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Biochemical Methodology

530 BCH

Eman Alshehri
KING SAUD UNIVERSITY, BIOCHEMISTRY DEPARTMENT

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Chemicals	• Tris hydroxyl methyl) amino methane • Tris base • Tris (hydroxyl methyl)-amino methane hydrochloride (Tris-HCl) • HCl • NaCl • Acetic acid • Sodium acetate • Di-potassium hydrogen orthophosphate • Potassium dihydrogen orthophosphate • NAD^+ -oxidized Nicotinamide adenine Dinucleotide • NADH - reduced Nicotinamide adenine Dinucleotide • Sodium pyruvate • Sodium lactate • Sodium Carbonate $NaCO_3$ • sodium carbonate anhydrous • sodium hydroxide pellets • Ammonium sulfate • Urea • Oxalic acid • SDS • Glycerol • Glycine • Acrylamide • Bisacrylamide • 2-mercaptoethanol • Bromophenol blue • Ammonium persulfate • Commasie blue • Methanol • Bovine Serum Albumin • Ethanol • Phosphoric acid • TEMED- N,N,N,N- tetramethylethyldiamine • Bradford Reagent – if available • Folin • Proteins Marker – For electrophoresis • Anion Exchange – DEAS Kit • Sephadex G-25 • EDTA • Sodium tartarate • CuSO4.5H2O
Equipment	• Centrifuge tubes 50 ml, 15 ml • Eppendorf tubes and rack • Dialysis tape • Anion exchange colum • Cuvettes – Quartz • Cuvettes – Plastic • Plastic Pasteur pipette • Micropipette 10-100, and 100-1000 and Tips • SDS PAGE electrophoresis tank- Power Supply-Comb 1.5 mm- 2 glass slides 1.5 mm • Magnetic stirrer

Instruments	• Blender • Balance • Centrifuge • Spectrophotometer • Water Bath • PH meter • Shaker
Glassware	• Beakers • Volumetric flasks 50 ml, 100 ml, 500 ml, 1000 ml • Measuring Cylinders 10, 50, 100, 200 ml • Test tubes • Brown Bottles • Buchner funnel • Glass wool

Solution	Preparation
Tris buffer (0.1 M, pH 7.4) :	Dissolve 12.114 g of Tris in 50 ml distilled water, adjust pH with concentrated HCl to pH 7.4, add distilled water to make the total volume 1000 ml.
9%Normal saline:	Dissolve 1.8 g of NaCl in 200 ml distilled water.
Tris-HCl buffer(0.01M, pH 8.6) :	Dissolve 0.1576 g of Tris base in 50 ml distilled water, adjust pH with concentrated HCl to pH 8.6 , add distilled water to make the total volume 100 ml.
0.1 M acetic acid solution:	Add 8.8164g of acetic acid in 1000 ml of distilled water.
Acetate buffer(0.1M,pH 4).	Mix 420 ml of acetic acid and 0.615 g of sodium acetate, adjust the final volume to 500 ml with distilled water, adjust the final pH using pH meter.
Acetate buffer(0.1M,pH 5).	Mix 185 ml of acetic acid and 2.615 g of sodium acetate, adjust the final volume to 500 ml with distilled water, adjust the final pH using pH meter.
1M Di-potassium hydrogen orthophosphate:	Dissolve 87.09 g of di-potassium hydrogen orthophosphate in 500 distilled water.
1M Potassium dihydrogen orthophosphate:	Dissolve 68.045 g of potassium dihydrogen orthophosphate in 500 distilled water.
Potassium phosphate buffer(0.1M,pH 6).	Add 13.2 ml of 1M dipotassium hydrogen orthophosphate to 86.8 ml of potassium dihydrogen orthophosphate, adjust the final volume to 200 ml with distilled water, adjust the final pH using pH meter.
Potassium phosphate buffer(0.1M, pH 7).	Add 61.5 ml of 1M dipotassium hydrogen orthophosphate to 38.5 ml of 1M potassium dihydrogen orthophosphate, adjust the final volume to 200 ml with distilled water, adjust the final pH using pH meter.
5 mM NAD^+ solution:	Dissolve 0.16 g of NAD^+ in 50 ml distilled water.
5 mM NADH solution:	Dissolve 0.16 g of NADH in 50 ml distilled water.
22.7 mM Sodium pyruvate solution:	Dissolve 0.12g of sodium pyruvate in 50 ml distilled water.
22.7 mM Sodium lactate solution:	Dissolve 0.1022 g of sodium lactate in50 ml distilled water.
18 mM Sodium Carbonate(Na_2CO3) solution:	Dissolve 0.0953 g of $NaCO_3$ in 50 ml distilled water.
0.5 M Sodium Chloride (NaCl)solution:	Dissolve 1.461 g of NaCl in 50 ml of distilled water
Sodium Bicarbonate stock solution (18 mM $NaCO_3$,0.5 M NaCl):	Mix 5o ml of $NaCO_3$ solution and 50 ml of NaCl solution together.
2 M urea solution:	Dissolve 0.0113g of urea in and make up to 50 of distilled water.
0.2 mM oxalic acid solution :	Dissolve 0.0018 g of oxalic acid and make up to 500 of distilled water .
1.5M Tris-HCl (PH 8.8), 100ml	18.15g of Tris base + 50ml distilled water + add Conc. HCl slowly to PH 8.8 (~3.2ml HCl)

	Allow solution to cool at room temp. PH will increase, add distilled water to make the total volume of 100ml.
0.5M Tris-HCl (PH 6.8), 100ml:	6.05g of Tris base + 5050ml distilled water + add Conc. HCl slowly to PH 6.8 (~7.4ml HCl) Allow solution to cool at room temp. PH will increase, add distilled water to make the total volume of 100ml.
10% SDS:	Weight 10g SDS + add distilled water to make the total volume of 100ml
50% glycerol, 100ml:	Pour 50ml 100% glycerol, then make the total volume 100 by adding 50ml distilled water
Electrophoresis buffer, Running buffer pH 8.3:	Weight 30g of Tris - base+ 144 g glycine +10 g SDS Then add distilled water to make the total volume 1 liter
Acrylamide stake solution, 100ml:	Weight 30g acrylamide + 0.8g bisacrylamide Then add distilled water to make the total volume 100ml
5X buffer, Sample buffer:	Weight 0.6ml 1M tris-HCl (PH 6.8) + 5ml 50% glycerol + 2ml 10% SDS + 0.5ml 2-mercaptoethanol + 1ml 1% bromophenol blue + 0.9ml distilled water, put it on steir then put it in folded tube with foil, then put it in the refrigerator for 5 days
10% Ammonium persulfate, 5ml: *prepare fresh*	Weight 0.5g ammonium persulfate + 5ml distilled water
Commasie blue, Staining solution for gel:	Weight about 1g commasie blue + 450ml methanol + 450ml distilled water + 100ml acetic acid
Commasie blue, Destaining solution for gel:	100ml methanol + 100ml acetic acid + 800ml distilled water

Extraction and Purification of Lactate Dehydrogenase Tissues

Aim of the experiment:

1. Learn basic techniques for protein purification.
2. Prepare crude extract.
3. Attempt purification of LDH.

Procedure:

Step one: Preparation of crude extract

Note: keep the tissues and fractions ice cold whenever possible

1. Make sure the tissue is free of adhering fat and membranes, and wash it in normal saline
2. Weight the tissue before starting homogenization.
3. Use a razor blade or scissors to dice the tissue into small pieces.
4. Homogenize the tissue with cold Tris buffer (0.1 M, pH 7.4), (20% weight/volume).
5. Record the volume (ml) of homogenate.
6. Pour the homogenate into centrifuge tubes then centrifuge the homogenate at 3000 X g for 15 minutes at 4 C°.
7. Filter the supernatant through cheesecloth to get the crude extract.
8. Record the volume (ml) of the crude extract.

Step two: Salt Fractionation (I):

1. Add solid ammonium sulphate $(NH_4)_2SO_4$to the crude extract to get a 40% saturation of the solution. The required amount of ammonium sulphate can be calculated from the table shown below. Crude extract (40% $(NH_4)_2SO_4$ fractionation at 40 C°; 22.6 grams/100 ml of the solution)
2. Add the salt slowly with constant stirring at 4 C° for 30 minutes.
3. Centrifuge at 14 500 g for 15 minutes.
4. Record the volume (ml) of the supernatant label it as fraction 1 (F1). Discard the precipitant.

Step 3: Salt Fractionation (II)

1. To the F1 add ammonium sulphate at 4 C° to get 60% saturation. $(NH4)2SO4$ (12.0 g/100 ml).
2. Add the salt slowly with constant stirring at 4 C° for 30 minutes.
3. Centrifuge at 14 500 g for 15 minutes at 4 C°.
4. Suspend the precipitant in 20 ml of iced cold Tris-buffer
5. Record the final volume (ml), and label it as fraction 1 (F2).
6. Keep the supernatant, and record the volume (ml) and label it as Fraction 3 (F3).

Step Four: Desalting

5. Suspend F2 in 20 ml of cold Tris buffer
6. Centrifuge at 3000 X g for 10 minutes at C°.
7. Record the volume of the supernatant, label it as fraction 4 (F4) and discard the pellets.
8. Desalt the F4 using Sephadex G-25 column eluting it with 30 – 35 ml 0f 0.1 M Tris, pH7.4
9. Label the resulting fraction as Fraction 5 (F5).

   Note: The sample volume should not be greater than one-fifth that of the volume of gel in the column so that the change can be accomplished in one pass.
   Dissolve an ammonium sulfate precipitate with a minimal amount of buffer, then dilute with an equal volume of buffer prior to desalting on a column. Protein concentrations should not be greater than30mg/ml.

Step Five: Anion Exchange Chromatography

1. Apply F5 on DEAE –Sepharose CL-6B at 40 C° equilibrated with 0.1 M Tris, pH 7.4
2. Elute with a linear salt gradient (Starting with 250 ml 0.1 M Tris, pH 7.4, final buffer 250 ml 0.1 M Tris, pH 7.4 containing 0.4 M NaCl)
3. Collect the fractions (5 ml).
4. Read the absorbance of each fractions
5. Plot the absorbance at 280 nm against the fraction number.
6. Assay for LDH activity and protein concentration
7. Pool the fractions with highest LDH activity
8. Plot the absorbance at 280 nm against the fraction number as shown for a hypothetical protein.

Purification Table

Purification procedure	Volume (ml)	Protein concentration (mg ml^{-1})	Total protein (mg)	Specific activity (units mg protein^{-1})	Total activity	Fold purity	% yield
Crude Extract						1	100
Ammonium sulfate precipitation 40% (sat)							
Ammonium sulfate precipitation 60% (sat)							
Dialysis							
DEAE ION exchange							

Table: A protein purification balance sheet provides the data necessary to measure the efficacy of the chromatographic (electrophoretic) technique used. It contains information on the volume, the protein concentration and the biological activity (an assay can be used to determine the presence of the target protein) relative to the amount of protein present (specific activity). It provides data on the yield (percentage recovery i.e. how much remains after a chromatographic technique when this is compared with the amount present in the starting material) and the degree (fold) of purification (a measure of the increase in specific activity after the chromatographic procedure).

$$\text{Specific activity (SA) (units mg}^{-1}\text{ protein)} = \frac{\text{target protein (total units* of activity in a fraction)}}{\text{total protein in a fraction protein (mg)}}$$

$$\text{Total protein (TP)} = \text{protein mg ml}^{-1} \times \text{volume (ml)}$$

$$\text{Total activity (TA)} = \text{units* mg}^{-1}\text{ protein (SA)} \times \text{total protein (TP)}$$

$$\text{Degree of purification (fold purity)} = \frac{\text{SA Step 2}}{\text{SA Step 1}}$$

$$\text{Yield (\% recovery)} = \frac{\text{TA Step 2}}{\text{TA Step 1}} \times 100$$

Enzyme Kinetics

Assay of Lactate Dehydrogenase Activity:

The assay mixture contains 2.7 ml of 0.1 M of Tris, PH7.4, 0.1 ml of 22.7 mM sodium pyruvate and 0.1 ml of 5 mM NADH (Prepared fresh). Start the reaction by adding of 0.1 ml of diluted enzyme then monitor the decrease in absorbance at 340 nm as a function of time. Calculate the total number of units as shown below. Plot the enzyme activity against the fraction number as shown for a hypothetical protein.

> **Note:** the reference and the control all components except the NADH and pyruvate respectively.

Total number of units=
(ΔA sample/min - ΔA control /min) X 1 unit X DF X V / 2.0733A/min X 0.1

- ΔA/minute is the change in absorbance expected for the disappearance of one micromole of NADH in 3.0 ml.?
- DF is dilution factor of the enzyme
- Volume is the total volume in ml of enzyme for a given purification step
- 0.1 ml is the volume of enzyme assayed.

The effect of substrate concentration:

Two kinds of substrates are used; one for liver and kidney enzymes and another for muscle enzyme. These substrates are pyruvate for kidney and liver, Lactate for muscle. The concentration of Lactate is varying from 5 to 13 mM, while for pyruvate is ranging from 0.05 to 0.5 mM. Reaction carried out in quartz cuvette of 3 cm light path containing 1.8 ml of 0.1 M Tris, pH 7.4, 0.1 to 1 ml of 1.5 mM sodium pyruvate and 0.1 ml of 5.0 mM NADH (***prepared fresh***) and 0.1 ml of enzyme extract and the difference in the volume were compensated by adding distilled water.

The reaction starts by addition enzyme extract and mixing by inverting the cuvette. The final volume of the reaction mixture is 3 ml. The blank contained all components except NADH. For muscle LDH, reaction carried out in quartz cuvette of 3 cm light path containing 0.2 ml of sodium bicarbonate, 0.4 ml of NAD^+, different volumes from lactate stock solution (0.1 M) and the difference in the volume were compensated by adding 0.1 M Tris, pH 7.4, The reaction starts by addition 0.1 ml enzyme extract and mixing by inverting the cuvette. The final volume of the reaction mixture was 3 ml. The blank contained all components except NAD^+.

Liver and Kidney					
[S] mM	Substrate – pyruvate volume ml	diS. wate ml	0.1 M Tris, pH 7.4	5 mM NADH	Activity
0.05	0.1	0.9	1.8 ml	0.1 ml	
0.1	0.2	0.8	1.8 ml	0.1 ml	
0.15	0.3	0.7	1.8 ml	0.1 ml	
0.2	0.4	0.6	1.8 ml	0.1 ml	
0.25	0.5	0.5	1.8 ml	0.1 ml	
0.3	0.6	0.4	1.8 ml	0.1 ml	
0.35	0.7	0.3	1.8 ml	0.1 ml	
0.4	0.8	0.2	1.8 ml	0.1 ml	
0.45	0.9	0.1	1.8 ml	0.1 ml	
0.5	1	-	1.8 ml	0.1 ml	

The effect of inhibitors:

Urea has been used as a competitive inhibitor that competes with lactate on the active site of LDH and its concentration was 2 M. For LDH in kidney and liver, oxalate was used also as a competitive inhibitor that compete with pyruvate on the active site of LDH in both tissues, oxalate concentration was 0.2 mM . Same enzyme assay except adding of inhibitors were employed here.

Liver and Kidney					
[S] mM	Substrate-pyruvate	diS. wate ml	0.1 M Tris, pH 7.4	5 mM NADH	0.2 mM Oxalate
0.05	0.1	0.9	1.8 ml	0.1 ml	0.1 ml
0.1	0.2	0.8	1.8 ml	0.1 ml	0.1 ml
0.15	0.3	0.7	1.8 ml	0.1 ml	0.1 ml
0.2	0.4	0.6	1.8 ml	0.1 ml	0.1 ml
0.25	0.5	0.5	1.8 ml	0.1 ml	0.1 ml
0.3	0.6	0.4	1.8 ml	0.1 ml	0.1 ml
0.35	0.7	0.3	1.8 ml	0.1 ml	0.1 ml
0.4	0.8	0.2	1.8 ml	0.1 ml	0.1 ml
0.45	0.9	0.1	1.8 ml	0.1 ml	0.1 ml
0.5	1	-	1.8 ml	0.1 ml	0.1 ml

The effect of pH on enzyme activity:

Enzymes are active only within a limited range of PH. But the limits may be wide, e.g. pH 5 to 10, or narrow over 1 pH unit. Within the range there will be an optimum at which the maximum activity is attained. This could be a short range in itself. Six different PH buffers ranged from 4 to 9 have been used (PH 4,5 were Acetate buffers, PH 6,7 were potassium phosphate buffers and Tris buffer for PH 8,9 for preparation see appendix).

The effect of Temperature on enzyme activity:

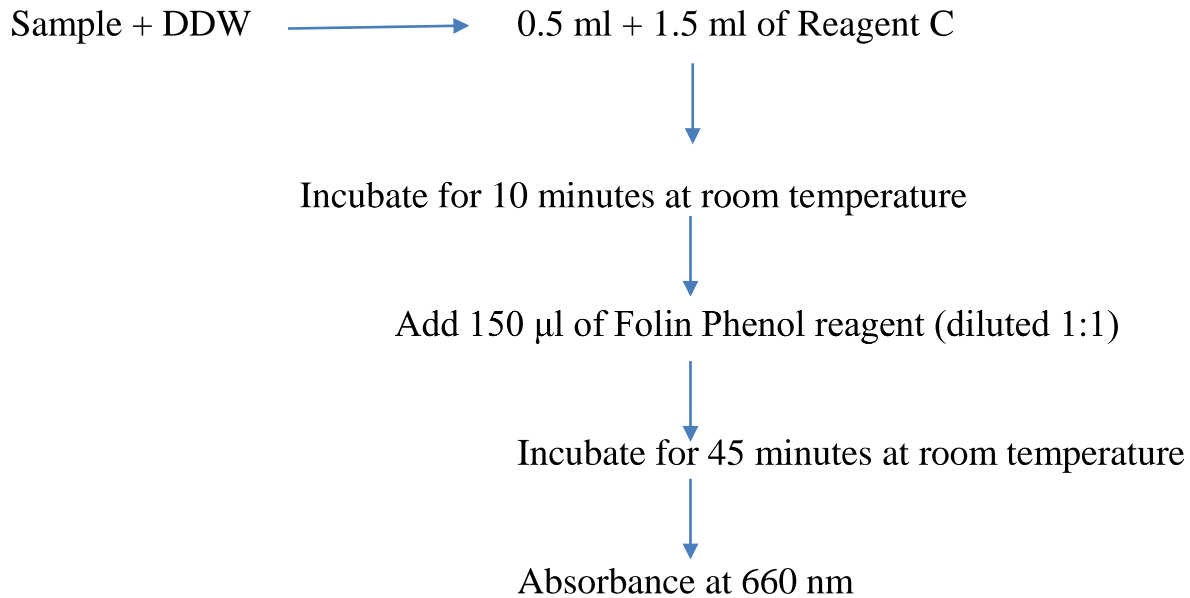
Temperature affects enzyme activity in much the same way as it affects other chemical reactions. Thus it is important when carrying out an enzyme assay to ensure that the temperature remains constant, and also that you know exactly what it is. Enzyme activity has been studied at different temperature 20, 30, 40, 50, 60 and 70 °C.

Determination of Protein Concentration

Protein concentrations are determined according to Lowry et al with bovine serum albumin as standard.

1. Add 1.5 ml from each sample to 3 ml of doubled distilled water.
2. Add 1.5 ml from reagent C and 0.5 ml from (sample and DDW) has incubated for 10 minutes at room temperature.
3. Add 150 µl from diluted folin phenol reagent and incubate for 45 minutes at room temperature
4. Measure the absorbance by spectrophotometer at 660 nm.

Procedure



Reagent A:

Reagent A	500 ml
Na_2CO_3	10 g
NaOH	2 g
Sodium tartarate	0.8 g
SDS	5.0 g

Reagent B:

$CuSO_4.5H_2O$ → 2 g/50 ml

Reagent C:

1ml of Reagent B+ 100 ml of Reagent A (Prepare fresh before use).

Standard for protein determination using BSA in 0.1 M Tris, pH7.4

Stock: 1 mg/ml BSA in 0.1 M Tris, pH7.4 (1000 µg/ml)

Working standard: 0.2 ml of stock A + 1.8 ml of 0.1 M Tris, pH7.4 (100 µg/ml)

	Volume of standard/stock (µl)	Concentration µg	Volume of DDW (µl)	Reagent C (ml)	Folin Phenol reagent (µl)	660 nm
	Working standard					
	100	10	400			
	200	20	300			
	400	40	100	1.5	150	
	Stock					
	50	50	450			
	100	100	400			
	200	200	300			
	Blank	-	400			

SDS-Poly acrylamide gel electrophoresis (SDS-PAGE)

In this system, two sequential gels are actually used; the top gel, called the stacking gel, is slightly acidic (pH 6.8) and has a low acrylamide concentration to make a porous gel. Under these conditions proteins separate poorly but form thin, sharply, defined bands.

The lower gel, called the separating (resolving gel) is more basic (pH 8.8), and has a higher polyacrylamide concentration which causes the gel to have narrower channels or pores.As a protein, concentrated into sharp bands by stacking gel, travels through the separating gel, the narrower pores have a sieving effect, allowing smaller proteins to travel more easily, and rapidly than larger proteins.

Gel preparation

- Separation gel contents

components	Amount
1.5 M Tris-HCL PH 8.8	2 ml
H2O	2.8 ml
10% SDS	80µl
10% Ammonium persulphate (fresh)	100 µl
TEMED	20 µl
Acrylamide stock	3.2 ml

- Stacking gel contents

components	Amount
1.5 M Tris-HCL PH 6.8	1 ml
H2O	3 ml
10% SDS	80 µl
10% Ammonium persulphate (fresh)	100 µl
TEMED	20 µl
Acrylamide stock	1 ml

- SDS-PAGE Running buffer pH 8.4 (5 X)

components	Amount
Tris	15 g
Glycine	72 g
SDS	5 g
Made up to 1L with distilled water	

- SDS- PAGE Disruption buffer

components	Amount
20% (w/v) SDS	1 ml
1M Tris HCL pH	0.5 ml
Glycerol	1 ml
B- mercaptoethanol	0.5 ml
Bromophenol blue	0.01 g
Made up to 10 ml with distilled water	

Sample Preparation

For best results, all samples should be in identical, low ionic strength buffers.

1) Mix 40 µl of each sample with 10 µl of disruption buffer.
2) Heat in a boiling water bath in for 2 min. in most cases, brief boiling 3 min improves denaturation, but it may also cause the protein to precipitate.

Electrophoresis

1) Remove the comb and clamp the gel to the electrophoretic apparatus.
2) Fill thr top electrolyte compartment with running buffer.
3) Check for leaks from thr top into bottom compartment, if there are no leaks, fill the bottom compartment.
4) With a plastic Pasteur pipette, thoroughly rinse each well in the stacking gel with running buffer.
5) Apply the sample by using a micropipette to carefully add up to 25 µl of protein to bottom of a well.
6) Replace the cover of the electrophoretic cell, with the (+) symbol on the cover connected to the (+) on the cell, so that the anode (+) is the bottom electrode.
7) Apply 15 mA/gel until the proteins are well into the stacking gel, then 35mA/gel until the tracking dye reaches the bottom of the gel (about 45 min).
8) Always turn down the power and unplug the wires from the power supply before removing the cover.

Periodic Table of the Elements

Atomic Number
Symbol
Name
Atomic Mass

1 IA 1A	2 IIA 2A	3 IIIB 3B	4 IVB 4B	5 VB 5B	6 VIB 6B	7 VIIB 7B	8	9 VIII 8	10	11 IB 1B	12 IIB 2B	13 IIIA 3A	14 IVA 4A	15 VA 5A	16 VIA 6A	17 VIIA 7A	18 VIIIA 8A
1 H Hydrogen 1.008																	2 He Helium 4.003
3 Li Lithium 6.941	4 Be Beryllium 9.012											5 B Boron 10.811	6 C Carbon 12.011	7 N Nitrogen 14.007	8 O Oxygen 15.999	9 F Fluorine 18.998	10 Ne Neon 20.180
11 Na Sodium 22.990	12 Mg Magnesium 24.305											13 Al Aluminum 26.982	14 Si Silicon 28.086	15 P Phosphorus 30.974	16 S Sulfur 32.066	17 Cl Chlorine 35.453	18 Ar Argon 39.948
19 K Potassium 39.098	20 Ca Calcium 40.078	21 Sc Scandium 44.956	22 Ti Titanium 47.867	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.845	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546	30 Zn Zinc 65.38	31 Ga Gallium 69.723	32 Ge Germanium 72.631	33 As Arsenic 74.922	34 Se Selenium 78.972	35 Br Bromine 79.904	36 Kr Krypton 83.798
37 Rb Rubidium 85.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224	41 Nb Niobium 92.906	42 Mo Molybdenum 95.95	43 Tc Technetium 98.907	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.906	46 Pd Palladium 106.42	47 Ag Silver 107.868	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.711	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.904	54 Xe Xenon 131.294
55 Cs Cesium 132.905	56 Ba Barium 137.328	57-71	72 Hf Hafnium 178.49	73 Ta Tantalum 180.948	74 W Tungsten 183.84	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.217	78 Pt Platinum 195.085	79 Au Gold 196.967	80 Hg Mercury 200.592	81 Tl Thallium 204.383	82 Pb Lead 207.2	83 Bi Bismuth 208.980	84 Po Polonium [208.982]	85 At Astatine 209.987	86 Rn Radon 222.018
87 Fr Francium 223.020	88 Ra Radium 226.025	89-103	104 Rf Rutherfordium [261]	105 Db Dubnium [262]	106 Sg Seaborgium [266]	107 Bh Bohrium [264]	108 Hs Hassium [269]	109 Mt Meitnerium [278]	110 Ds Darmstadtium [281]	111 Rg Roentgenium [280]	112 Cn Copernicium [285]	113 Nh Nihonium [286]	114 Fl Flerovium [289]	115 Mc Moscovium [289]	116 Lv Livermorium [293]	117 Ts Tennessine [294]	118 Og Oganesson [294]

Series															
Lanthanide Series	57 La Lanthanum 138.905	58 Ce Cerium 140.116	59 Pr Praseodymium 140.908	60 Nd Neodymium 144.242	61 Pm Promethium 144.913	62 Sm Samarium 150.36	63 Eu Europium 151.964	64 Gd Gadolinium 157.25	65 Tb Terbium 158.925	66 Dy Dysprosium 162.500	67 Ho Holmium 164.930	68 Er Erbium 167.259	69 Tm Thulium 168.934	70 Yb Ytterbium 173.055	71 Lu Lutetium 174.967
Actinide Series	89 Ac Actinium 227.028	90 Th Thorium 232.038	91 Pa Protactinium 231.036	92 U Uranium 238.029	93 Np Neptunium 237.048	94 Pu Plutonium 244.064	95 Am Americium 243.061	96 Cm Curium 247.070	97 Bk Berkelium 247.070	98 Cf Californium 251.080	99 Es Einsteinium [254]	100 Fm Fermium 257.095	101 Md Mendelevium 258.1	102 No Nobelium 259.101	103 Lr Lawrencium [262]

Alkali Metal	Alkaline Earth	Transition Metal	Basic Metal	Semimetal	Nonmetal	Halogen	Noble Gas	Lanthanide	Actinide

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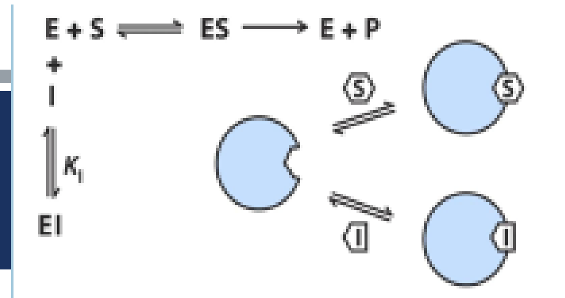
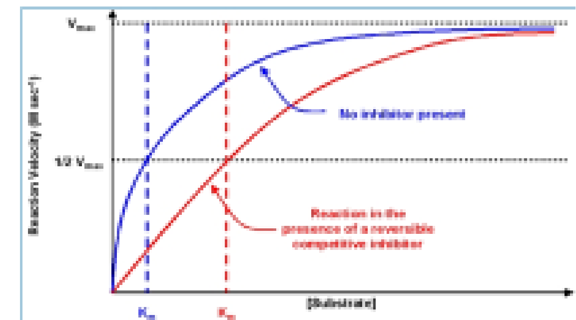
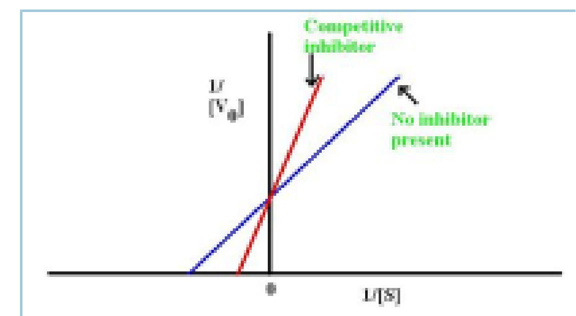
- ***Reversible inhibitors***

COMPETITIVE INHIBITORS

- As the name implies, the inhibitor compete with the substrate for active site of the enzyme.
- The substrate and inhibitors have resemble structures
- Have the same Vmax (with I OR without I)
- Km high and affinity low (with I)
- This type of inhibition can be overcome by sufficiently high concentrations of substrate by out-competing the inhibitor

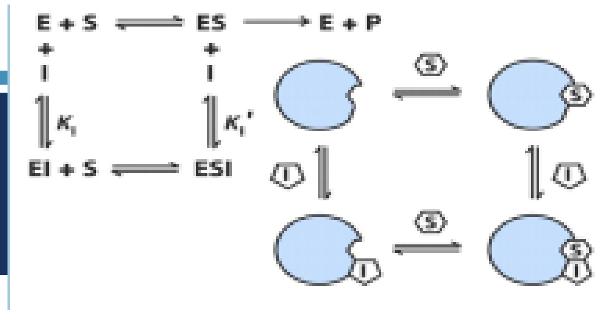


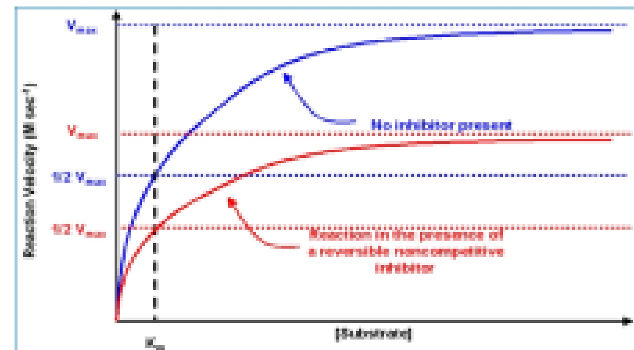


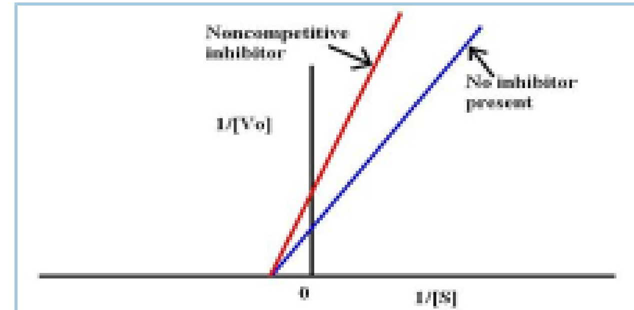


NONCOMPETITIVE INHIBITORS

- The inhibitors bind with site on the enzyme other than active site
- They can bind with E or ES complex.
- Have the same Km (with I OR without I)
- low Vmax (with I)







UNCOMPETITIVE INHIBITORS

- The inhibitor binds only to the substrate-enzyme complex
- Both Vmax and Km are low (with I)