University of Baghdad
College of Sciences
Chemistry Department

Laboratory Manual of Biochemistry

For 2 ed year

Second cours

Biology

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Biochemistry Lab. Exp.1 **Assis. Teach. shaema sadoon**

Qualitative evaluation of proteins

Proteins are polymers of about 20 different types of amino acids which connected with each other by “peptide bonds".

(H-N-CO-)

Functions:

1. Enzymes: all enzymes are proteins and these acts as biocatalysts in the cells

2. They are the constituents of bio membranes (carriers, transporters or Translators).

3. Antibodies

4.Some of proteins acts as hormones, such as insulin

5.Transport proteins like hemoglobin and ferritin

6.Structural proteins, e.g: hair and nail

7.Storage proteins, e.g: casein in milk

The proteins have four structures:

1-primary structure

2-secondary structure

3- tertiary structure

4-quaternary structure

The proteins have bonds:

♦ peptide bonds

♦ Sulfur bonds R-S-R

♦ Hydrogen bond

♦ Van der waals

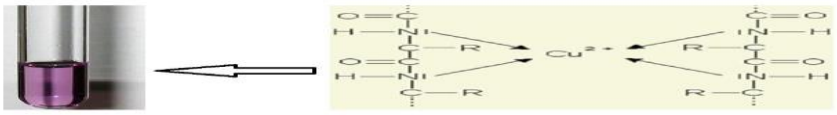
♦ Ionic bonds

♦ Dipol-dipol

Exp.Name: The biuret test for peptide bonds

Alkaline copper sulphare reacts with compounds containing two or more peptide bonds to give aviolet coloured comphex. The deoth of the coloue obtained is a measure of the number of peptide bonds present in the protein.the name of the test comes from the compound biuret which gives a typical reaction.

Copper coordination complex



♦The reaction is not absolutely specific for peptide bonds , since any compound containing two carbonyl groups linked through nitrogen or carbon atoms will give a positive result.

CO-C-CO or CO-N-CO

Method :

10 drops + 5 drops + 3 drops → violet complex

Albumin $CuSO_4$ 40%NaOH

Biochemistry Lab. Exp.2 Assis. Teach. shaema sadoon

Exp. Name: Precipitation Reactions of Protein

Aim: to precipitate and separate the protein

Using of some chemical compounds such as; strong acids, strong bases, heavy metals may results in losing the structure, shape and bioactivity of proteins, this process is .called denaturation

Denaturation: is the alteration of a protein shape through some form of external stress (for example, by applying heat, acid or alkali), in such a way that it will no .longer be able to carry out its cellular function

Denaturation Factors

: Denaturation can also be caused by

Changes in Temperature.1 .

Changes in pH.2 .

Using chemical compounds.3 .

U.V or X-rays.4 .

Precipitation Reactions of Proteins

-Precipitation by heat 1 :

:Principle

Increasing the Temp. will cause breaking in hydrogen and ionic bonds which causes increasing in the precipitation rate of proteins (decreasing its solubility)

:Materials

Albumin (0.5 gm of albumin soluble in 100 ml of 0.9% 0.5% Nacl)

Method:

drops of Albumine 10 $\xrightarrow{\Delta}$ يترسب البروتين اويتعكر

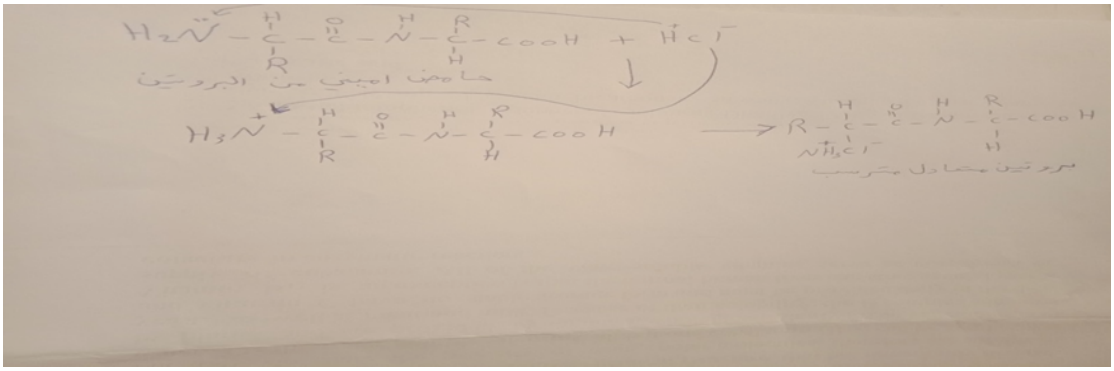
10 min (In boiling water bath)

When albumin solution is heated, white coagulum is ♦ obtained because albumin is denatured by heat (Albumin .is a coagulable protein)

: Precipitation by extreme pH -2

:Principle

changes in the pH can affect the chemistry of the amino .acids and their residues



: Method

→ drops + 5drops 10 تعكر المحلول → drops 5→يذوب الراسب

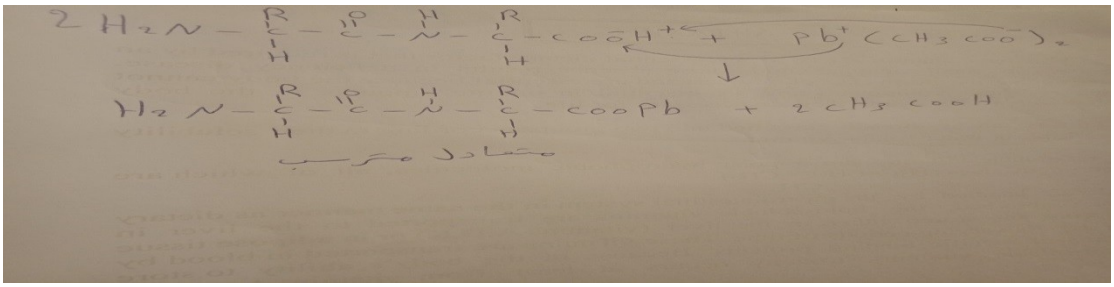
Albumin HCl(1N) NaOH(1N)

: precipitation by heavy metals -3

: Principle

At PH 7 and above proteins are usually negatively charged ,the positively charged metal ion neutralizes this charge and the protein coms out of solution. Precipitation by heavy metals is,therefore,most effective at neutral to slightly alkaline PH values,although the solution must not be too alkalin otherwise there is a risk of precipitation of .metal hydroxides

Note : The precipitate is frequently soluble in excess of the heavy metal solution since the excess ions confer a .stabilizing positive charge on the particles



Heavy metals: copper sulphate,lead acetate ,mercuric nitrate♦ .,$HgCl_2$,$CdSO_4$, $ZnSO_4$

The heavy metals have high molecular weight and carry a ♦ .positive charge

The charge of the protein in alkalin medium is <u>negative</u>♦ (PH>7)

: Method

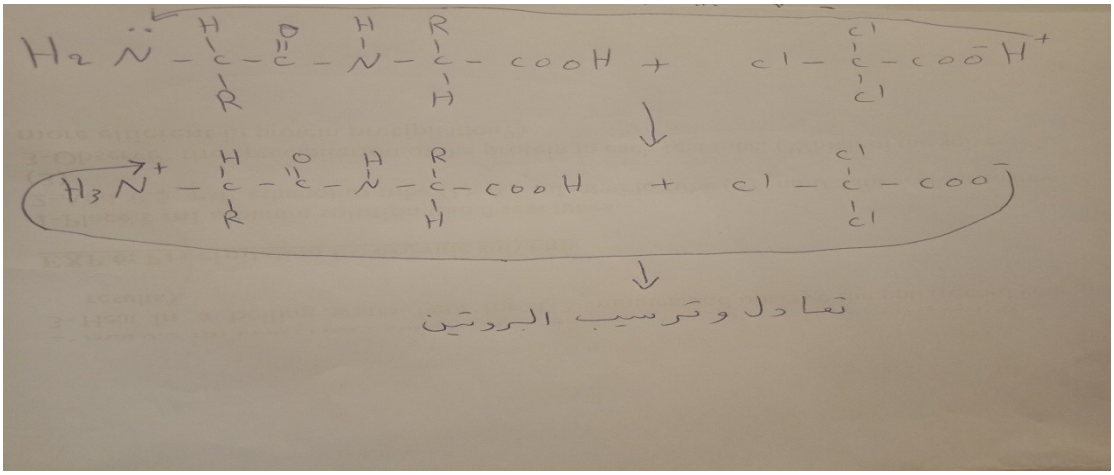
→ drops + 4-5drops 10 تعكر المحلول(راسب ابيض)

Albumin AgNO3

:Precipitation by acidic reagents -4

:Principle

These compounds carry a large negative charge, which neutralize positively charged proteins to form an insoluble .salt



Acidic reagents:sulphosalicylic acid,picric acid,tannic acid, ♦ trichloroacetic acid(TCA)

The charge of the protein in acidic medium is <u>positive</u>♦ (PH<7)

: Method

→ drops + 4-5drops 10 (راسب ابيض)تعكر المحلول

Albumin TCA

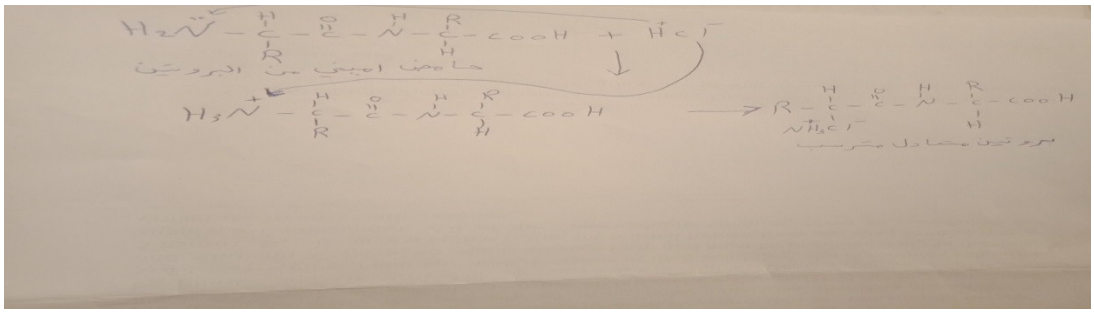
Biochemistry Lab. Exp.3 Assis. Teach. shaema sadoon

Exp. Name: Precipitation Reactions of Protein

:Precipitation by mineral acids -5

:Principle

These compounds carry a large negative charge, which neutralize positively charged proteins to form an .insoluble salt



Mineral acid :conc.HCl,conc.H_2SO_4,conc.HNO_3♦

The charge of the protein in acidic medium is positive♦ (PH<7)

: Method

→ drops + 4-5drops 10 (راسب ابيض)تعكر المحلول

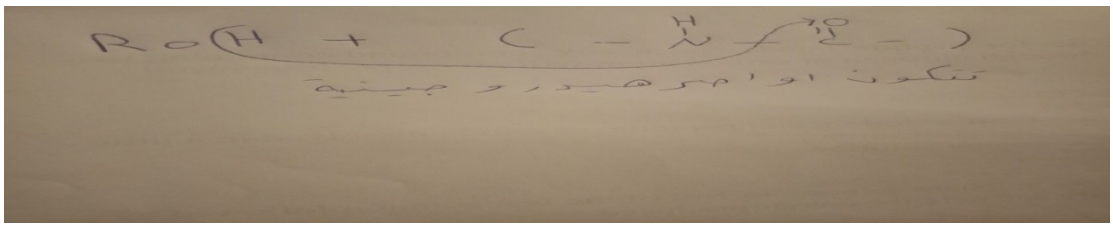
Albumin HCl

Precipitation by organic solvents -6

:Principle

By using organic solvents such as; ethanol, acetone or ether, the protein will be precipitated as a result for the

.hydrogen bonds formed



Method:

→ drops + 4-5drops 10 تعكر المحلول(راسب ابيض)
Albumin ethanol

Precipitation by mineral salts (salting in and salting out) -7:

:Principle

The increasing in protein solubility when low concentrations of salt is added to a protein solution, is called **"salting in"**, While the decreasing in protein solubility (increasing precipitation) when high concentration of salt is added to a protein solution is called **''salting out''**. Saturated ammonium sulphate{$(NH_4)2SO_4$}, saturated magnesium sulphate {$MgSO_4$},saturated sodium chloride{NaCl} will causes precipitation of protein (salting out), while 0.9% sodium .chloride NaCl will dissolve the protein (salting in)

The degree of protein precipitation with salts depends on ♦ the nature of the protein,its molecular weight, salt .concentration and type

Method:

→ drops + 4-5 drops 10 محلول رائق **((salting in**
Albumin 0.9%NaCl
→ drops + 4-5 drops 10 تعكر المحلول(راسب ابيض) **(salting out)**
Albumin NaCl

:Precipitation at the Isoelectric point -8

:Principle

♦ The Isoelectric point (PI) is the pH at which the protein carries no net electrical charge (positive charges= negative charges).

♦ The proteins precipitate at their PI.

♦ Different proteins can be separated according to their PI values.

Biochemistry Lab. Exp.4

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Exp. Name: Quantitative test for proteins

Aim: Biuret quantitative test

Principle:

Compounds containing two or more peptide bonds form a characteristic purple color when treated with dilute alkaline copper sulphate .The color is due to coordination complex of copper and four nitrogen atoms and is related to the familiar blue color obtained with copper sulphate and ammonia.

Materials:

♦ Stock standard protein (10 mg/ml)

♦ Biuret reagent

♦ Sodium chloride 0.9%

Method:

1- In a five clean and dry test tubes prepare (2ml) of the following protein concentrations **(0.0 , 1, 2, 4 , 6, 8 mg/ml)** using sodium chloride (0.9%) for the dilutions.
2- In another test tubes add (2ml) of a prorein solution with unknown concentration.
3- Add 3ml biuret reagent to each test tube.
4- Mix well and warm at 37 C˚ for 10 mins .
5- Cool and read the absorbance at 540 nm .
6- Plot the known protein concentrations (as mg/ml) on the a-axis versus the absorbance .Then calculate the concentration of the unknown sample.

Note:

♦ **Stock solution:** is a concentrated solution or standard solution that will be diluted to some lower concentrations for actual use.

♦ **Blank solution**: is a solution that containing no analyte that would be measured.

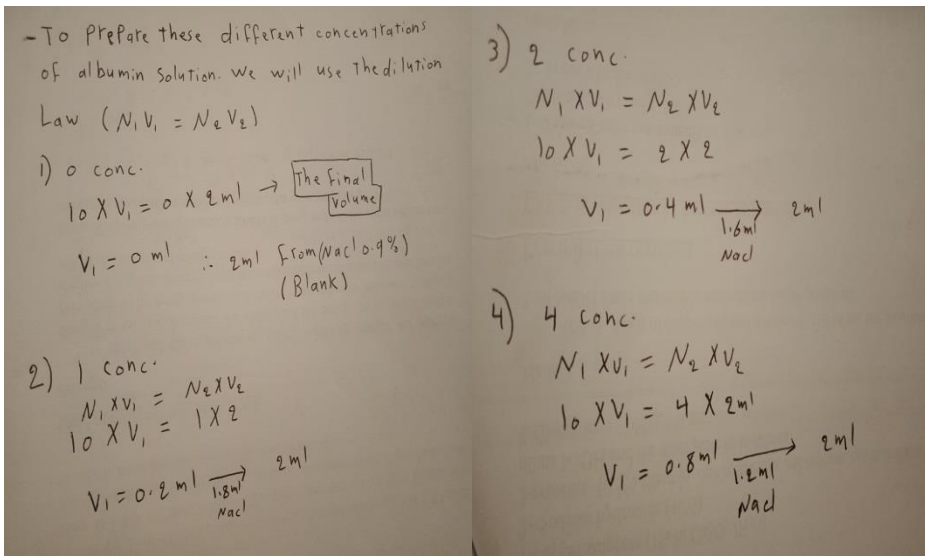
♦The absorbance will be measured for the colored solution.

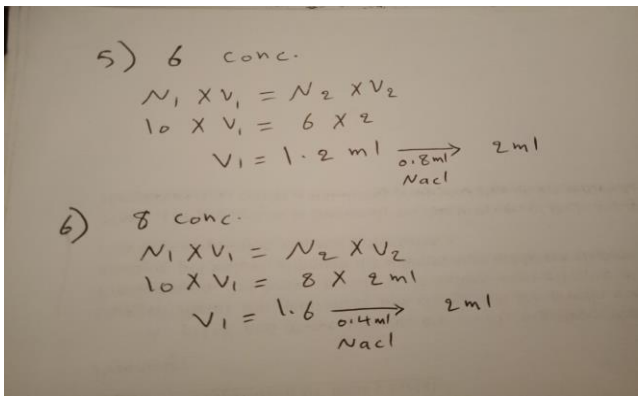
♦ **Absorbance:** is a measure of the quantity of light absorbed densityby a colored solution, it is also known as" optical density"

♦ Biuret test consists of ($CuSO_4$ +NaOH).

♦ In order to study the quantitative test for proteins , we shall prepare different concentrations of protein such as “Albumin” from its stock solution

♦By using dilution law we will prepare the following concentrations of Albumin. Stock standard protein (10 mg/ml)





Tube no.	Conc.of solution	V1 Vol.of stock	V2 Vol.of NaCl 0.9%	Total vol.
1	0	0 ml	2 ml	2 ml(blank)
2	1	0.2 ml	1.8 ml	2 ml
3	2	0.4 ml	1.6 ml	2 ml
4	4	0.8 ml	1.2 ml	2 ml
5	6	1.2 ml	0.8 ml	2 ml
6	8	1.6 ml	0.4 ml	2 ml
7	Un.	2 ml of un.	0	2 ml(un)
Add 3ml biuret reagent to each test tube				
Mix well and warm at 37 C˚ for 10 mins				
Cool and read the absorbance at 540 nm				

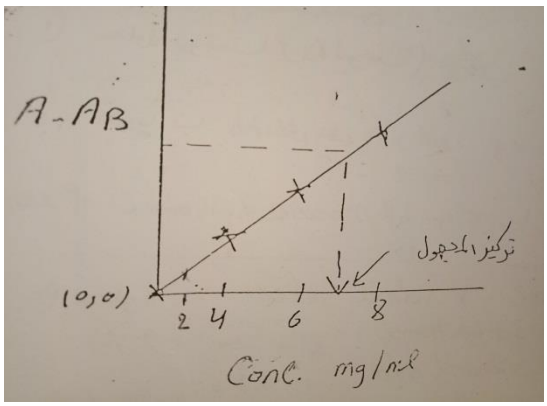
Tube no.	Conc. mg/ml	A	A-AB
1	0(Blank)		
2	1		
3	2		
4	4		
5	6		
6	8		
7	Un.		

Note:

Conc. = concentration of protein

A = absorbance

AB = absorbance of blank



Enzymes

♦ Enzymes are proteins molecules that act as biocatalysts which accelerate the biochemical reactions in the cells .

♦ Enzymes differ from most other catalysts by being much more Specific.

♦There are two types of enzymes :

1.Endoenzymes; the enzymes that acts in the cells .

2.Exoenzymes; the enzymes that acts out of the cells .

♦ Each enzyme needs a molecule upon which the enzyme act on and it is Called suustrate.

♦ Enzymes may require non-protein organic compounds to do Their function, these organic compound are called coenzymes or prosthetic groups.

♦ Because enzymes are proteins and they have all the properties of proteins, they extremely unstable and may be denatured more or less easily by changes in environment.

♦Since the denaturation alters the biological properties of proteins , the activity of enzymes is influenced markedly by many environmental variables such as; Temperature, pH, the concentration of enzyme and substrate, and the presence of inhibitors .

♦Enzymes have a specific site on its surfaces which called "active site".

♦ The active site represent the site by which the substrate binds with the enzyme.

♦The enzyme (E) and substrate (S) combine with each other to form an unstable enzyme-substrate complex (ES) for the formation of product (P).

♦Some enzymes need metal ions which serves as an activators Mg^{+2} is the common one

♦ Some enzymes are inhibited by various substances such as heavy metal ions (Hg^{2+}),(Pb^{2+}),(Ag^{+}).

The factors that affect on the enzymatic reactions:

1- Concentration of substrate: with a given quantity of enzyme the reaction velocity increases with increasing substrate conc. until alimiting value is reached " enzyme saturated with substrate''.

2- Concentration of enzyme: in the presence of excess substrate the velocity of reaction is proportional to the conc. of enzyme .

3- pH: Generally, an enzyme will be active over a relatively narrow range of pH. The maximum value is referred as the "optimum pH''.

4-Temperature: the point at the accelerating effect of temp. increase is balance by the denaturation of the enzyme is called the "optimum Temperature''.

5-Concentration of products : the Accumulation of the product may retard the enzymatic activity . This may be caused by the combination of product with the active site, thus removing enzyme from the reaction. In the living system, this type of inhibition is generally prevented by a quick removal of products formed.

EXP name : Enzymatic activity of α – Amylase

1- Prepare six test tubes as followes

Contents	1	2	3	4	5	6
0.4%Starch	3 ml	3 ml	3 ml	3 ml	3 ml	4.5 ml
H_2O	1 ml	1.5 ml	--	--	--	--
1% Na_2CO_3	--	--	1 ml	--	--	--
1% NaCl	--	--	--	1 ml	--	--
$Na_2C_2O_4$	--	--	--	--	1 ml	--
α – Amylase	0.5 ml	--	0.5 ml	0.5 ml	0.5 ml	--

2-Incubate all tubes at 37 ˚C for 15 mins ,stop the enzymatic reaction by placing the tubes in a boiling water bath for 5 mins.then cool.

3- Transfer a drop of content of each tube onto a white tile and add a drop of iodine solution to each aliquot and notice the blue color complex.

4- Add 0.5 ml Benedict's qualitative reagent to each test tube and heat in a boiling water bath for 5 mins .Notice the red cuprous oxide precipitate in some of the tubes.

Biochemistry Lab. Exp.6

Assis. Teach. shaema sadoon

Exp. Name: The effect of substrate concentration on enzyme activity .

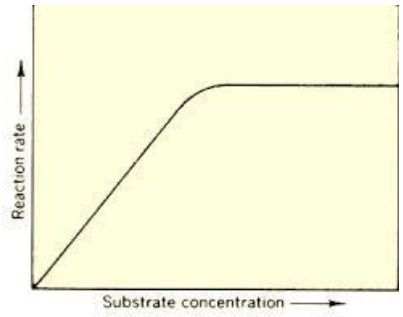
Exp. Aim:

1-To find the effect of substrate concentration on the rate of the enzymatic reaction.

2-To find Km and Vmax by using michaelis- menten equation and line weaver Burk equation.

Concentration of substrate

As we mentioned before, with a given quantity of enzyme the reaction velocity increases with increasing substrate conc. until a limiting value is reached " enzyme saturated with substrate''.



■In order to study the effect of substrate concentration on the velocity of the enzymatic reaction, we shall prepare different concentrations of substrate such as "starch" from its stock solution.

■ Stock solution: is a concentrated solution or standard solution that will be diluted to some lower concentrations for actual use.

■ Blank solution: is a solution that containing no analyte that would be measured.

■ By using dilution law we will prepare the following concentrations of starch.

 # To prepare these different concentrations of starch solution,we will use the dilution Law ($N_1V_1=N_2V_2$)

1) first conc. 0.05% starch in 2ml → the final volume

$N_1V_1=N_2V_2$ → 1% xV_1= 0.05 x 2ml → V1=0.1ml of 1% starch solution

Tube No.	Starch Conc.	Strach Vol.(ml)	H2O Vol.(ml)	NaCl(ml)	α-amylase (ml)	Incubate All tubes at 37 C° For 10 min. then complete other addition	NaOH(ml)	DNS(ml)	Let all tubes Boiling For 3 mint. then Read the absorbance at 540 nm
1	0.05 %	0.1	1.9	0.5	0.5		0.5	0.5	
2	0.1 %	0.2	1.8	0.5	0.5		0.5	0.5	
3	0.2 %	0.4	1.6	0.5	0.5		0.5	0.5	
4	0.4 %	0.8	1.2	0.5	0.5		0.5	0.5	
5	0.8 %	1.6	0.4	0.5	0.5		0.5	0.5	
6	1 %	2	0	0.5	0.5		0.5	0.5	
7	0 (Blank)	0	2	0.5	0.5		0.5	0.5	

The role of the used materials:

♦ Starch: substrate

♦ H_2O: for dilution purpose

♦ NaCl: activator for the α-amylase enzyme

♦ α-Amylase: Enzyme

♦ NaOH: to stop the enzymatic reaction

♦ 3,5 Dinitrosalcylic acid (DNS): oxidizing agent

♦ In the presence of α- amylase the starch will be converted to glucose which will be oxidized by DNS and give us a colored solution.

♦ The absorbance will be measured for the colored solution.

♦ Absorbance: is a measure of the quantity of light absorbed by a colored solution, it is also known as optical density.

Tube No.	Starch Conc.= [S]	A	A-AB
1	0.05%		
2	0.1 %		
3	0.2 %		
4	0.4 %		
5	0.8 %		
6	1 %		
7	0 (Blank)		

where [S] = the conc. of substrate

A = absorbance

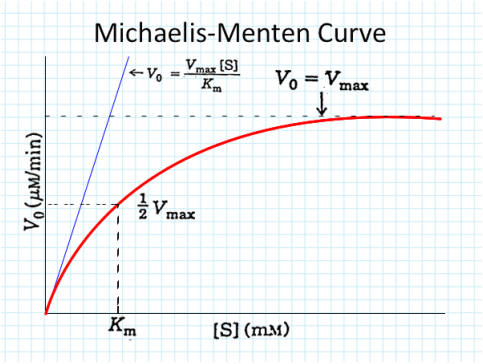
AB = absorbance of blank

Michaelis-Menten Equation

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

♦ **Km**: is the substrate concentration at which the enzymatic reaction velocity equal half of its maximum velocity. This constant is related to the affinity of the enzyme for the substrate.

So, if an enzyme has a small Km , it achieves maximal catalytic efficiency (Vmax) at a low substrate concentration.
Higher Km = lower the affinity = higher [S] required to reach 1⁄2 Vmax.
♦ **Vmax:** is the maximum velocity that the enzymatic reaction reaches when an enzyme saturated with the substrate As [S] is first increased, the initial rate or velocity (V_0) increases with increasing substrate concentration. In the continuous ,increasing of [S]
V_0 increases less and less. Finally, V_0 doesn't increase anymore and velocity reaches its maximum (Vmax), At this point, the enzyme is saturated with substrate, S.



Line weaver - Burk equation

♦ We can calculate Km and Vmax by using Line weaver – Burk equation.
♦ Line weaver - Burk equation is the reciprocal for Michaelis- Menten Equation.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

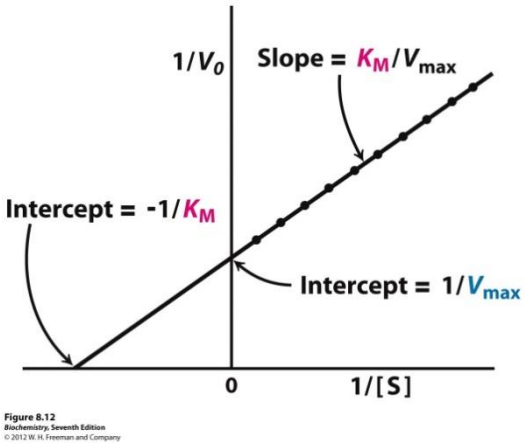


Figure 8.12
Biochemistry, Seventh Edition

Tube No.	Starch Conc.= [S]	1/[S]	A	A-AB	1/ A-AB
1	0.05%				
2	0.1 %				
3	0.2 %				
4	0.4 %				
5	0.8 %				
6	1 %				
7	0 (Blank)				

Biochemistry Lab. Exp.7

Assis. Teach. shaema sadoon

Exp. Name :

The **effect of pH** on enzyme activity.

Exp. Aim:

To find the effect of changes in pH on the activity of the enzyme.

THE EFFECT OF PH

♦ The structure of the enzyme has a great influence on the activity of the enzyme. In other words, changes in the structure of the enzyme affect the rate of chemical reactions.

♦ When the pH value of the reaction medium changes, the shape and structure of the enzyme will change. For example, pH can affect the ionization state of acidic or basic amino acids.

♦ There are carboxyl functional groups on the side chain of acidic amino acids, as well as, there are amine-containing functional groups in the side chain of basic amino acids.

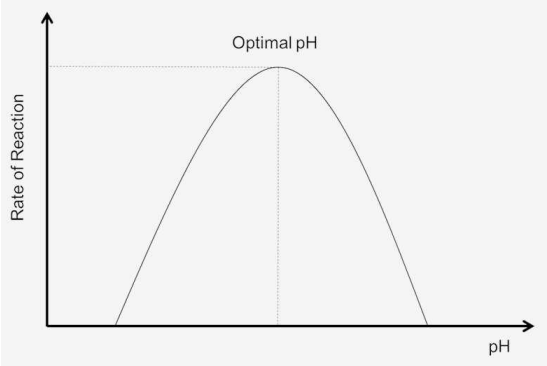
♦ If the ionized state of amino acids in the protein is changed, the ionic bonds that maintain the three-dimensional shape of the protein will change. This may lead to changes in protein function or inactivation of enzymes, in other words the denaturation will occur.

♦ The changing in pH not only affects the activity of the enzyme, but also affects the charge and shape of the substrate, so that the substrate cannot bind to the active site, or cannot be catalyzed to form a product.

♦ If the level of pH changes significantly, the enzyme and substrate may be denatured. In this case, the enzyme and the substrate do not recognize each other, so there will be no reaction.

♦ All enzymes have an ideal pH value, which is called optimal PH.

♦ Optimum pH is the point where the enzyme have the highest value of activity.



Under the optimum pH conditions, each enzyme showed the maximum activity, and when the pH value deviates from the ideal conditions, the activity of the enzyme slows down and then stops.

♦ For example, the optimum pH of an enzyme that works in the acidic environment of the human stomach is lower than that of an enzyme that works in a neutral environment of human blood.

♦The enzyme has an active site at which the substrate bind to and the shape of the active site will change with the change of pH value.

Method :

Materials	Tube (1⟶5)	Tube 6 (blank)
Starch	2 ml	2 ml
Buffer PH(**3.6** ,**4.4**, **5.2** ,**7** , **7.4**)	1 ml	1 ml
Incubation 3min at 37C°		
α- amylase	0.1 ml	-----
Incubation at 37C° for 15 min		
NaOH	0.5 ml	0.5 ml
DNS	0.5 ml	0.5 ml
Boiling for 3min ,then read the Absorbance at (540 nm)		

Tube No.	PH	A	A-AB
1	3.6		
2	4.4		
3	5.2		
4	7		
5	7.4		
6	Blank		

Exp. Name :

The **effect Temperature** on enzyme activity.

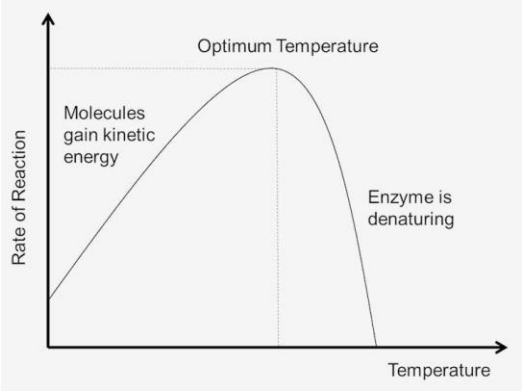
Exp. Aim:

To find the effect of changes in Temperature on the activity of the enzyme.

THE EFFECT OF TEMPERATURE

♦ Collisions between all molecules increase as temperature increases. This is due to the increase in velocity and kinetic energy that follows temperature increases.
♦ Enzyme activity increases as temperature increases, and in turn increases the rate of the reaction. This also means activity decreases at colder temperatures.
♦ All enzymes have a range of temperatures when they are active, but there are certain temperatures where they work optimally.
♦ The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because most enzymes rapidly become denatured at temperatures above 40°C, most enzyme determinations are carried out somewhat below that temperature.
♦ Some enzymes lose their activity when frozen.

♦ The conditions under which a particular enzyme is most active are called the optimum conditions. When an enzyme is most active the rate of the biological reaction it catalyzes is highest.
♦ The optimum temperature is the temperature at which the enzyme reaches its maximum activity (Temp. at which the enzyme is more active).



♦ As the temperature is increased enzyme activity increases to a maximum value at the optimum temperature (around 37oC for most human enzymes). As the temperature is increased above the optimum temperature enzyme activity decreases.

♦ At low temperatures enzyme activity is low because the enzyme and substrate molecules have less kinetic energy so there are fewer collisions between them.

♦ At the optimum temperature, the kinetic energy in the substrate and enzyme molecules is ideal for the maximum number of collisions.

♦ At high temperatures the shape of the enzyme is altered so that it is no longer complementary to its specific substrate. This effect can be permanent and irreversible and is called denaturation.

Method :

Materials	Tube (1→5)	Tube 6 (blank)
Starch (0.5%)	2ml	2ml
NaCl (0.9%)	0.5ml	0.5ml
H_2O	0.5ml	1ml
α- amylase	0.5ml	----
Incubation for 10min at different temp.(4 , 25 ,37, 60 , 100 C˚)		
NaOH	0.5ml	0.5ml
DNS	0.5ml	0.5ml
Boiling for 3min ,then read the Absorbance at (540nm)		

Tube No.	Temp.	A	A-AB
1	4		
2	25		
3	37		
4	60		
5	100		
6	Blank		

Biochemistry Lab. Exp.9

Assis. Teach. shaema sadoon

EXP Name: Assay of Urease Enzyme Activity

Urease is a hydrolytic enzyme that catalyzes the urea into carbon dioxide and ammonia .The enzyme commission number is 3.5.1.5. This reaction follows

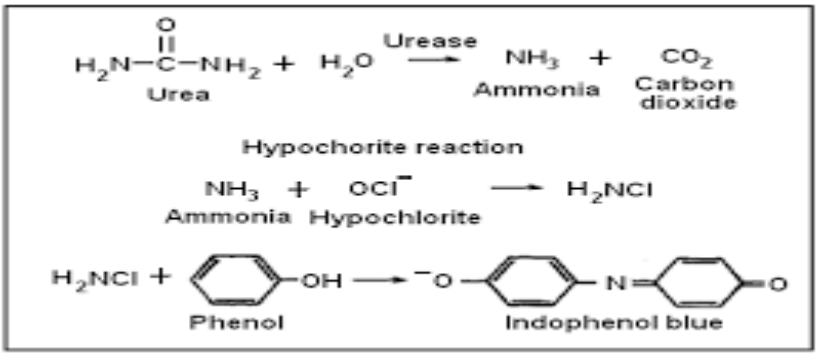
$$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$$

Every organism decomposes nucleic acids and proteins, generating nitrogenous waste because nucleic acids and proteins contain nitrogen. Mammals, amphibians and some invertebrates excrete nitrogenous waste as urea , which is produced in the liver .Urea is an especially good compound for disposing of nitrogen because it is water – soluble and less toxic than ammonia – the excretory produce of fish, for example. Human urine contains 2% urea.

Many species of bacteria produce urease , including Helicobacter pylori, the bacterium responsible for stomach ulcers. Bydoing this,H.pylori raises the PH of the gastric juice from about PH 3 to PH7 ,the optimal PH for its growth.

Principle:

The method is based on the Berthelot reaction . Alkaline phenol and sodium hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration present .The blue color formed is intensified by the use of sodium nitropusside as a catalyst.



Reagents:

1- Enzyme substrate (25 mM urea in 100 mM phosphate buffer PH6.8):

-Add 13.121 g of $Na_2HPO_4.7H_2O$ and 7.044 g of NaH_2PO_4 to 800 ml of distilled water in a suitable container. Adjust solution to PH 6.8 using HCl or NaCl .Add distilled water until volume is 1L.

- Dissolve 1.50 g of urea in1L of 0.1M phosphate buffer PH 6.8.

2- Urease enzyme from beans: Grind 5 g of dry beans and add 5ml of 100 mM phosphate buffer PH 6.8.

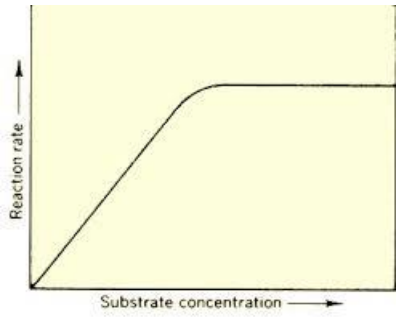
3- Reagent A : contained 10 g phenol and 50 mg of sodium nirtoprusside in 500ml of distilled water.

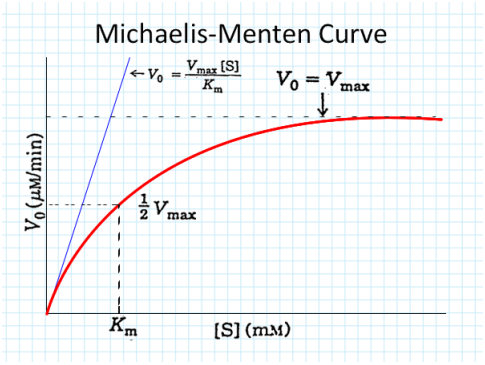
4- Reagent B: contained of 5.0 g sodium hydroxide and 8.4 ml of sodium hypochlorite in 500 ml of distilled water.

Procedure:

No.of tube	1				2	3	4	5	6
	Final urea concentration (Mm)	Volume of urea (ml)	Volume of buffer (ml)	Final volume					
1	0.00	0	2	2	Pre incubation at 37˚C for 5 minutes	Add 0.1 of urease extract and incubate at 37˚C for 10 minutes	Add 2 ml of Reagent A + 2ml of Reagent B	To develop the color, incubate at 37˚C for 5 minutes	Recor the intensity of the color at ʎ630nm
2	5	0.4	1.6	2					
3	10	0.8	1.2	2					
4	15	1.2	0.8	2					
5	20	1.6	0.4	2					
6	25	2	0	2					

Tube No.	Final urea concentration (Mm)	A	A-AB
1	0.00		
2	5		
3	10		
4	15		
5	20		
6	25		
7	0 (Blank)		


Reaction rate
Substrate concentration


Michaelis-Menten Curve
$\leftarrow V_0 = \frac{V_{max}[S]}{K_m}$
$V_0 = V_{max}$
V_0 (μM/min)
$\frac{1}{2}V_{max}$
K_m
[S] (mM)

Biochemistry Lab. Exp.10

Assis. Teach. shaema sadoon

EXP Name: Estimation of ascorbic acid in lemon juice

Ascorbic is found in fruit, particularly citrus fruits , and vegetables . a quantitatively significant dietary is ascorbate added to other foods as a preservative .It cannot by synthesized by man ,other primates ,or the guinea pig.

Ascorbate can be reversibly oxidized in biological systems to dehydroascorbate and ,although its functions in man are not certain ,it probably acts as a hydrogen carrier .It seems to be necessary for normal collagen formation**.**

Principle:

Vitamin C (L-ascorbic acid) gets oxidized to its dehydro form by air especially at alkaline PH. However, it is stable in an acidic solution . Therefore ,vitamin C is extracted in metaphosphoric acid or in a mixture of metaphosphoric and dilute acetic acid or by oxalic acid and acetic acid. Its estimation in the extract is carried out by titrating it against 2,6-dichlorphenol indophenol solution. Oxidized form of this dye is blue in colour in an alkaline medium and red in an acidic medium . Reduced form of the dye ,on the other hand, is colourless and its termed its leuco form .The redox reaction accuring during the titration is:

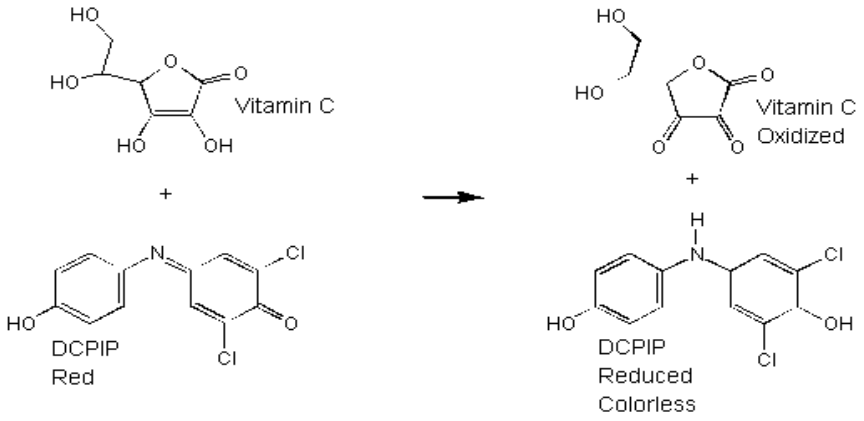


Figure 1. Oxidation of Vitamin C with 2,6-dichlorophenolindolphenol (DCPIP).

Reagents:

1- Lemons, orange juice ,green papper ,black currant juice.
2- **2,6- dichlorophenol indophenol solution:** Dissolve 52 mg of sodium salt of the dye and 42 mg of sodium bicarbonate in water . Make up the final volume to 500 ml.
3-**Standard vitamin C solution:** Dissolve 20 mg of vitamin C in 1L distilled water.

4- Acetic acid glacial.

Procedure:

2.5 ml (standard solution) + 0.5 ml Acidic acid glacial ----→ V standard
(Titration with dye)

2.5 ml (lemon juice) + 0.5 ml Acidic acid glacial ----→ V test
(Titration with dye)

2.5 ml (distilled water) + 0.5 ml Acidic acid glacial ----→ V blank
(Titration with dye)

Calculation :

[Vitamin C] mg/100ml ={(V test – V blank)/(V standard – V blank)} x (concentration of standard/ Vol. test) x 100

V standard = The volume of dye consumed by titration with standard solution.

V test = The volume of dye consumed by titration with lemon juice.

V blank= The volume of dye consumed by titration with distilled water.

Vol. test = The volume of orange juice used to measure the concentration of the vitamin C.