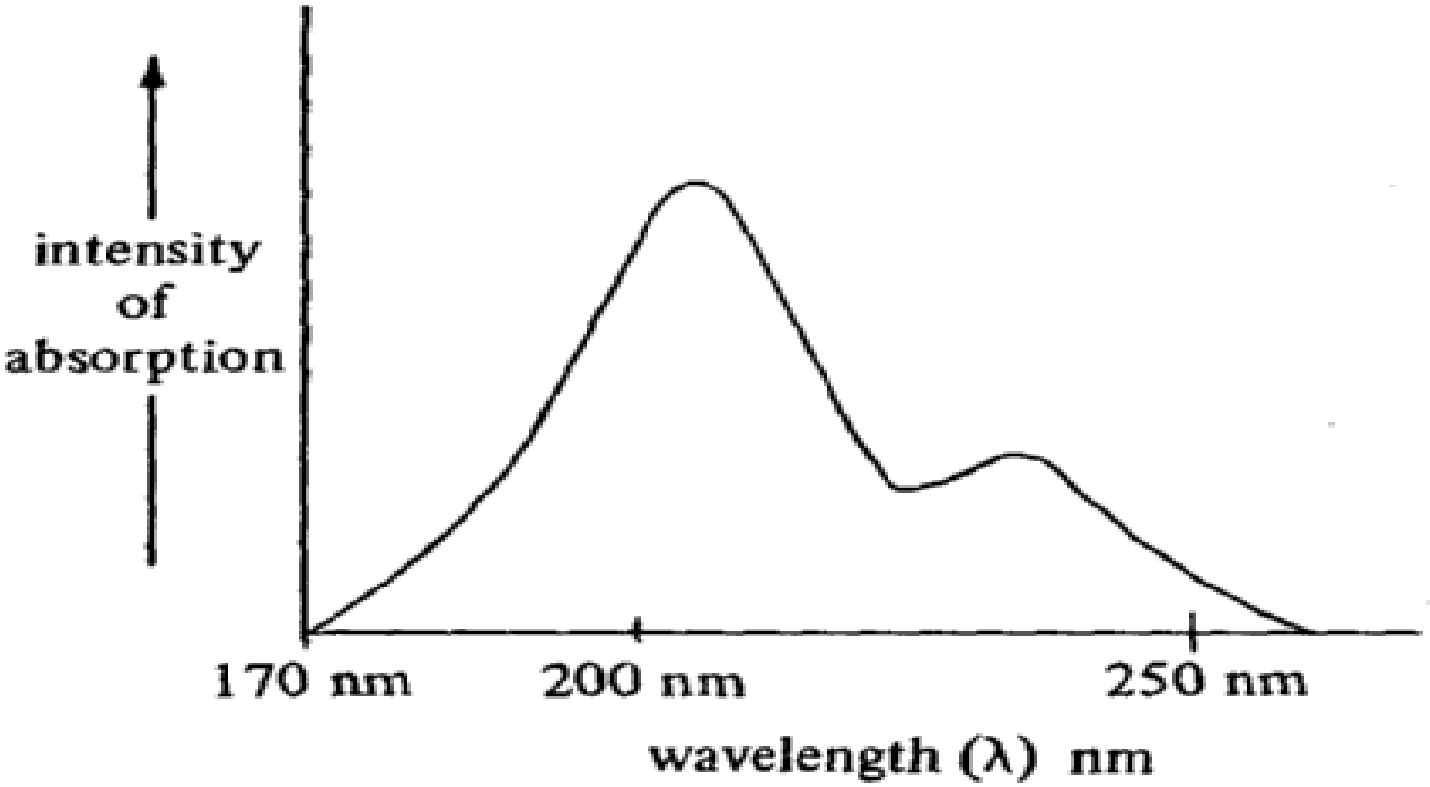
intensity of the absorption due to a ππ* type transition is always 10 to 100 times more intense than nπ* type absorptions. The spectrum of a compound which has both ππ* and nπ* transition is shown below. The position of maximum absorption of each band (called λ_{max}) corresponds to the wavelength of light necessary for the transitions. The width of the bands is, in part, due to instrumentation.





Prof. J. Al-Zehouri

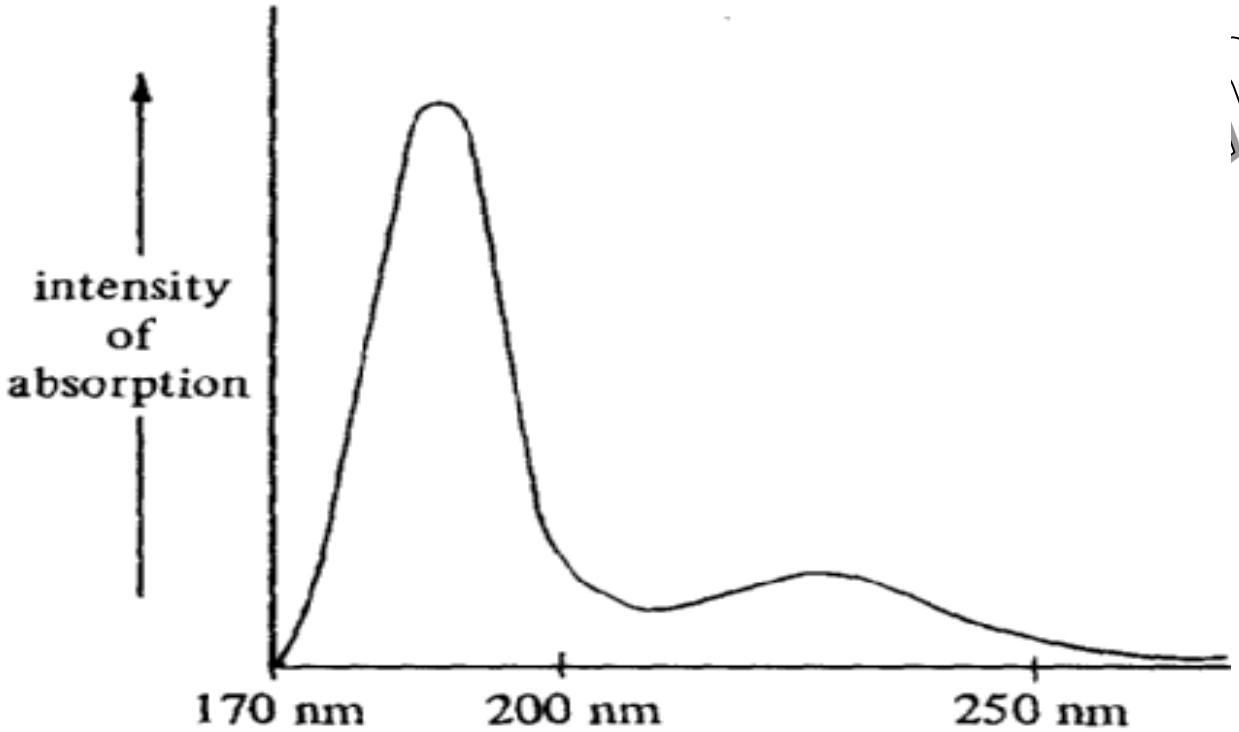
Q For the following spectrum, indicate the λ_{max} for each peak.



A $\lambda_{max} = 205$ nm.

$\lambda_{max} = 235$ nm.

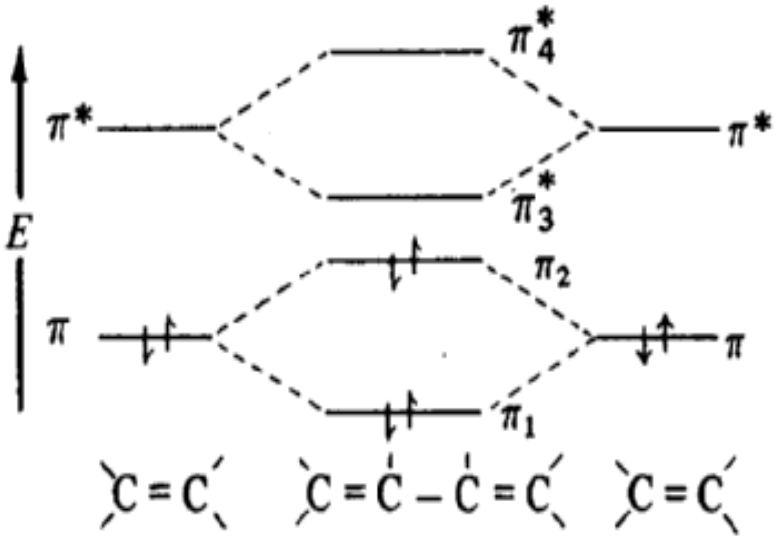
Q The spectrum for a compound which has both $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions is given below. Give λ_{max} for the band resulting from each type of transition.



A $n \rightarrow \pi^* \lambda_{max} = 230$ nm.

$\pi \rightarrow \pi^* \lambda_{max} = 190$ nm.

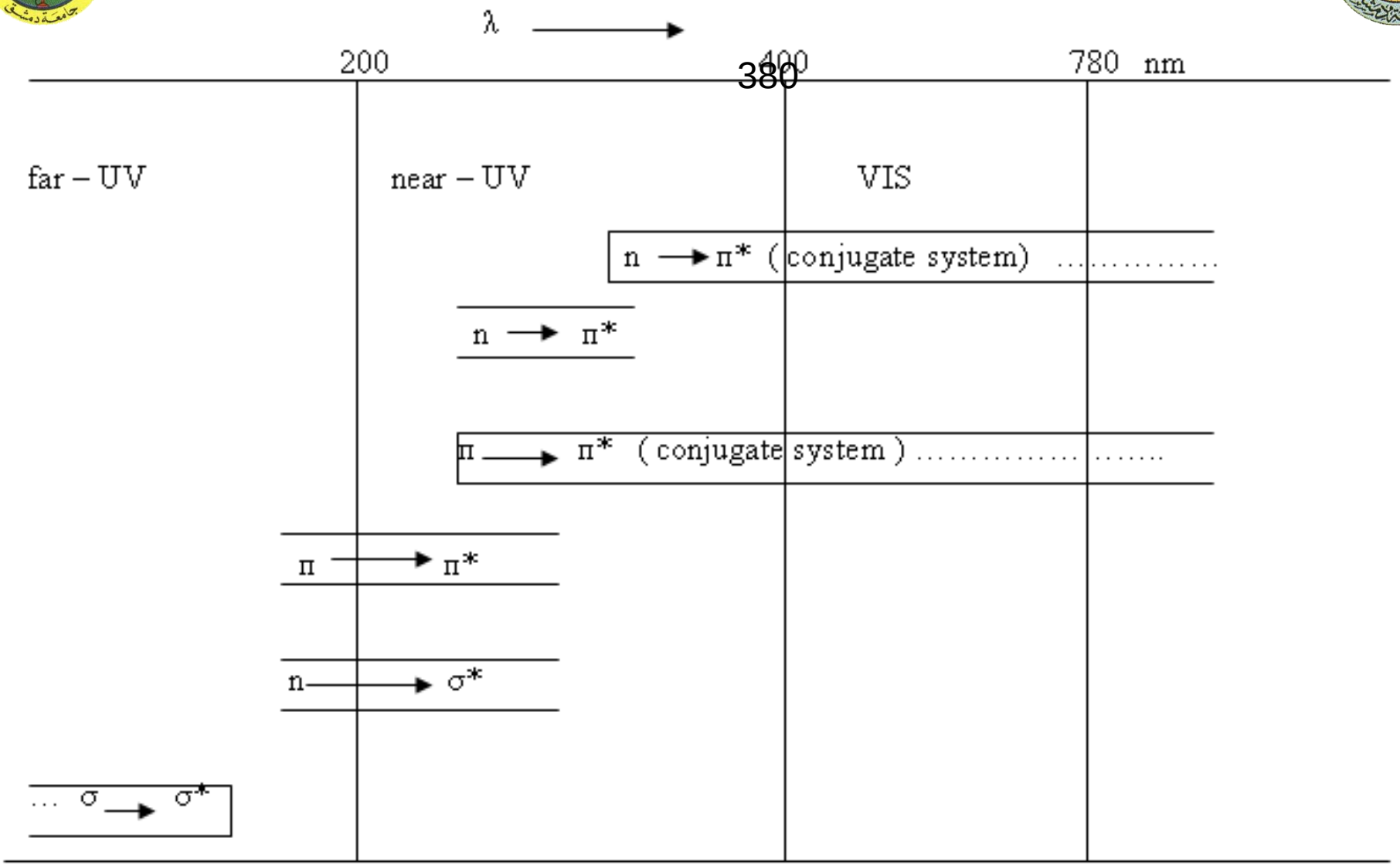
In conjugated systems, such as $C=C-C=C$, π orbitals from each double bond interact to form a new set of bonding and anti-bonding orbitals. This interaction is illustrated below.



التأثير الترافقي

تداخل المدارات π, π* يؤدي لتكوين مدارات جديدة بحيث تصبح المدارات π أقرب للمدارات π * مقارنة بالوضع قبل التبادل وهذا يعني أن طاقة الأنتقال ل ππ* تصبح أقل عما كانت عليه قبل التبادل

As the conjugated system in a molecule becomes longer (involves more atoms with π bonds), the difference in energy between the ground states and the excited states for the $\pi \rightarrow \pi^*$ transitions becomes less. Consequently, as the conjugated system increases in length the energy required for a $\pi \rightarrow \pi^*$ transition becomes less and absorption will occur at longer wavelength.



Absorption ranges of different electronic transition

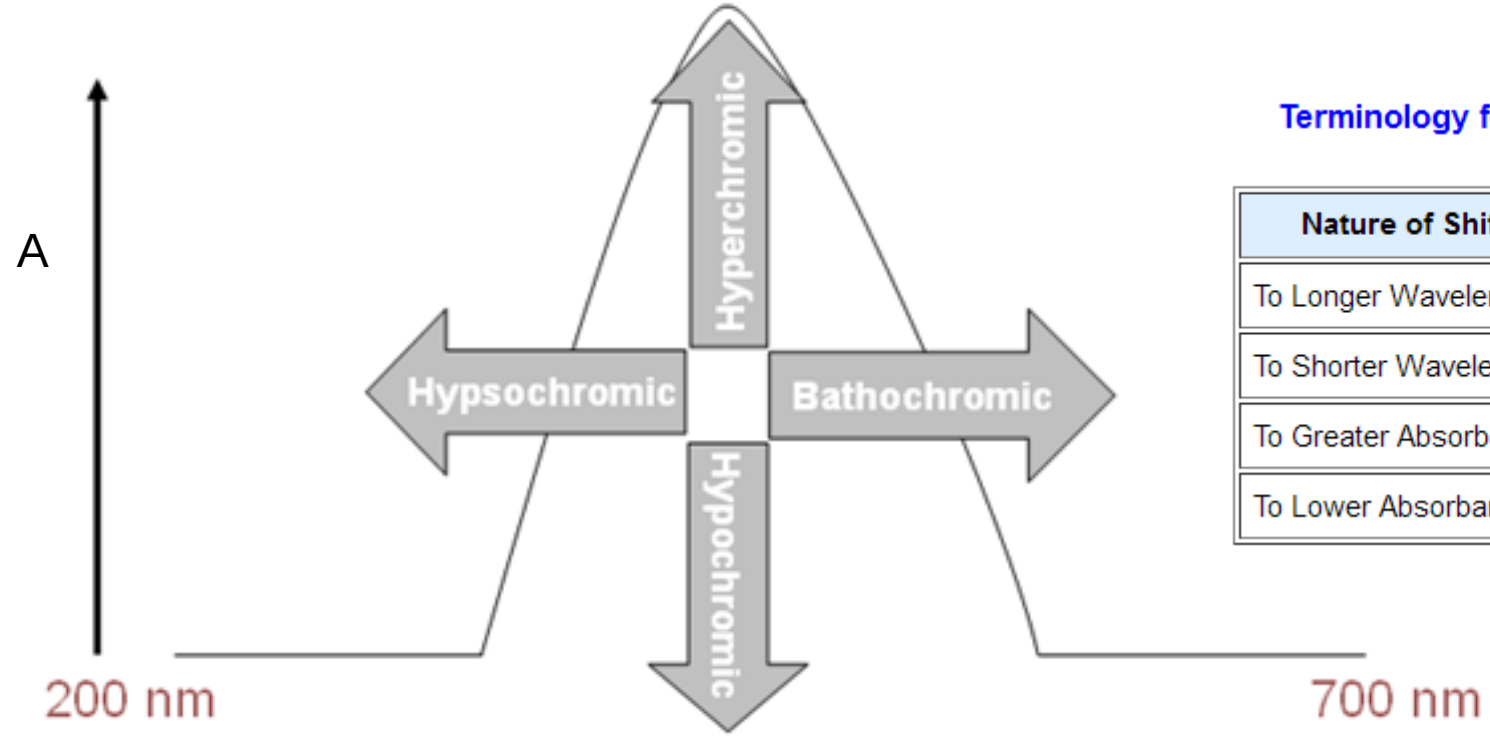
Terminology for absorption shifts

i. Bathochromic shift (red shift) – a shift to longer λ; lower energy

ii. Hypsochromic shift (blue shift) – shift to shorter λ; higher energy

iii. Hyperchromic effect – an increase in intensity

iv. Hypochromic effect – a decrease in intensity



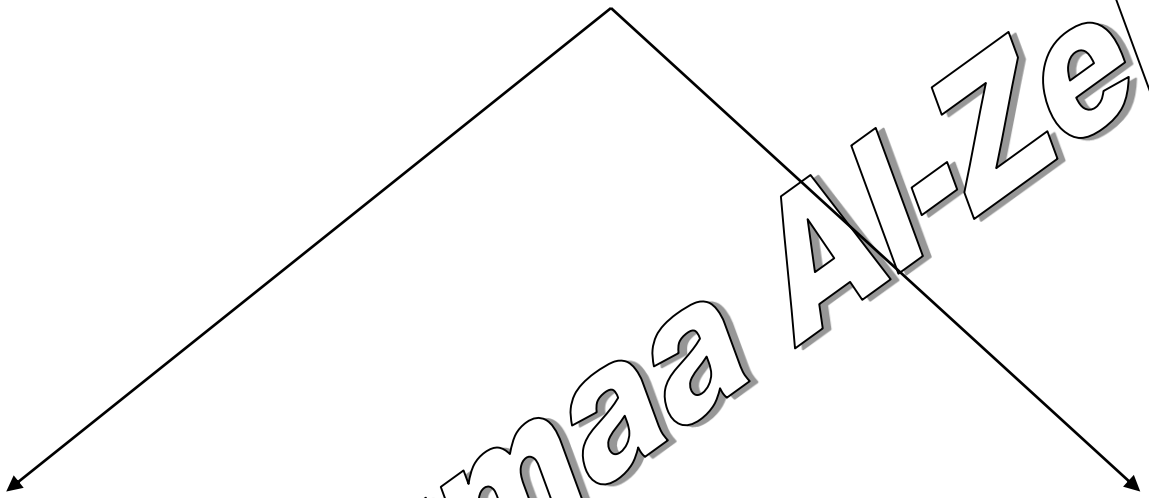
Terminology for Absorption Shifts

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

المذيبات المستخدمة في التحليل الطيفي

- ***اهم الشروط الواجب توافرها في المذيبات المستخدمة في تحضير المحاليل أن لا تمتص الضوء في المجال الطيفي الذي تقاس عنده العينة .***
- ***تستخدم المذيبات الهيدروكربونية المشبعة في المجال فوق البنفسجي***
- ***يستخدم الماء كمذيب للمواد غير العضوية في المجال المرئي***
- ***أختيار المذ يب المناسب مهم جداَ في المجال فوق البنفسجي لأنه قد يؤثر على طيف الأمتصاص نتيجة للتفاعل الذي قد يحدث بينه وبين المادةالمذابة .***

Factors which effect on the Spectra

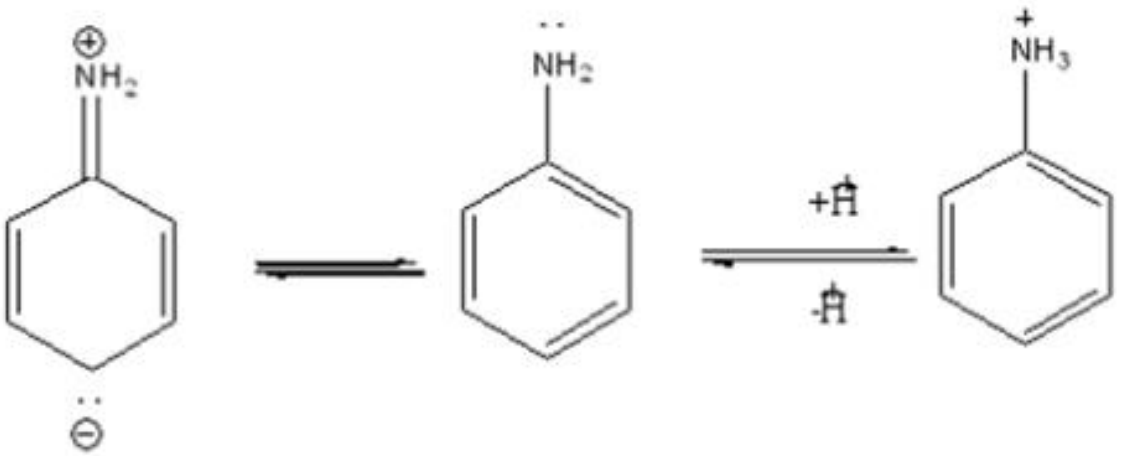


Solvent effect

pH-effect

line

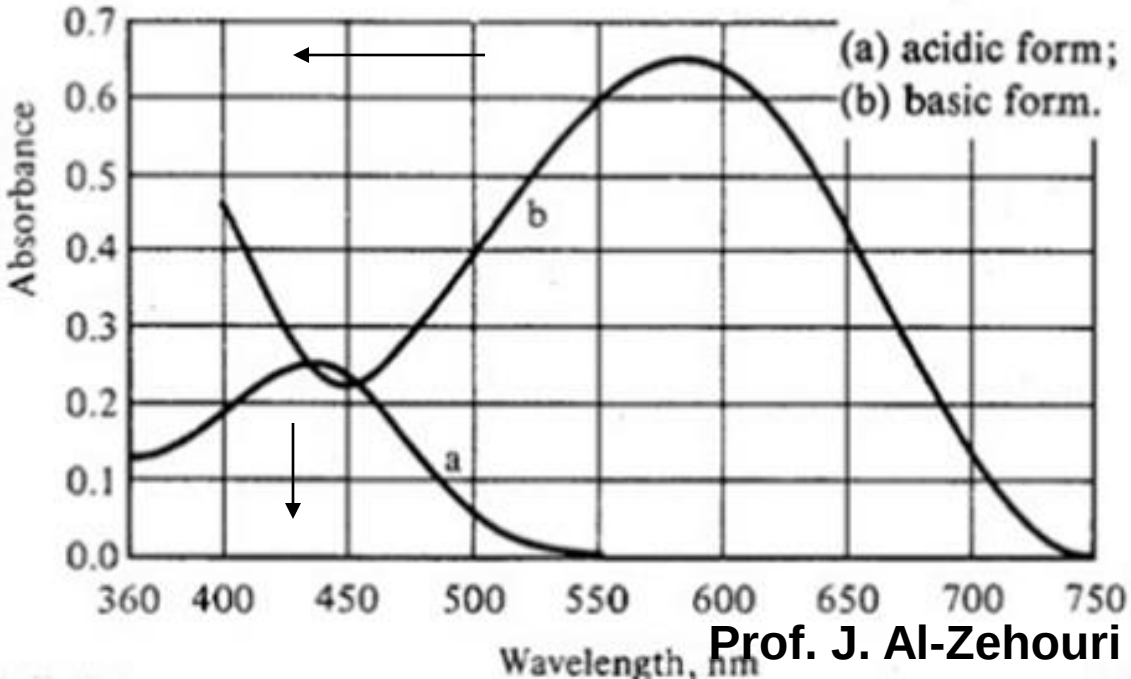
pH –effect



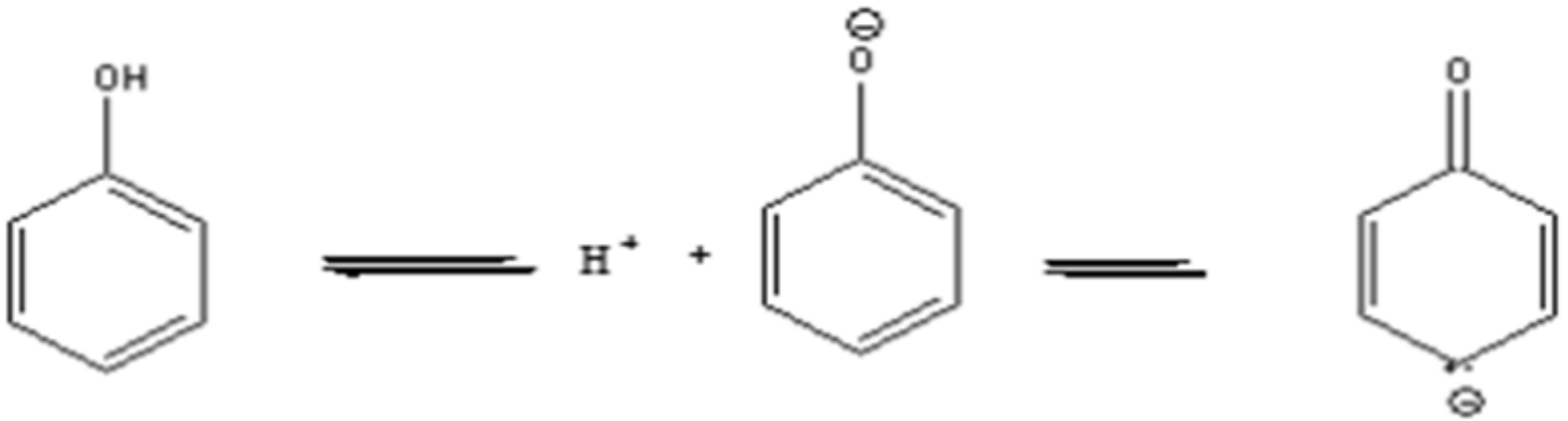
alkaline medium

acid medium

Acid medium shows Hypsochromic shift and Hypochromic effect



Phenol pH –effect



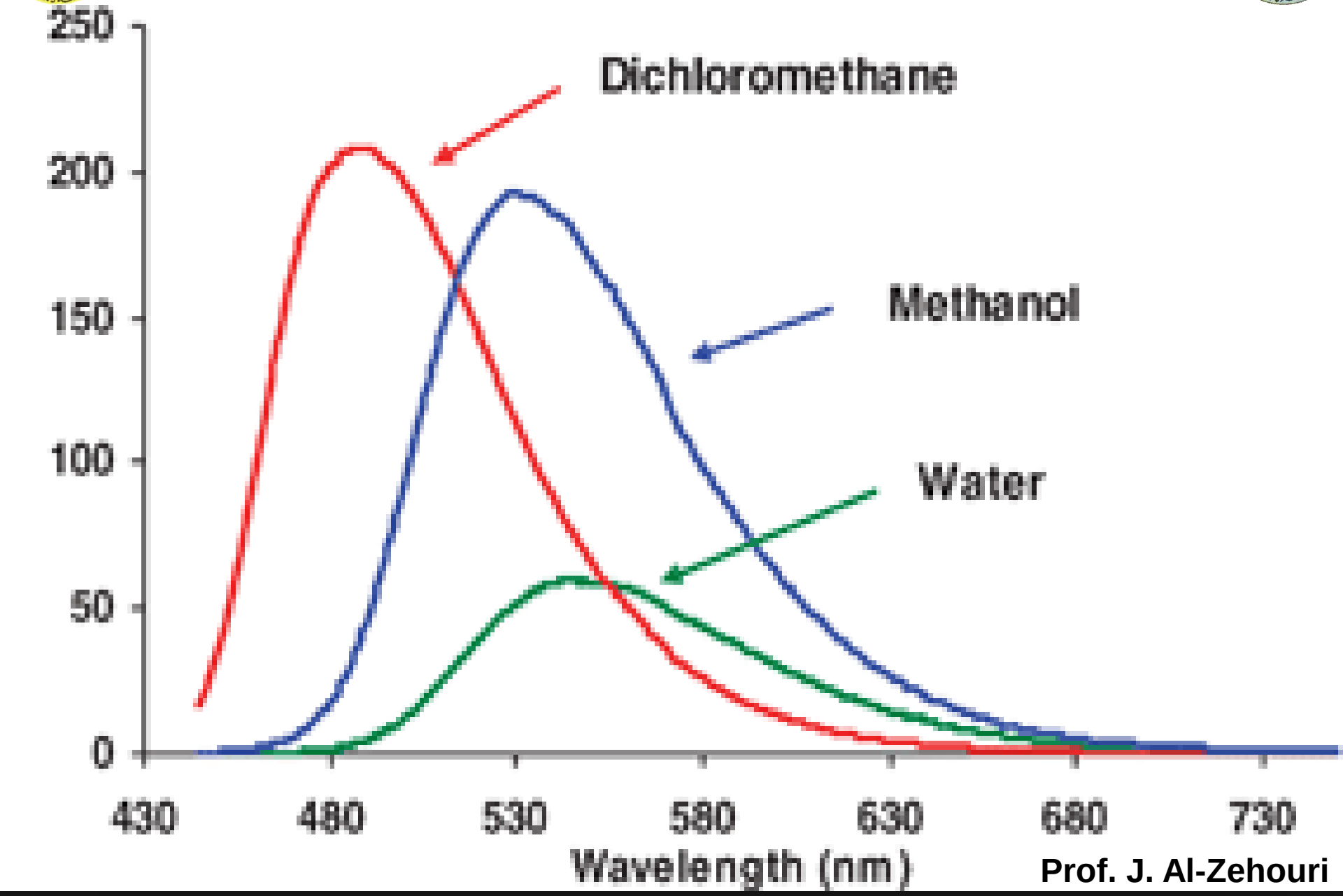
acid medium

alkaline medium

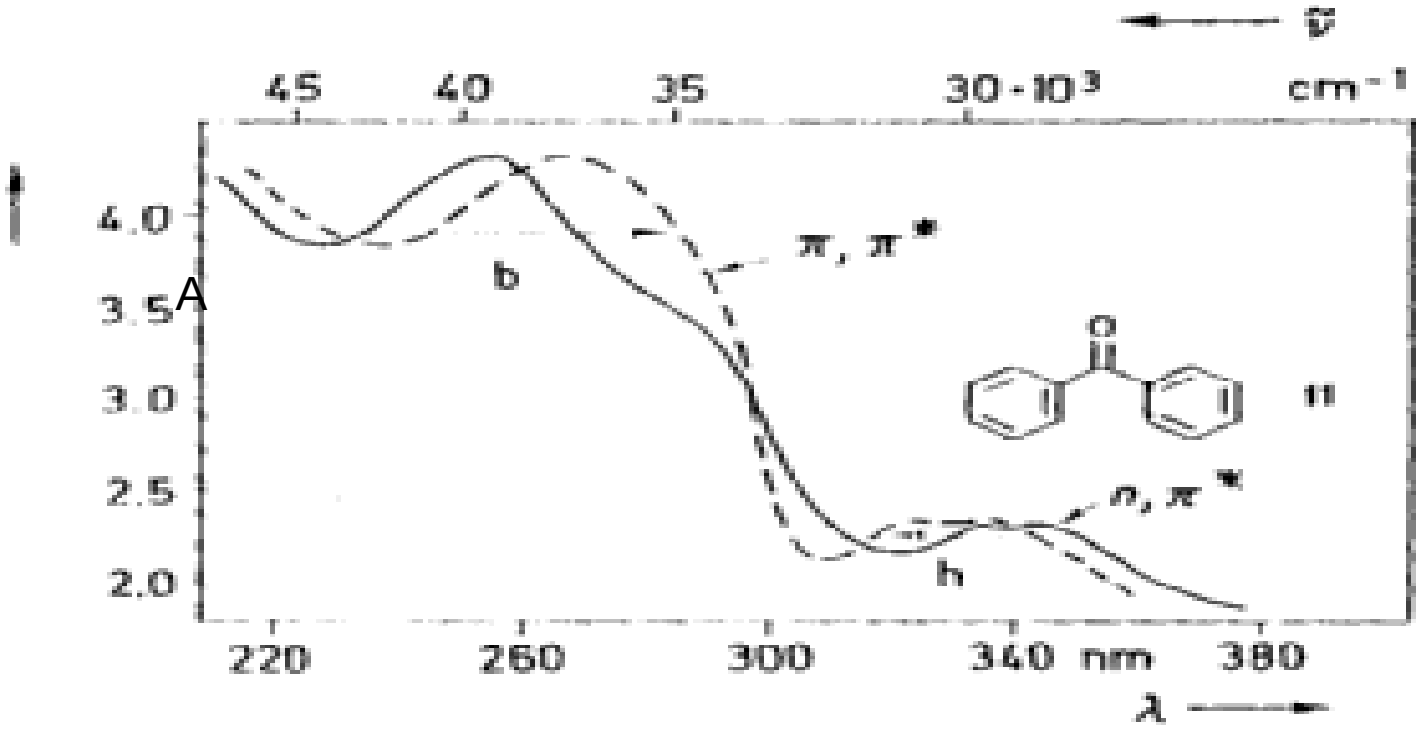
Alkaline medium shows Bathochromic shift and Hypochromic effect

254 nm
270 nm

Solvent effect



Absorption spectra of Benzophenon



——— in Cyclohexan

– – – – in Ethanol

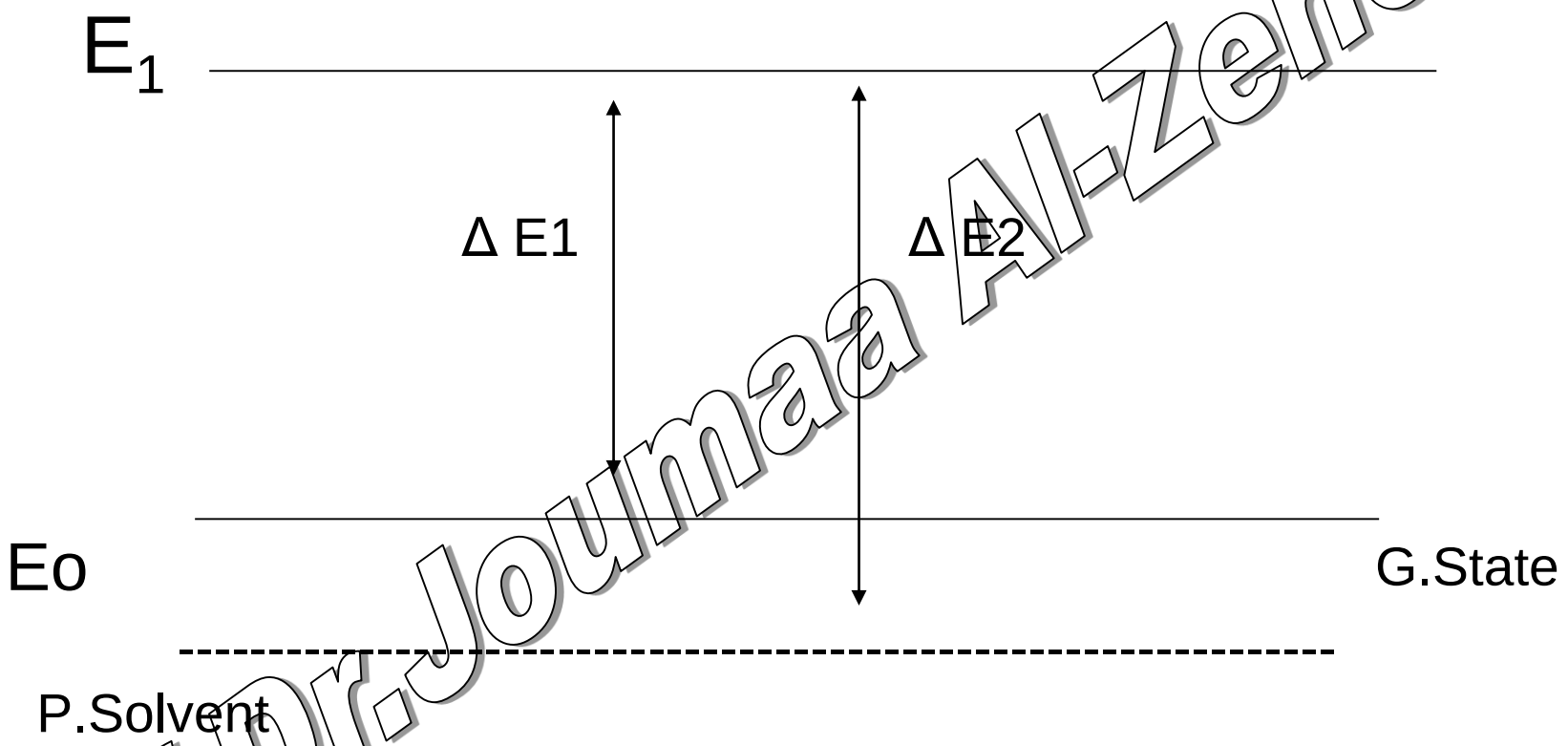
b Bathochromic effect (with increase the polarity)

h Hypsochromic effect (with increase the polarity)

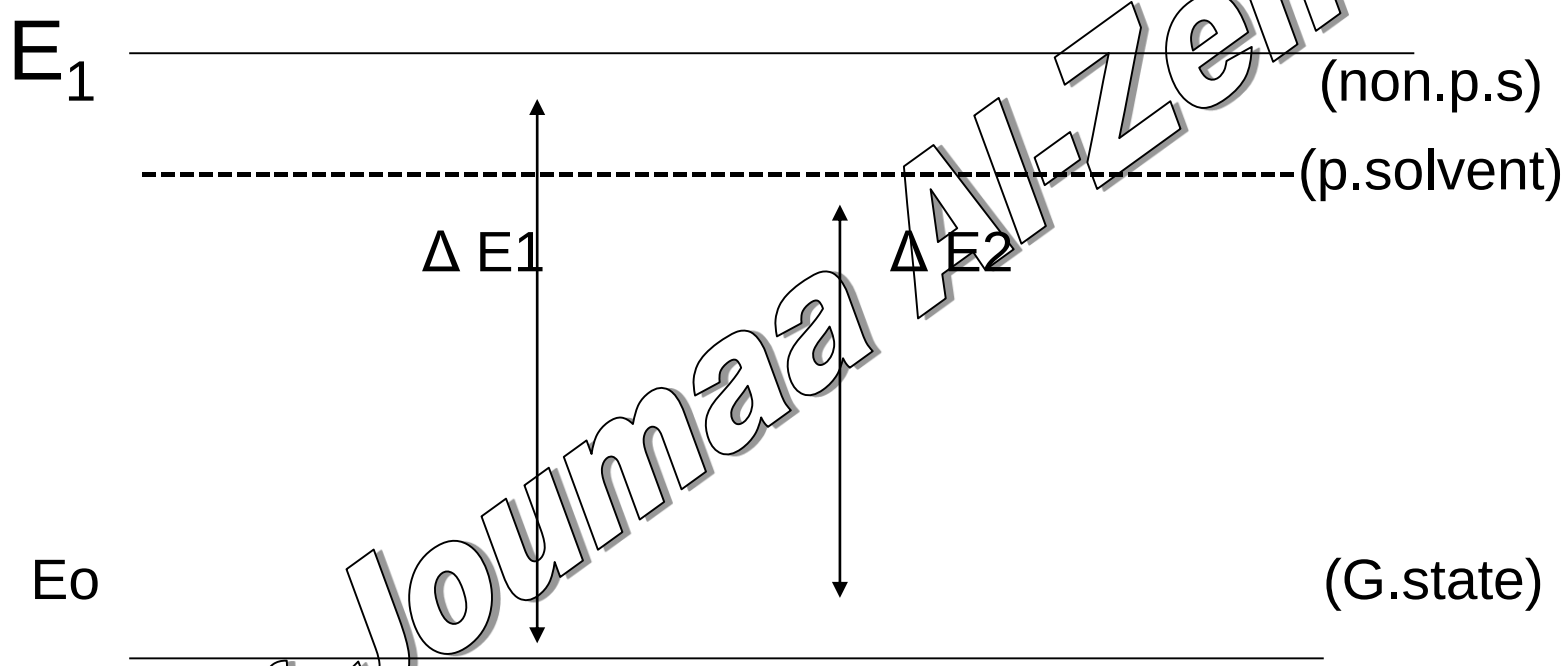
Result

- Solvent polarity ↑ → ↓ λ max by nπ*
- Solvent polarity ↑ → ↑ λ max by ππ*

Effect of polarity at n π*



Effect of polarity at π π*



Molecular absorption methods

UV/Vis quantitative analysis

The major use for this spectral region.

Wide applicability

- thousands of methods
- most common type of assay

High sensitivity

- detection limits of 10^{-4} - 10^{-6} M

Molecular absorption methods

Quantitative Analysis

Reasonably selective

Relatively specific methods can or have been developed.

Significant efforts have been made to apply the approach to a wide range of materials.

Good accuracy - 1 - 5% range

Relative simple and inexpensive

$$Log\ 1/T = A$$

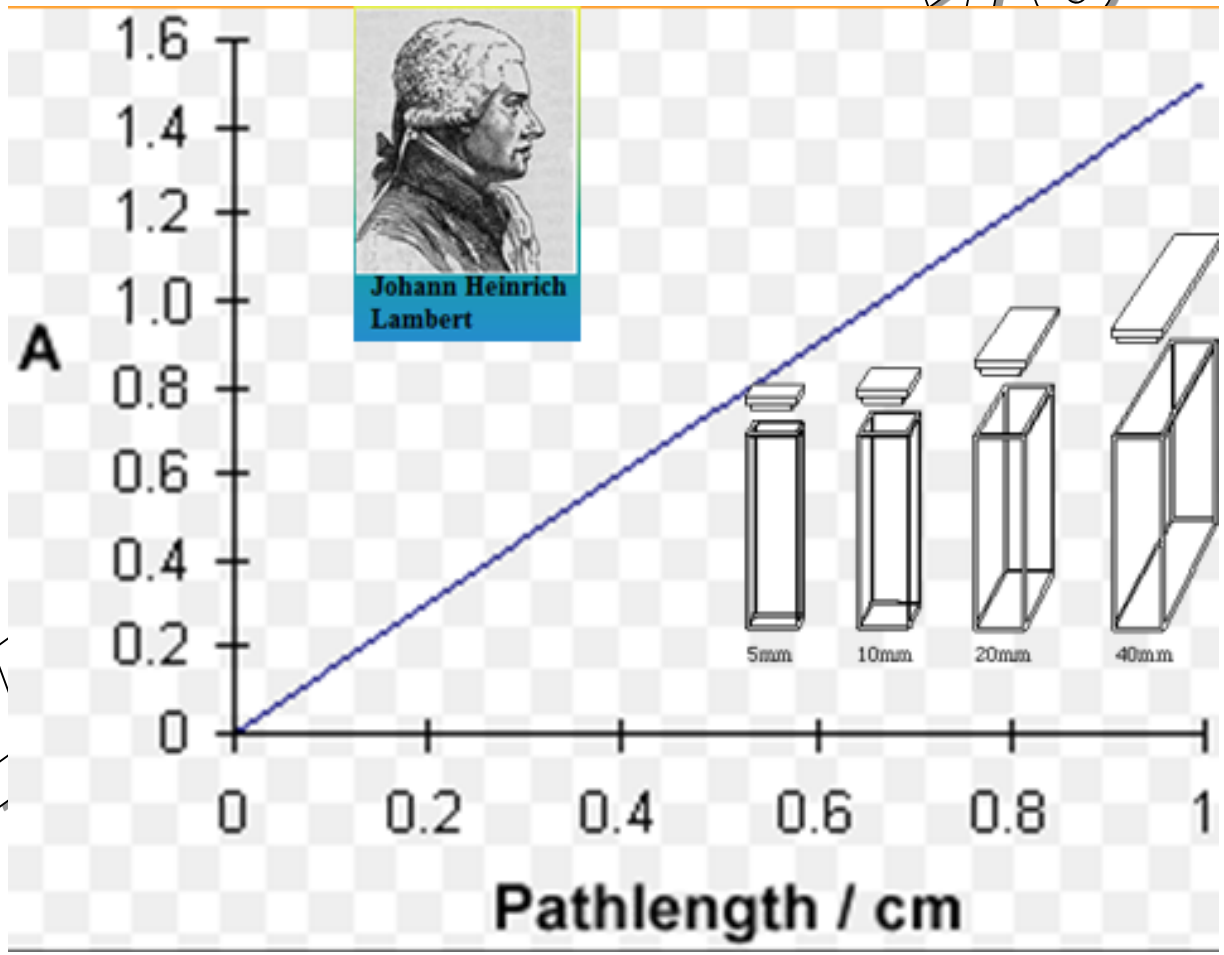
$$A = I_0 - I \quad \text{or} \quad P_0 - P$$

$$T = \frac{I}{I_0} = \frac{P}{P_0}$$

ann Heinrich **Lambert** (France 1760) :

Lambert's law stated that absorbance of a material sample is directly proportional to its thickness (path length)

Aα b

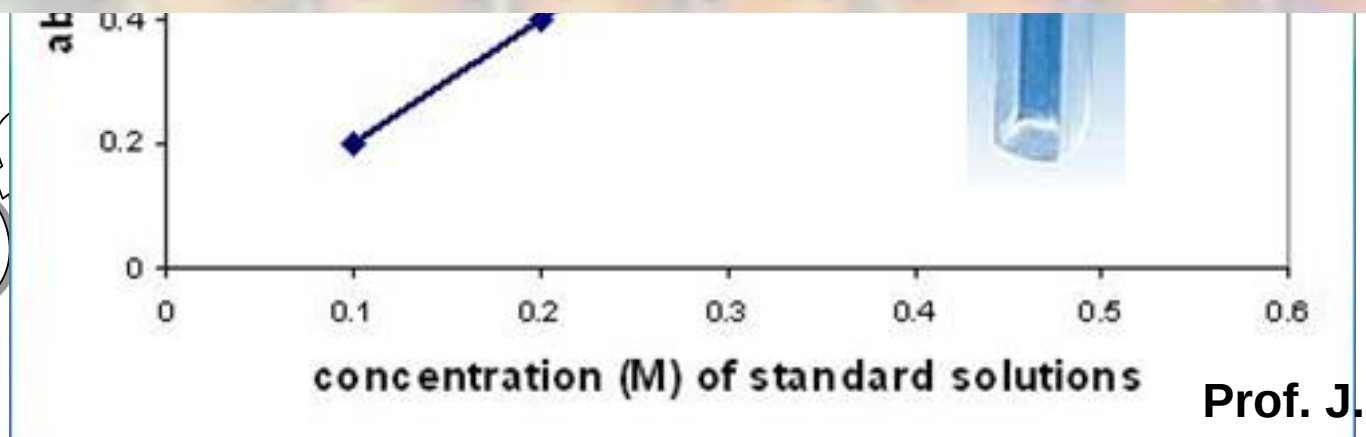


C = constant

Prof. J. Al-Zehouri

ugust Beer (German) 1852

Beer's law stated that absorbance is proportional to the concentrations of material sample.



mbert Beer s Law

Aα b (Lambert)

A α C (beer)

A α C α b

A = constant X C X b

harmonious

A = constant X Concentration

A(1%,1cm) g%

ε mol/l

A(1%,1cm)=Absorption of 1 g dissolved in 100 ml using 1 cm cell at certain wave length

ε= Absorption of M.W dissolved in 1000 ml at certain wave length

Sample cells

standard liquid cuvette

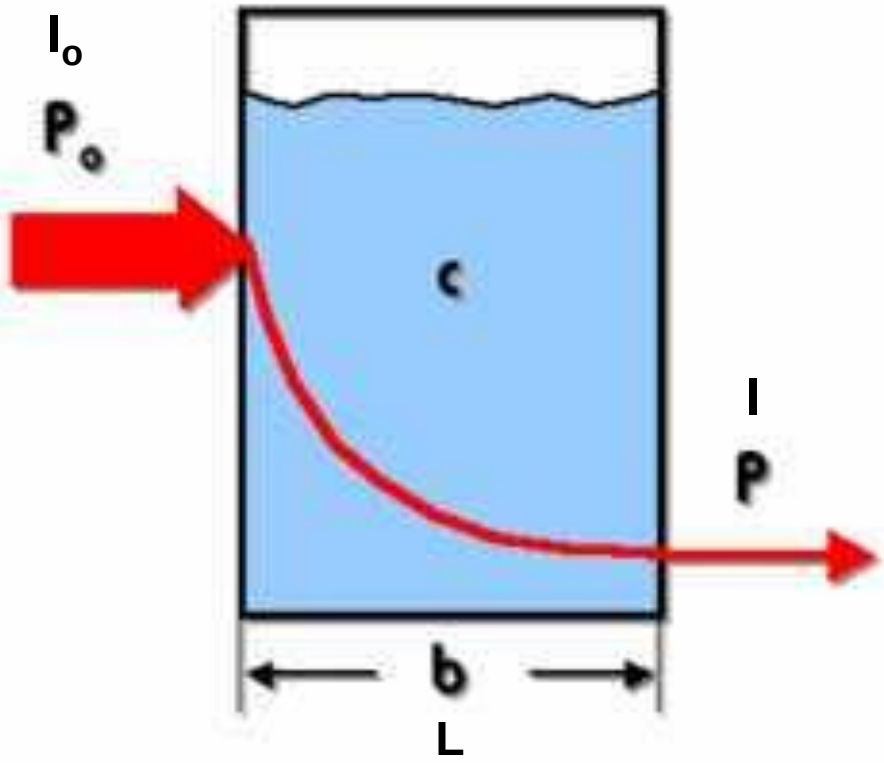
liquid sandwiched between two NaCl plates for IR

sample cell for gases

Prof.J.Al-Zehouri

Quantitative Analysis

$\frac{P}{P_o}$ is a measure of the light that passes through the solution - transmitted



$\frac{P}{P_o}$ is also called the transmittance (T)

$-\log(T) = A$

A - absorbance

$A = abc$

Lambert-Beer Law

- $-\log P/P_o = \varepsilon c b (L)$
- P = transmitted light (Radiation), Power=Transmitted intensity, I
- P_o = Incident light (radiation), Power=Incident intensity ,I_o
- C = concentration in moles/l
- ε = molar absorptive= Molar extinction coefficient ,k
- b (L, d) = Path length (thickness) length of cell through which light passes.

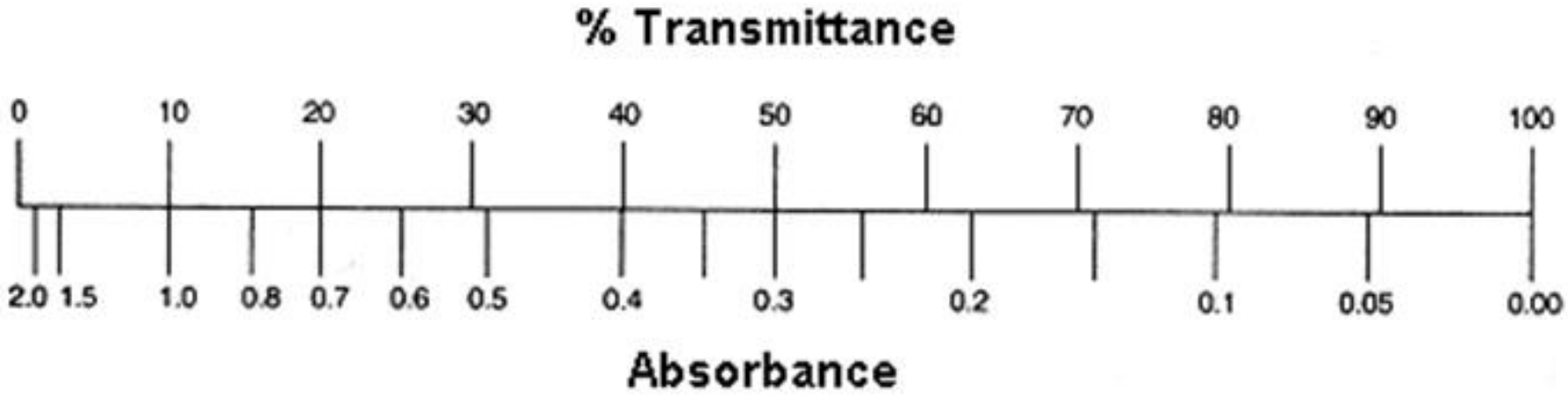
Absorbance vs. Transmittance

$$A = \log_{10} P_0 / P$$

$$A = \log_{10} 1 / T$$

$$A = \log_{10} 100 / \%T$$

$$A = 2 - \log_{10} \%T$$



Transmittance T = Optical density D = Extinction E

Summary of equations

$$-\log \frac{P}{P_o} = abc$$

$$\log \frac{P_o}{P} = abc$$

$$-\log(T) = abc$$

$$-\log(T) = A$$

$$A = abc$$

$$A = \varepsilon bc$$

Example one

Calculate the absorbance of a solution having a %T of 89 at 400 nm.

%T = T x 100 so:

$$T = 89 / 100 = 0.89$$

$$A = -\log(T) = -\log(0.89)$$

$$= 0.051$$

Example 2

If the % transmission of a solution is 90% at 310 nm what is the absorbance at that wavelength?

A. $-\log p/p_o$ = Absorbance

$-\log 0.90$ = Absorbance

0.05 = Absorbance

All conditions must be held constant.

Variations in solvent, temperature, pH, time of reaction and other factors used to prepare the sample can alter the complex and how/where it absorbs.

Instrumental conditions must also be the same of all standards and unknowns - wavelength, slits, cuvettes, ...

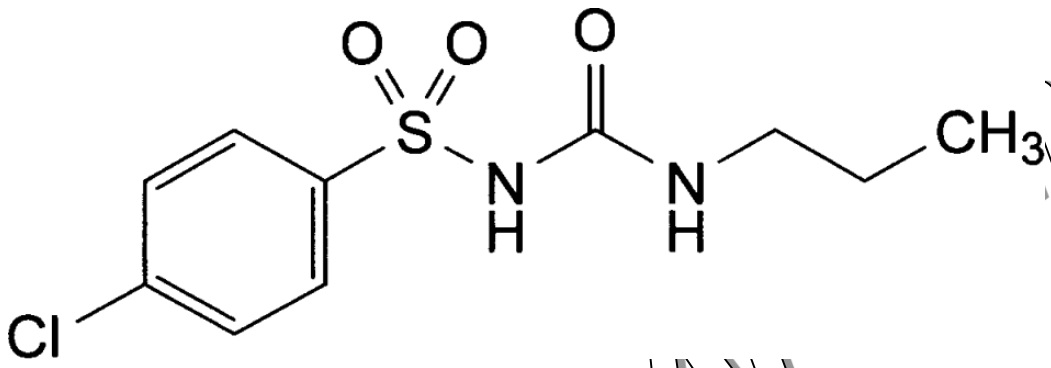
Biochemistry Example assay

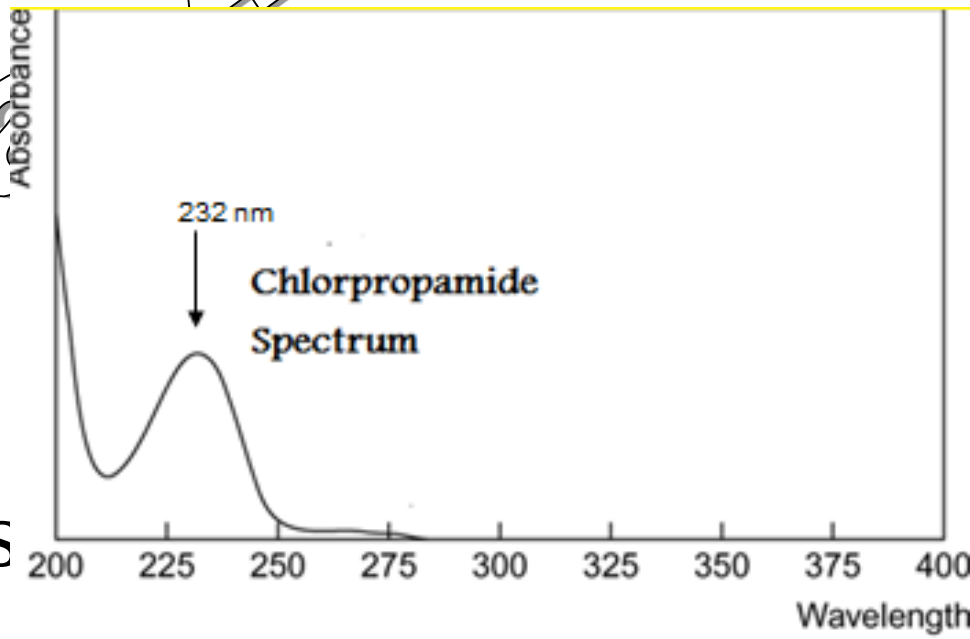
Q. Throw the determination of Glucose in patient serum we found the sample absorption 0.85 and the standard absorption (100mg/dl) 0.36 at 500 nm.

What is the Glucose concentration.

A. 0.85 / 0.36 X 100 = 236 mg/dl

Chlorpropamide





Action and use

Hypoglycaemic.

Preparation

Chlorpropamide Tablets

Chloropropamide Tablets 100 mg

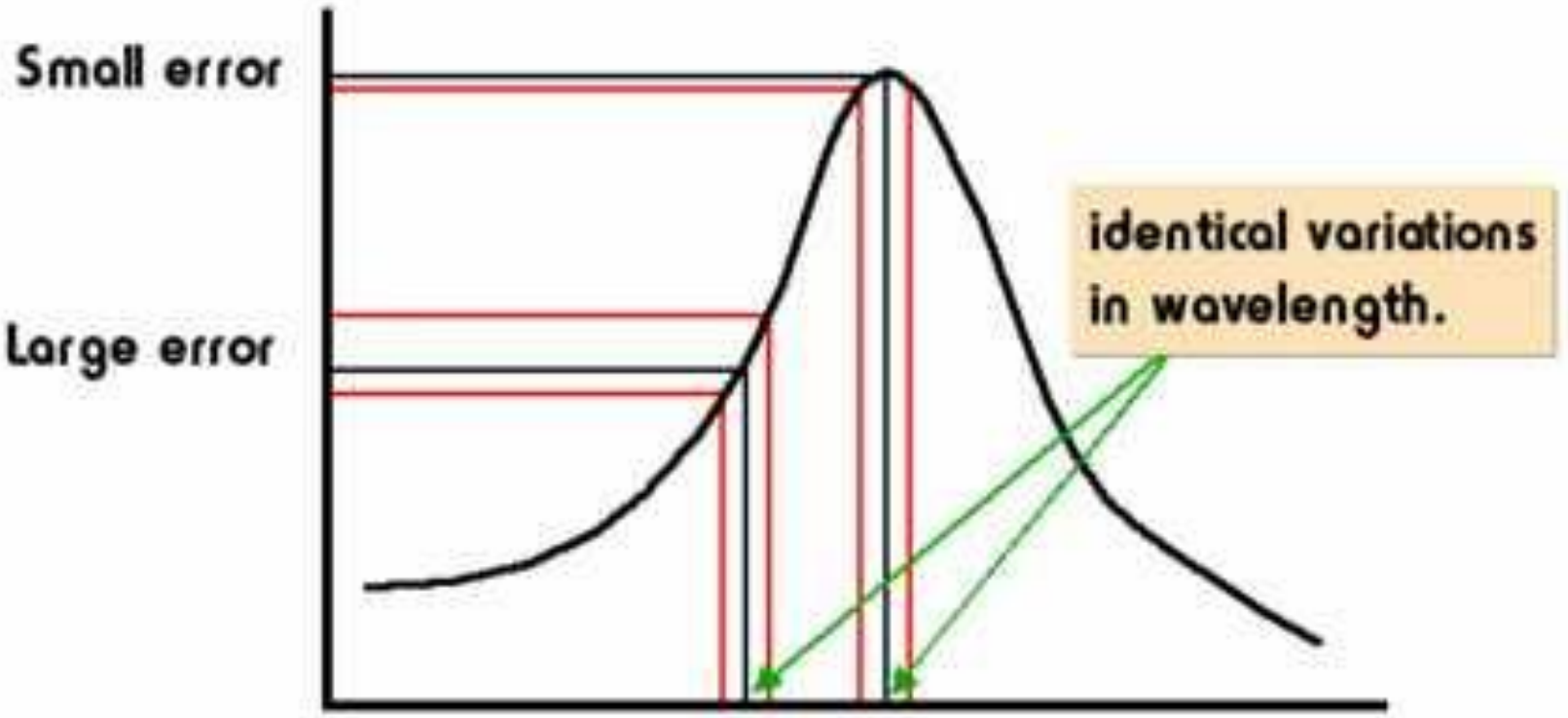
Assay : Weigh and powder 20 tablets, Shake a quantity of the powder containing 0.25 g of Chlorpropamide with 40 ml of methanol for 20 minutes, add sufficient methanol to produce 50 ml, mix ,filter and dilute 5 ml of the filtrate to 100 ml with 0.1 M hydrochloric acid. Dilute 10 ml of this solution to 250 ml with 0.1 M hydrochloric acid and measure the absorbance of the resulting solution at the maximum at 232 nm, appendix II B .Calculate the content of Chloropropamide taking 598 as the value of A(1%,1cm) at the maximum at 232 nm.

Chloropropamide

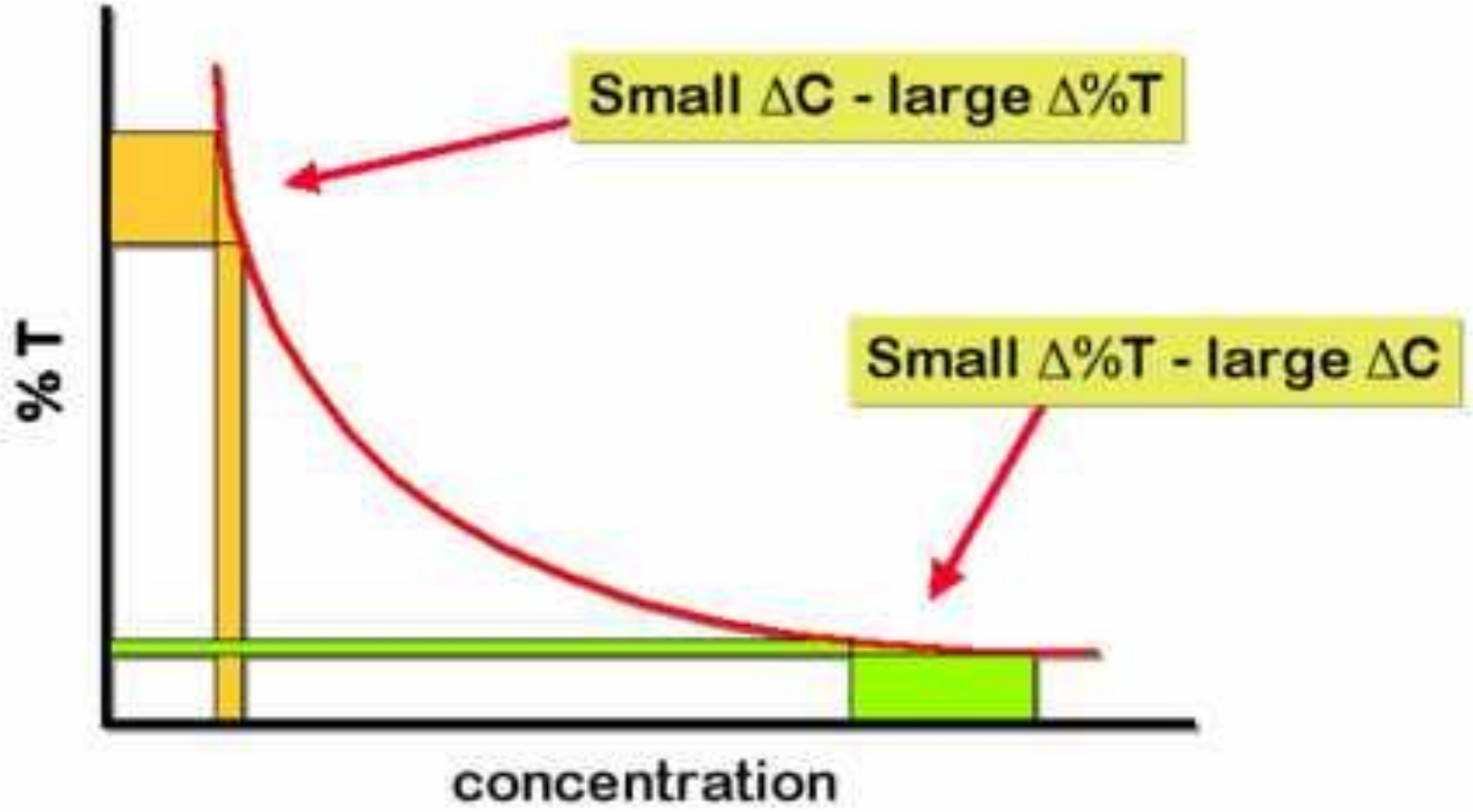
- Suppose the average weight for 1 tablet 300 mg .What is the weight tacked?
- If the abs. = 0.61 ,What is the Concentration of Chloropopamide.?
- What is the tablet content ?

Quantitative analysis

You want to make your measurement at a λ_{max} to minimize errors and achieve maximum sensitivity.



Measuring absorbance



Measuring absorbance

Errors may also be made during the measurement of absorbance.

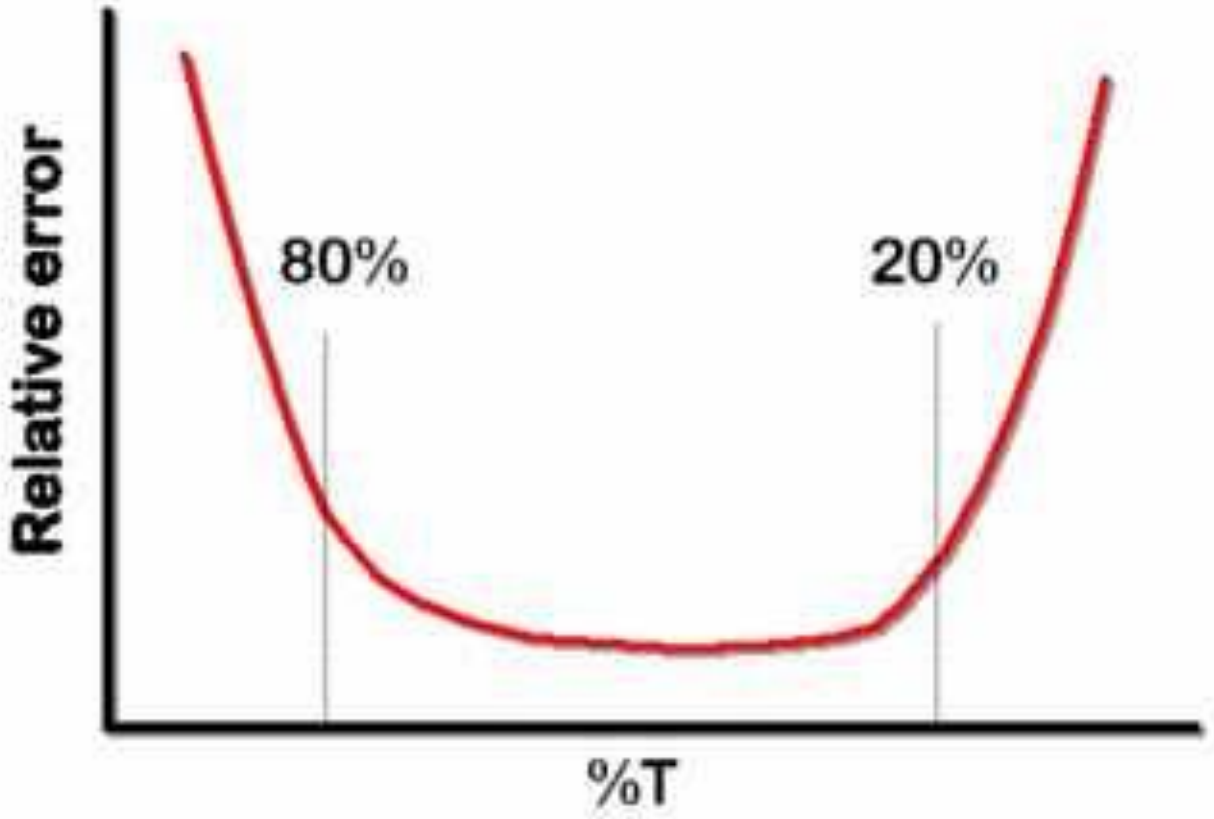
At low c - a small change in concentration can result in a large change in %T.

At high c - changes in %T are very small.

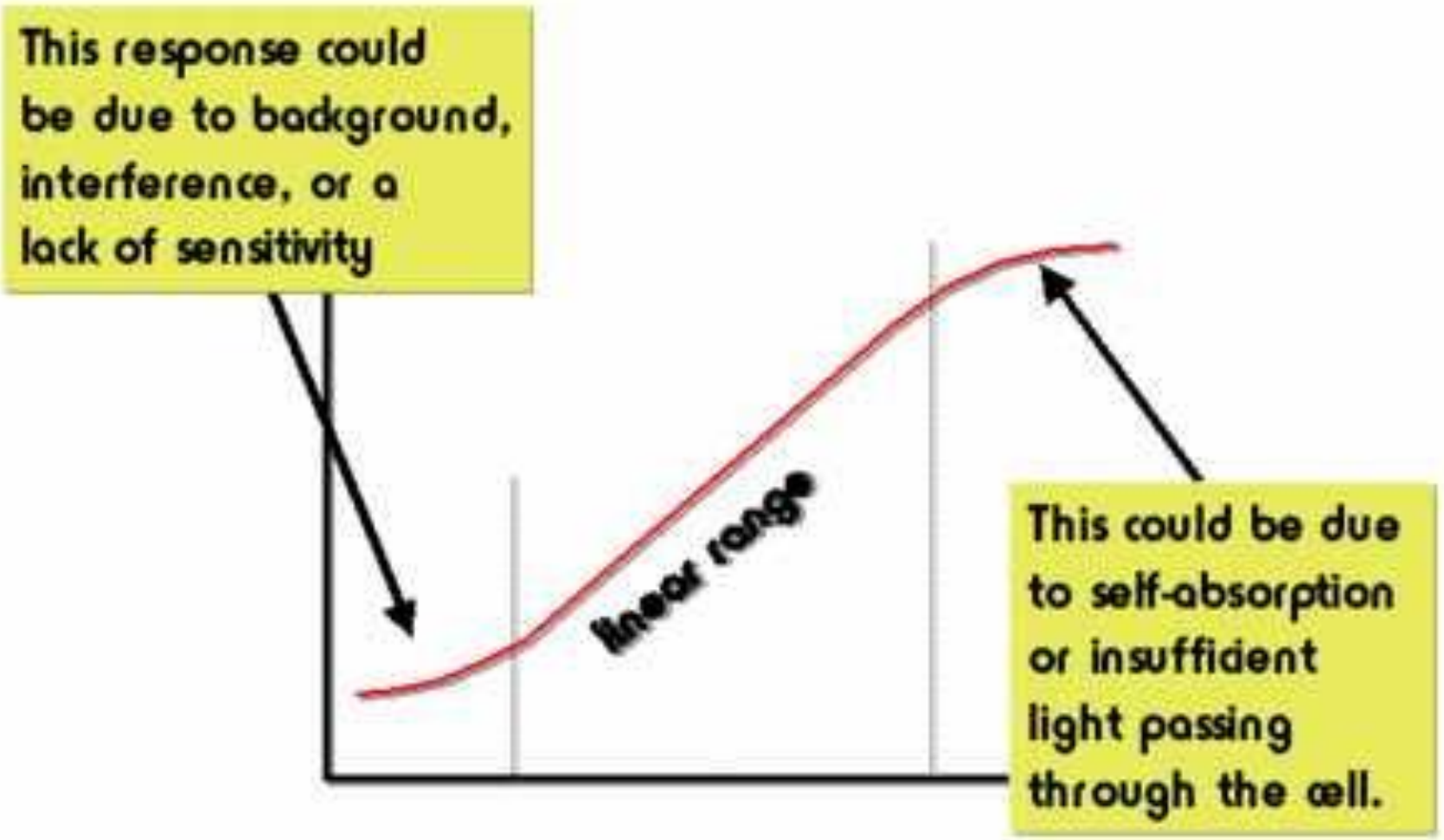
It is best to stay in a range of 80-20%T to minimize measurement errors.

Measuring absorbance.

Relative error vs. %T

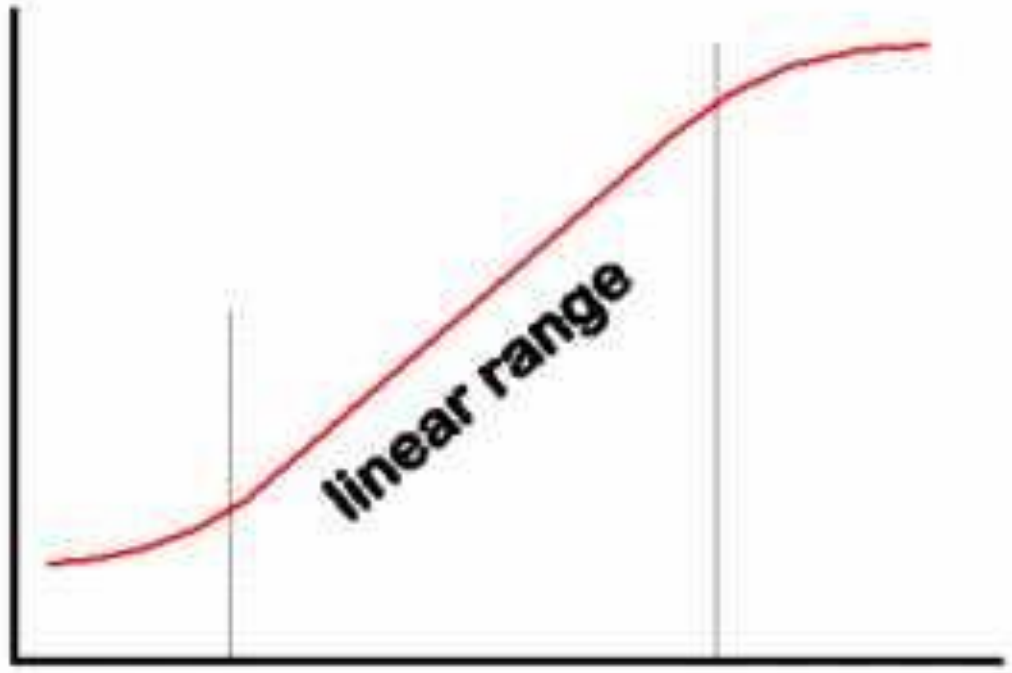


Measuring absorbance



Quantitative analysis

The relationship between concentration and absorption must be established.



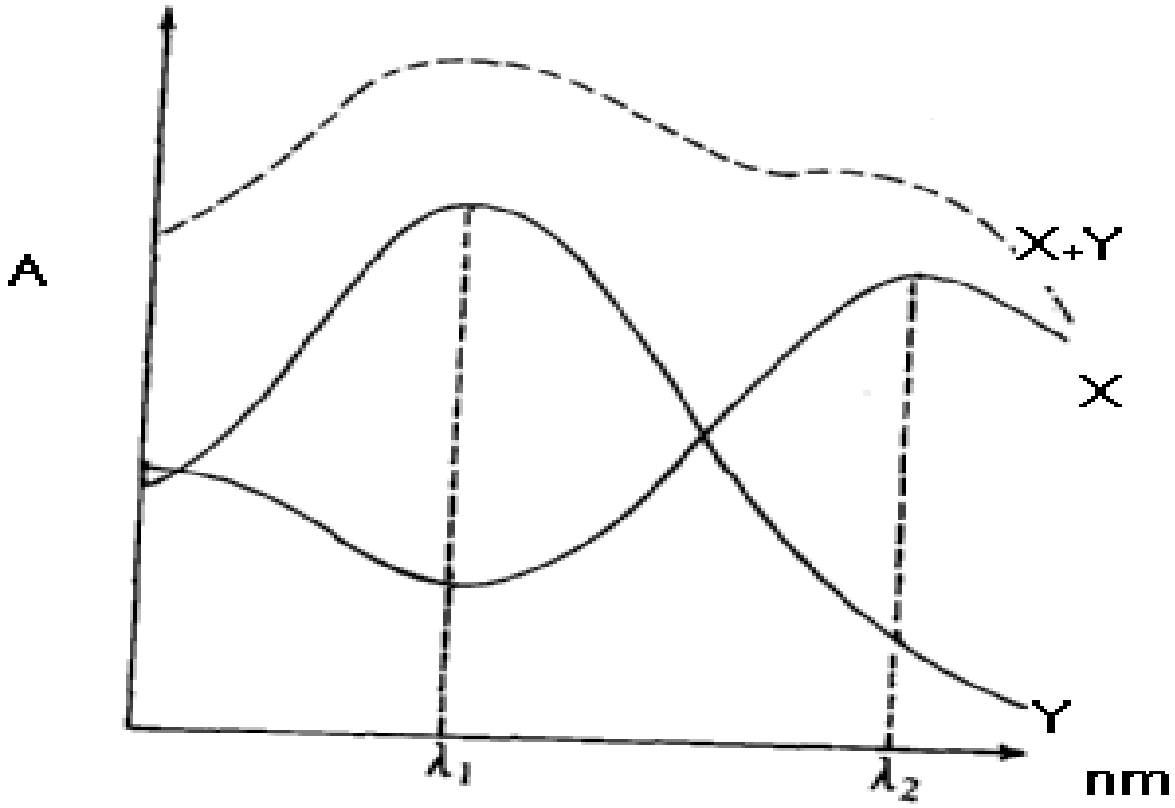
Your method should only be used in this range.

Beer ُs Law and Multicomponent Samples

Beer ُs law can be extended to samples containing several absorbing components provided that three are no interactions between the components.

For a two-component mixture of X and Y , the total absorbance A_{tot}, is :

$A_{tot} = Ax + Ay$



Measuring multiple species

When two or more species absorb light at the same wavelength, the resulting absorbance is the sum of all absorbances.

$$A_T = a_1 b_1 c_1 + a_2 b_2 c_2$$

Since they are in the same sampling cell, then:

$$A_T = (a_1 c_1 + a_2 c_2)\, b$$

In order to determine both the nickel and cobalt in a water sample using optical absorption, the use of standard solution of cobalt concentration 0.15 mol /'s, and another nickel in the same concentration, then determine the absorption of each solution separately values, as well as the sample mixture solution at a wavelength of 390 nm and 510 nm. If you know that the absorption values were as follows:

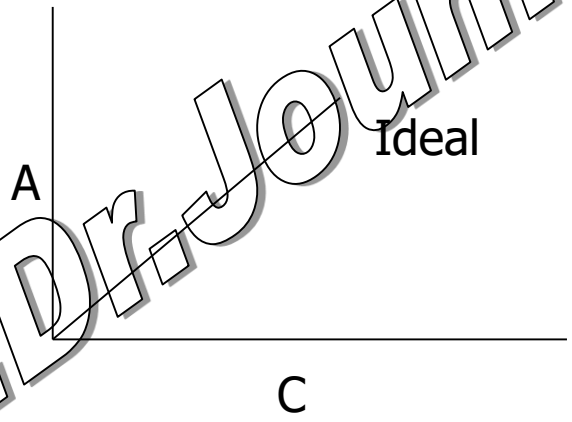
- ***absorption of cobalt standard solution at 390 nm = 0.02***
- ***absorption of cobalt standard solution at 510 nm = 0.62***
- ***absorption of Nickel standard solution at 390 nm = 0.74***
- ***absorption of Nickel standard solution at 510 nm = 0.054***
- ***absorption of Mixture solution at 390 nm = 0.88***
- ***absorption of solution at 510 nm = 0.410***

Required :

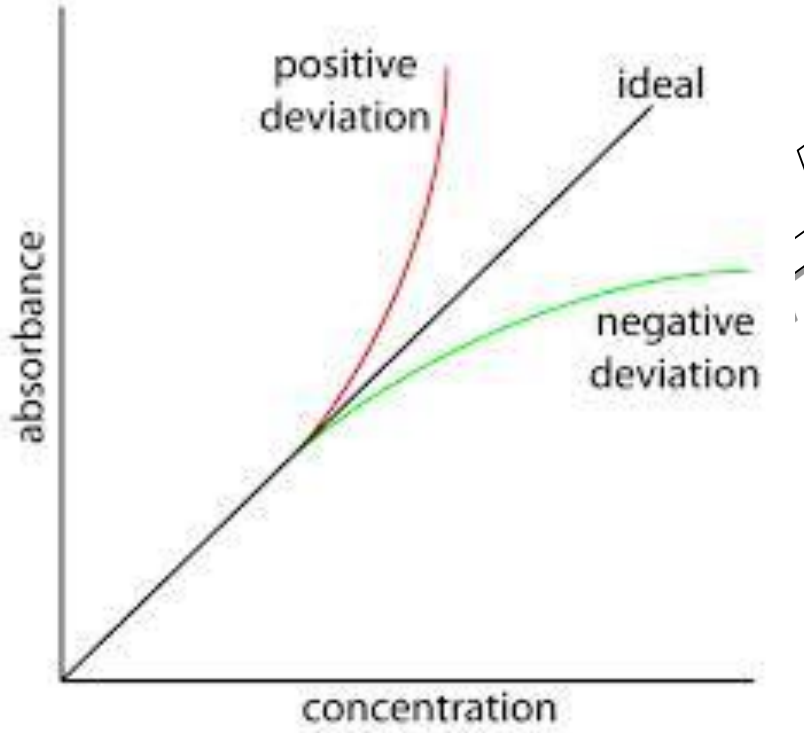
Determine the molar concentration of each of cobalt and nickel in the sample mixture. Required :

Limitations to Beer ُs Law

According to Beer ُs law , a calibration curve of absorbance versus the concentration of analyte in a series of standard solutions should be a straight line .



Deviations from linearity



- Fundamental Limitation to Beers Law
- Chemical Limitation to Beers Law
- Instrumental Limitations to Beer s Law

Fundamental Limitations

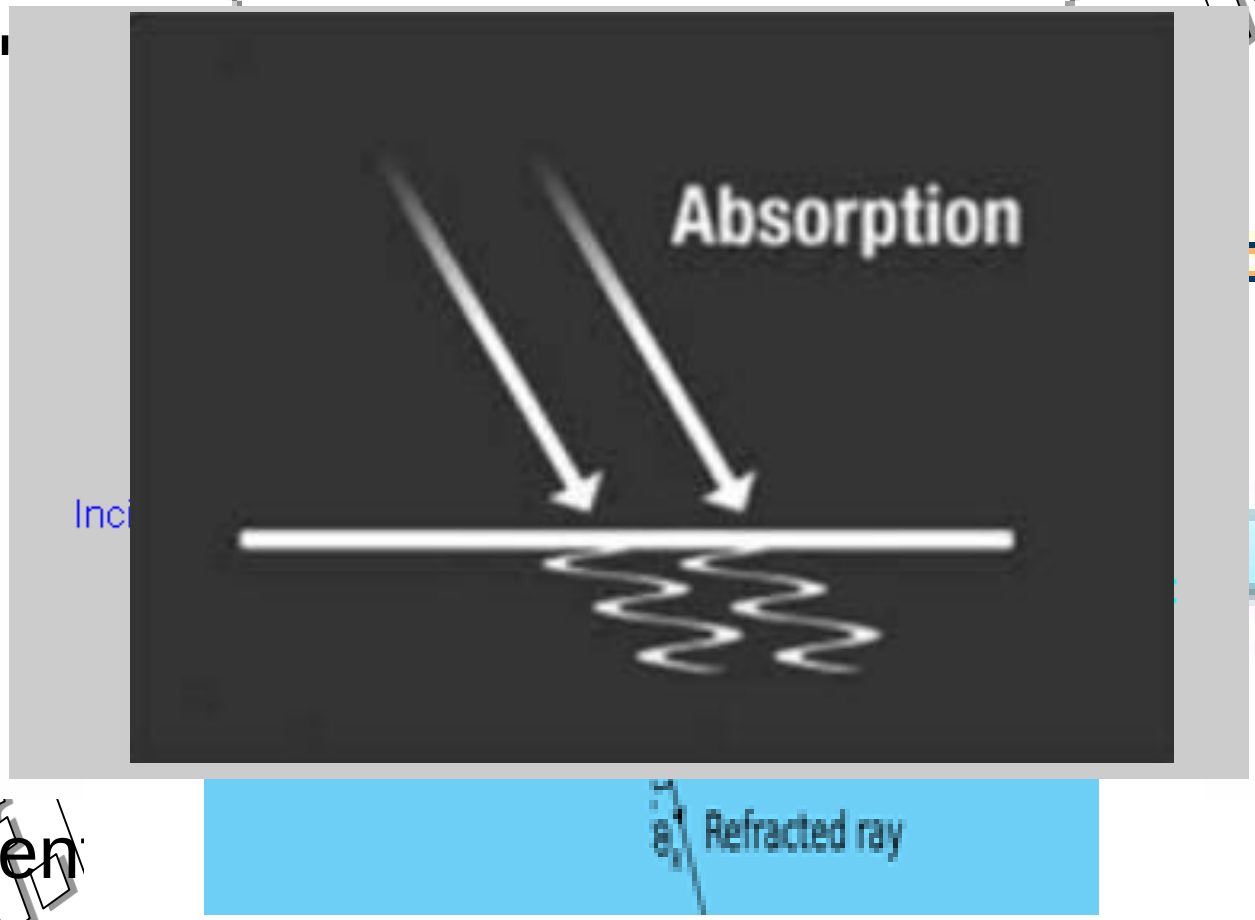
- Beer s Law is a limiting law that is valid only for low concentrations of analyte.
- For sufficiently low concentration of analyte , the refractive index remains essentially constant, and the calibration curve is linear.
- Since the refractive index varies with the analyte sُ concentration ,the value of A will Change Therefore we must avoid the high concentration of analyte solution. (less than 0.01M)

Paracetamol = 151,2 g

1.51 g/l

Fundamental Limitations

In Hig
1- Inc
2- Re
3-Ref
4- Sc
5- Tra
6- Ab
Soluti
If solven

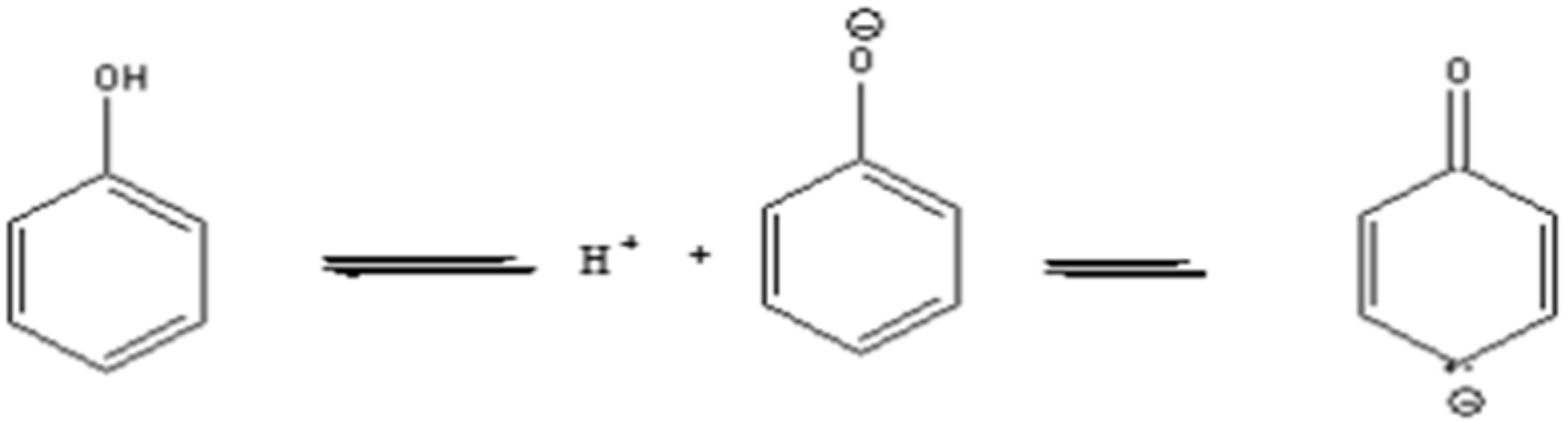


light P

Chemical Limitations

- Chemical deviation from Beer s law can occur when the absorbing species is involved in an equilibrium reaction.

Phenol pH –effect



acid medium

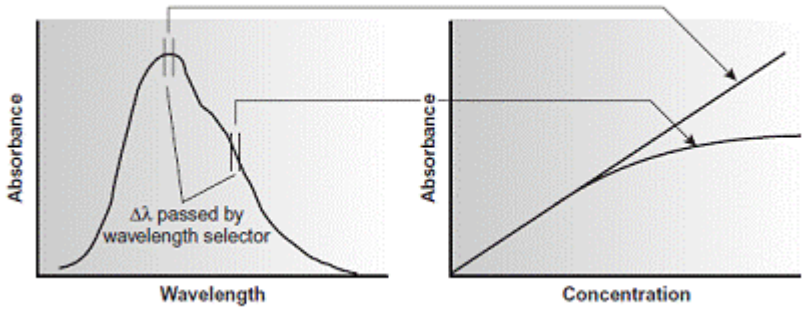
alkaline medium

Alkaline medium shows Bathochromic shift and Hypochromic effect

254 nm
270 nm

Instrumental Limitations

- Using polychromatic radiation always gives a negative deviation from Beer s law.
- Stray radiation → less absorbance → negative deviation.



Effect of wavelength on the linearity of a Beer's law calibration curve.

Stray radiation

Any radiation reaching the detector that does not follow the optical path from the source to the detector.

Instrumental methods

All of the methods have the same general components

Source, Wavelength selector

Sample cell, Detector

Read-out

The actual arrangement of the components will vary based on the method.

Appendix II B. Ultraviolet and Visible Absorption Spectrophotometer

Apparatus Spectrophotometers suitable for measuring in the ultraviolet and visible range of the spectrum consist of an optical system capable of producing monochromatic radiation in the range of 200 nm to 800 nm and a device suitable for measuring the absorbance.

Instruments for Optical Molecular Spectroscopy

The equipment used share any common features regardless of the λ being measured.

Each will have a

- light source
- sample cell
- λ selector
- detector

Sample cells

Cell materials

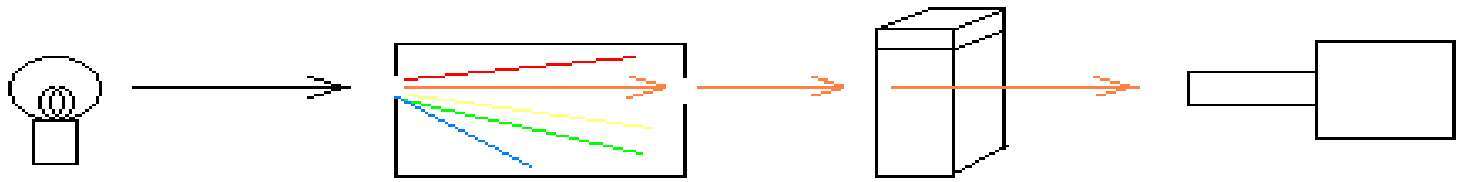
UV	quartz, fused silica
Visible	glass, plastic (UV cells can be used)

material	nm range
silica	150 - 3000
glass	375 - 2000
plastic	380 - 800

Absorbance

Common arrangement for UV/Vis

Scheme of an Absorption Spectrophotometer



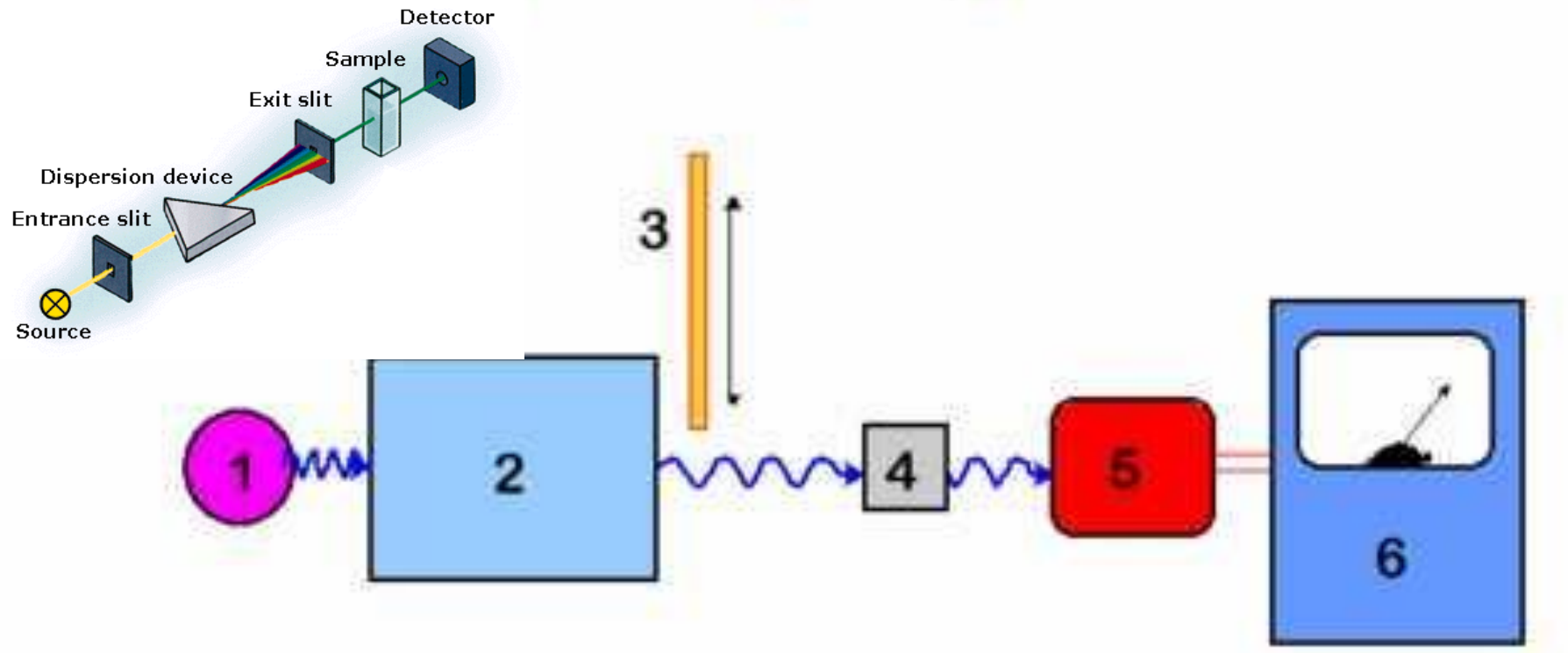
Single beam spectrophotometer

This type of instrument works with a single light path.

One must account for variations in detector response and source output for each λ..

It is best when working with single λ methods and individual analytes..

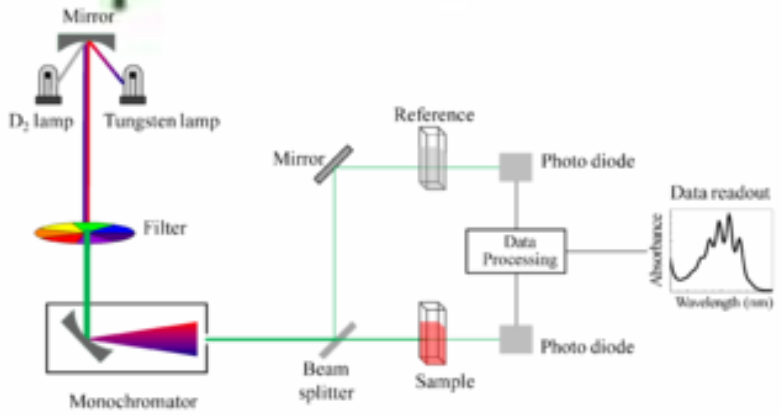
Single beam spectrophotometer

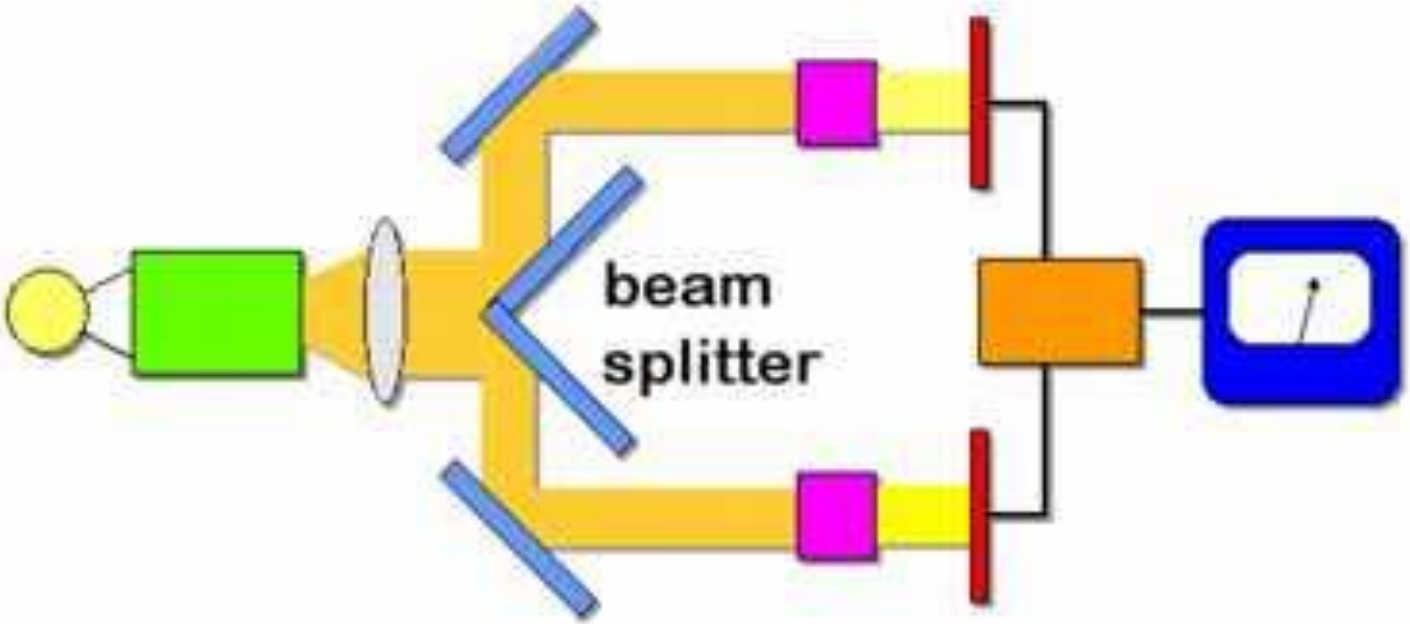


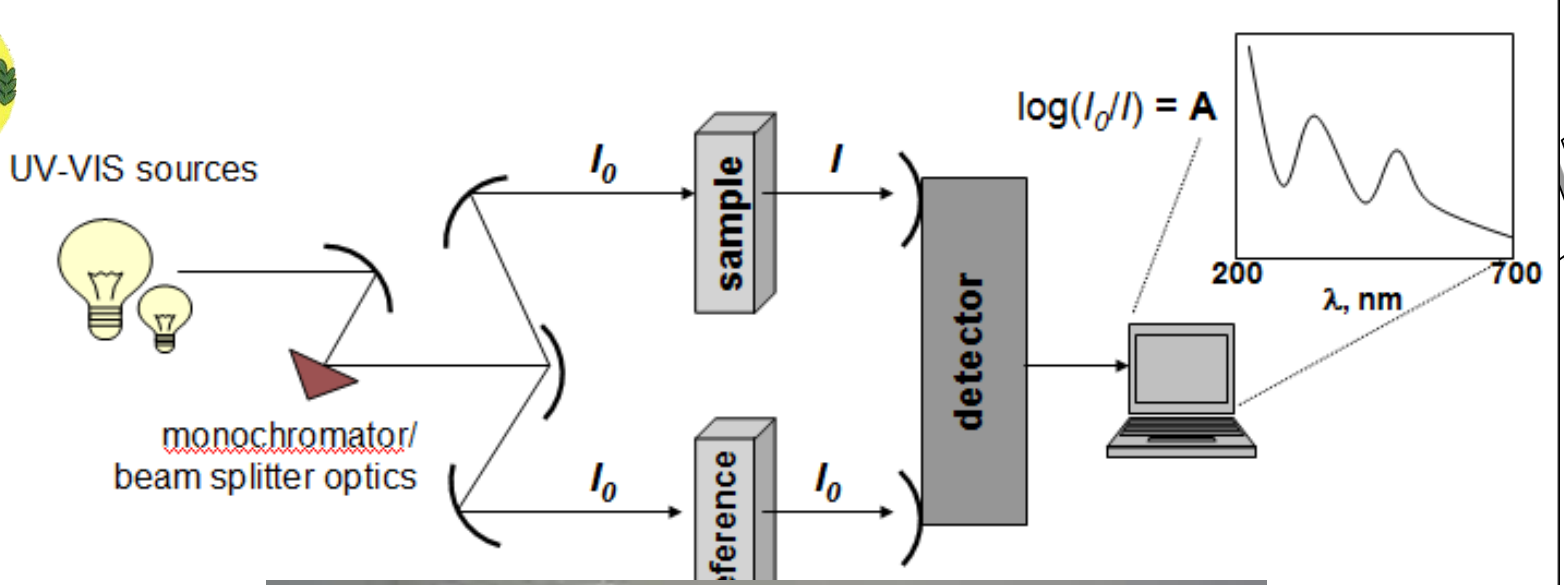
1 - light source
2 - wavelength selector
3 - shutter
4 - sample cell
5 - detector
6 - readout

Double beam spectrophotometer

Double beam in space.

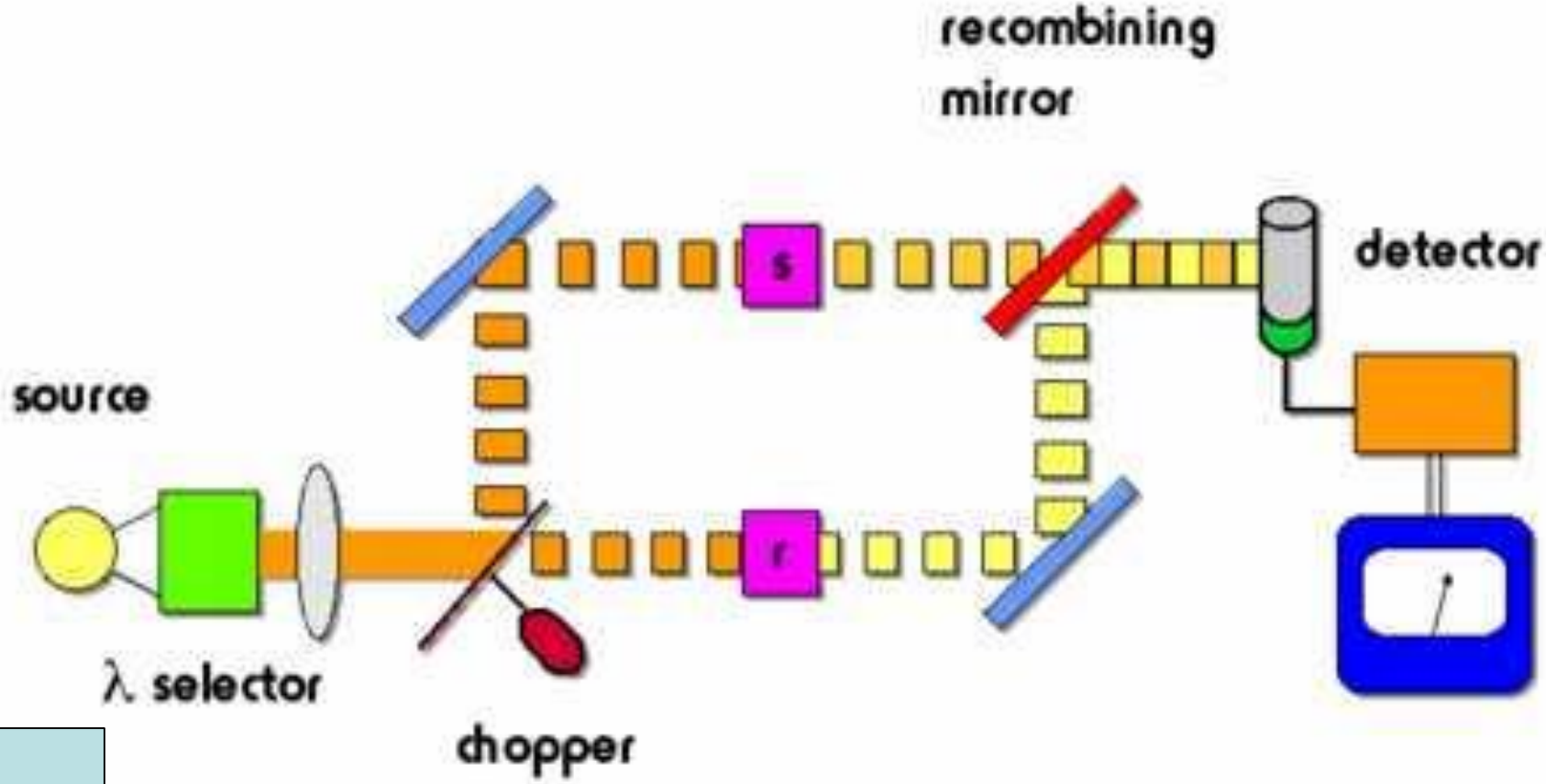





UV-VIS sources
monochromator/
beam splitter optics
I_0
I
sample
I_0
I_0
reference
detector
$\log(I_0/I) = A$
200
700
λ, nm

Double beam spectrophotometer

DB in time.



Double beam spectrophotometer

While a double beam in time instrument can reduce much of our noise and make it possible to obtain entire spectra, there are still problems.

كامل

The major one is that you can't look at anything that changes at a rate near or faster that the chopper rate.

With a typical instrument - no kinetic studies are possible.

Sources

For a general purpose instrument, we need a way to produce a broad range of λ with reasonably uniform intensity.

We can seldom obtain uniform intensity but most instruments can account for this.

Lets review some of the more common sources.

UV source

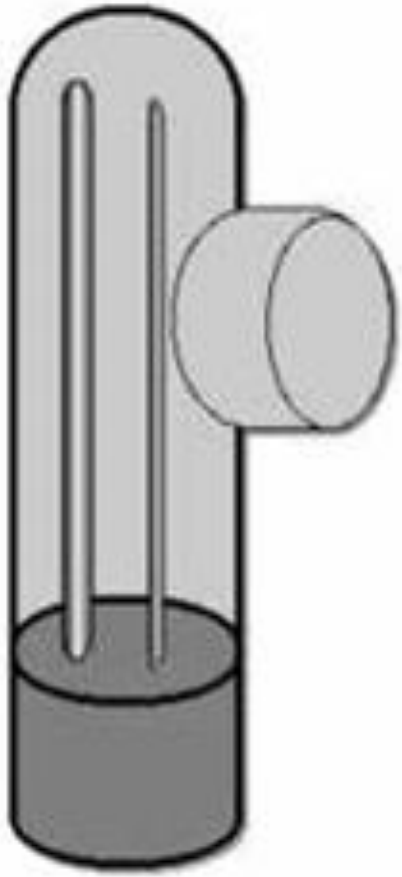
D_2 lamp

D_2 + electrical energy

↓

$D_2^* \longrightarrow D_2 + h\nu$

λ range: 200 – 380 nm



Visible source TUNGSTEN LAMP

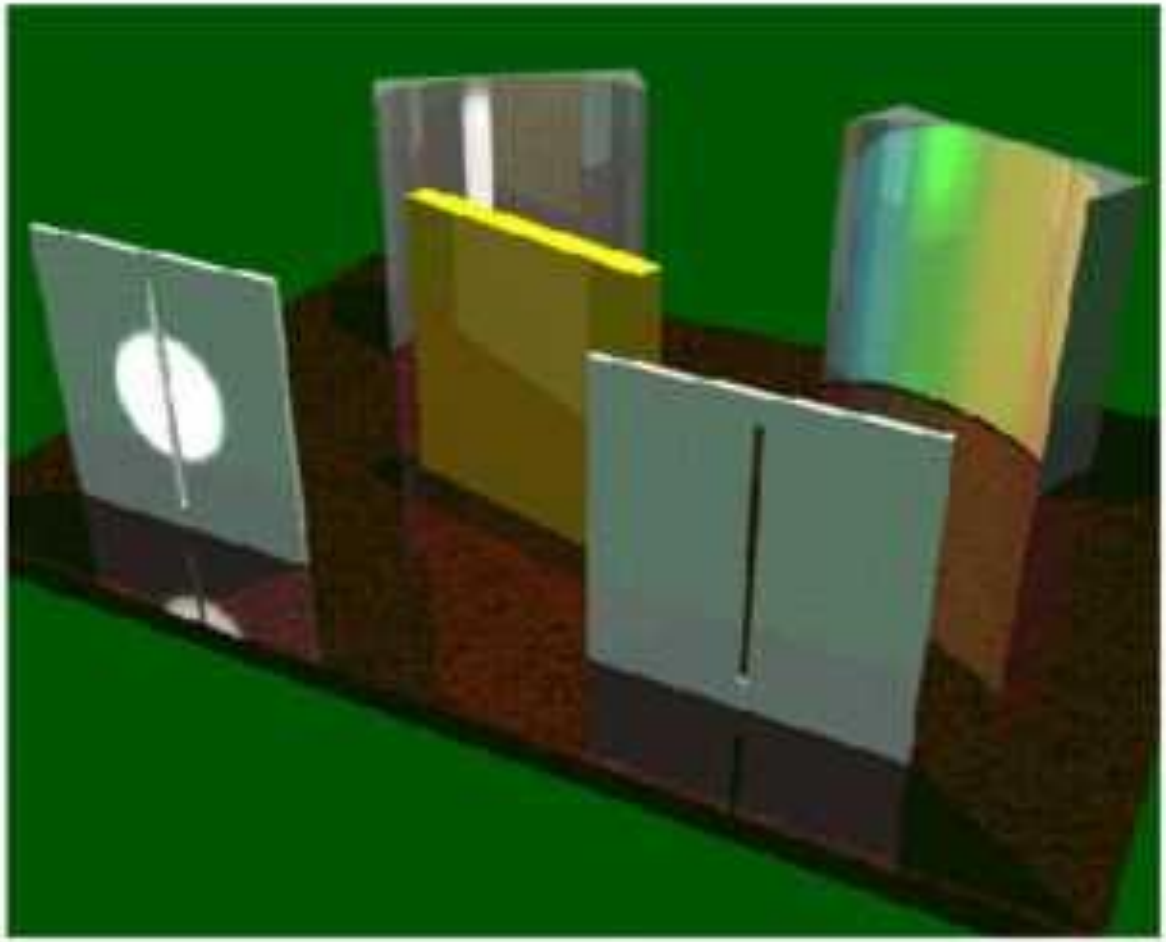
The tungsten lamp is similar to a normal light bulb.

λ range: 350-2200 nm

Useful in visible and near IR range.

Wavelength selectors

As with a prism, we still need the proper lenses and slits in order for a grating حاجز to work as a monochromator.



Lens materials

The material used for construction of lenses must allow light to pass.

This is a function of the λ range that the instrument is designed to work with.

Visible - ordinary glass or silica

UV - fused silica or quartz

Lens materials

The material used for construction of lenses must allow light to pass.

This is a function of the λ range that the instrument is designed to work with.

Visible - ordinary glass or silica

UV - fused silica or quartz

Detectors

OK, now we need a way of detecting any light that has made it though our system.

The purpose of a detector is to convert our response into a measurable signal.

The approach taken varies based on the type of light that is being used.

الغاية من المتحري هو تحويل الأستجابة لأشارة مقروءة

Detectors

Common detectors

detector type	λ range nm	property measured	typical use
Phototube	150-1000	current	UV
Photomultiplier	150-1000	current	UV/Vis
Solid state Photodiodes	350 - 3000	varies	varies

Detectors

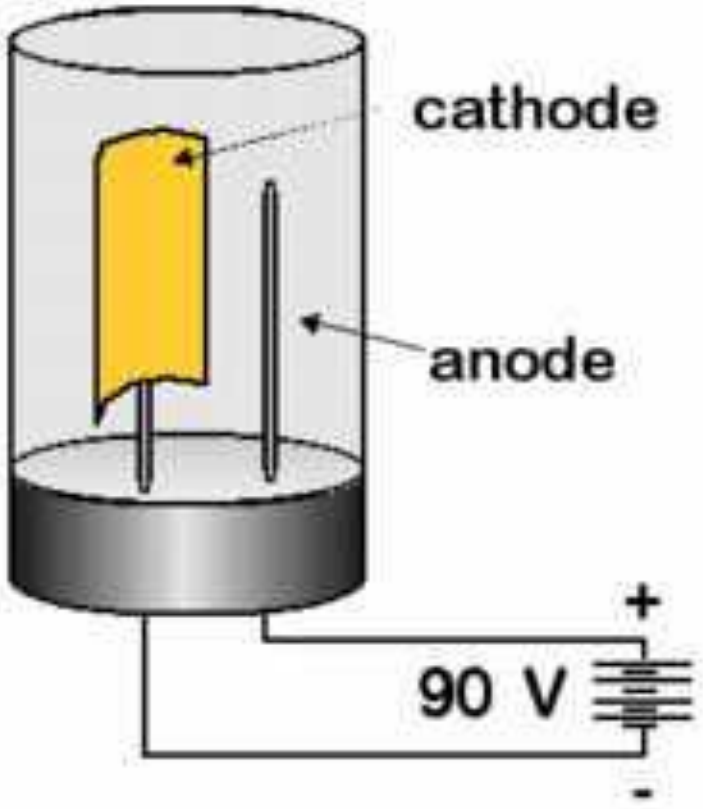
يتكون من سطح معدني على شكل مصعد حساس للاشعة حيث يعطي اليكترونات عند سقوطها ، حيث تنجذب هذه الأخيرة إلى المصعد محدثة تيار كهربائي بين القطبين حيث تتناسب قوة هذا التيار مع شدة الأشعة الساقطة على الخلية الضوئية . وعادة مايكون المهبط من المعادن سهلة التأين مثل المعادن القلوية والقلوية الأرضية .

Phototube Works via the photoelectric effect.

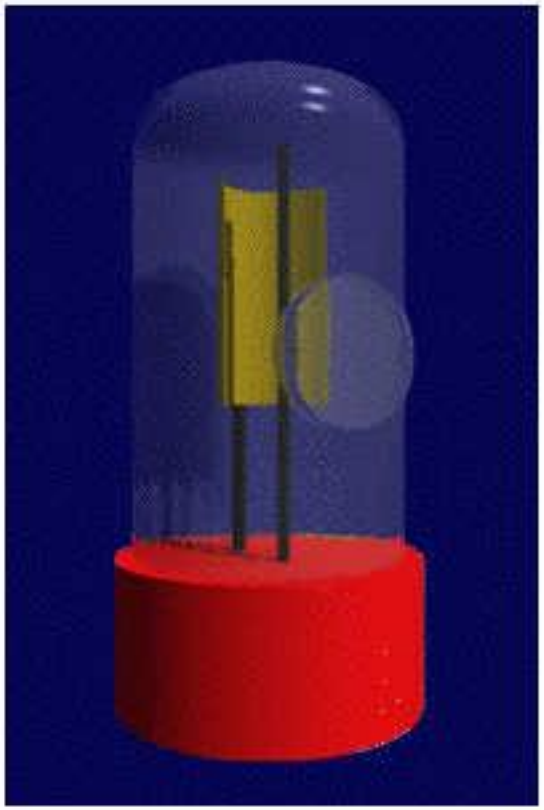
A photon hits the cathode which is covered with a photoemissive surface.

You get a current that is proportional to the intensity of the photons.

Tubes are subject to a small 'dark current' due to thermal effects.



Phototube



Detectors

الأنبوب الضوئي المضاعف

وهو الأكثر شيوعاً حيث يكون المهبط على شكل dynode الذي يضاعف الأليكترونات ولذا فهو أكثر حساسية

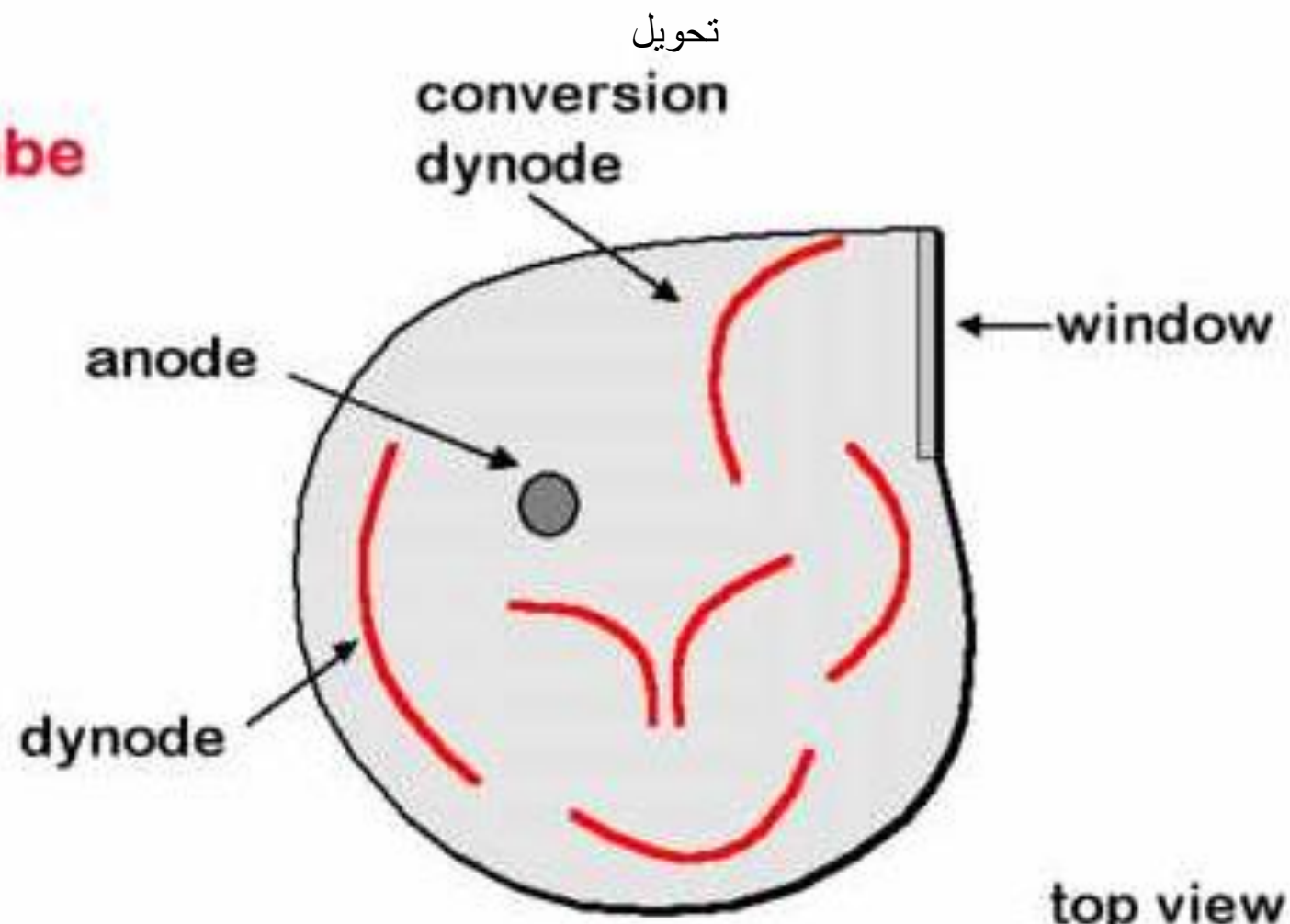
Photomultipliers (PM)

These detectors are similar to a phototube in that you still have an initial cathode where an incoming photon will cause an electron to be ejected.

Unlike a phototube, a PM will amplify your signal using a series of 'dynodes.'

Detectors

PM Tube



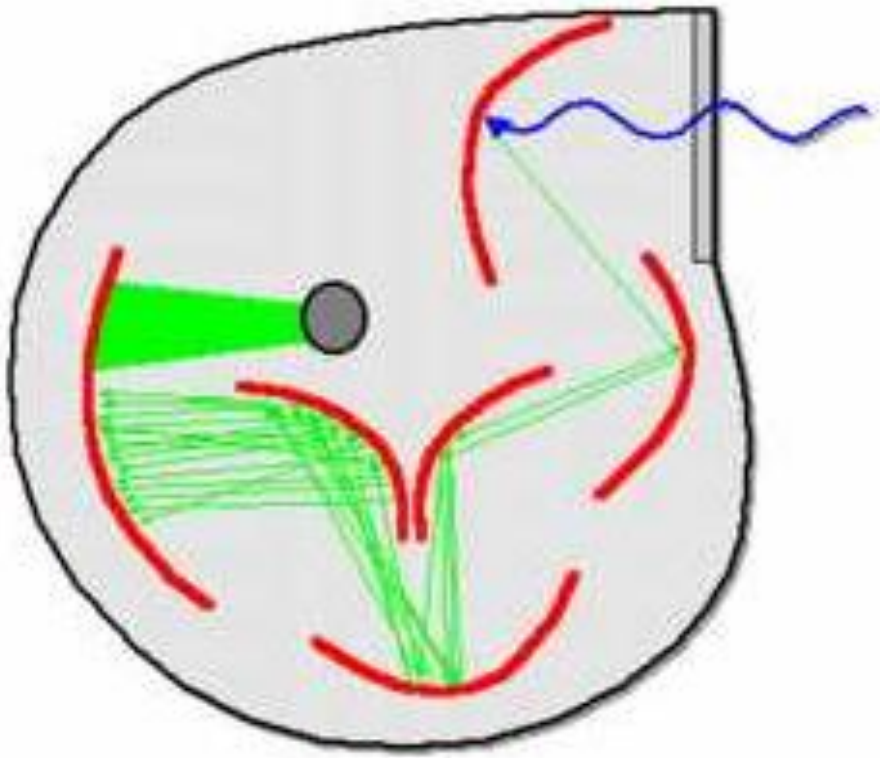
Detectors

PM Tube

A single electron is ejected at the conversion dynode.

Subsequent dynodes are ~90V more positive which results in the e^- being accelerated and ejecting additional electrons.

Amplifications of 10^6-10^7 are obtained.



Detectors

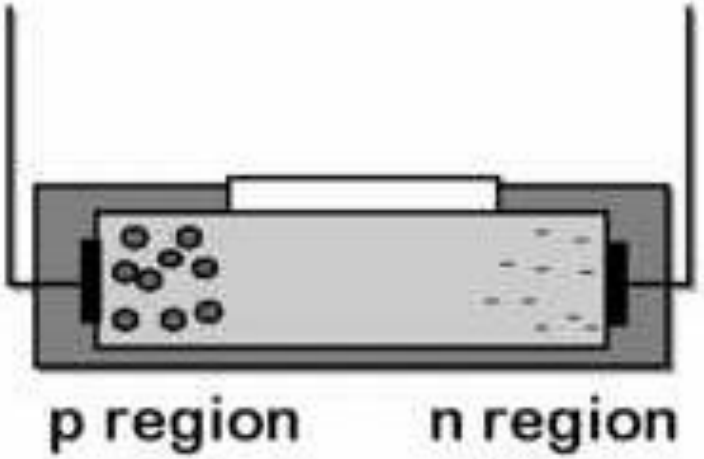
Solid state - photodiodes

When a potential is applied to a doped Si crystal, we can obtain two regions

n - electron rich

p - + hole rich

Once established, no current flows.



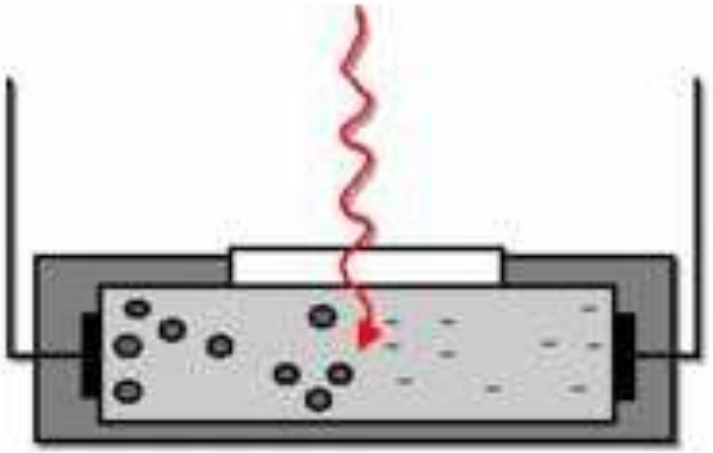
Detectors

Photodiodes

When exposed to light, this disturbs things and allows a current to flow.

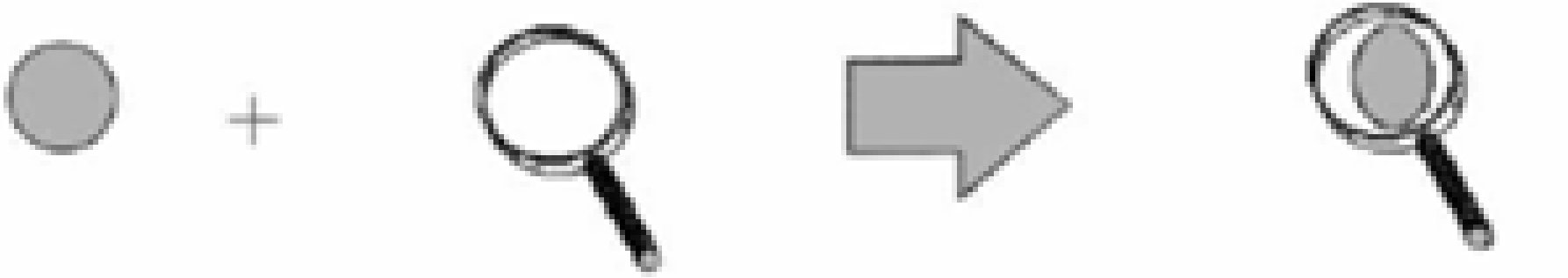
The current is proportional to the amount of light.

A photodiode is more sensitive than a phototube and costs less than a PM tube.



Chemical Derivatization

PRINCIPLE :

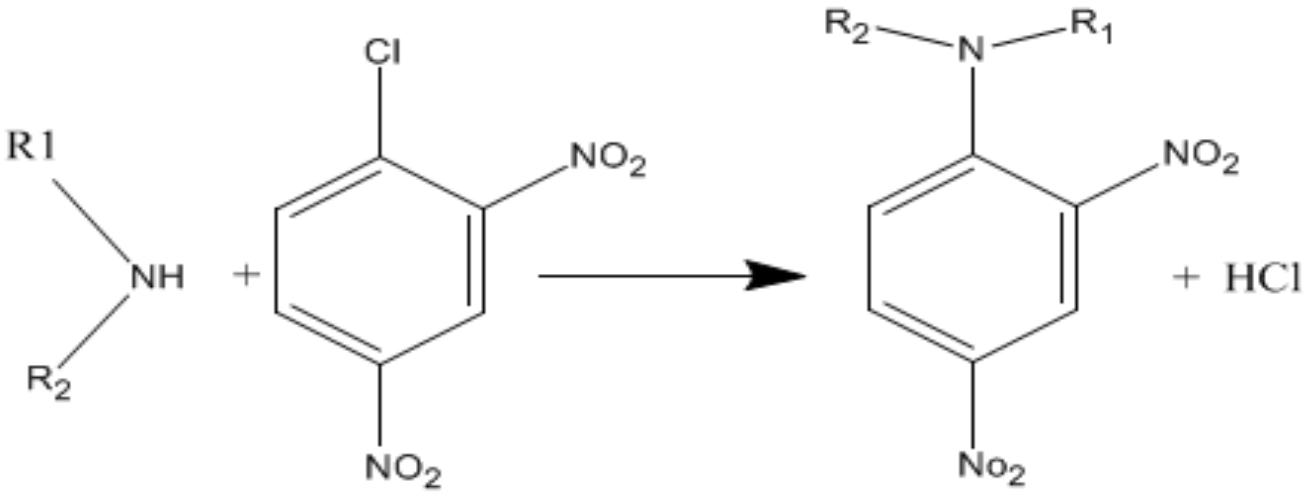


Chemical Derivatization (Amines)

Method(3): *1-Chloro-2-4-dinitrobenzene*

Principle:

Reaction with 1-Chloro-2-4-dinitrobenzene to give a N-alkyl 1-2,4-dinitroaniline : yellow color (450 nm)



Chemical Derivatization (Amines)

Method(4): Hantzsch reaction

Condensation of amine with acetyl acetone and Formaldehyde

Arzneimittelforschung. 2001 Feb;51(2):97-103.

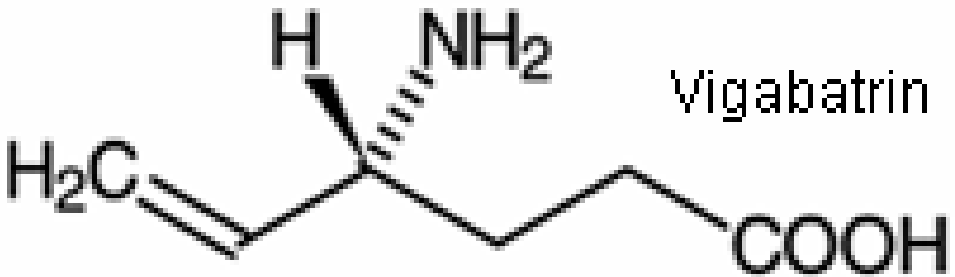
...ation of the antiepileptics vigabatrin and gabapentin in dosage forms and biological fluids usin... reaction.

al-Zehouri J

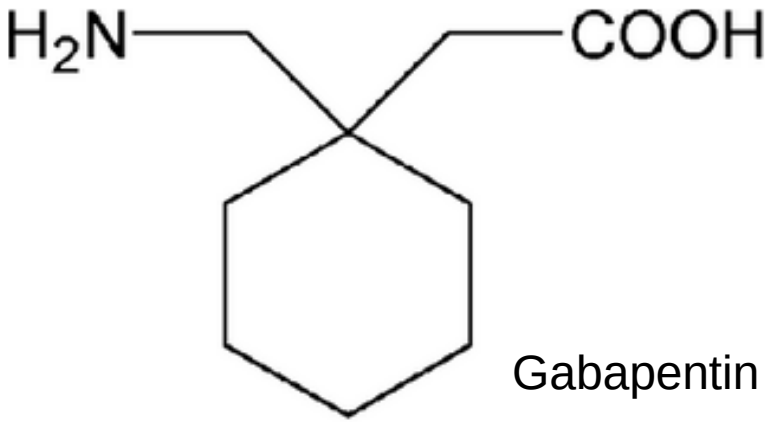
Author information

Abstract

A selective and sensitive method was developed for the determination of the anticonvulsants vigabatrin (I) (CAS 60643-86-9) and gabapentin (II) (CAS 60142-96-3). The method is based on the condensation of the drugs through their amino groups with acetylacetone and formaldehyde according to Hantzsch reaction yeilding the highly fluorescent dihydropyridine derivatives. The yellowish-orange color was also measured spectrophotometrically at 410 nm and 415 nm for I and II, respectively. The absorbance-concentration plots were rectilinear over the ranges 10-70 micrograms/ml and 20-140 micrograms/ml for I and II, respectively. As for the fluorescence-concentration plots, they were linear over the ranges 0.5-10 micrograms/ml and 2.5-20 micrograms/ml with minimum detection limits (S/N = 2) of 0.05 microgram/ml (approximately 2.1 x 10(-8) mol/l) and 0.1 microgram/ml (approximately 5.8 x 10(-7) mol/l) for I and II, respectively. The spectrophotometric method was applied to the determination of I and II in their tablets. The percentage recoveries +/- SD (n = 6) were 99.45 +/- 0.13 and 98.05 +/- 0.53, respectively. The spectrofluorimetric method was successfully applied to the determination of I and II in spiked human urine and plasma. The % recoveries +/- SD (n = 5) were 98.77 +/- 0.29 and 98.39 +/- 0.53 for urine and 99.32 +/- 0.74 and 98.90 +/- 0.96 for plasma, for I and II, respectively. No interference was encountered with the co-administered drugs: valproic acid (CAS 99-66-1), diphenylhydantoin (CAS 57-41-0), phenobarbital (CAS 50-06-6), carbamazepine (CAS 298-46-4), clonazepam (CAS 1622-61-3), clobazam (CAS 22316-47-8) or cimetidine (CAS 51481-61-9). A proposal of the reaction pathway is suggested. The advantages of the proposed methods over existing method are discussed.



Vigabatrin



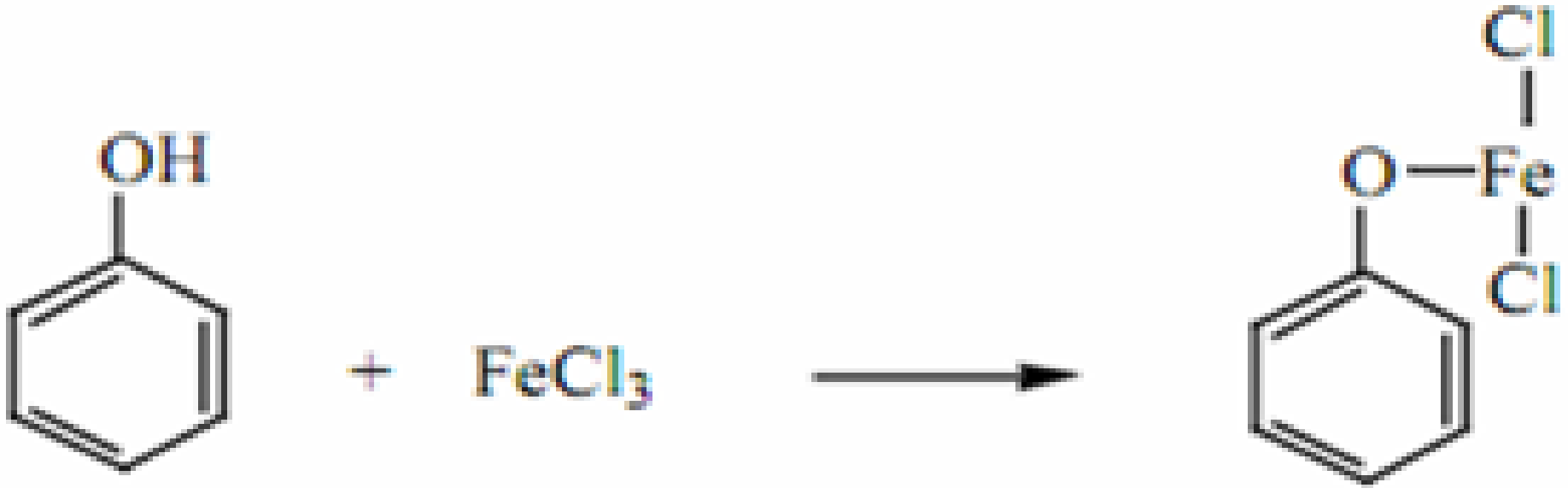
Gabapentin

Prof. J. Al-Zehouri

Phenols

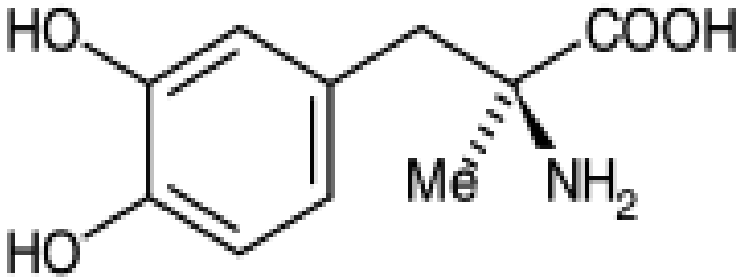
- Ar-OH + Ferric Chloride

Violet color (530-550



Methyldopa

Vis after Derivatization



238.2 C10H13NO4.1½H 2O

Action and use Antihypertensive.

Preparation

Methyldopa Tablets

Vis after Derivat...

Principle of assay

The reaction between the Iron ion and Phenol group in Methyldopa give complex with violet color has λmax at 545 nm , convenient to the visible assay.

Methyldopa Tablets

Vis after Dervatisation

ASSAY :

Weigh and powder 20 Tablets. Dissolve a quantity of the powder containing the equivalent of 0.1 g of anhydrous methyldopa as completely as possible in sufficient 0.05 M sulphuric acid to produce 100 ml and filter. To 5 ml of the filtrate add 2 ml of iron sulphate-citrate solution ,8 ml of glycine buffer solution and sufficient water to produce 100 ml.Measure the absorbance of the resulting solution at the maximum at 545 nm.

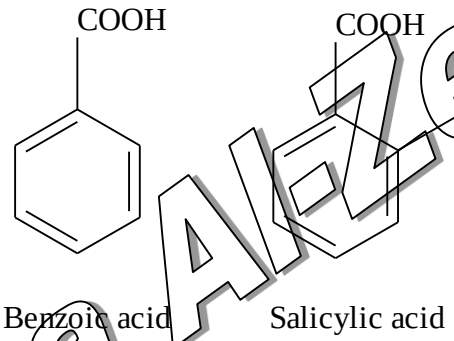
Repeat the procedure using 5 ml of 0.10%w/v solution of methyldopa BPRS in place of 5 ml of the filtrate, beginning at the words add 2 ml of……..

Calculate the content of C10H13NO4 using the declared content of C10H13NO4 in methyldopa BPRS.

Compound Benzoic acid oi
Bp2007

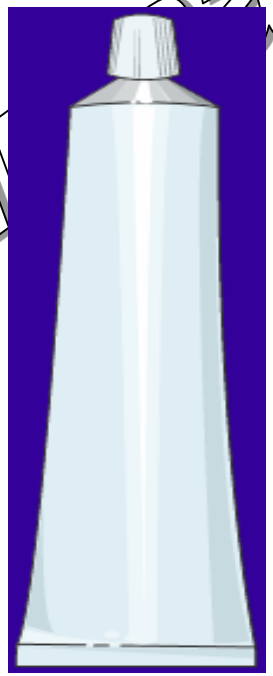
Vis after Dervatisation&Titration

- Benzoic acid 6%
- Salicylic acid 3%

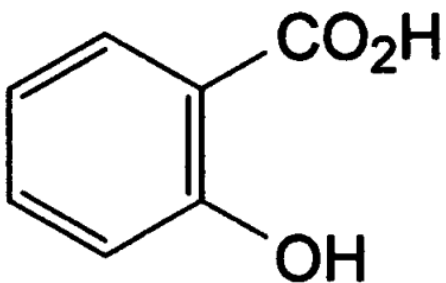


Benzoic acid Salicylic acid

Use : Antiacne

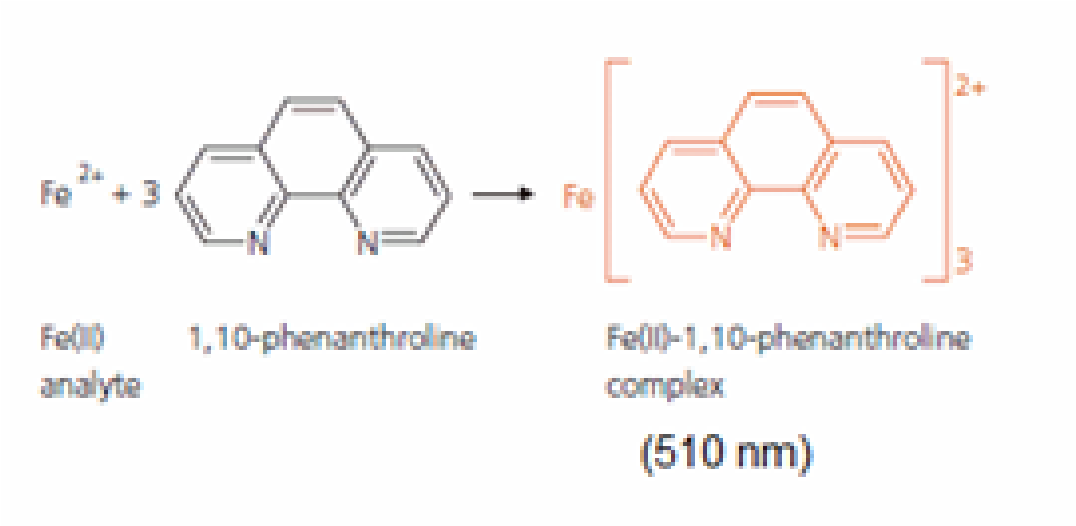


Cool the titrated solution obtained in the Assay for benzoic acid, dilute to 250 ml with *water* and filter. To 5 ml of the filtrate add sufficient *iron(III) nitrate solution* to produce 50 ml. Filter, if necessary, to remove haze and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using *iron(III) nitrate solution* in the reference cell. Calculate the content of C7H6O3 from the absorbance obtained by repeating the operation using 5 ml of a 0.024% w/v solution of *salicylic acid* and beginning at the words 'add sufficient *iron(III) nitrate solution* ...'.



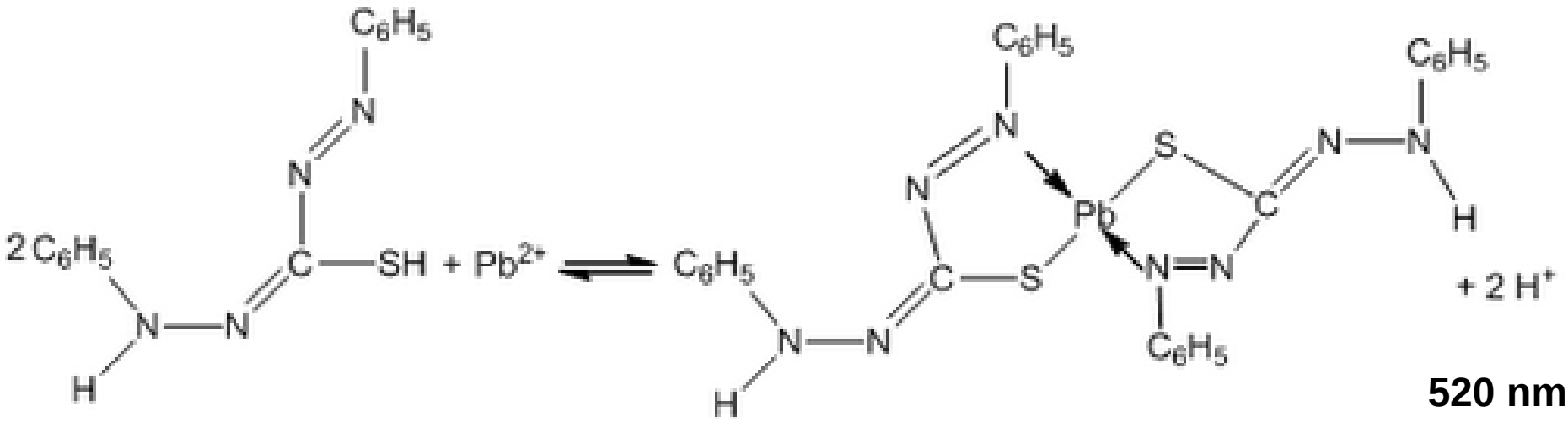
Complexation reactions

- Analysis of metals and metalloids in water, pharmaceutical preparations and other matrices.
- Once the metal ions bind the ligand specific colour changes are observed which can be quantitated as a function of the amount of metal ions present.



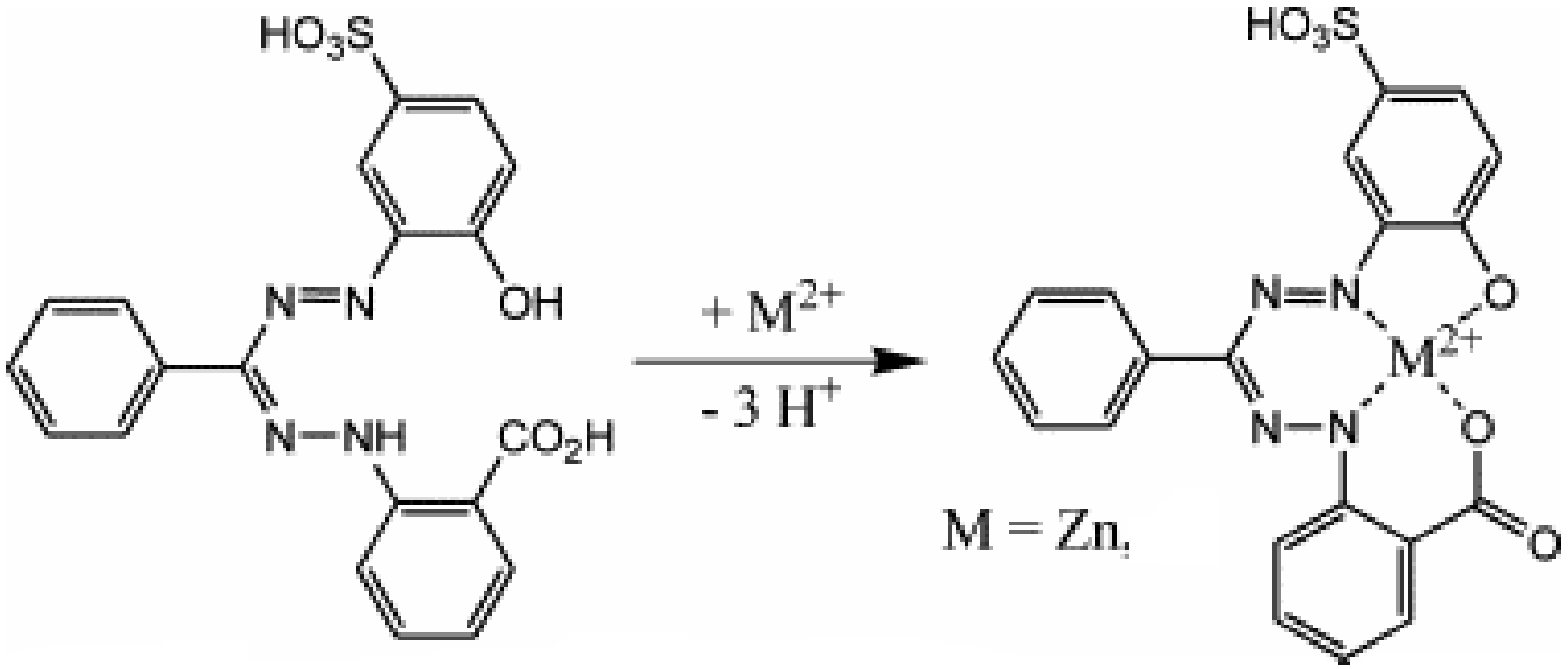
Dithizone (Pb)

Reaction of complex formation of dithizone with Pb 2+



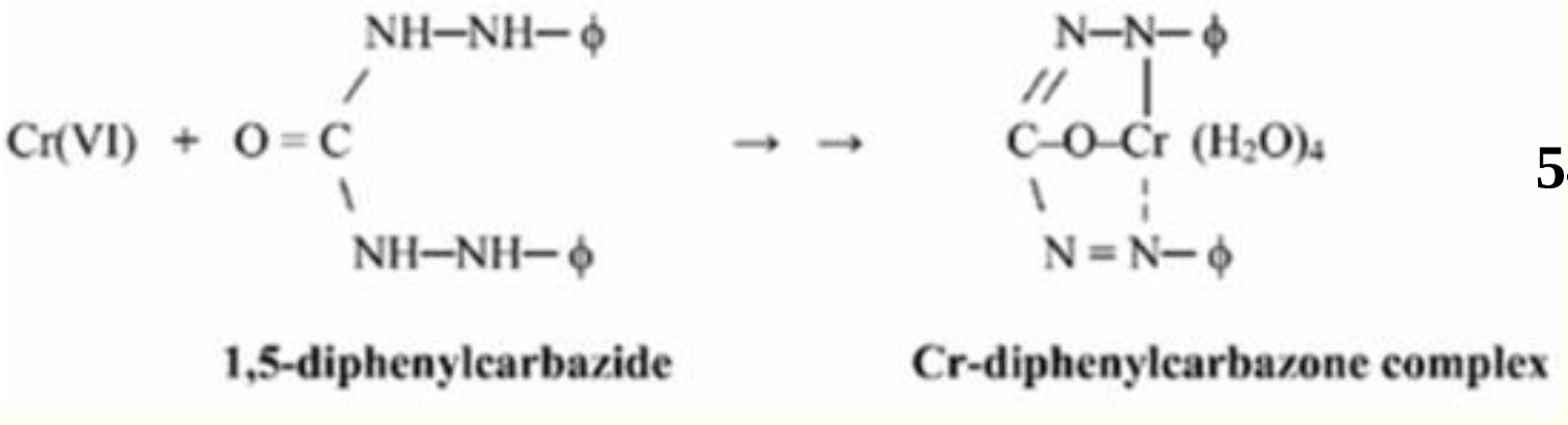
520 nm

Zincon (Zn)



620 nm

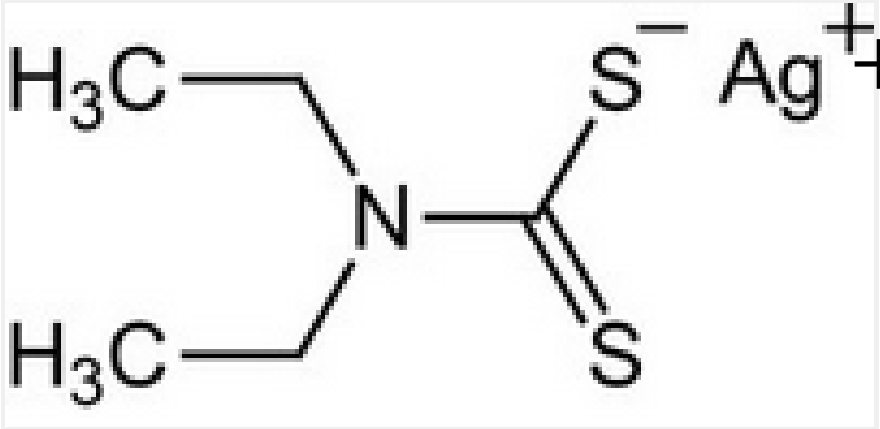
Diphenylcarbazide (Cr)



540 nm

The chemical reaction between chromium (VI) and diphenylcarbazide, which results in chromium-diphenylcarbazone.

Silver diethyldithiocarbamat (AS)

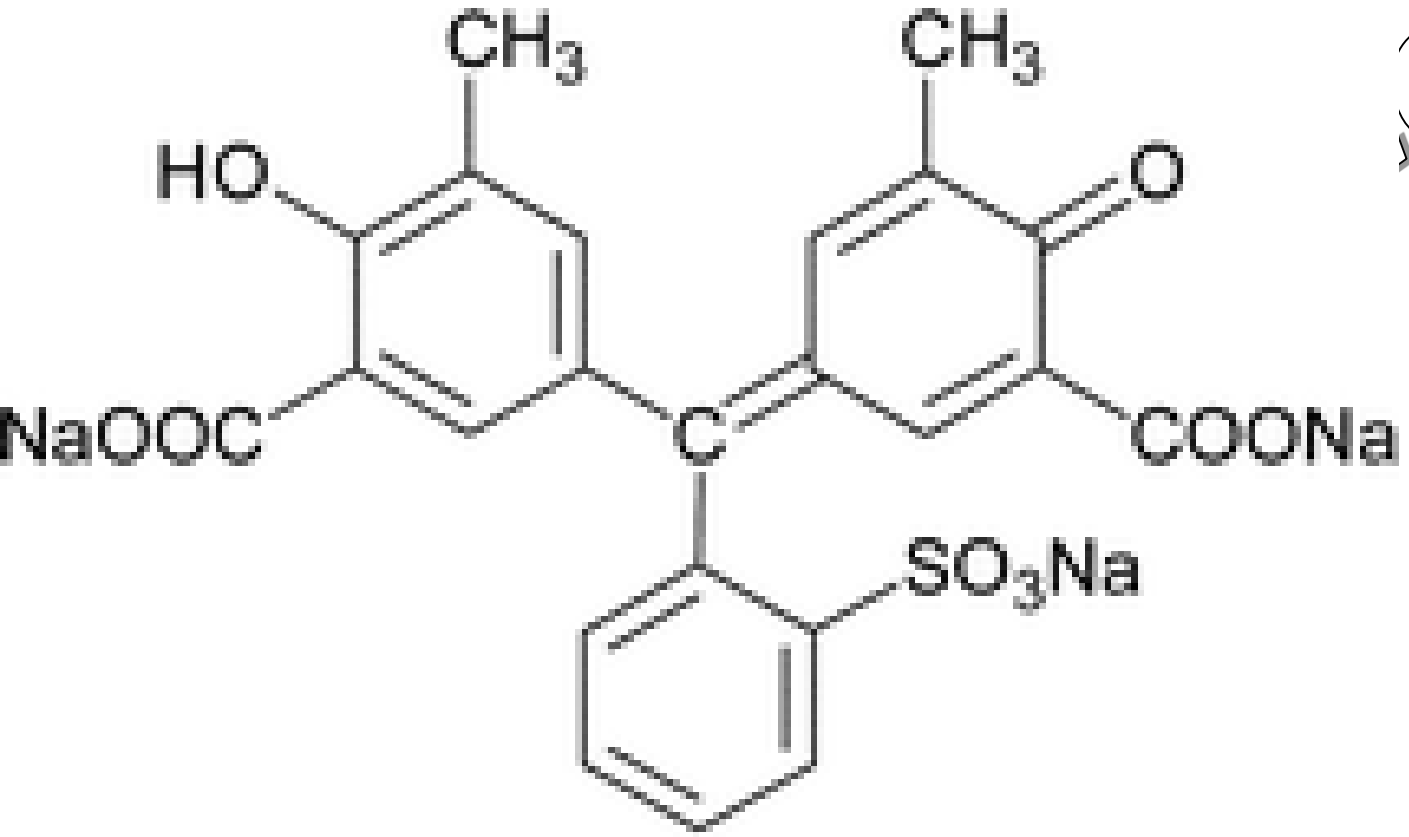


+ As ⟶ Complex

620 nm

Silver diethyldithiocarbamat

Eriochrome Cyanine R

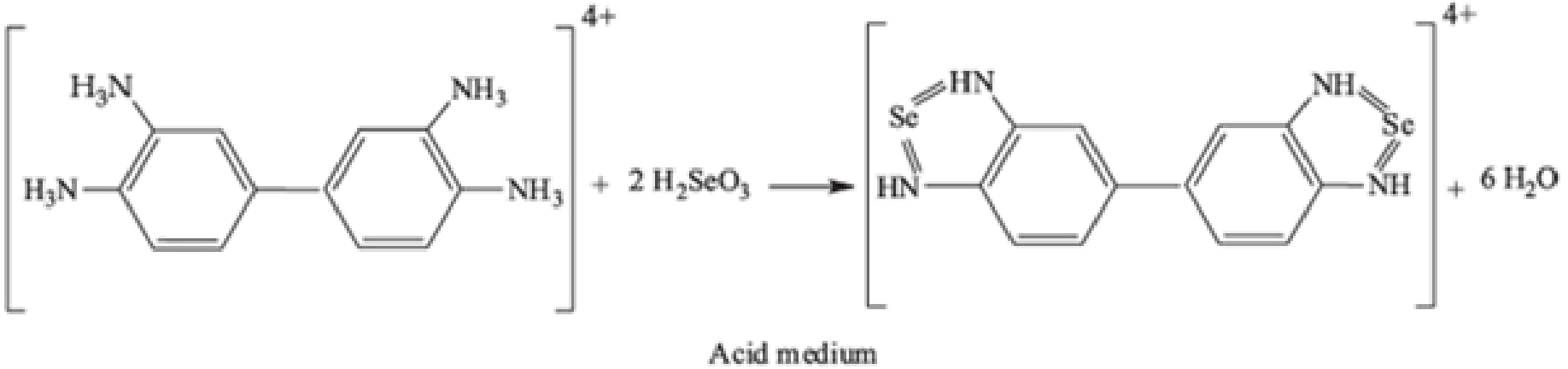


Eriochrome Cyanine R.

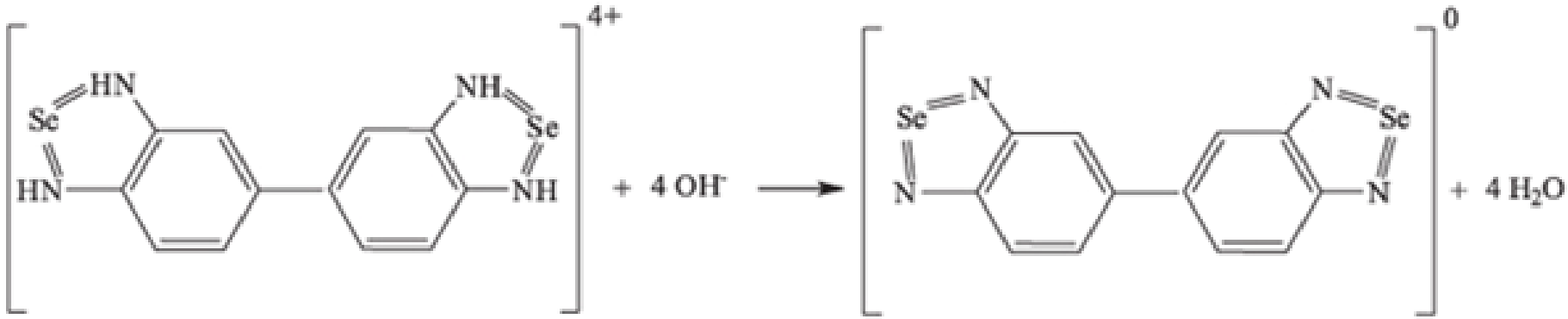
+ Al → complex

535 nm

Diaminobenzidine (Se)

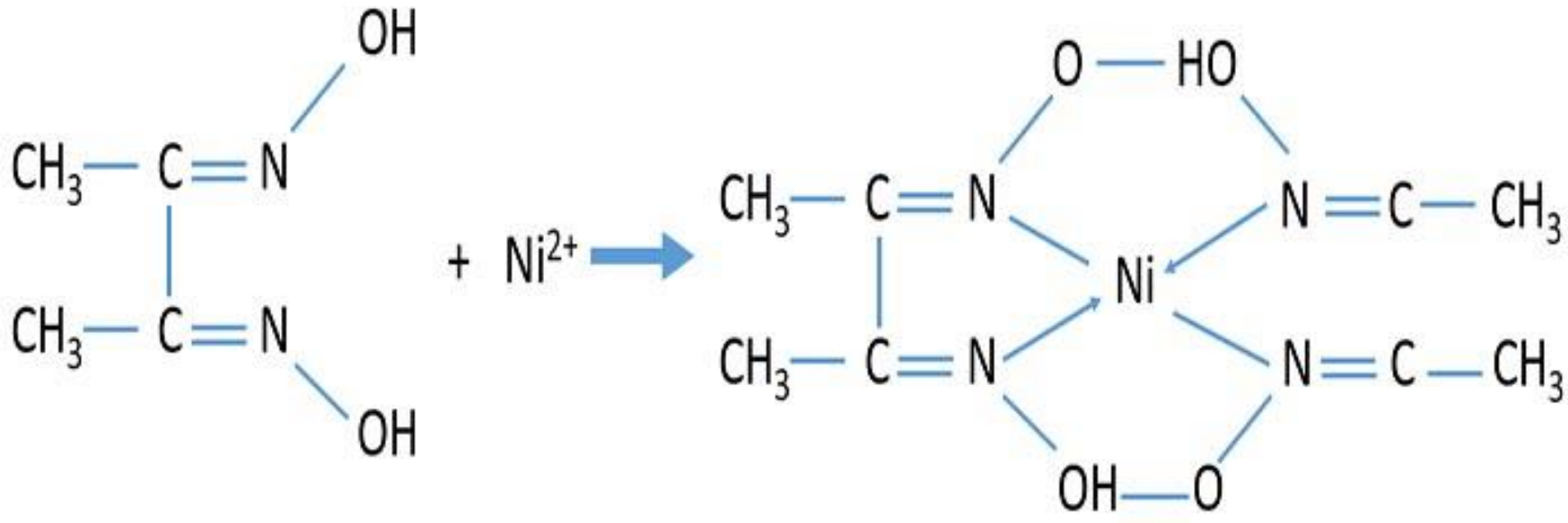


Acid medium



Neutral or alkaline medium

Dimethylglyoxime (Ni)



Dimethylglyoxime aqueous

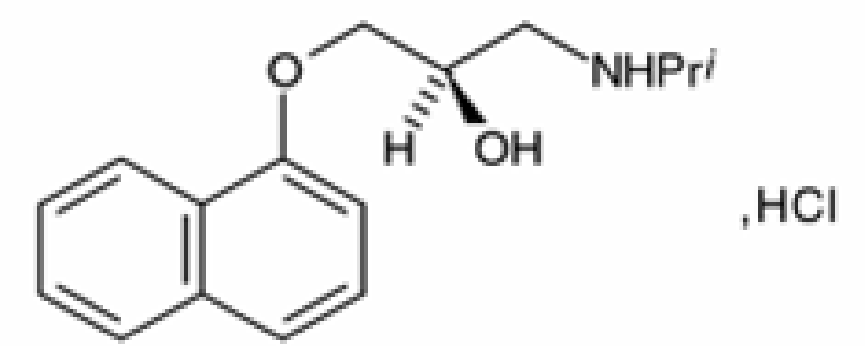
Nickel dimethylglyoxime complex

Pharmaceutical Application

- UV (A1%,1cm)
- UV (Std)
- UV after extraction
- UV & Titration
- Vis.
- Vis. after Dervatisation
- Vis. after Dervatisation & Titration

Propranolol Hydrochloride

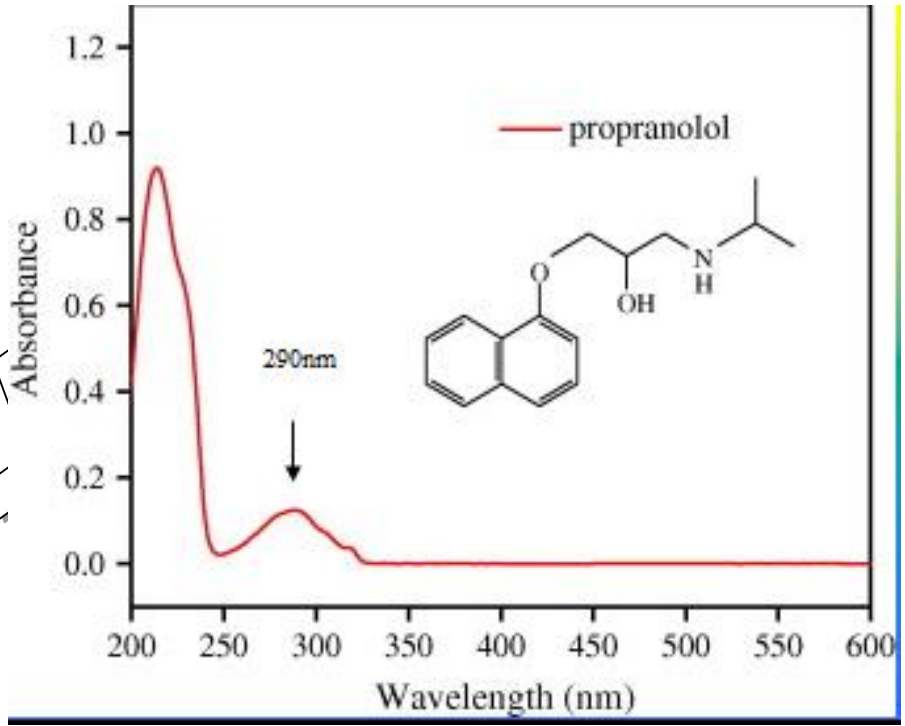
UV (A1%,1cm)



C16H21NO2,HCl

MW = 295.8

Use :Beta Blocker (Antihypertensive agent)



UV (A1%,1cm)

Propranolol Tablets 80 mg

Assay : Weigh and powder 20 tablets. Shake a quantity of the powder containing 20 mg of Propranolol Hydrochloride with 20 ml of water for 10 minutes. Add 50 ml of methanol, shake for further 10 minutes, add sufficient methanol to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with methanol and measure the absorbance of the resulting solution at the maximum at 290 nm. Calculate the content of C16H21NO2,HCl taking 206 as the value of A(1%,1cm) at the maximum at 290 nm .

1- If the tablet average weigh 300 mg . What is the sample taken ? (Answer = 75 mg)

2- If A = 0.83 What is % content ? (Answer = 100.75%)

3- What is the Tablet practical content? (Answer =80.58 mg)

التركيز العملي = 0.00403 التركيز النظري = 0.004 **Prof.J.Al-Zehouri**

300 Contain 80

X 20

X= 75 mg

T.C = 20/100 X 10/50 X 100 / 1000 = 0.004 g%

P.C = 0.83 /206 =0.00403

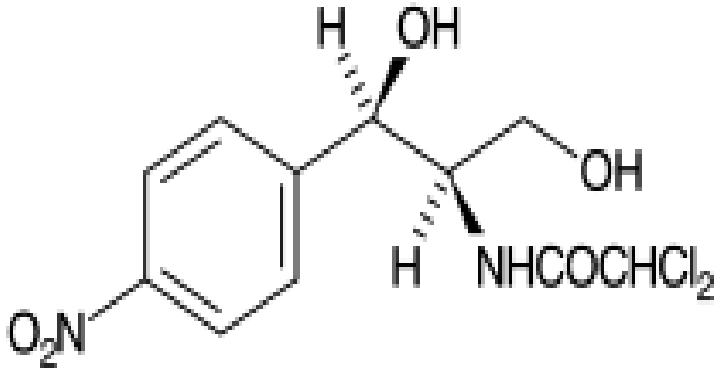
% yeild = 0.00403/0.004 X 100 = 100.7 %

- 80 100

X 100.7

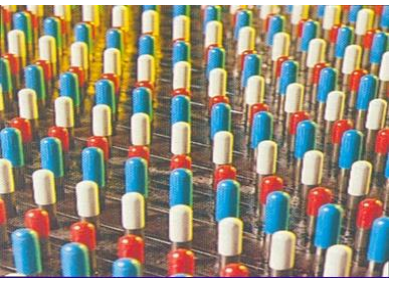
X = 80.. 56 mg

Chloramphenicol



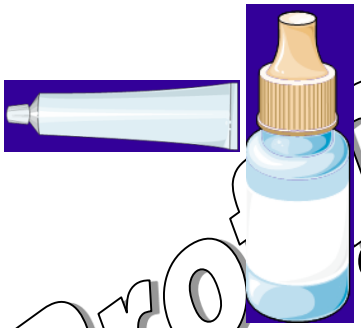
UV after Extraction

323.1 C11H12Cl2N2 O5



Action and use Antibacterial.

Preparations

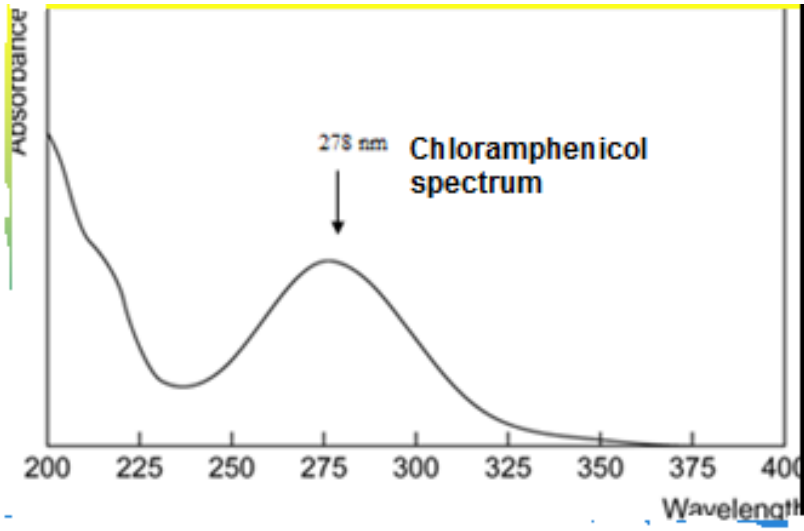


Chloramphenicol Capsules

Chloramphenicol Ear Drops

Chloramphenicol Eye Drops

Chloramphenicol Eye Ointment



Henderson- Hasselbalch equation

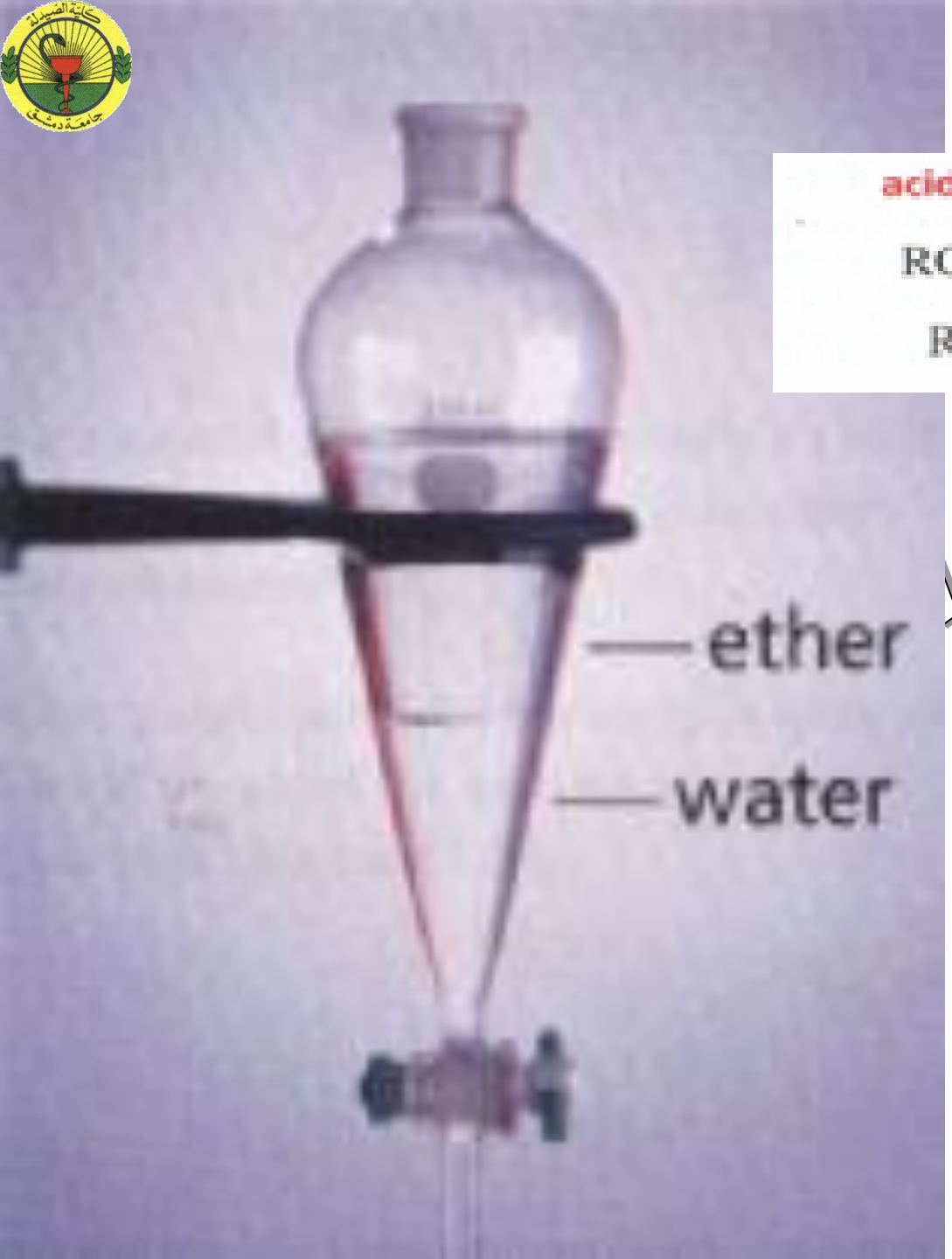
$$pH = pKa + \log \frac{\text{Concentration of Salt}}{\text{Concentration of acid}}$$

$pH = pka + \log A^-/HA$ (acid)

$pH = pKa + \log B/BH^+$ (base)

$$pH = pKa + \log \frac{[\text{nonprotonated species}]}{[\text{protonated species}]}$$

- A compound will exist primarily in its acidic form if the pH of the solution is less than the compound sُ pKa.
- A compound will exist primarily in its basic form if the pH of the solution is greater than the compound sُ pKa.



acidic form		basic form
$RCOOH$	$\rightleftharpoons$	$RCOO^- + H^+$
$R\overset{+}{N}H_3$	$\rightleftharpoons$	$RNH_2 + H^+$

For the most effective separation, the pH of the water layer should be at least two units away from the pKa values of the compounds being separated. Then the relative amounts of the compounds in their acidic and basic forms will be at least 100:1

Prof J. Al-Zehouri

Chloramphenicol Eye Ointment 2%

UV after Extraction

Assay :

Suspend a quantity containing 10 mg of Chloramphenicol in 50 ml of Benzene and extract with successive quantities of 50,50,50 and 30 ml worm water .Combine the extracts, dilute to 200 ml with water, mix well and filter, discarding the first 20 ml of the filtrate. Dilute 10 ml of the filtrate to 50 ml with water and measure the absorbance of the resulting solution at the maximum at 278 nm .

278 nm

1. What is the sample weight taken ? (Answer = 0.5g)
2. If A(1%,1cm) = 297 and A=0.3 What is the % Concentration? (Answer= 101%)

التركيز النظري = 0.001

التركيز= 0.00101

3. If the DISTRUBUTION Constant 4/1 water/benzene What is the resulting extraction % ? (Answer 99.84%)

170 v

Calculate A1%,1cm for :

A- compound with an absorption of 0.524 and a concentration of 0.002% W/V

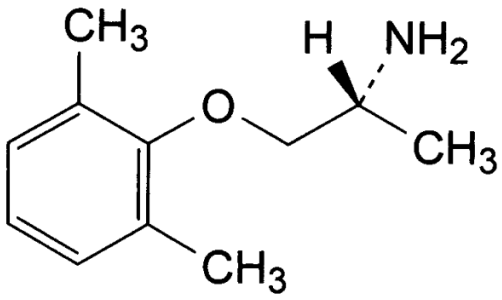
Answer = 26

B- A compound with an absorption of 0.715 and a concentration of 10 µg/ml

Answer = 715

UV (Std)

Mexiletine Hydrochloride



and enantiomer , HCl

C11H17NO,HCl 215.7 *5370-01-4*

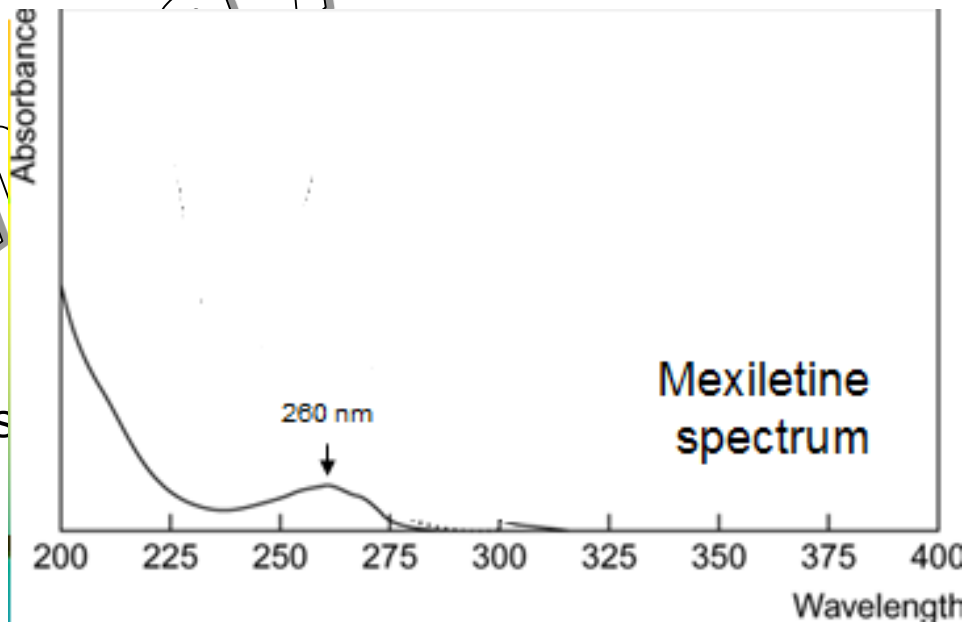
Action and use

Anti-arrhythmic.

Preparations

Mexiletine Capsules

Mexiletine Injection



مضاد لأضطرابات نظم القلب

UV (Std)

MEXITIL INJECTION

(250 mg/10 ml)

(Acceptable range 92.5 to 107.5% of the stated amount.)

Assay :

Dilute a volume 2 ml to 100 ml with 0.01 M HCl .

Measure the absorbance at 260 nm . (A = 0.55)

Calculate % content taking 0.232 as the absorbance of a 20 mg % solution of standard Mexilitine HCl

VIS

Rifampicin

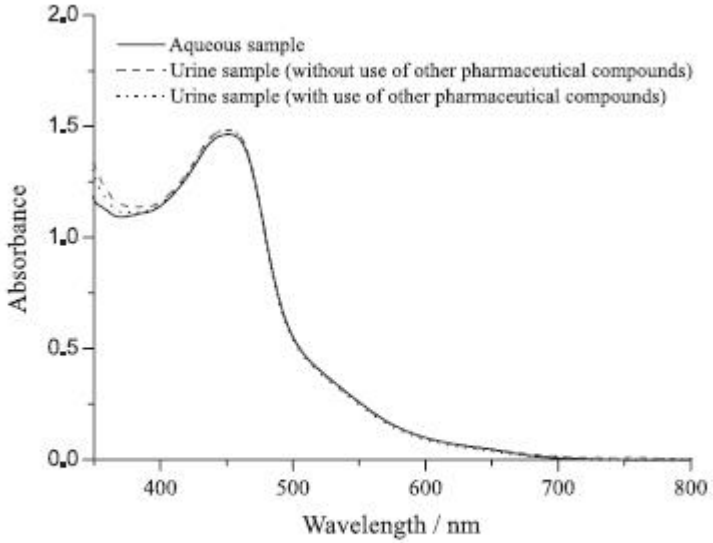
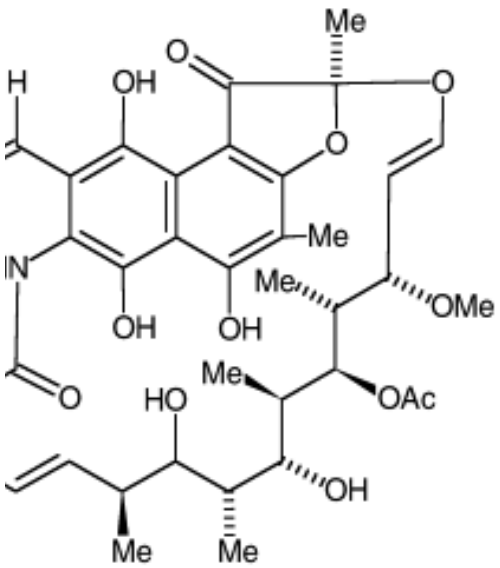


Figure 6. Absorption spectra of urine samples spiked with rifampicin and isoniazid and a synthetic mixture of these analytes after colorimetric reaction.



C43H58N4O12

823

se Antituberculous.

VIS

VIS

Rifampicin Capsules

Visible-spectroscopy method

Assay :

طريقة أخذ العينة

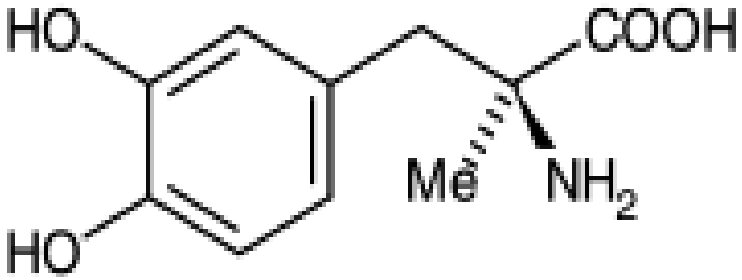
Shake a quantity of the mixed contents of 20 capsules containing 0.1g of Rifampicin with 80 ml of methanol , add sufficient methanol to produce 100 ml and filter. Dilute 2 ml of the filtrate to 100 ml with phosphate buffer pH 7.4 and measure the absorbance of the resulting solution at the maximum at **475 nm**. Calculate the content of C43H58N4O12 taking 187 as the value of A(1%,1cm) at 475 nm . (A = 0.38)

Answer = 101.6%

التركيز النظري 0.002، التركيز العملي 0.00203

Methyldopa

Vis after Derivatization



238.2 C10H13NO4.1½H 2O

Action and use Antihypertensive.

Preparation

Methyldopa Tablets

Vis after Derivat

Principle of assay

The reaction between the Iron ion and Phenol group in Methyldopa give complex with violet color has λmax at 545 nm , convenient to the visible assay.

Methyldopa Tablets

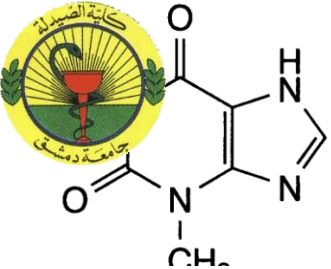
Vis after Dervatisation

ASSAY :

Weigh and powder 20 Tablets. Dissolve a quantity of the powder containing the equivalent of 0.1 g of anhydrous methyldopa as completely as possible in sufficient 0.05 M sulphuric acid to produce 100 ml and filter. To 5 ml of the filtrate add 2 ml of iron(II)sulphate-citrate solution ,8 ml of glycine buffer solution and sufficient water to produce 100 ml.Measure the absorbance of the resulting solution at the maximum at 545 nm.

Repeat the procedure using 5 ml of 0.10%w/v solution of methyldopa BPRS in place of 5 ml of the filtrate, beginning at the words add 2 ml of……..

Calculate the content of C10H13NO4 using the declared content of C10H13NO4 in methyldopa BPRS.



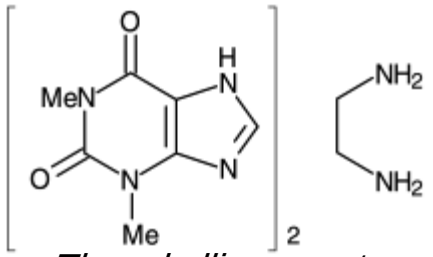
Aminophylline

Slig

very soluble	less than 1
freely soluble	from 1 to 10
soluble	from 10 to 30
sparingly soluble	from 30 to 100
slightly soluble	from 100 to 1000
very slightly soluble	from 1000 to 10 000
practically insoluble	more than 10 000

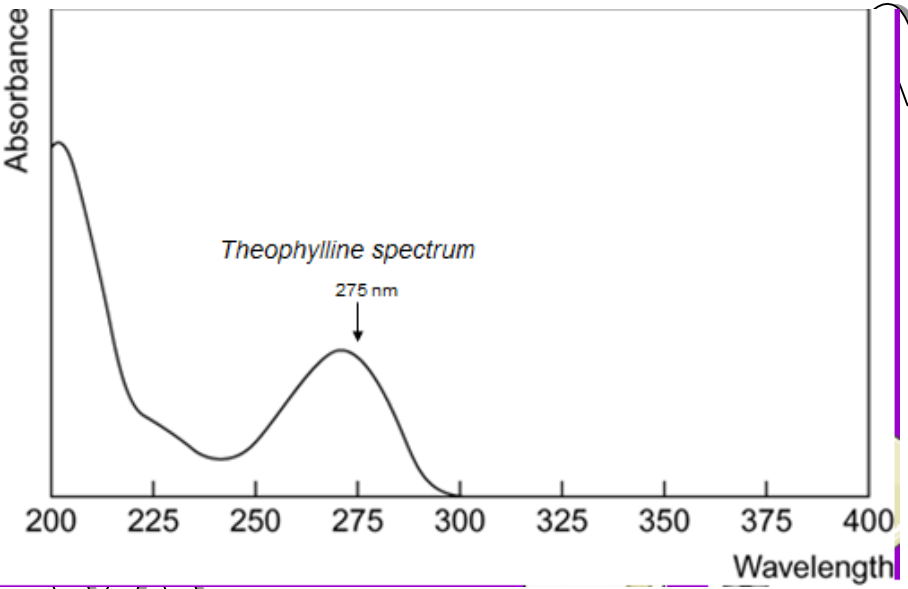
Aminophylline

UV+ Titration



Theophylline spectrum

Theophylline-Ethylenediamine



Ethylenediamine + H_2SO_4

Aminophylline Tablets

UV+ Titration

Content of theophylline, $C_7H_8N_4O_2$

80.6 to 90.8% of the stated amount of Aminophylline.

Content of ethylenediamine, $C_2H_8N_2$

Not less than 10.9% of the stated amount of Aminophylline.

Assay

For theophylline Weigh and powder 20 tablets. Shake a quantity of the powder containing 80 mg of Aminophylline with a mixture of 20 ml of 0.1M *sodium hydroxide* and 60 ml of *water* for 10 minutes, add sufficient *water* to produce 200 ml, mix and filter. Dilute 5 ml of the filtrate to 250 ml with 0.01M *sodium hydroxide* and measure the *absorbance* of the resulting solution at the maximum at 275 nm, Appendix II B. Calculate the content of $C_7H_8N_4O_2$ taking 650 as the value of $A^{1\%}_{1\,cm}$ at the maximum at 275 nm.

For ethylenediamine Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.3 g of Aminophylline with 20 ml of *water*, heat to 50° for 30 minutes and titrate with 0.05M *sulphuric acid VS*, using *bromocresol green solution* as indicator, until the color changes from blue to green. Each ml of 0.05M *sulphuric acid VS* is equivalent to 3.005 mg of C2H8N2.

lole Ethylenediamine ≡ 1 Mole H_2SO_4

Indicator	Color			pH Range
	acidic	endpoint	basic	
bromocresol green	yellow	green	blue	4.0-5.6
methyl red	red	yellow	yellow	4.4-6.2
bromothymol blue	yellow	green	blue	6.2-7.6
phenolpthalein	colorless	light pink	red	8.0-10

ent

Weak base vs strong acid , titration jump from 4 to 6

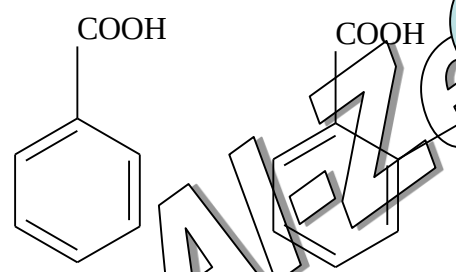
Pharmaceutical Application (Pharmaceutical Dosage Form 2 API)

- Compound Benzoic acid ointment
- Aspirin and Caffeine Tablets
- Zinc and Salicylic Acid Paste

Compound Benzoic acid ointment Bp2007

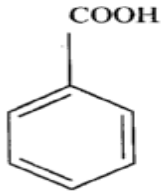
- Benzoic acid 6%
- Salicylic acid 3%

Use : Antiacne



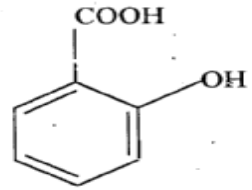
Vis after Dervatisation&Titration

e in water



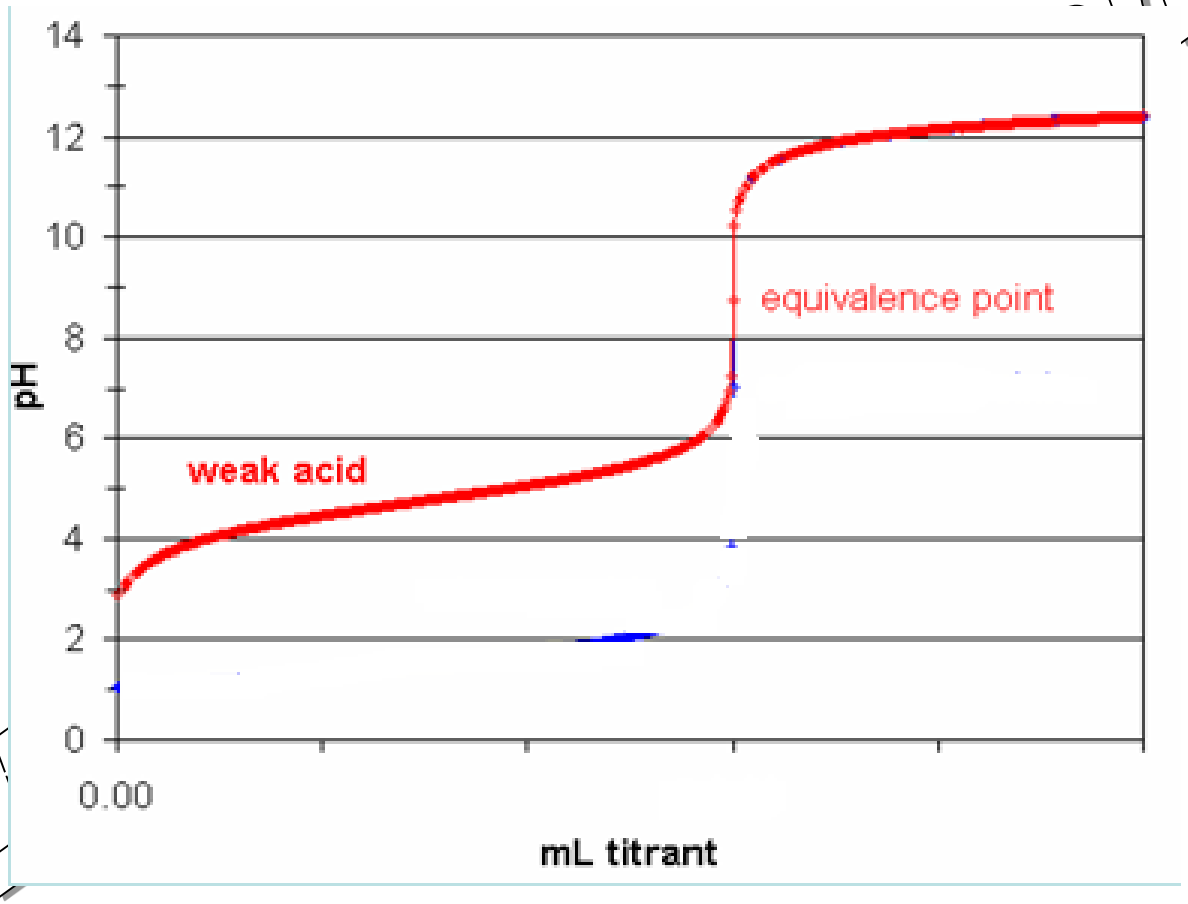
Benzoic acid

pKa=5.18



Salicylic acid

pKa= 3.97



Derivatization,Vis and titration

DEFINITION

Compound Benzoic Acid Ointment contains **6.0% w/w of Benzoic Acid and 3.0% w/w of Salicylic Acid in a suitable emulsifying basis.**

Content of benzoic acid, C7H6O2

5.7 to 6.3% w/w.

Content of salicylic acid, C7H6O3

2.7 to 3.3% w/w.

ASSAY

For benzoic acid

To 2 g add 150 ml of *water* , warm until melted and titrate with 0.1M *sodium hydroxide VS* using *phenolphthalein solution R1* as indicator. Reserve the solution for the Assay for salicylic acid. After the subtraction of 1 ml for each 13.81 mg of C7H6O3 found in the Assay for salicylic acid, each ml of 0.1M *sodium hydroxide VS* is equivalent to 12.21 mg of C7H6O2.

For salicylic acid

Cool the titrated solution obtained in the Assay for benzoic acid, dilute to 250 ml with *water* and filter. To 5 ml of the filtrate add sufficient *iron(III) nitrate solution* to produce 50 ml. Filter, if necessary, to remove haze and measure the *absorbance* of the resulting solution at the maximum at 530 nm, Appendix II B, using *iron(III) nitrate solution* in the reference cell. Calculate the content of C7H6O3 from the absorbance obtained by repeating the operation using 5 ml of a 0. 024% w/v solution of *salicylic acid* and beginning at the words 'add sufficient *iron(III) nitrate solution* ...'.

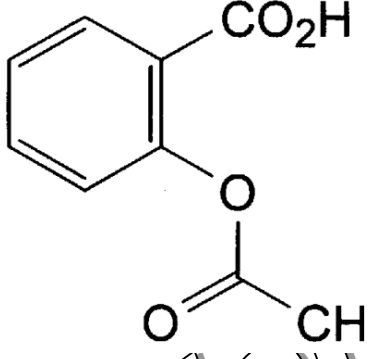
mpound Benzoic Acid Ointment contain 3% Salicylic acid & 6% Benzoic acid , the assay will done as the following :

very soluble	less than 1
freely soluble	from 1 to 10
soluble	from 10 to 30
sparingly soluble	from 30 to 100
slightly soluble	from 100 to 1000
very slightly soluble	from 1000 to 10 000
practically insoluble	more than 10 000

following information :A(S) = 0.38 , A(ST)= 0.39Calculate the % content of S.A and B.A

Aspirin and Caffeine Tablets (BP)

Aspirin and Caffeine Tablets



DEFINITION

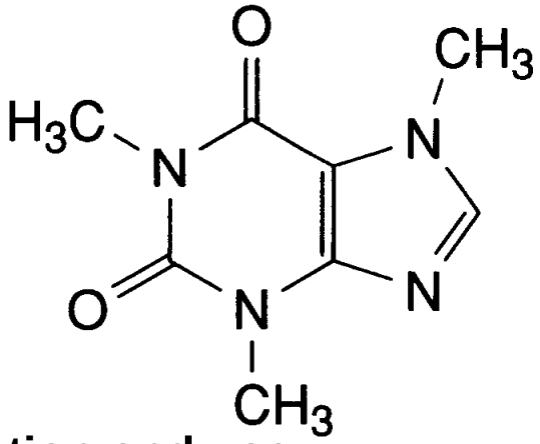
Aspirin and Caffeine Tablets contain, in each, 350 mg of Aspirin and 30 mg of Caffeine.

Content of aspirin, $C_9H_8O_4$

330 to 370 mg.

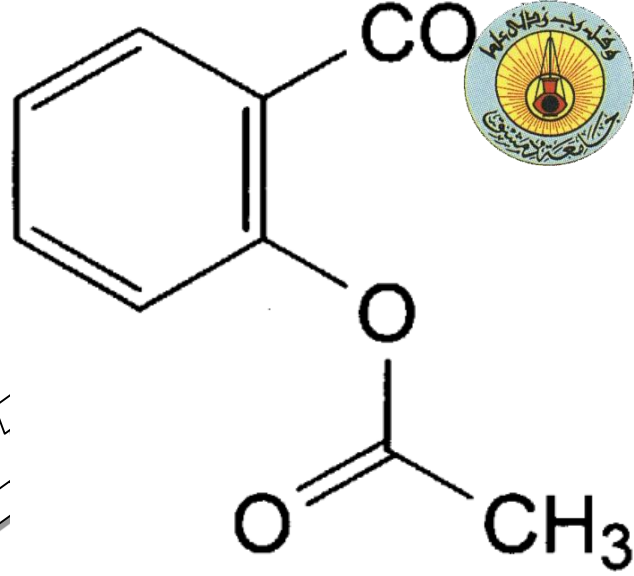
Content of caffeine, $C_8H_{10}N_4O_2$

27.5 to 32.5 mg.



Action and use

Central nervous stimulant.



1- Aspirin :(as Raw material)

$C_9H_8O_4$ 180.2 *50-78-2*

ASSAY

In a flask with a ground-glass stopper, dissolve 1.000 g in 10 ml of *alcohol R*. Add 50.0 ml of *0.5 M sodium hydroxide*. Close the flask and allow to stand for 1 h. Using 0.2 ml of *phenolphthalein solution R* as indicator, titrate with *0.5 M hydrochloric acid*. Carry out a blank titration. 1 ml of *0.5 M sodium hydroxide* is equivalent to 45.04 mg of C9H8O4.

Back-titration

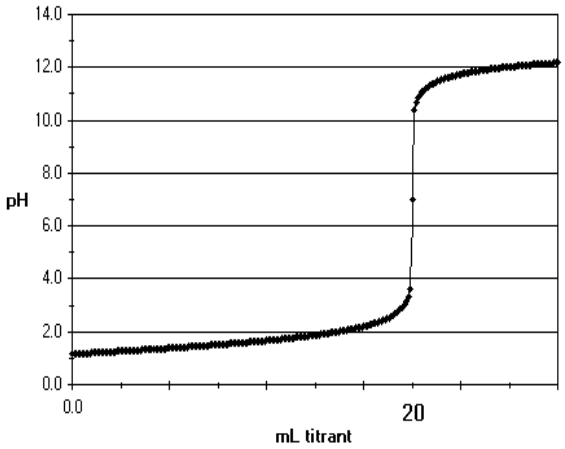
Asprin

$NaOH + HCl \rightarrow NaCl + HOH$

1 M ASPIRIN ≡ 2 M NaoH

180.2 g 2000 ml NaOH 1M

180.2 g 4000 ml NaOH 0.5 Mol



2- C

C_8H

ASS

D

anh

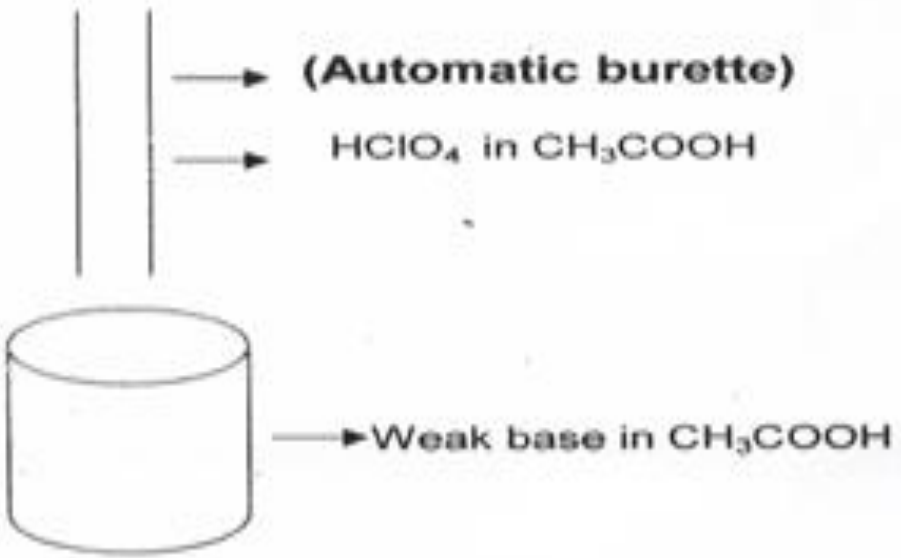
ml c

R. T

dete

(2.2

equ



* Burette : $HClO_4 + CH_3COOH \longrightarrow CH_3COOH_2^{\oplus} + ClO_4^{\ominus}$
Acétacetium ion

* Conical : $R_1R_2N: + CH_3COOH \rightarrow R_1R_2N^{\oplus}H + CH_3COO^{\ominus}$
Acetate ion

* Reaction : $CH_3COOH_2^{\oplus} + CH_3COO^{\ominus} \longrightarrow 2CH_3COOH$

* Indicato: $IND + CH_3COOH_2^{\oplus}ClO_4^{\ominus} \rightleftharpoons IND^{\oplus}ClO_4^{\ominus} + CH_3COOH$

Crystal Violet
Naphtolbenzein
Malachite Green

10

ne

y

SAY (as finished product)

Weigh and powder 20 tablets.

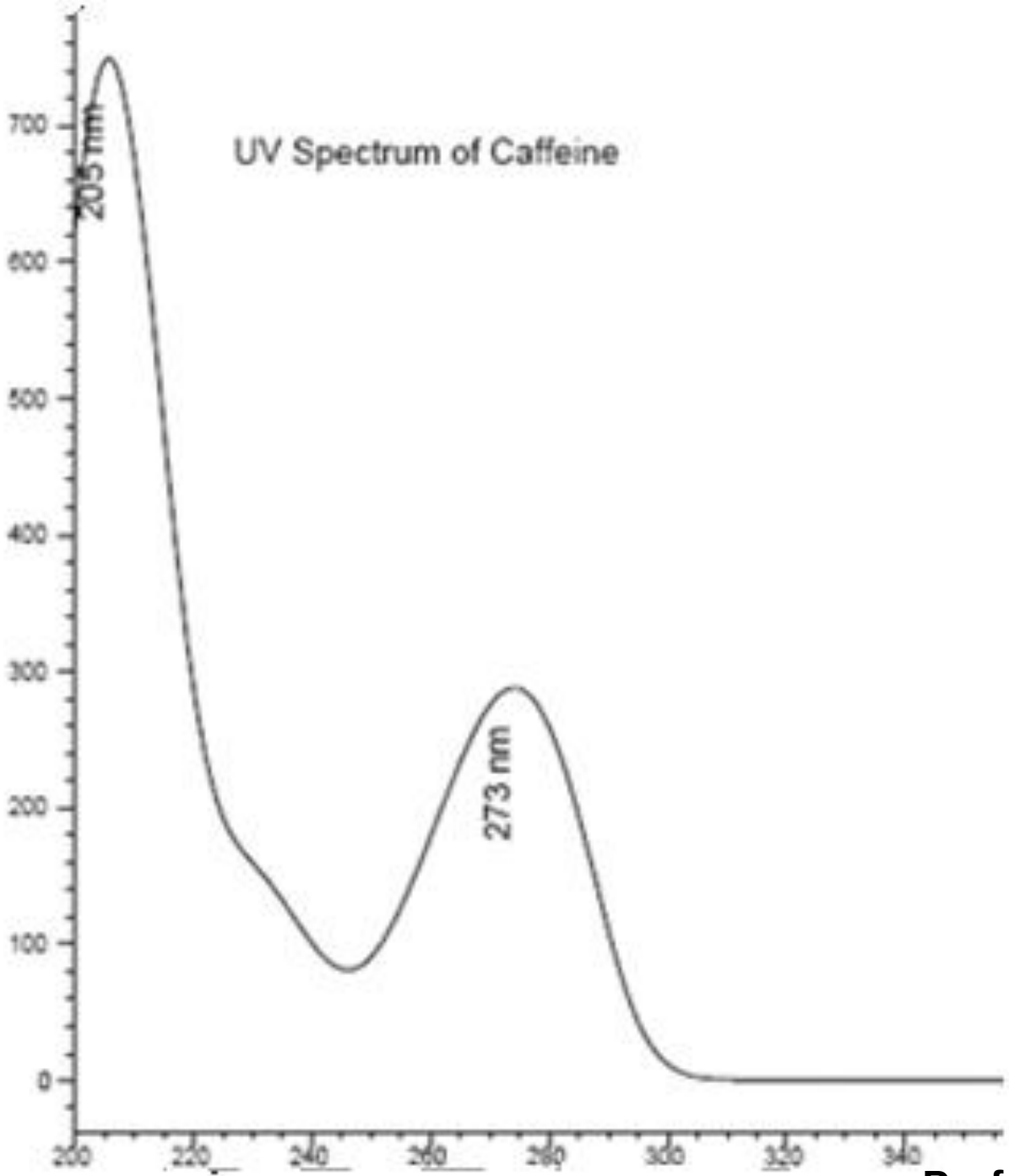
For aspirin

To a quantity of the powder containing **0.7 g** of Aspirin add 20 ml of *water and 2 g of sodium citrate and boil under a reflux condenser for 30 minutes. Cool, wash the condenser with 30 ml of warm water and titrate with 0.5M sodium hydroxide VS using phenolphthalein solution R1 as indicator. Each ml of 0.5M sodium hydroxide VS is equivalent to* ***45.04 mg*** *of* $C_9H_8O_4$.

caffeine

To a quantity of the powder containing **30 mg** of Caffeine add 200 ml of *water and shake for 30 minutes. Add sufficient water to produce* ***250*** *ml and filter. To* ***10 ml*** *of the filtrate add 10 ml of 1M sodium hydroxide and extract immediately with five 30 ml quantities of chloroform, washing each extract with the same 10 ml of water. Filter the combined chloroform extracts, if necessary, through absorbent cotton previously moistened with chloroform.*

Evap
disso
possi
neces
produ
neces
resul
nm, A
of CE
A(1%



o
he
73
nt
of
m.

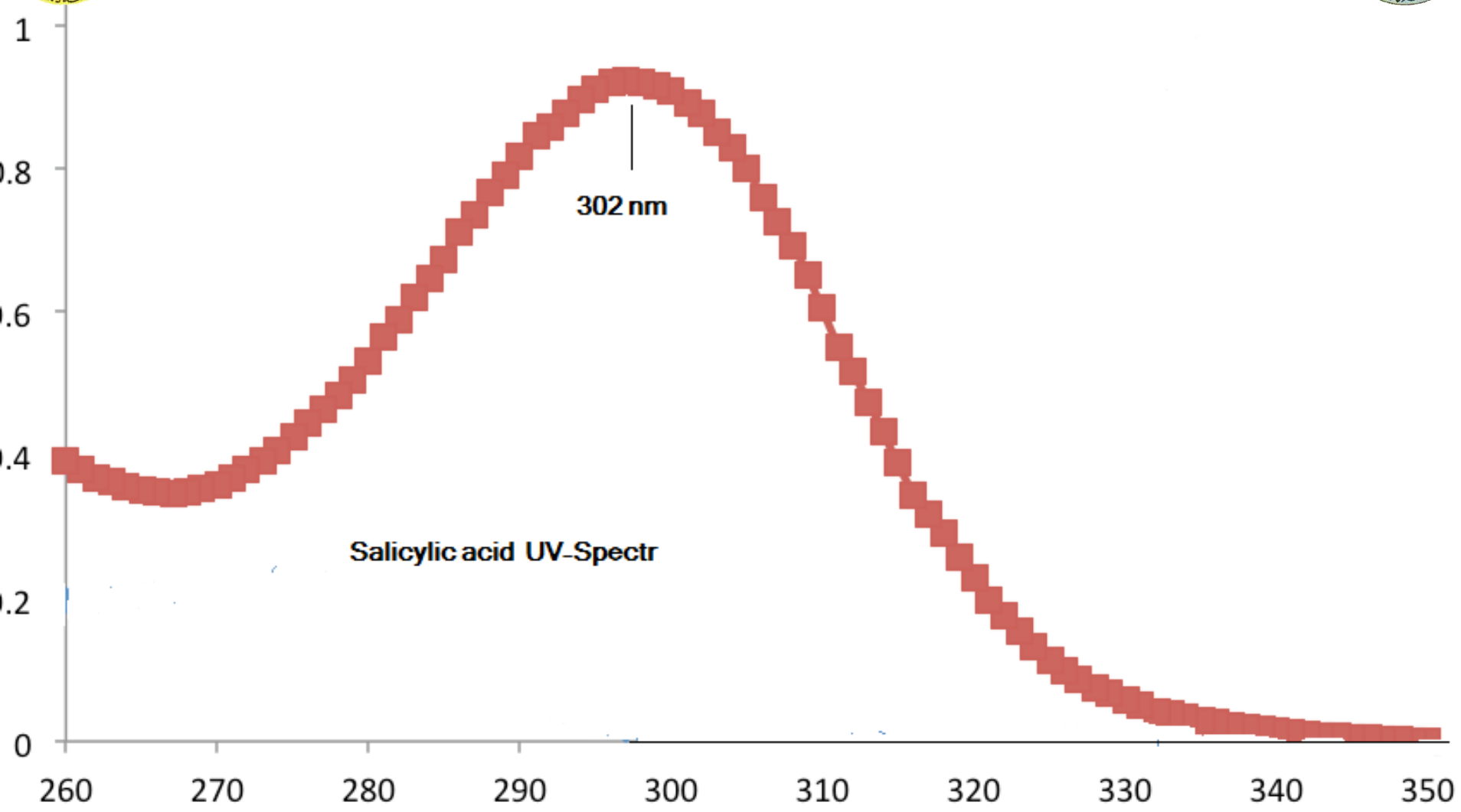
- If Vs= 15.5 & F=1

calculate the % of Aspirin & the practical tablet contains

-If A= 0.63

calculate the % of caffeine & the practical tablet contains

Zinc and Salicylic Acid Paste



1.9 to 2.1% w/w.

UV+ EDTA titration

SAY
for salicylic acid

Shake **0.5 g** with 10 ml of 1M *hydrochloric acid and 10 ml of ether until fully dispersed. Decant and reserve the aqueous layer. Extract the ether layer with two further 10 ml quantities of 1M hydrochloric acid, combine the aqueous extracts with the reserved aqueous layer, wash with 10 ml of ether and reserve for the Assay for zinc oxide. Combine the ether extracts, add 15 ml of petroleum spirit (boiling range, 40° to 60°) and extract with successive quantities of 20 ml, 10 ml and 10 ml of a mixture of equal volumes of ethanol (90%) and 1M sodium hydroxide. Dilute the combined extracts to 100 ml with 2M hydrochloric acid, further dilute 15 ml of the resulting solution to 50 ml with the same solvent and measure the absorbance of the final solution at the maximum at 302 nm, Appendix II B. Calculate the content of $C_7H_6O_3$ taking 260 as the value of A(1%, 1 cm) at the maximum at 302 nm.*

For zinc oxide

To the combined aqueous extracts obtained in the Assay for salicylic acid add 20 ml of 1M *sodium hydroxide and 50 mg of xylenol orange triturate. To the resulting solution add sufficient hexamine to change the colour of the solution to red and then a further 3 g of hexamine and* ***titrate with 0.1M disodium edetate VS. Each ml of 0.1M disodium edetate VS is equivalent to 8.139 mg of ZnO.***

- If Vs= 14.1 & F=1

calculate the % of Zinc Oxide & the practical paste contains

-If A= 0.78

calculate the % of Salicylic acid & the practical paste contains


Thank you

Q&A