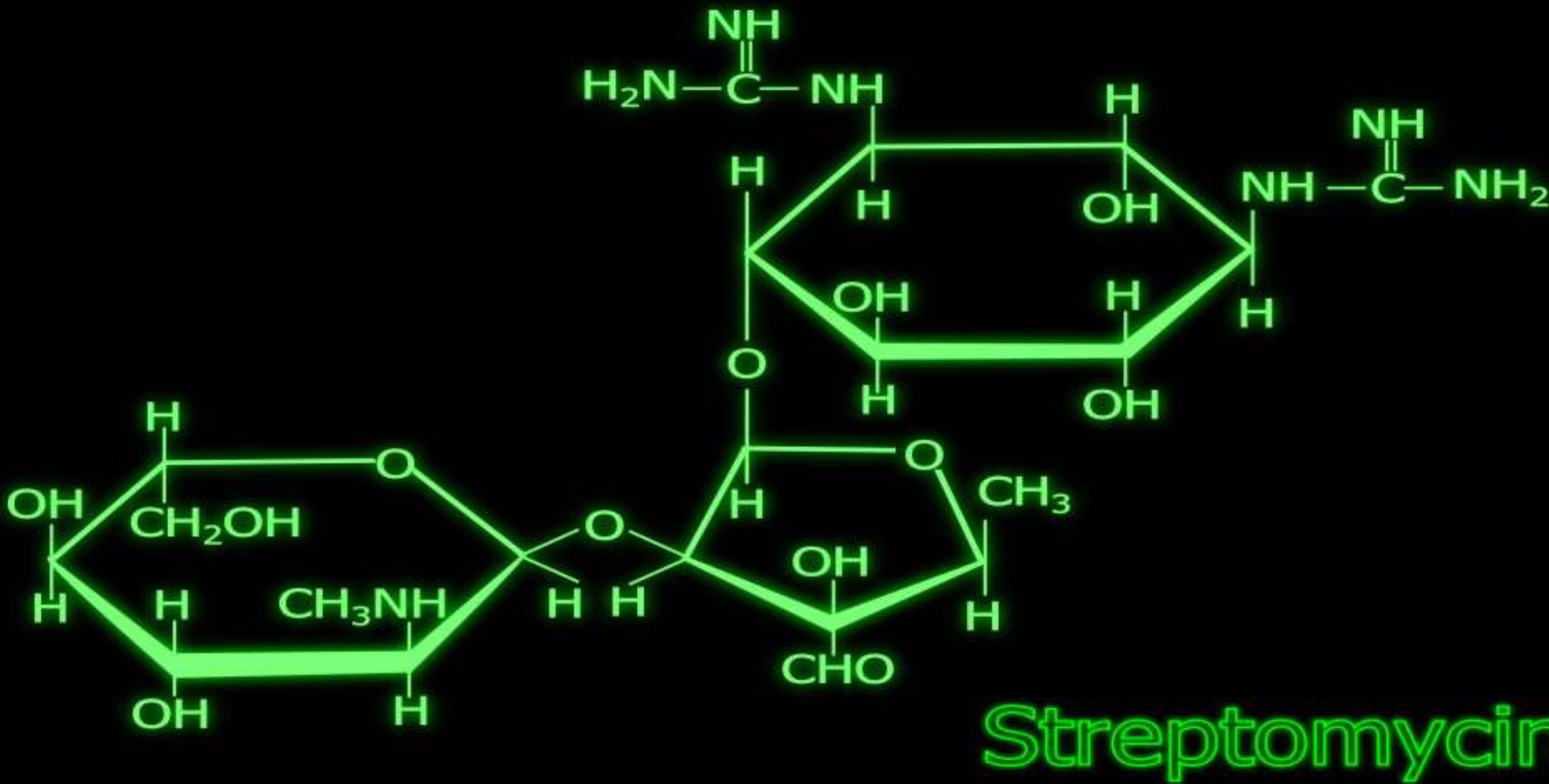
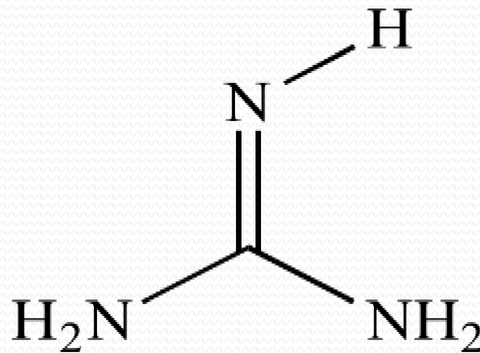
Assay of streptomycin

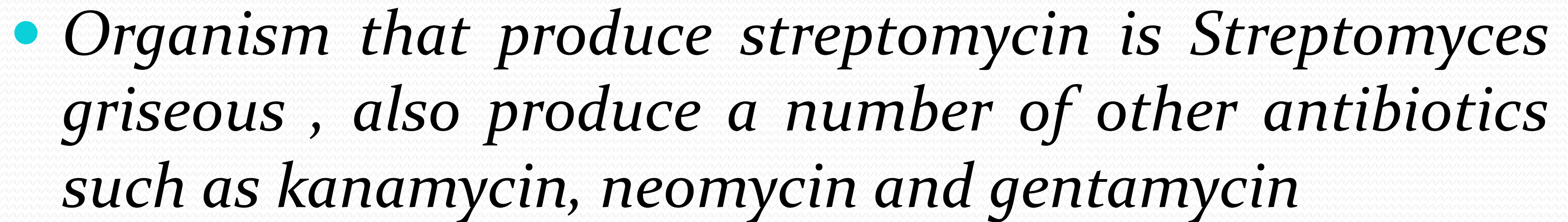
- *Streptomycin sulfate is a white odorless powder that is hygroscopic but stable toward light and air ,its freely soluble in water forming solution that are slightly acidic or nearly neutral .*
- *Its very slightly soluble in alcohol and in soluble in most organic solvents.*



- streptobiose amine ((N-methyl- L-glucosamine + L-streptose))
- streptidine

- *Acid hydrolysis yield streptidine and streptobiose amine which is a combination of N-methyl- L-glucosamine and L-streptose .*
- *It act as triacidic base through the effect of its strong basic guanidine groups, and the more weakly basic N-methyl amino group.*
- *Aqueous solution may be stored at room temperature for one week without loss of potency .*





Organism that produce streptomycin is Streptomyces griseous , also produce a number of other antibiotics such as kanamycin, neomycin and gentamycin

- ***Mechanism of action***:
- *ST. bound to ribosome at 30s subunit and inhibit the initiation of protein synthesis ,or may cause coding for insertion of wrong amino acid in protein molecule .*
- *ST. exert bactericidal action unlike most other antibiotic that interfere with protein synthesis whose action are bacteriostatic. It's used in the treatment of TB. But it's used as adjuvant treatment*

Oxidized nitroprusside method

- *Guanidine and many of its other derivatives react with oxidized nitroprusside reagent to give a color which vary from orang to red depend up on particular compound .*
- *In ST. and di-hydro ST. The streptidine portion of which are guanido structure so both form orange color with this reagent .*
- *This method is suitable for both ST. and di hydro-ST. .*

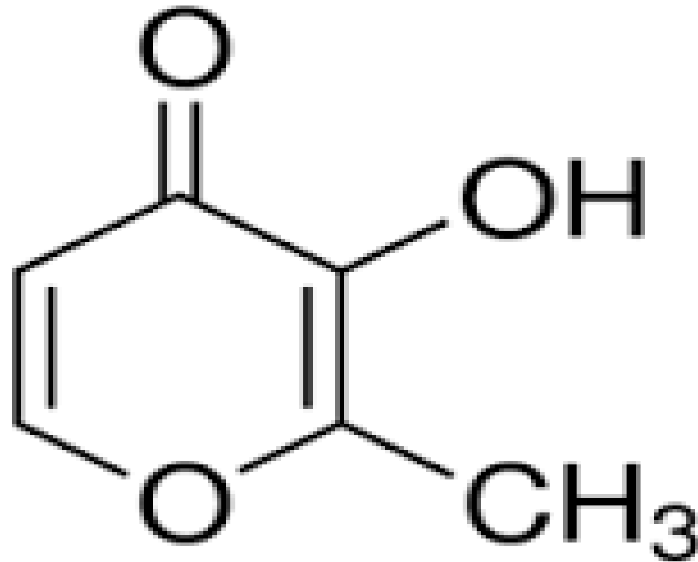
- *Procedure:*

	I	II	III	IV	V	VI	UN	BL
Stock	*1ml*	*2*	*3*	*4*	*5*	*6*	-	-
DW	*9*	*8*	*7*	*6*	*5*	*4*	-	*10*
UNK	-	-	-	-	-	-	*10ml*	-
Oxid. Nitro.pros.	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*

- *Wait for 3 min. read at 490 nm within 30 min not more*

Maltol method for the assay

- *Alkaline hydrolysis of ST. produce maltol, the maltol product is derive from the streptose portion*



- *Maltol react with ferric ion to give purple-red color*
- *So by hydrolysis using NaOH and heating in boiling waterbath for 10 min.*
- *ST. decomposes to streptidine and di-saccharide portion of molecule (streptobose amine).then hydrolysis of streptobios amine to 2 sugar moieties (streptose +hexose).*
- *Streptose by NaOH rearrangement by cleavage to 2 portion ,then reattachment of 2 portion after electronic re arrangement to give maltol*
- *Then 2 molecules of maltol attach to form chelate with ferric ion , this give violet color which could be use as a base for determination of conc. Of ST.*
- *Di hydro-ST dose not give maltol compound so cant be assayed by this method*

Procedure

Stock	std.I	II	III	IV	V	VI	UNK	BLK
	1	2	3	4	5	6	10	-
DW	9	8	7	6	5	4	-	10
1N NaOH	2	2	2	2	2	2	2	2
Heat (boiling) in water bath for 10 min. then cool , then add								
1.2N HCL	2	2	2	2	2	2	2	2
0.25% $FeCL_3$	5	5	5	5	5	5	5	5

- ***Then complet the volume to 25 ml by D.W and read at A 550 nm***
- ***Shaking is important after each addition***
- ***HCLis used to neutralized excess NaOH***
- ***Ferric chloride required an acidic medium to form a complex***

Quantitative analysis
(Beer's law)

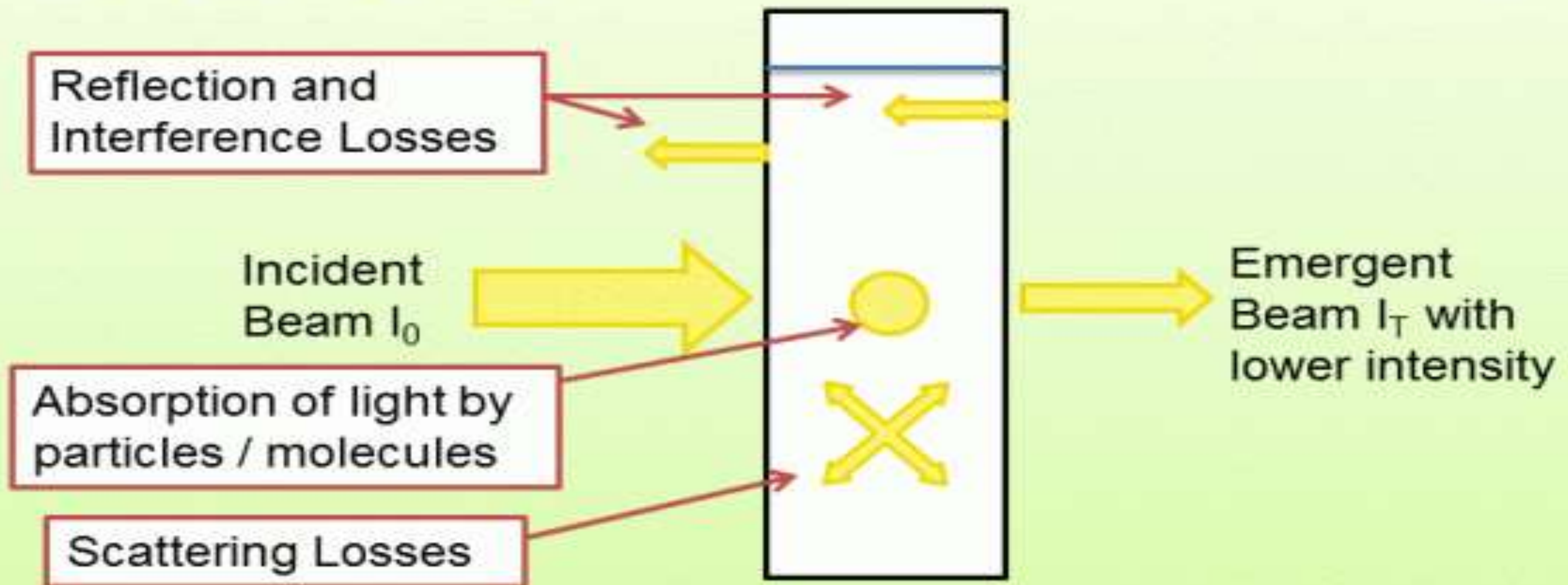
Objective

- 1-To demonstrate if a colored solution obeys Beer's law at the wave length of maximum of absorption(λ max) , and if so;

- 2- to find the concentration of an unknown colored solution from a Beer's law plot.

Many compounds absorb ultraviolet or visible light. When a beam of monochromatic radiation I_0 is directed at a solution, some will be absorbed, some transmitted I_T, and some reflected I ref.

I ref is compensated by the blank



Relationship between absorbance and transmittance

$T = I_t / I_o$ (the fraction of light that passes through the sample)

where: I_o = intensity of the incident radiation entering the medium.

I_t = intensity of the transmitted radiation leaving the medium.

T is usually expressed as percent transmittance, %T: ((%T= T x 100))

The relationship between percent transmittance (%T) and absorbance (A) is given by the following equation:

$A = \log I_o / I_t$

$A = \log 1 / T$

$A = \log 100 / \%T$

$A = 2 - \log \%T$

Beer's law

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

Beer-Lambert Law

Two scientists had studied the factors that affect the extent of absorption of monochromatic radiation at certain wave length .

Lambert studied the effect of the thickness of the medium on absorption , he stated that the intensity of transmitted light is inversely proportional to the thickness of the medium.

Log $I_0/I_t \propto t$ ***A=K t***

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

Log $I_0/I_t \propto c$ ***A=K c***

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

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

K= constant

Combining both equations

A= K c t

$$\log_{10}\frac{I_o}{I} = \varepsilon\, l\, c$$

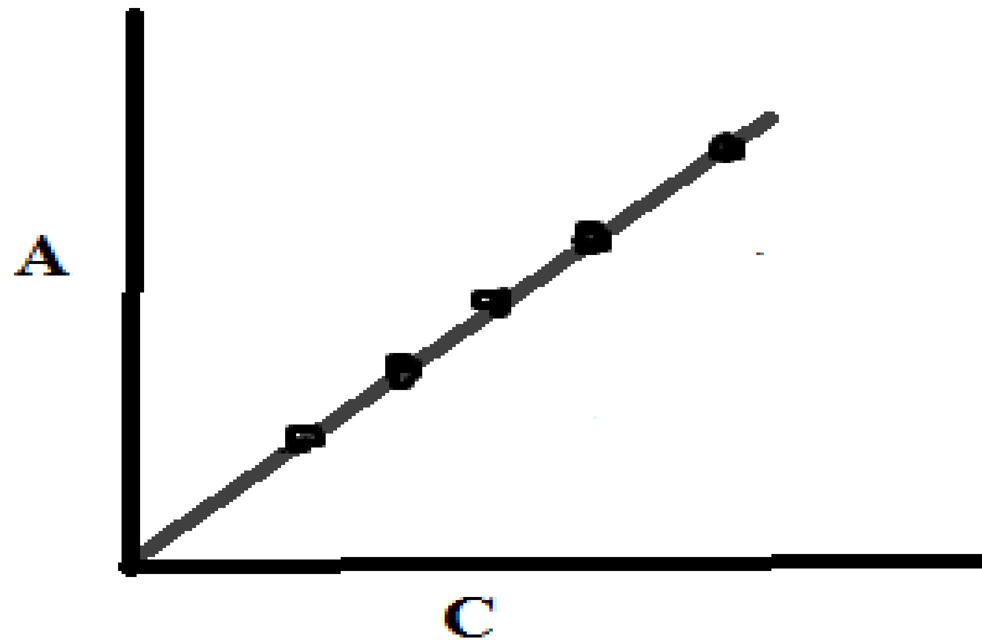
Greek letter, epsilon

concentration of solution (mol dm^{-3})

length of solution the light passes through (cm)

This means that the absorbance is directly proportional with the concentration of the sample absorbing the monochromatic radiation and the path length of sample (the path length of the cuvette).

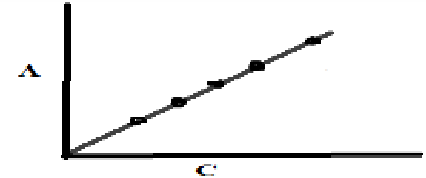
- **A,** is absorbance (no unite, since A=log Io/ I)
- **C,** is concentration of compound in solution, expressed in mol per litter
- **K,** is symbolized as ***ε*** and called the molar extinction coefficient or molar absorptivity (unit is L per mole per cm) if c is molar concentration. The larger the molar absorptivity, the more probable the electronic transition.
- ***t,*** length of the light path in cm.



A1/C1=A2/C2=A3/C3

Beer-Lambert Law

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



Beer's law is obeyed for dilute solutions (the concentration should be low).

Some solutions may not obey Beer's law as in sulfonamides (no straight line is obtained).

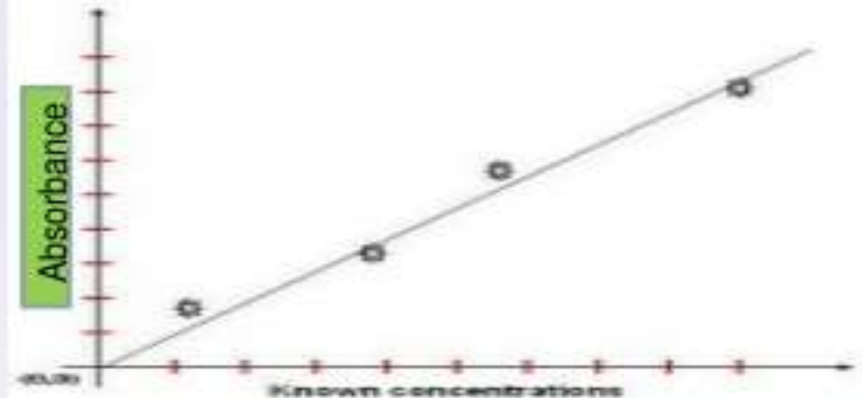
Purpose:

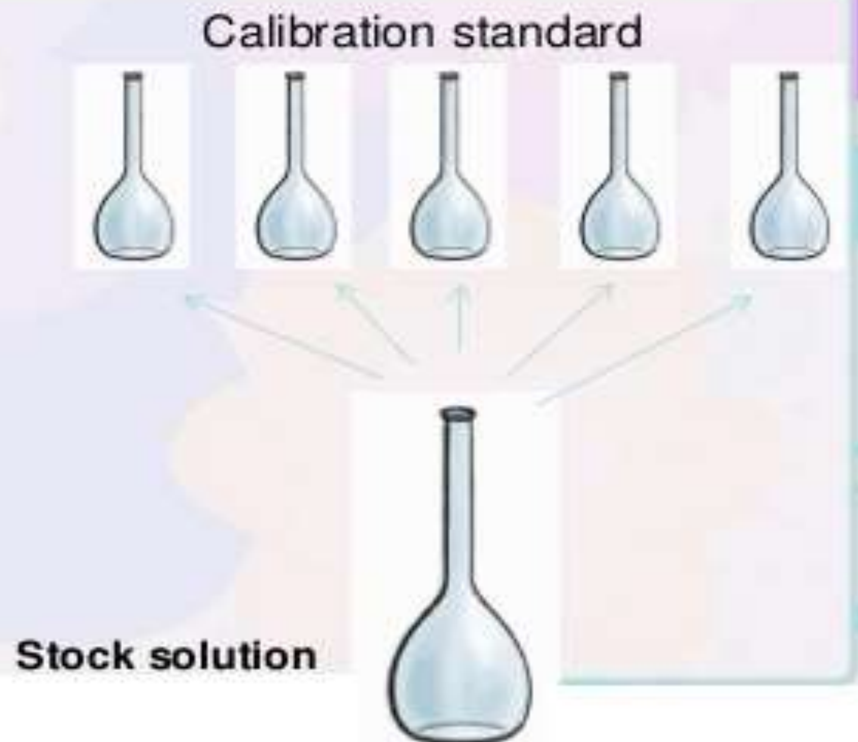
1- To demonstrate whether a solution obeys Beer's Law at ʎ max.

2- To find the concentration of an unknown solution from Beer's Law plot.

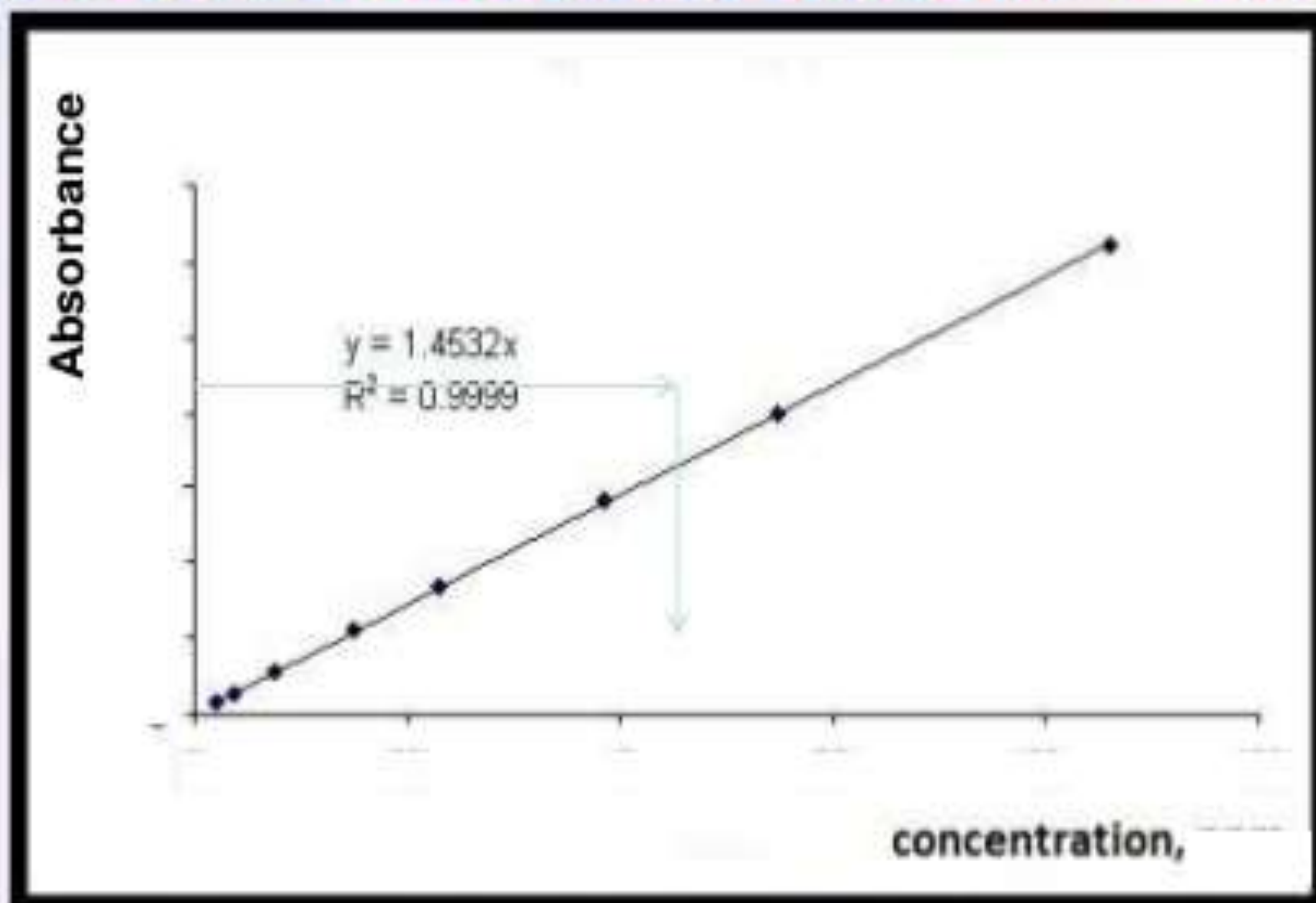
Calibration curve (standard curve)(Beer's plot)

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





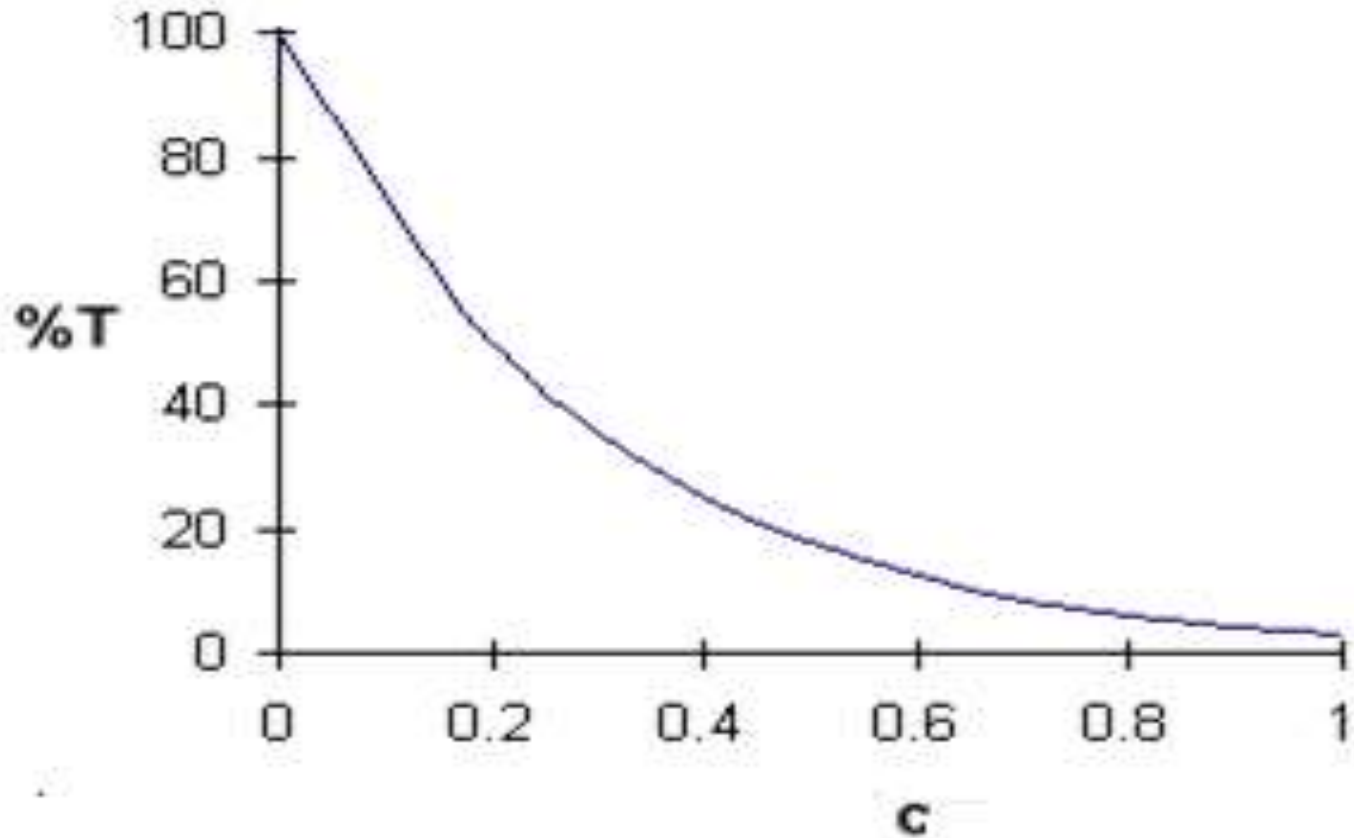
Standard Calibration Curve



How to measure the concentration of unknown?

- Practically, you have to measure the absorbance of your unknown. Once you know the absorbance value, you can just read the corresponding concentration from the graph.

The relation ship between %T and C is not linear unlike that between A and C



Notes

- It is preferred to express the Beer' plot using absorbance rather than %T . why?((H.W))

- Always read at ʎ max . why?

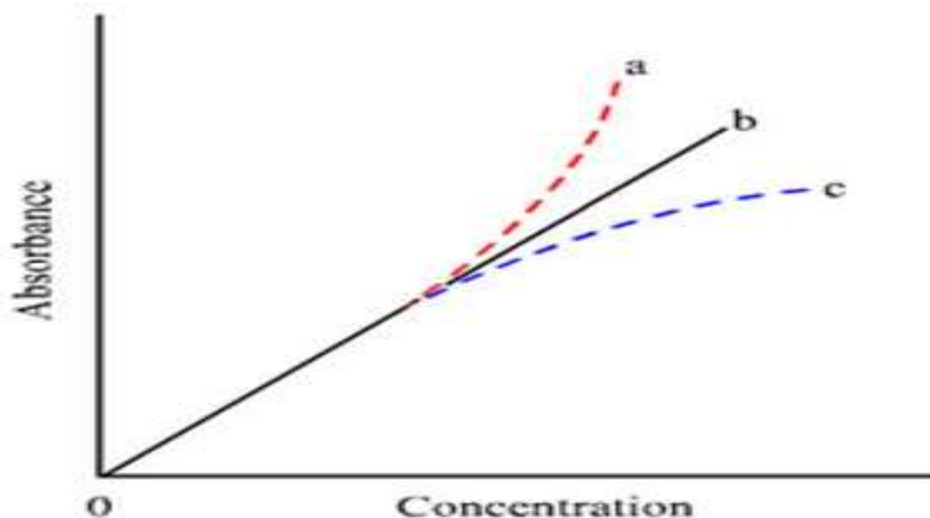
  Since, better sensitivity and reduced error in measurement.(maximum absorbance)

- Always read from lower conc. to higher conc.
- In this experiment we read at one wave length which is ʎ max therefore zeroing with blank is needed only once.

Deviations from Beer's law

Some solutions show deviations from Beer's law ex: at high concentration solutions, dimerization and polymerization occurs, these aggregates absorb at ʎ max different from that of the monomers. Where as at very low concentrations (very dilute solutions) deviation may also occurs.

Deviations are either positive or negative.



Today's experiment

- Name of experiment: assay of chromic nitrate solution

- Aim of experiment: determination of the concentration of unknown of chromic nitrate solution.
- From stock solution (0.05 M) prepare 5 standard solutions of different conc.

$$M1\ V1 = M2\ V2$$

- Read the A for them and draw Beer's plot.
- Read the absorbance of unknown then determine its conc. From the plot.

Name

Aim

No. of unk.

No. of instrument

Results:

1. plot (in detail)
2. Table A , C of standard solution (mathematical details required)
3. unk. A , C

Molar absorptivity

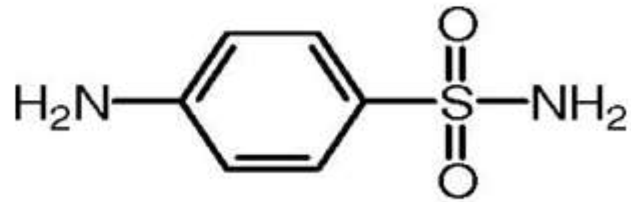
- Molar absorptivity is a constant for a particular substance, it is the probability of absorption of a sample at certain wave length. So if the concentration of the solution is halved the absorbance is halved also.

- A compound with a high molar absorptivity is very effective at absorbing light (of the appropriate wavelength), and hence low concentrations of a compound with a high molar absorptivity can be easily detected.

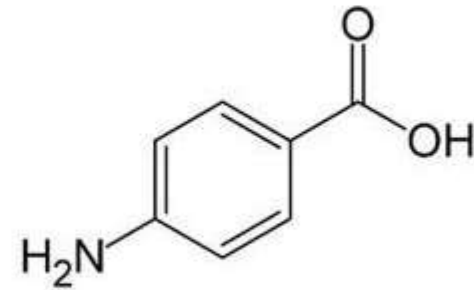
Colorimetric Assay of Sulfa Drugs

Bratton and Marshall Method

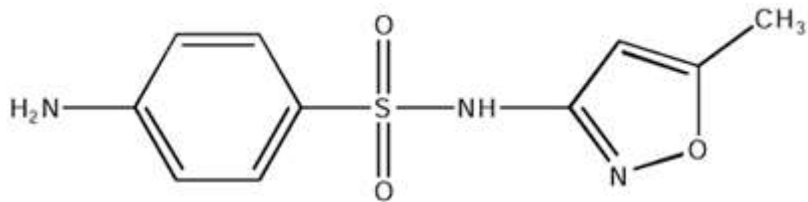
structures



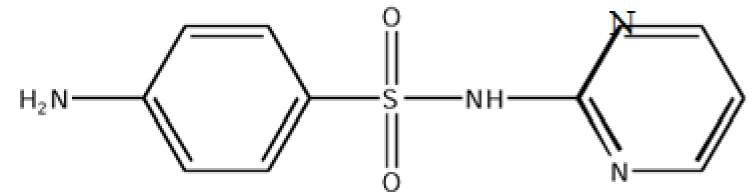
sulfanilamide



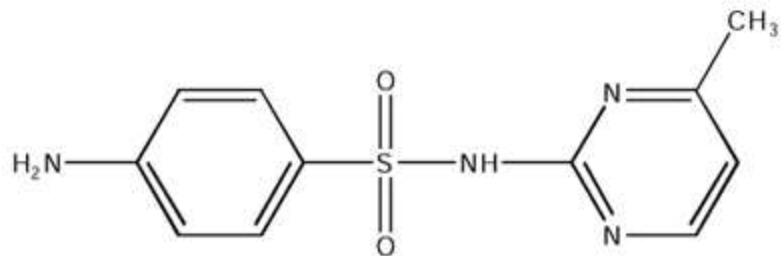
PABA



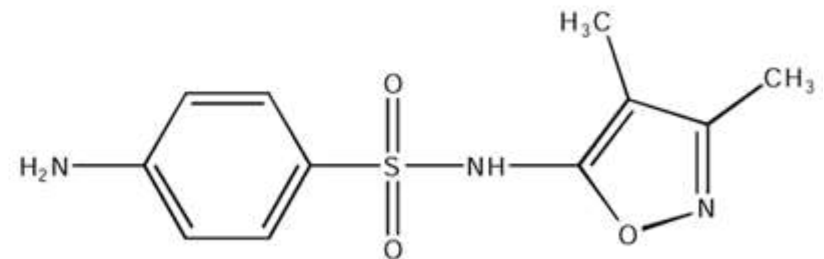
Sulfamethoxazole



Sulfadiazine



Sulfamerazine



Sulfisoxazole

Introduction

- The sulfonamide antibacterial drugs were first effective chemotherapeutic agents that could be used systemically for the cure of bacterial infections in humans.
- Today there are a few sulfonamides and especially sulfammethoxazole –trimethoprim combination that are used for opportunistic infections in patients with AIDs as treatment or prophylaxis, cerebral toxoplasmosis, also useful in urinary tract infections because of their high excretion fraction through the kidneys, and in burn therapy.
- Mechanism of action: sulfonamides are structural analogs of PABA ; they competitively inhibit the net biosynthesis of folate coenzymes that will inhibit bacterial growth and cell division. Thus Sulfonamides are bacteriostatics.

properties

- Most of sulfonamides are slightly soluble in water so the main disadvantage is their crystallization in renal tubules.
- Sulfonamides are weak acids and they form salts with bases example sodium salts which are very soluble in water.

Assay principle

- The method of analysis is based on the presence of the free primary aromatic amine group.
- Diazotization: is the reaction in which the free aromatic amine group reacts with nitrous acid (unstable formed by reaction of $NaNO_2$ with mineral acid) to form a diazonium salt and coupling the diazo-compound with N-(1-naphthyl)-ethylene diamine.
- A pink color is obtained and the intensity of the color is compared with standards produced by treating Known amount of sulfonamides in a similar manner and measured in the region of 540-550 nm.

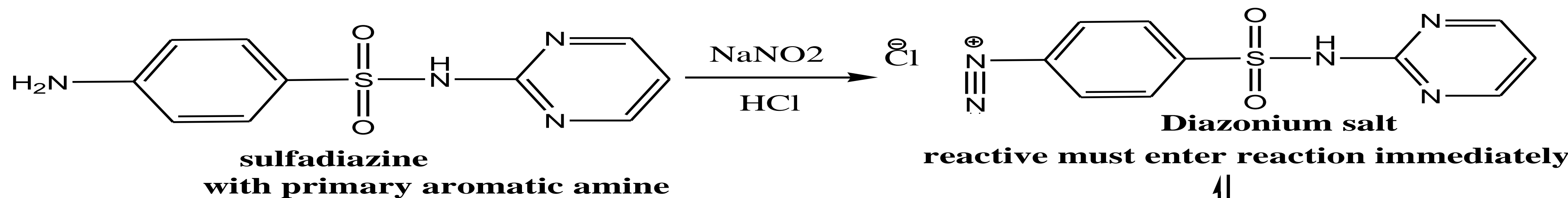
Assay principle

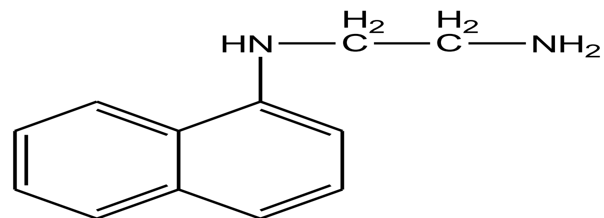
- Diazonium salts formed should used immediately or it may undergo decomposition.
- Diazonium salts are weak electrophiles that react with coupling reagents which are aromatic compounds containing strong electron releasing groups such as (OH, NH_2, NHR) rich in electrons leading to formation of azo bond and the resulting compound is an azo dye (colored compound due to the high conjugation).
- Excess nitrous acid is removed by addition of sulfamic acid.

Bratton Marshall Method

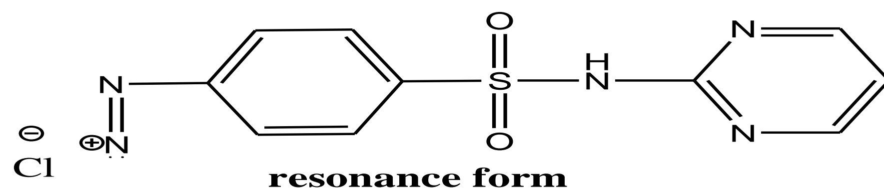
- sulfa drugs show deviation from Beer's law (the system does not obey Beer's law), as a result another method is used called Bratton Marshall method:

$$\textbf{Conc. of unknoun} = \frac{\textbf{A of unknoun* conc. Of standard}}{\textbf{Absorbance of standard}}$$





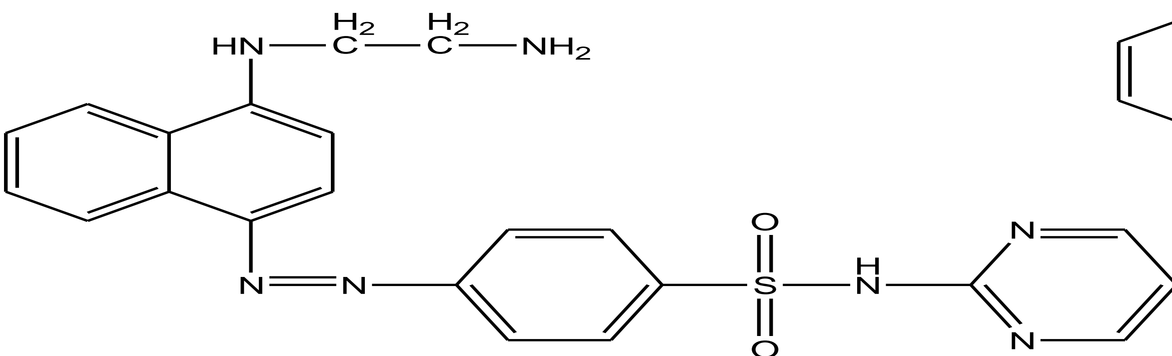
+

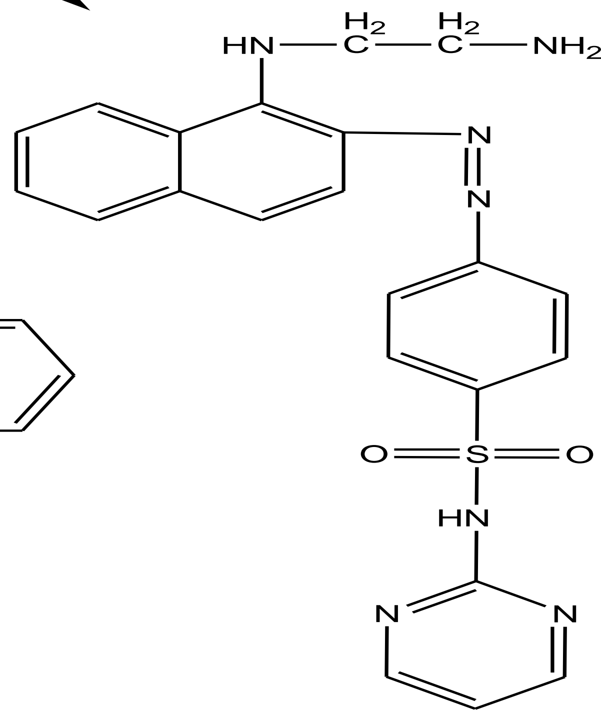


Coupling reagent

para position

ortho position



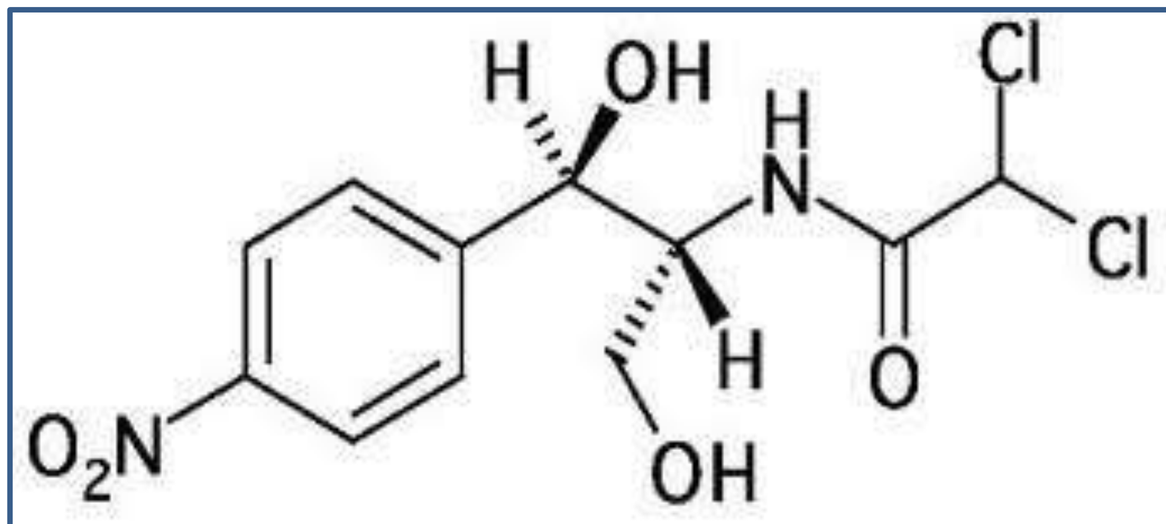


Azo dye
pink color

Assay of Chloramphenicol

Colorimetric Method

Structure



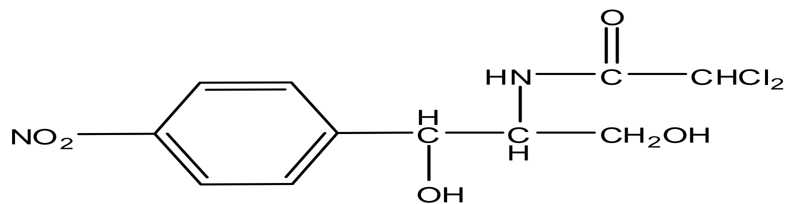
Properties

- A broad spectrum bacteriostatic antibiotic.
- White crystalline compound that is very stable in the bulk state and in solid dosage forms. In solutions, however, it slowly undergoes various hydrolytic and light induced reactions. The rates of these reactions depend on pH, heat, and light.
- Very soluble in alcohol and other polar organic solvents but only slightly soluble in water.
- It has no odor but has a very bitter taste.
- Structurally it possesses two chiral carbon atoms as a result four sterioisomers; only the D-threo isomer is biologically active.

- Unfortunately instances of serious blood dyscrasias and other toxic reactions limited its use for treatment of serious infections as in meningitis since it can penetrate into the central nervous system.
- Because it is bitter it is administered orally as capsules or as the palmitate ester at C3 (prodrug) which is insoluble in water and may be suspended in aqueous vehicles for liquid dosage forms.
- Parenterally it is used as an aqueous suspension or as a solution of the sodium salt of the succinate ester.
- Mechanism of action: inhibit protein synthesis in bacteria.

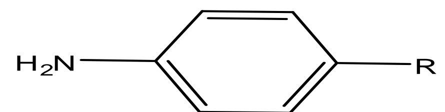
Assay principle

- The nitro group of chloramphenicol may be reduced to an amino group which can be diazotized and coupled with a reagent such as N-(1-napthyl)-ethylene diamine to form purple colored azo dye.
- Zinc-HCl acid has been used to reduce the nitro group in presence of heat.
- Waiting for two hours is needed for the reaction to complete then read A at 558 nm
- Prepare standard curve and measure the conc. of the unk.



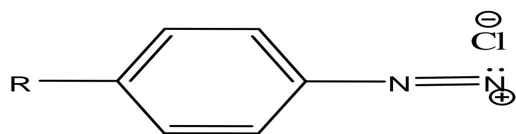
Zn/ HCl

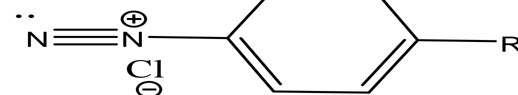
Heat



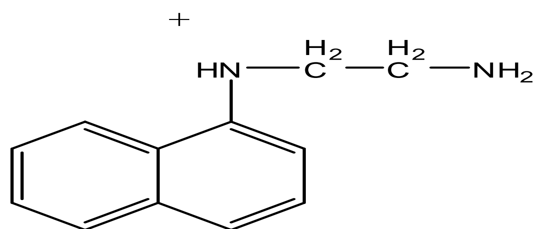
primary aromatic amine

NaNO2 + HCl





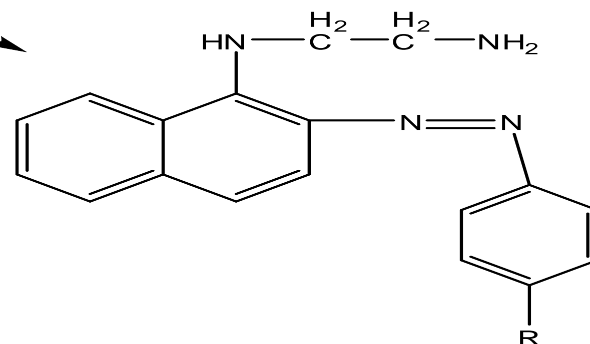
Diazonium salt

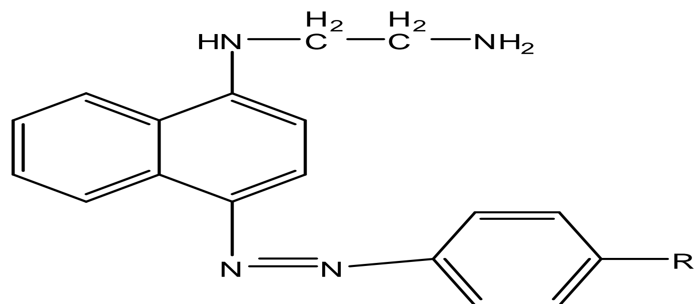


Coupling reagent

ortho position

para position





purple color azo dye

Advanced pharmaceutical analysis

Using instruments

Instrumental analysis

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

Introduction

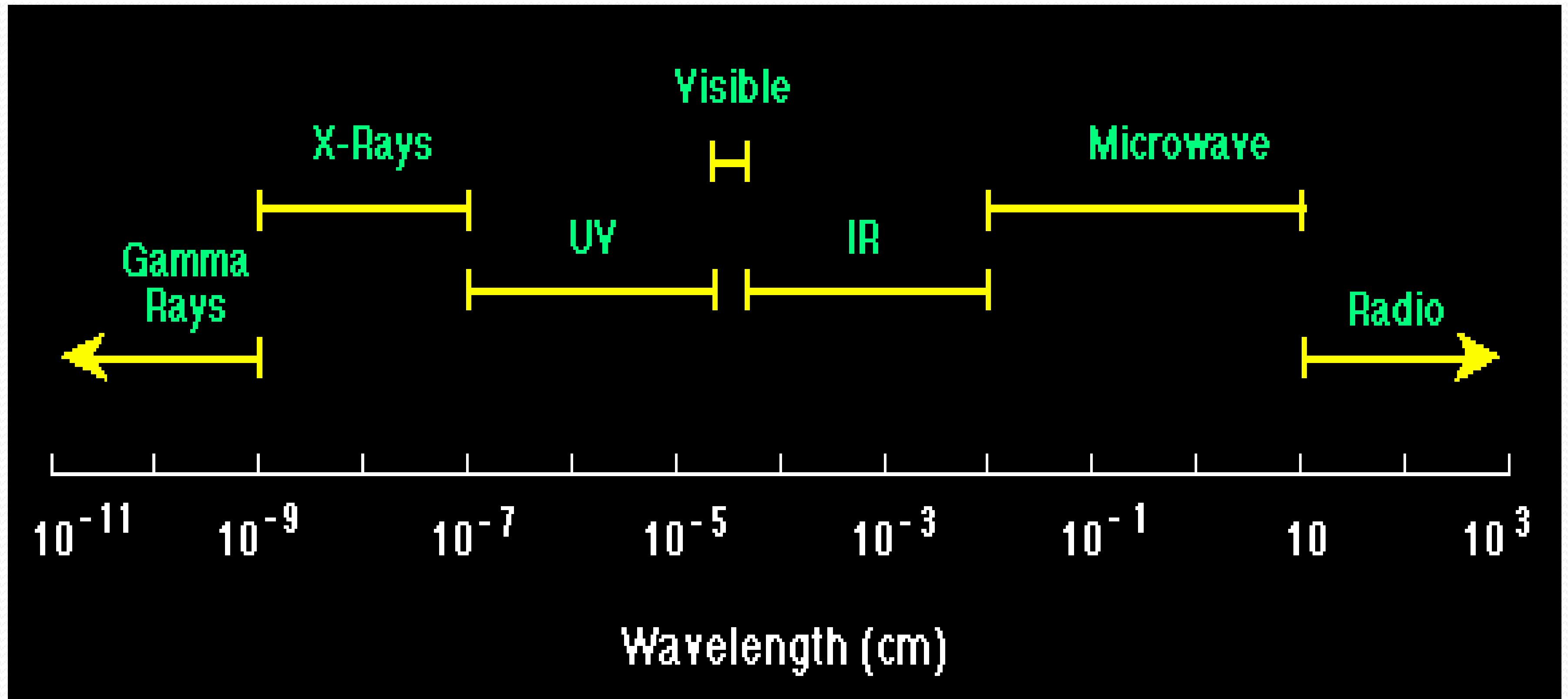
- Atoms and molecules show the ability for absorption of energy in the form of electromagnetic radiation.
- The type and quantity of the absorbed energy depends on

1. The chemical structure of these molecules.
2. The quantity of the absorbed energy depends on the number of the molecules absorbing this radiation.

Electromagnetic radiation

- Electromagnetic radiation: is a term applied to the energy diffused in the form of waves.
- Electromagnetic spectrum: is composed of wide range of radiation frequencies. It is possible to divide the spectrum into the following regions according to the wave length, energy, frequency, and the nature of interaction between the molecules and radiation (ionization, excitation, or vibration).

Electromagnetic spectrum

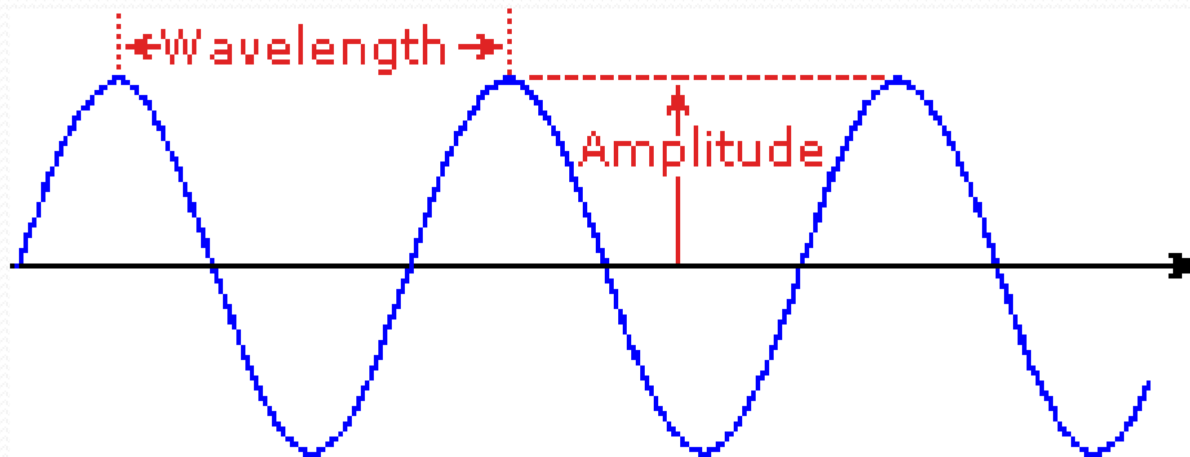


Electromagnetic spectrum

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

Definitions

- ***Wavelength*** is defined as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10^{-9} meters).
- (1mμ= 10A= 10^{-7} cm).
- ***Frequency*** is the number of wave cycles that travel past a fixed point per unit of time, and is usually given in cycles per second, or hertz (Hz).



Definitions

- The greater the frequency, the greater the energy is.
- The higher the frequency, the lower the wavelength is.

energy of the light

frequency of the light

$$E = h\nu$$

Planck's constant

wavelength

speed of light

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

frequency

UV-Visible Spectroscopy

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

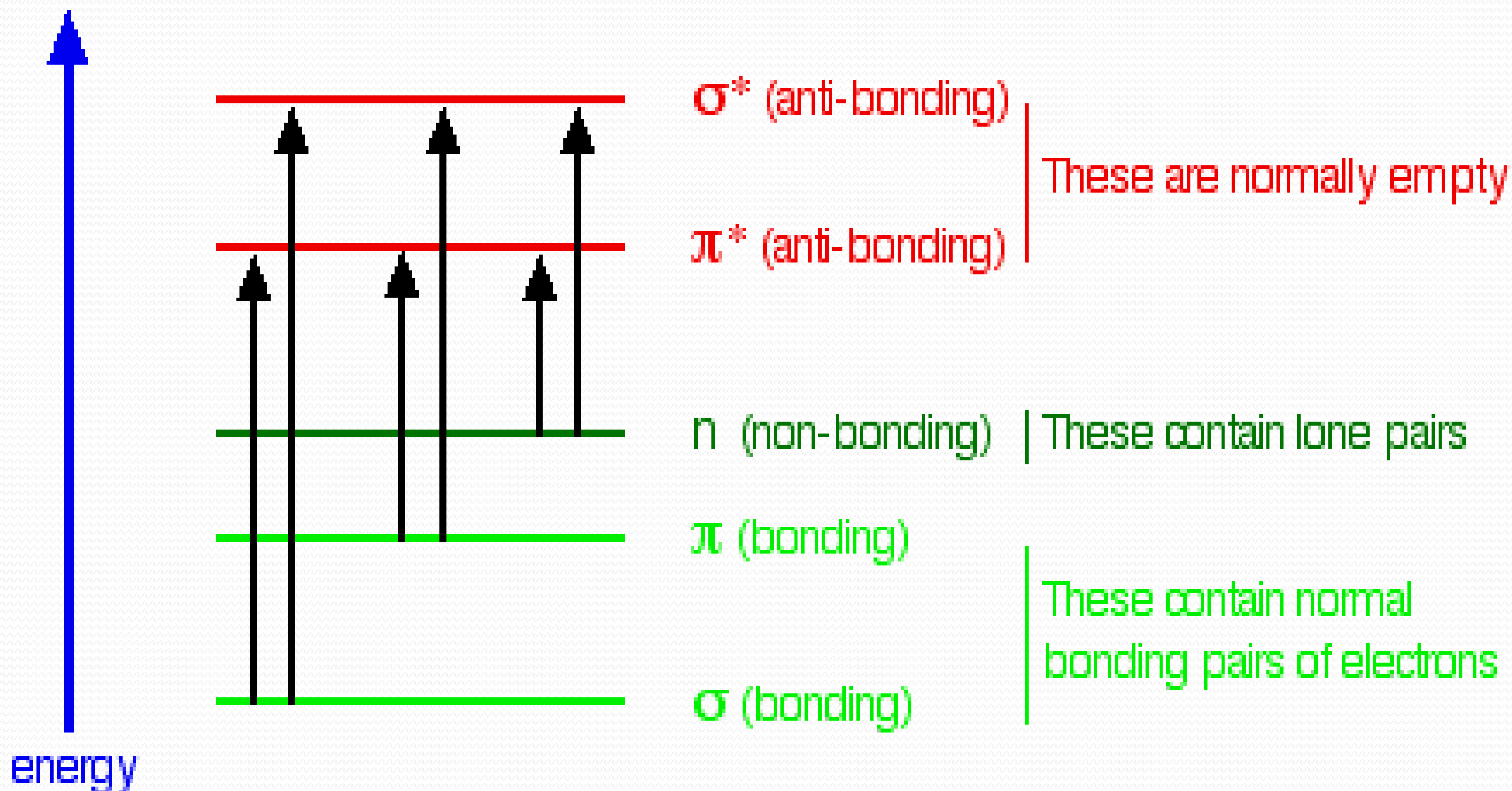
The principle

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

The principle

- An electron is excited from a full orbital **(low energy ground state orbital)** into an empty anti-bonding orbital **(high energy excited state orbital)**. Each jump takes energy from the light, and a big jump obviously needs more energy than a small one. Each wavelength of light has a particular energy associated with it.

The principle

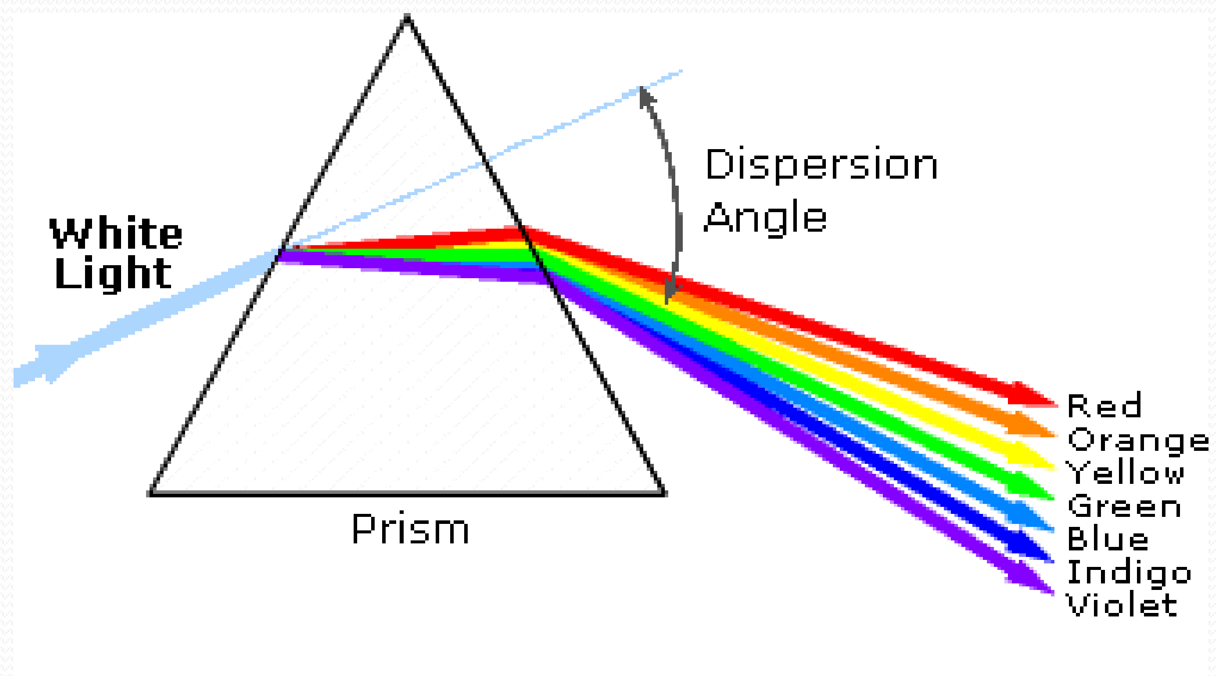


The principle

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

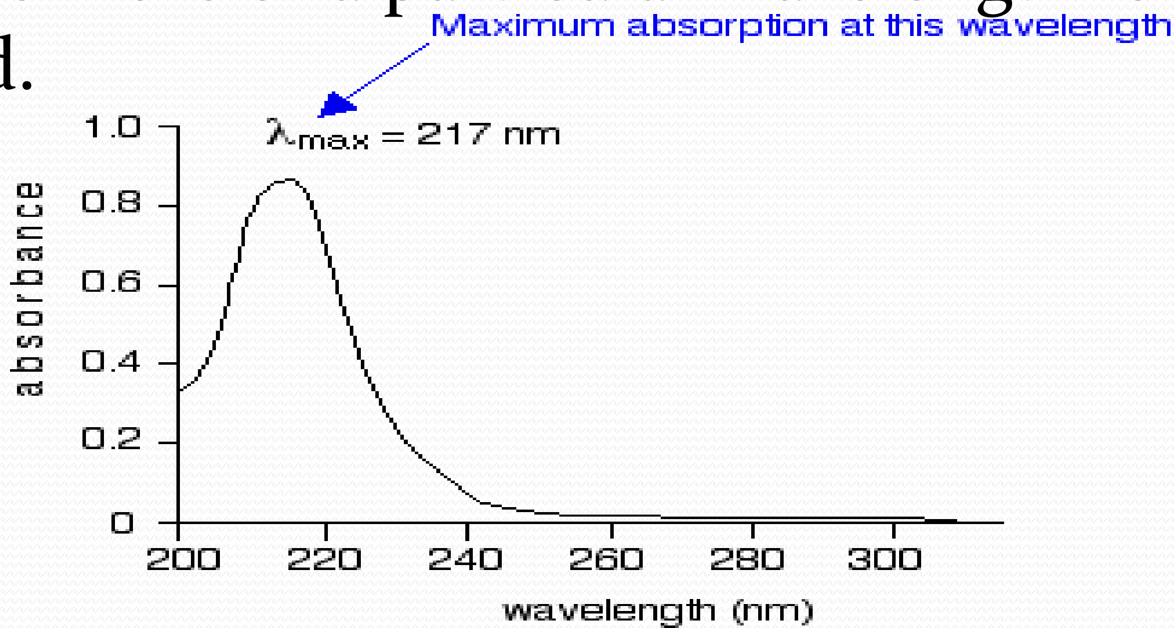
Visible light

- White light is a combination of lights of different wavelengths in the visible spectrum. Passing white light through a prism splits it up into the several colors of light observed in the visible spectrum between 400 nm and 780 nm.

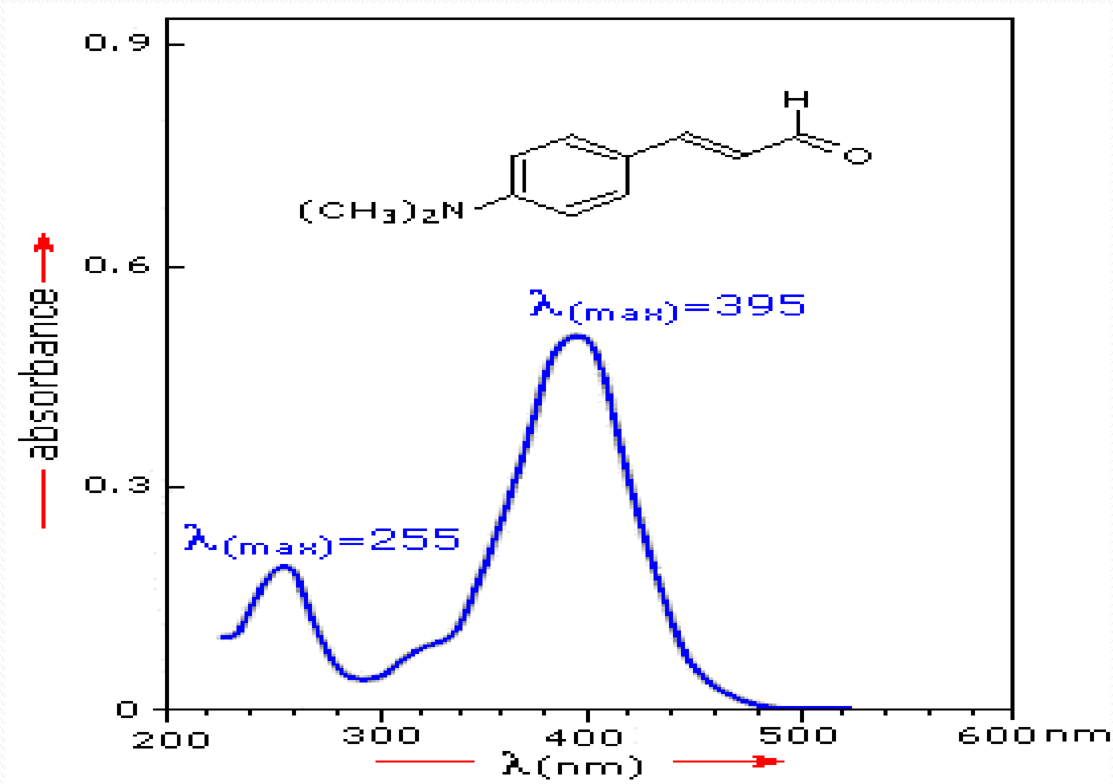


Absorption spectrum

- It is a spectrum of absorbance versus wave length or transmittance versus wave length.
- An example of simple UV-visible absorption spectrum for buta-1,3-diene is shown below. Absorbance (on the vertical axis) is just a measure of the amount of light absorbed. The higher the value, the more of a particular wavelength is being absorbed.



- ***Lambda max* (ʎ max):** is the wave length at which maximum absorption occurs (%T is minimum).
- While **ʎ min** is the wave length at which minimum absorption occurs (%T is maximum).
- Some compounds show more than one ʎ max.



Instrument parts

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

Definitions

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

Operating procedure:

1. Open the spectrometer and allow standing for 20 minutes (warming).
2. Set the wavelength dial on the Spec. to lambda required nm. With the sample chamber empty, until reads Zero %T which is the case when no cuvette is placed in sample holder.
3. Place your reference blank into the sample compartment until reads 100% T (A=0).
4. The sample is added now and the A or %T is recorded.
5. Whenever a change in wave length is made the 0%T and 100%T must then be reset since the amount of compensation varies with the wave length.

The purpose

- Qualitative

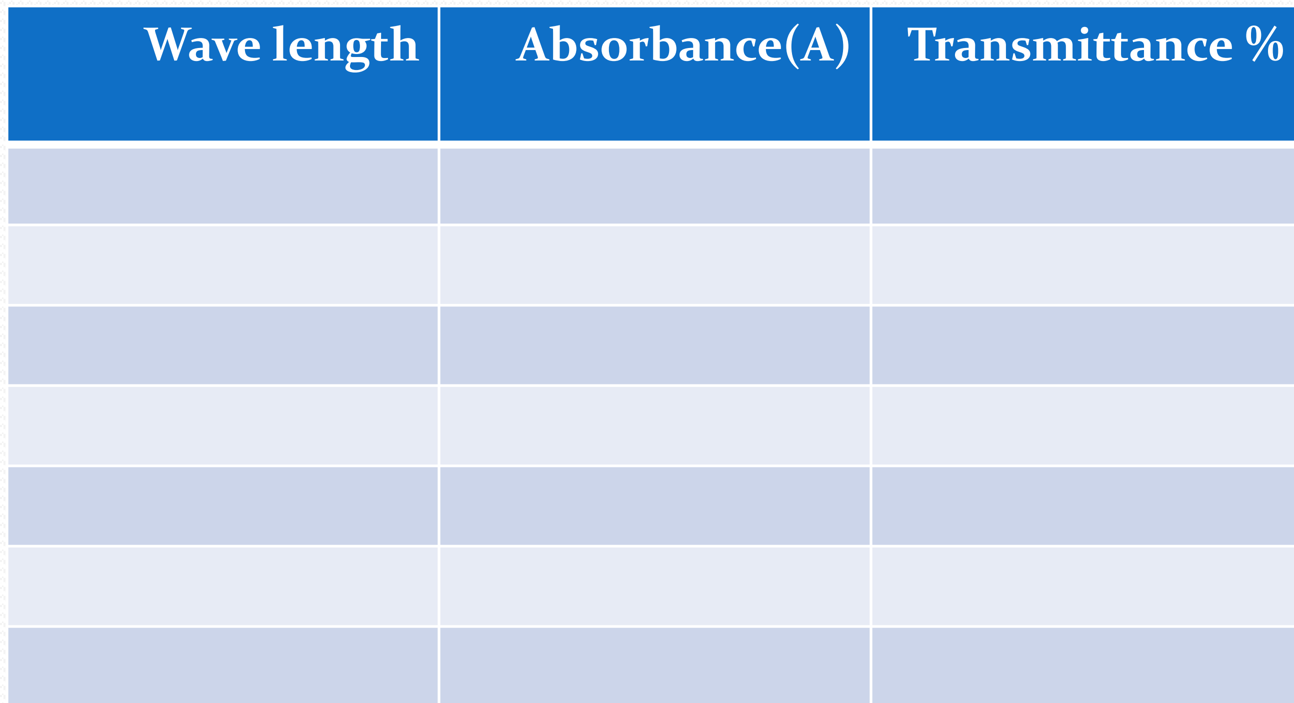
1. Determine the absorption spectrum of unknown compounds (qualitative analysis).
2. Determine the λ_{max} of a compound.

- Quantitative

3. Determine the concentration of a compound in solution using Beer-Lambert′s Law .

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

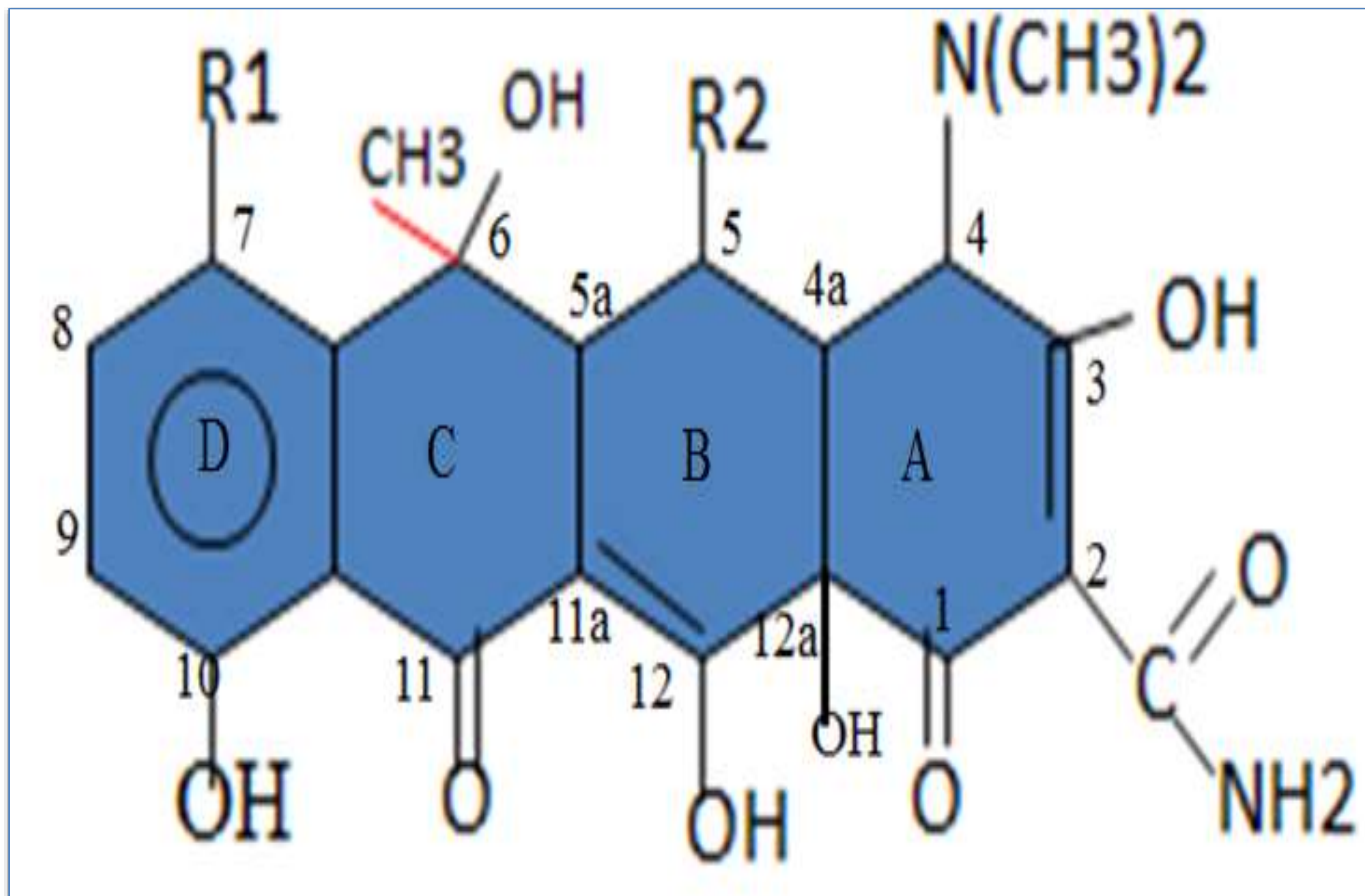
- By gradual scanning along wide range of wave lengths then reading A and %T
- Plot your data on graph paper (plot absorbance (A) on the y-axis as a function of wavelength (λ) on the x-axis).
- Plot %T on the y-axis as a function of wavelength (λ) on the x-axis.
- Establish the λ_{max} and the λ_{min}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



Wave length	Absorbance(A)	Transmittance %

Quantitative Analysis of Tetracycline

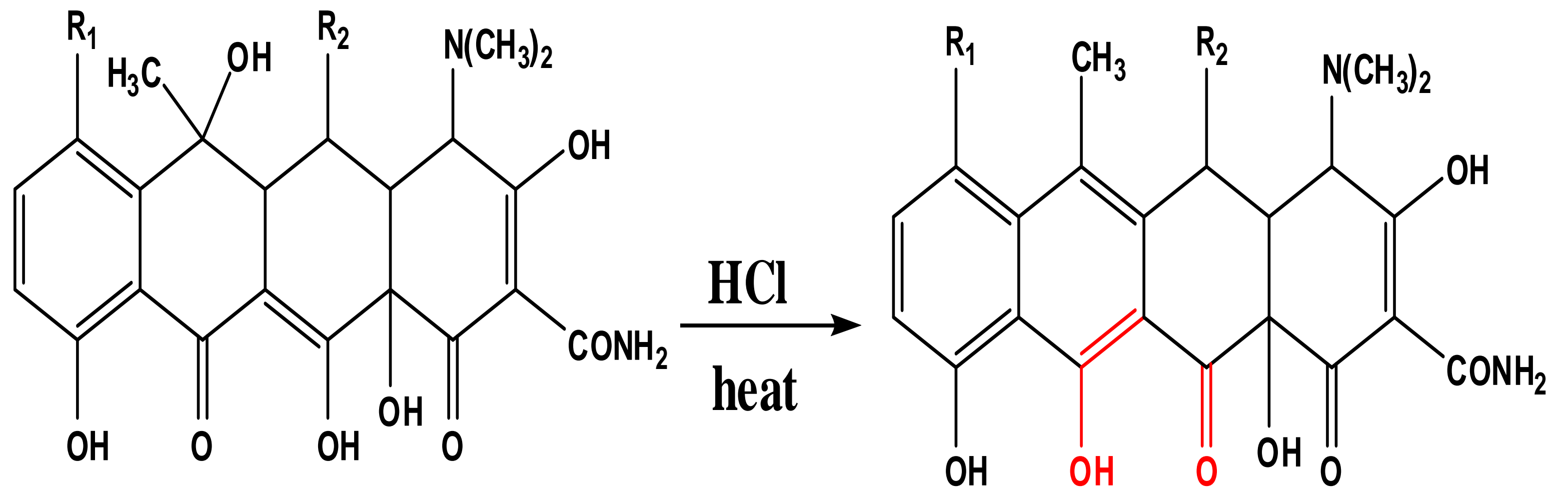
Acid and base colorimetric method



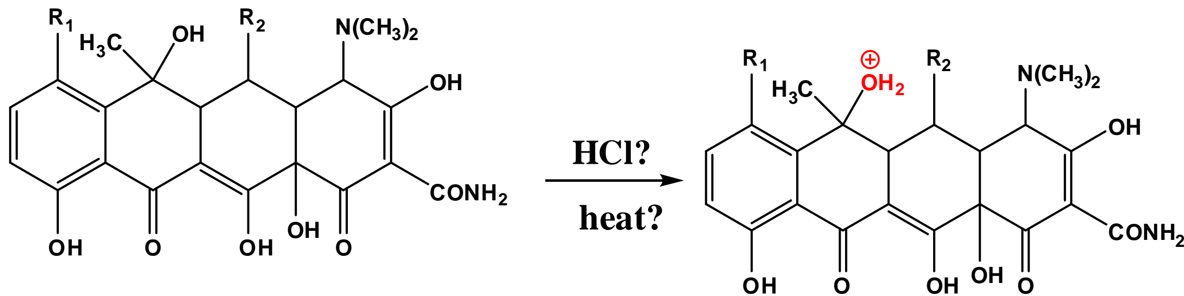
R1
CH3
OH
R2
N(CH3)2
7
6
5
4
8
5a
4a
OH
3
D
C
B
A
9
2
O
11a
1
12a
10
11
12
C
OH
OH
O
OH
O
NH2

- Strong acids and strong bases attack TC with a hydroxyl group on C6 causing a loss in activity through modification of the C ring.
- At pH less than 2, TC ***eliminates a molecule of water with concomitant aromatization of ring C*** forming the more energetically favored resonant system of the naphthalene group found in the ***inactive anhydrotetracyclines***.
- Bases promote a reaction between the 6- OH group and the ketone group at the 11-position, causing the bound between the 11 and 11a atoms to ***cleave*** forming the lactone ring found in the ***inactive isotetracycline***.

Acid colorimetric method chemical principle



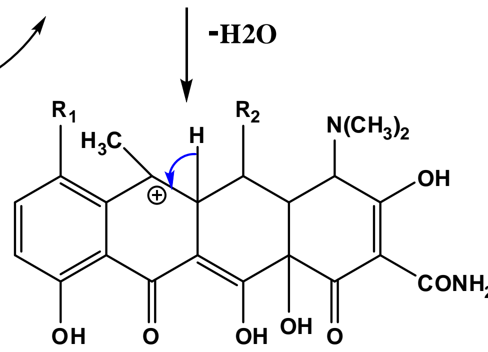
Mechanism of acidic method



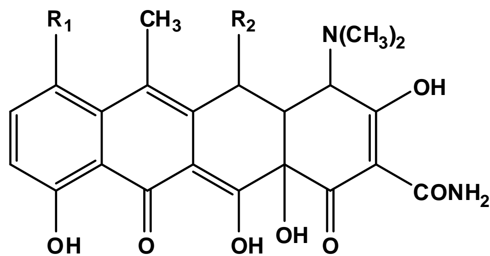
The hydroxyl group is a bad leaving group
but in presence of proton leads to

Formation of oxonium ion

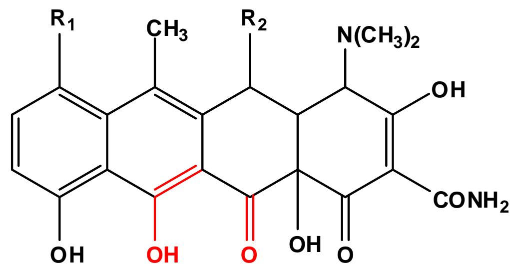
-H2O



Carbocation



tautomerism why?

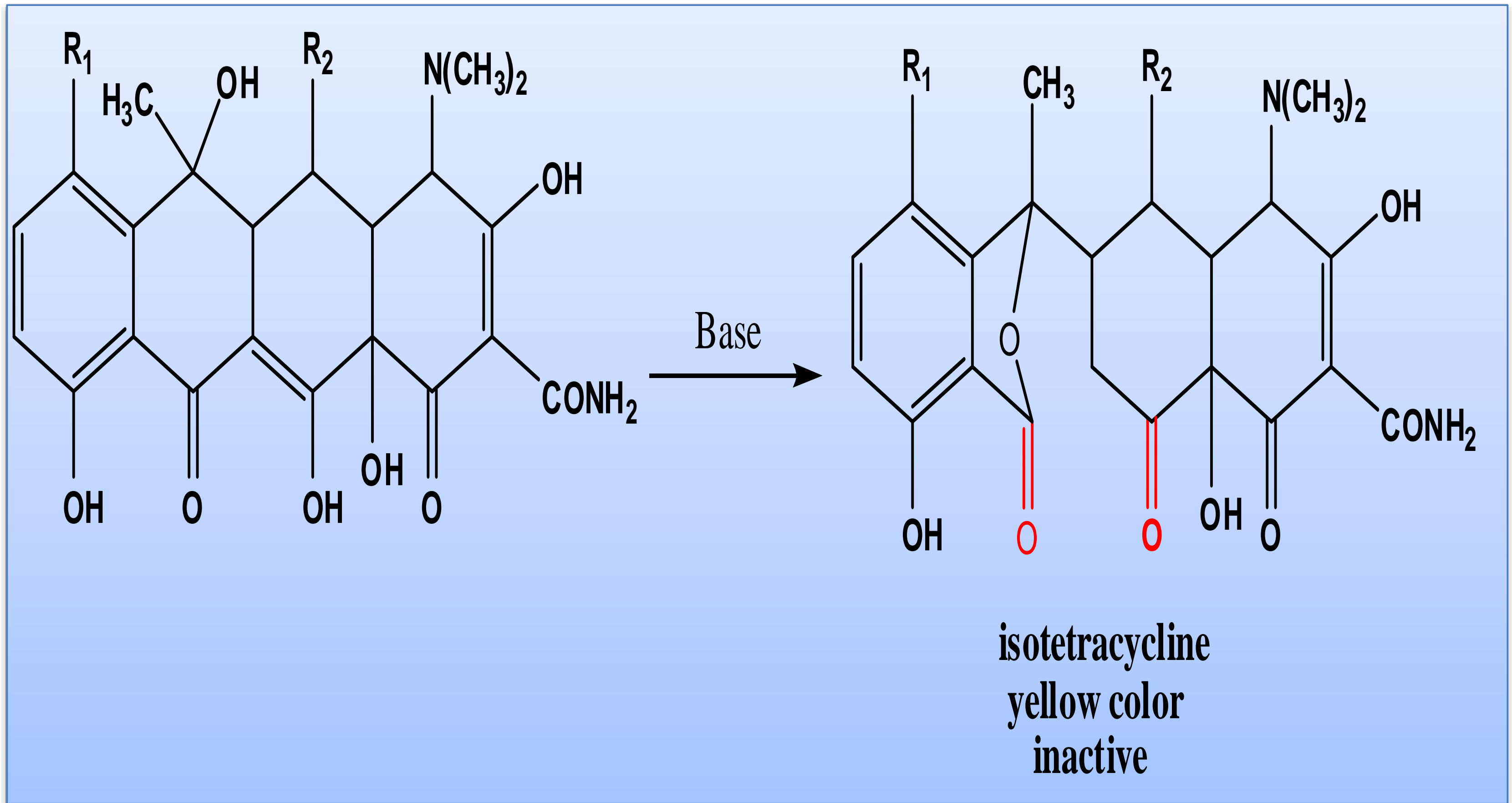


Anhydrotetracycline
deep yellow color (stable) why?
inactive

Acid colorimetric method

- This method is used for both tetracycline and chlotetracycline since when heated with HCl acid develop a stable yellow color while oxytetracycline doesn't give this reaction and it's solution remains colorless.
- In this method a blank is needed for each standard solution and for each a Known (why?).
- TC in presence of HCl (without heating) give pale yellow color and in order to cancel the effect of this from the std. that contain the anhydrotetracycline we use a blank for each.
- Anhydrotetracycline differs from TC in both molecular and structural formula (why?) and inactive as antibacterial.

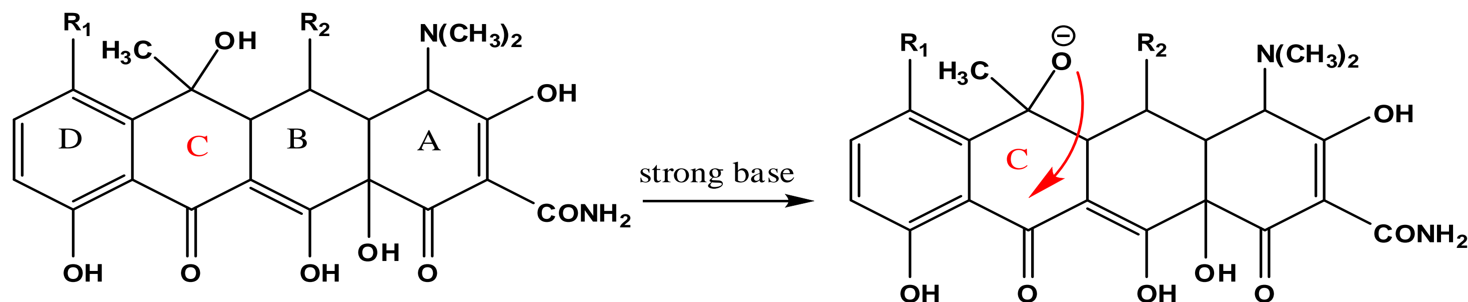
Base colorimetric method chemical principle



Base colorimetric method

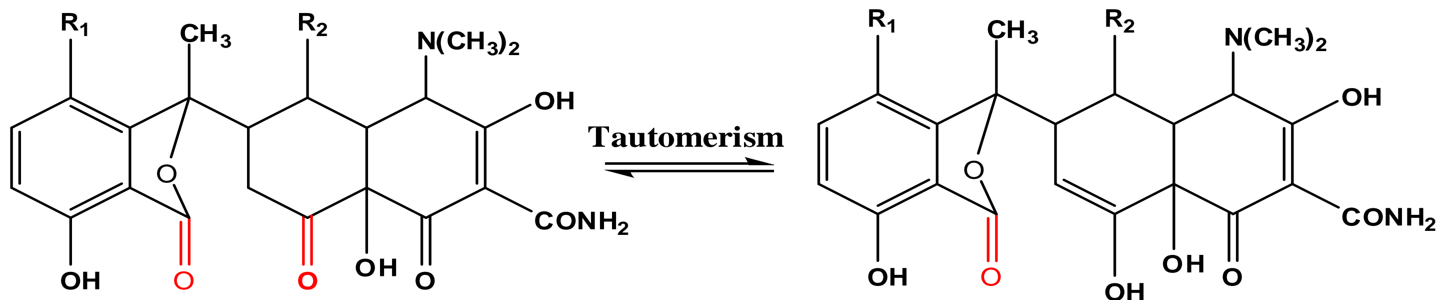
- This method is used for Tetracycline and oxytetracycline since the addition of alkali to their solutions produces a yellow color having an absorption maximum at 380 nm while chlortetracycline gives a yellow color that rapidly disappears and gives practically no absorption at 380 nm.
- The disadvantage of the method: reading of absorbance should be within exactly 6 minutes after the addition of the alkali because there is a slow but definite decrease in absorbance with time. (Intensity of color decreases with time).
- Isotetracycline has the same molecular formula as TC but different structural formula and it is inactive.

Mechanism of basic method



1. Formation of alkoxide anion
(strong) (unstable)

2. Attack of the carbonyl group and cleavage of 11-11a bound



isotetracycline
yellow color with absorption maximum at 380nm
inactive as antibacterial

Formation of lactone ring
(cyclic ester)

Procedure of acid method

From the stock solution 1mg/ml (freshly prepared) we prepare five standard solutions as follows:

	Standard					Blank					
	I	II	III	IV	V	I	II	III	IV	V	
Stock	0.5	0.7	1	1.3	1.5	0.5	0.7	1	1.3	1.5	ml
D.W.	1.5	1.3	1	0.7	0.5	1.5	1.3	1	0.7	0.5	ml
HCl	5	5	5	5	5	-	-	-	-	-	
D.W.	-	-	-	-	-	5	5	5	5	5	ml

Heat in water bath for 5 minutes then cool

HCl	-	-	-	-	-	5	5	5	5	5	ml

Complete the volume to 50 ml with D.W. and read A at ʎ max 440 nm

Procedure for unknown

Unknown	Unk. Blank
2ml	2ml
5ml HCl	5ml D.W.

Heat in water bath for 5 minutes then cool

-	5ml HCl

Complete the volume to 50 ml with D.W. and read A at ʎ max 440 nm

Report

- Name: Assay of tetracycline or chlortetracycline by acid colorimetric method
- Aim: Determination of unknown concentration
- No. of unk.
- No. of instrument
- Results:
- Table of std. A, C
- Mathematical details $C_1V_1=C_2V_2$
- Unk. A, C
- Plot (in details)

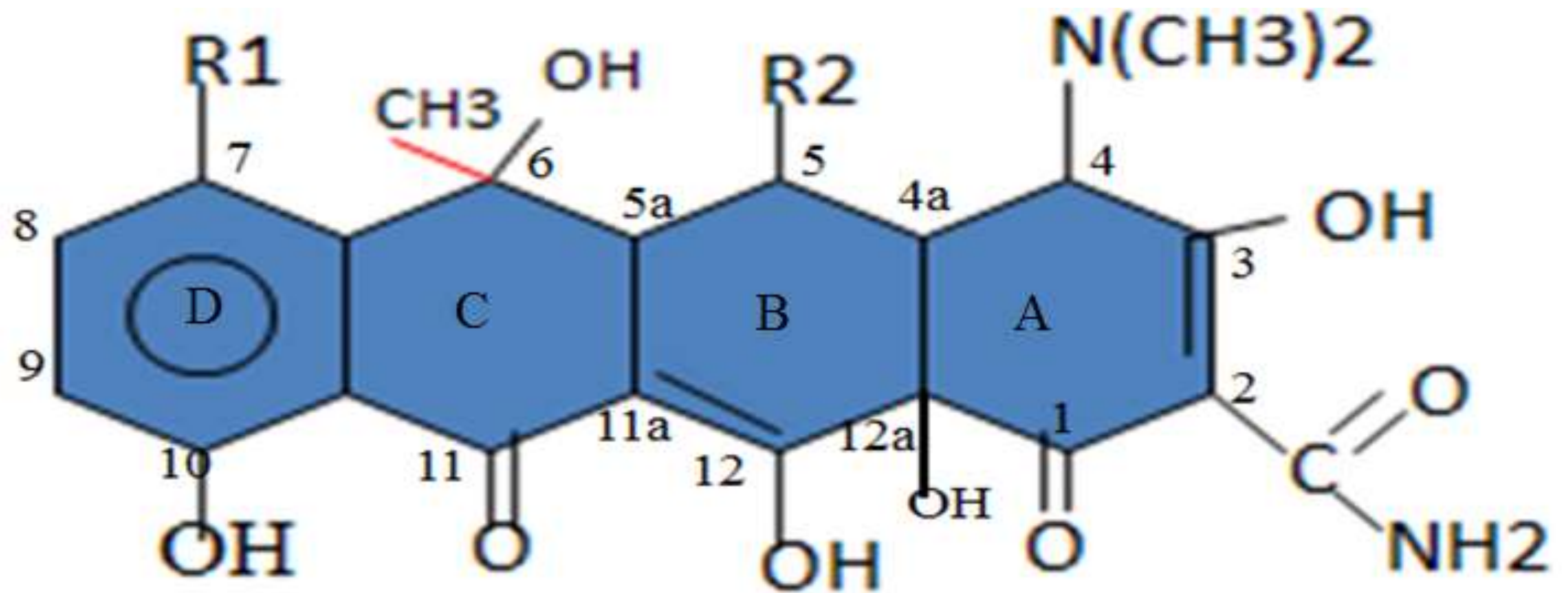
Determination of % purity

$$\frac{Practical}{Theoretical} \times 100\%$$

Quantitative Analysis
of Tetracycline

Colorimetric
method

Structure of Tetracyclines



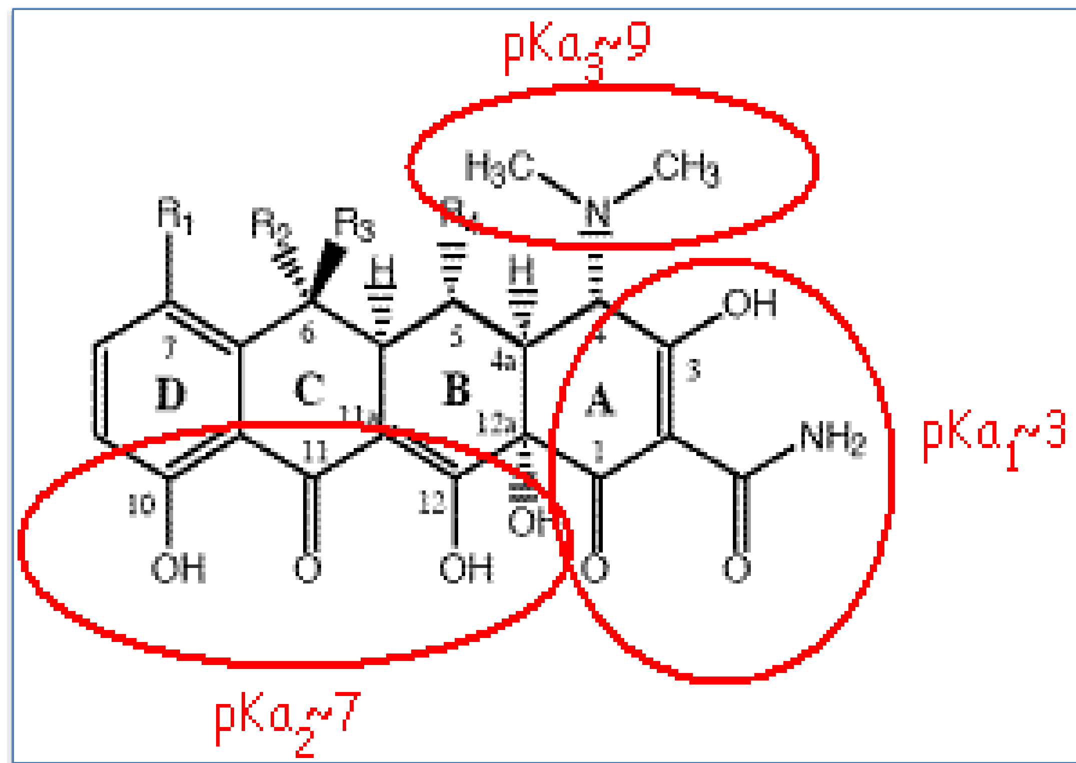
tetracyclines	R1	R2
tetracycline	H	H
chlortetracycline	Cl	H
Oxytetracycline	H	OH

Tetracycline

- Broad spectrum bacteriostatic antibiotic.
- Mechanism of action: inhibition of protein synthesis in bacteria.
- The important members of the group are derivatives of an octahydronaphthacene skeleton that consist of four fused six membered rings, so the group name is derived from this tetracyclic system.
- The conjugated systems exist in the structure from C10 through C12 and from C1 through C3.

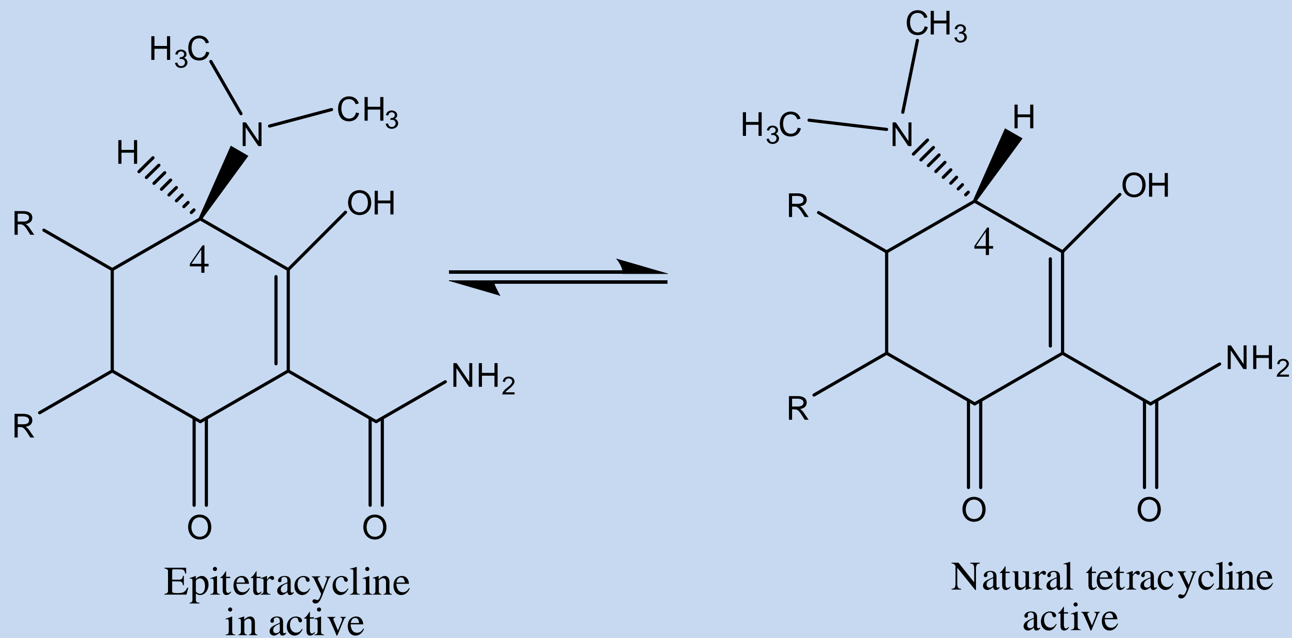
Physical and Chemical properties

- Physical:
- Yellow crystalline powder.
- Have a bitter taste.
- Light sensitive (darkens in light).
- Sparingly soluble in water.
- Odorless.
- Chemical:
- Amphoteric (react with acids and bases) why? Tetracycline contain basic group (dimethylamine).
- TC has three pKa values in aqueous solutions of the acid salts.(9.5, 7.7, 3.3)
- In neutral solutions TC exist mainly as zwitterion.


pKa3~9
H3C
CH3
N
R1
R2
R3
R4
H
H
OH
3
4
5
6
7
4a
D
C
B
A
11a
12a
NH2
pKa1~3
1
11
12
10
OH
O
OH
OH
O
O
pKa2~7

Tetracycline

- Tetracyclines form salts with acids and bases
- The hydrochloride salts are used commonly for oral administration and usually are encapsulated because they are bitter.
- Water soluble salts also are obtained from bases such as sodium and potassium salts but they are not stable in aqueous solutions.
- Divalent and polyvalent metals form insoluble salts.
- TC should not be given with antacid, milk, iron salts (at the same time) why? (stable chelate complexes with many metals calcium, magnesium, and iron are formed which are insoluble in water and not absorbed.
- Tetracycline shouldn't be given for children under 8 years of age due to its affinity for calcium which results in its incorporation into newly forming bones and teeth as tetracycline calcium orthophosphate complexes, these deposits in teeth cause a yellow discoloration that darkens over time (photochemical reaction).



Epimerization occurs at position 4 (asymmetric center) to form epitetracycline (this occurs in solutions of intermediate pH range). Under acidic conditions an equilibrium is established in about 1 day and the formed 4-epitetracycline is inactive isomer thus accounting for the decreased therapeutic value of aged solutions.

Colorimetric method

1- $FeCl_3$ Method.

(General method) why? for TC, CTC, OTC.

Formation of orange brown color why? 2 reasons

2- Acid Method.

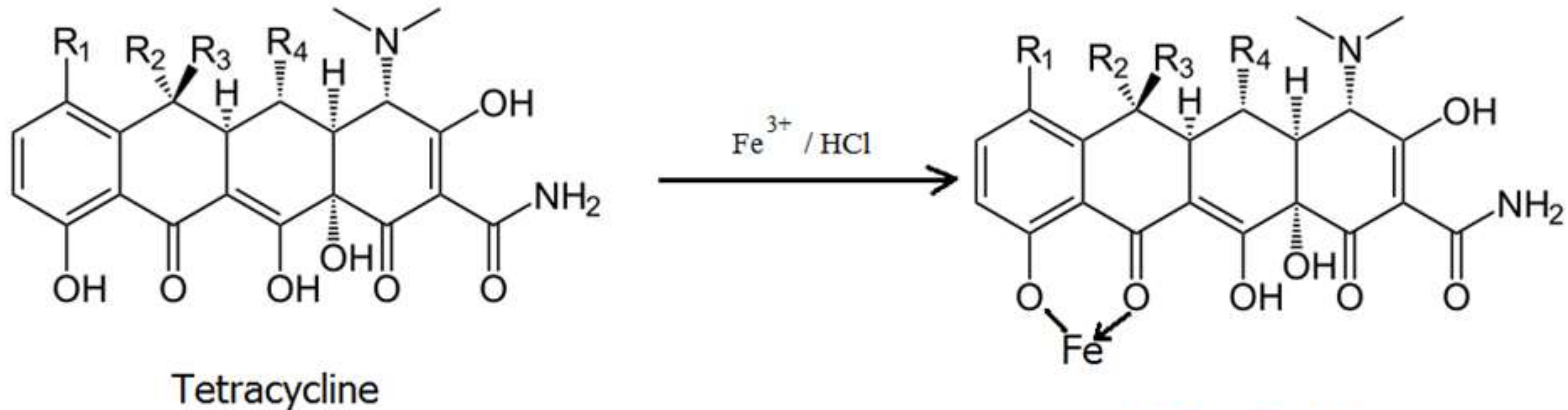
TC, CTC.

3- Base Method.

TC, OTC.

Chemical principle of the general method

- It depends on the reaction of tetracycline with the Ferric ion in acidic medium to form a soluble orange –brown chelate with metal ligand ratio 1:3



Today's experiment

- From the stock solution 0.25 mg/ml (freshly prepared) we prepare five standard solutions as follows:

Std.	I	II	III	IV	V	
stock	2	3	4	5	6	ml
0.01 N HCl	8	7	6	5	4	ml
0.05% $FeCl_3$	10	10	10	10	10	ml

Mix and stand at room temperature for 10 minutes. Why?

Orange-brown color appear then read at ʎ max 490nm

Procedure

- Unknown 10 ml + 10 ml 0.05% $FeCl_3$

Mix and stand at room temperature for 10 minutes then read at 490 nm.

- Blank 10 ml 0.01 N HCl + 10 ml 0.05% $FeCl_3$

Mix and stand at room temperature for 10 minutes then read at 490 nm.

Report

- Name: Assay of tetracycline ferric chloride colorimetric method
- Aim: Determination of unknown concentration
- No. of unk.
- No. of instrument
- Results:
- Table of std. A, C
- Mathematical details $C_1V_1=C_2V_2$
- Unk. A, C
- Plot (in details)