LAB (6):

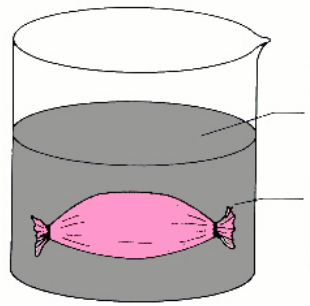
DIALYSIS

KAU-Faculty of Science- Biochemistry department

Analytical biochemistry lab (Bioc 343) 2012

T.A Nouf Alshareef

nf.shareef@hotmail.com



Introduction:

- Dialysis is non chromatographic separation method used to separates molecules according to size through the use of semi-permeable membranes.

- dialysis process occurs at the membrane surface, depend on conc. differences between two solutions on both side of the membrane.

- the porous membrane selectively allows smaller solutes to pass while retaining larger species

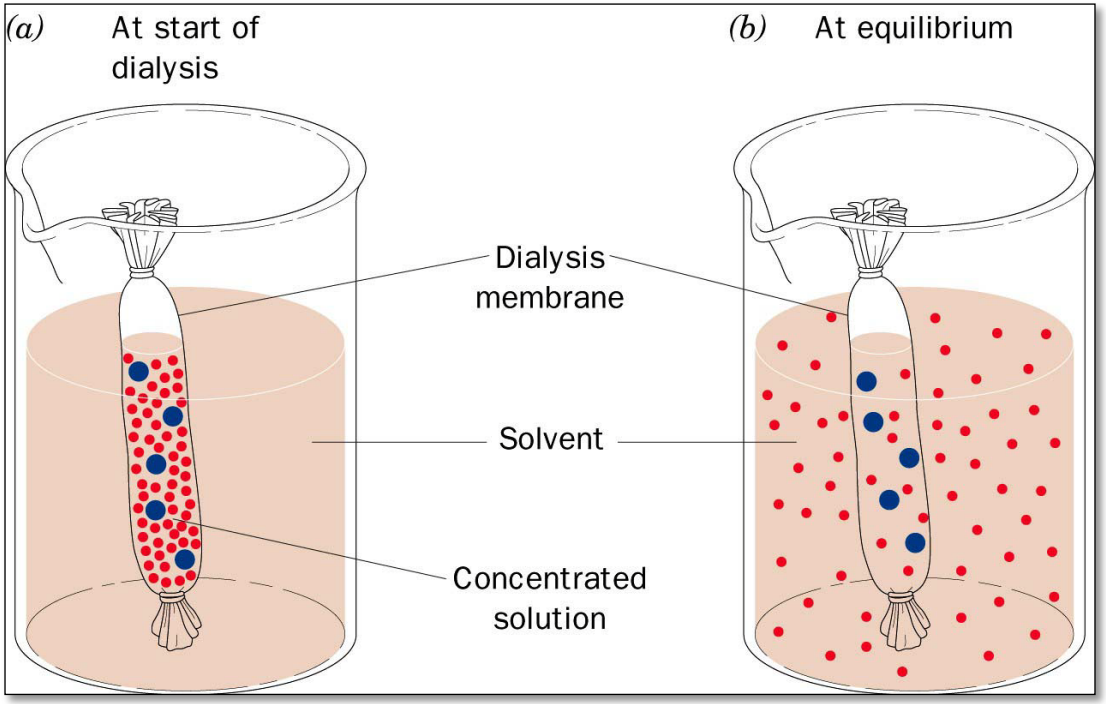
It is a simple process in which:

High conc. solution
"small solutes "

diffuse
semi-permeable membranes

Low conc. solution

until equilibrium



Factors affect on dialysis:

- Temperature, viscosity, and pressure gradient across the membrane.
- Solvent, pore size, and the nature of the membrane.
  (rate of dialysis is greatest in distilled water)

Advantages of lab dialysis:

- Very gentle conditions
- Easy operation
- Wide range of samples volume
- Many membrane types
- Inexpensive materials
- Disposable membranes & devices

Dialysis membrane materials:

- Synthetic or natural membranes are used for diffusion or osmosis.
- It allows the passage of small molecules but not larger ones (Filtration applications).
- Membrane materials: regenerated cellulose, cellulose acetate, polysulfone, polycarbonate, polyethylene, polyolefin, polypropylene, and polyvinylidene fluoride.
- Membrane size: wide range of pore size (MWCO: M.wt. cut-off). The main feature of this membrane is that it is porous.

Regenerated cellulose (Cellu·Sep®):

- is derived from cotton:
- has a symmetric pore structure allows small molecules to migrate in either direction.

Cellophane:

- is most commonly use dialysis material.
- is thin, transparent sheet made of regenerated cellulose.
- contains traces of sulpher compounds, metal ions and some enzymes.

- Dialysis is best carried out with freshly prepared tubing
  (wet is very susceptible to attack by micro-organisms).

- For storing:
  the tubing is best kept with a trace of benzoic acid in the solution

Dialysis membrane preparation:

- Boil the bag for 30 min in **alkaline EDTA**

  (activation of membrane and to make the dialysis sac soft)

- After boiling, the tubing is washed with distilled water and soaked in water.

Lab practice:

Aim:

- Separation of maltose from mixture solution using dialysis process.

- Our sample: (solution of starch, salivary amylase and maltose)
- dialysis bag: cellophane sealed with string by knotting.
- Solvent: distilled water.

Principle:

- Starch consists of:
  - Amylose: (M.wt 50,000 g)
  - Amylopectin: (M.wt 1000,000 g)

neither of them will pass through dialysis membrane

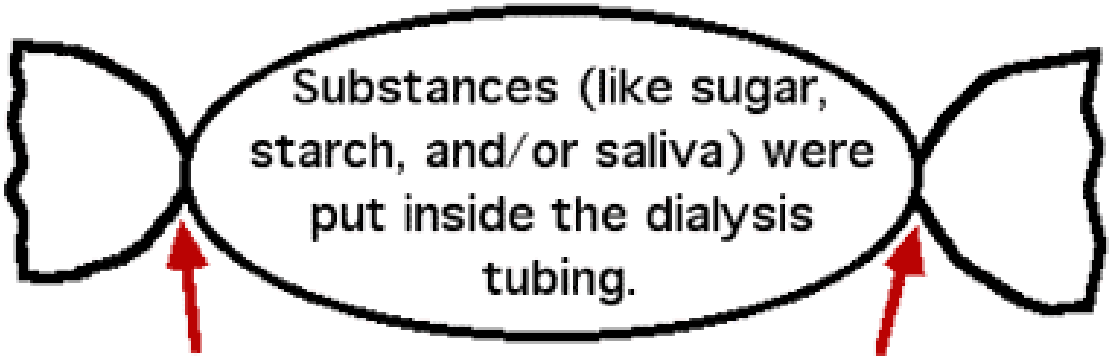
Starch —Salivary amylase→ Maltose (M.wt 360 g)

(Able to diffuses out into the dialyzate)

- Maltose (smaller than membrane pores) → moves freely across out of the membrane into the solvent

- Starch (Larger molecule) → is retained inside the dialysis bag

The dialysis tubing is sitting in a container of distilled water.



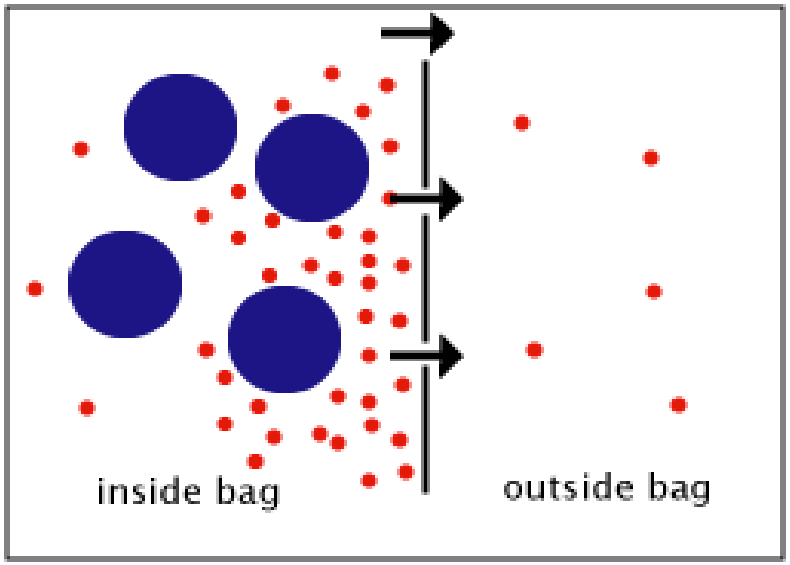
Dialysis tubing was tied shut, so that molecules can only escape through the pores in the membrane.

Dialysis process:

- the volume of the solvent outside the bag is greater than inside, over time, most of maltose will leave the bag (Osmosis).

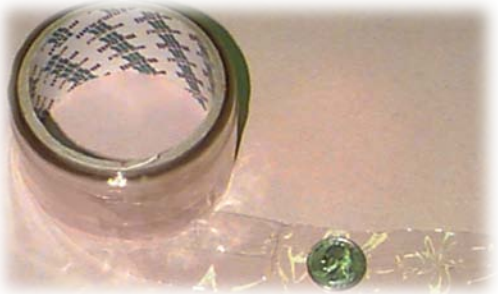
- The passage of maltose into or out of the bag is in the direction of decreasing concentration, therefore displaying diffusion.

- Equilibrium is an achieved when the concentration of maltose equal inside and outside of the bag.



Chemicals & other material:

- Salivary amylase (1 ml saliva diluted to 5 ml with distilled water)
- Iodine solution (5 mmol/L in 30 g/L Potassium iodide)
- Soluble starch (20 g/L)
- Sodium chloride solution (1g/L) buffered with 0.02 mol/L Sodium phosphate pH 6.8).
- Fehling’s solution.

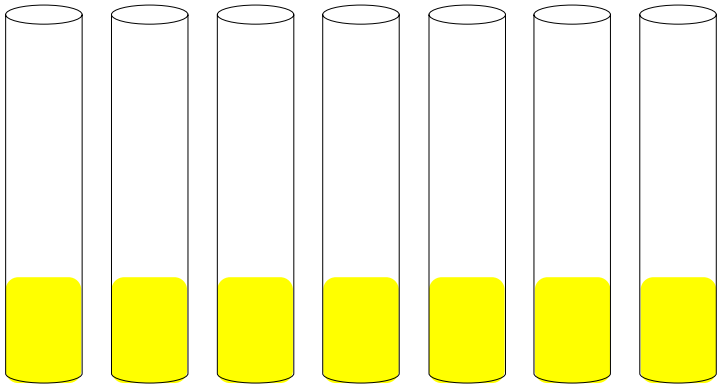


Procedure:

1- Label 14 small test tubes as the following:

add 10 drops of iodine solution

1S 2S 3S 4S 5S 6S 7S

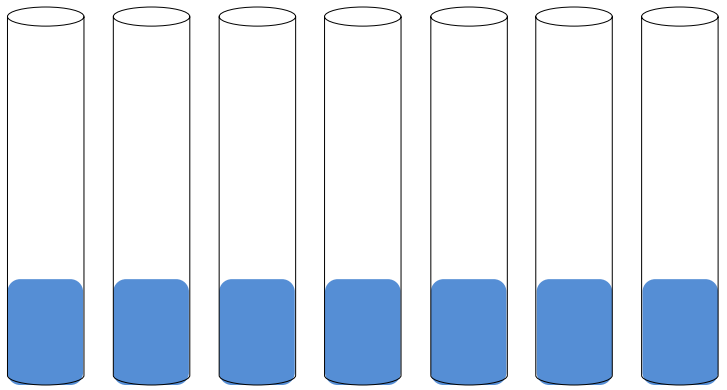


(Test for starch).

Starch + iodine ⟶ (Blue color).

1ml of Fehling's

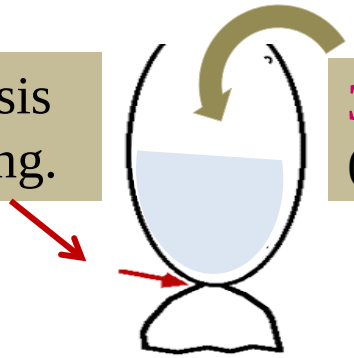
1M 2M 3M 4M 5M 6M 7M



(Test for maltose).

Maltose + Fehling's $\xrightarrow[5\text{ min}]{\text{Heating}}$ Red ppt

2- Seal one end of the dialysis bag (the bottom) with a string.

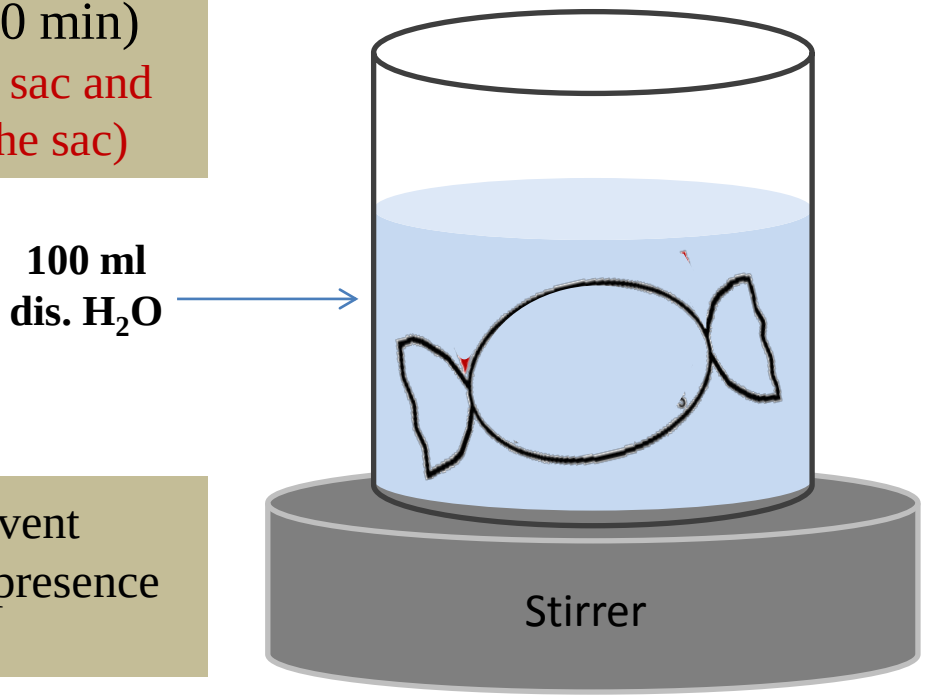


3- Fill the sac with the reaction mixture:
(2.5 ml starch, 0.5 ml buffer, 0.5 ml amylase)

4- Quickly (at 0 min) pipette 1.5 ml of reaction mixture (0.5 ml to tube 1S) and (1 ml to tube 1M)

5- Immerse the dialysis bag in beaker contain 100 ml dis. H_2O.

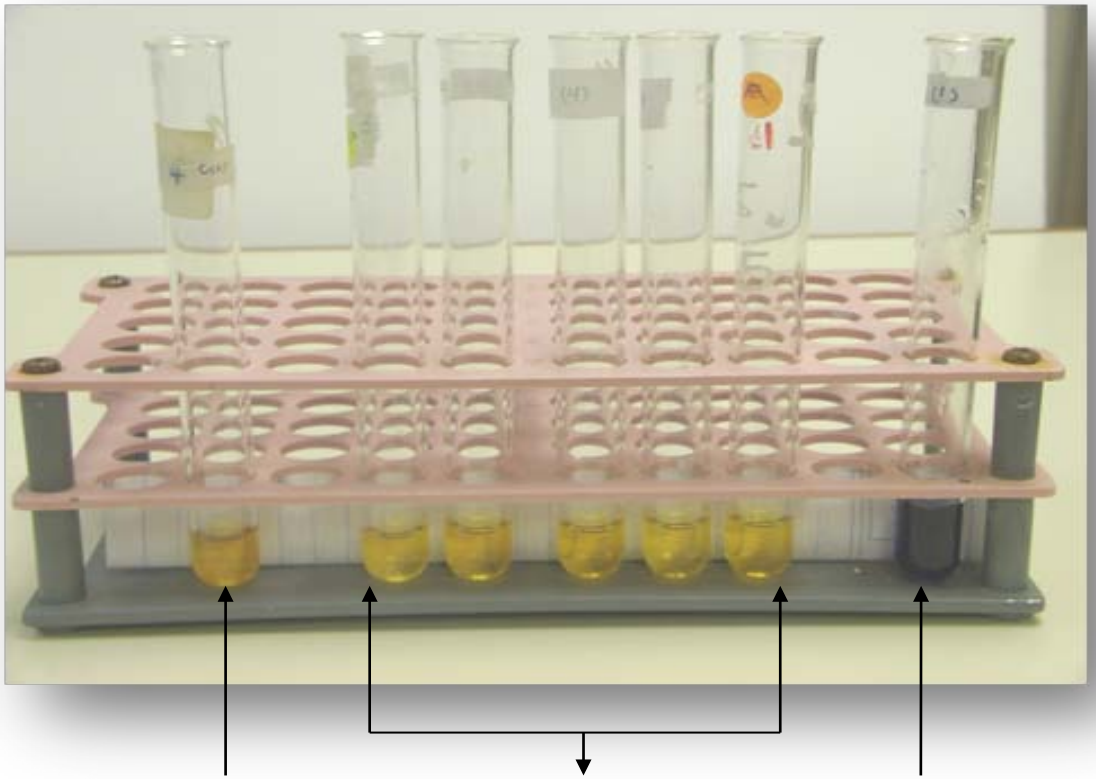
6- Place the beaker on a stir plate (stir 90 min)
(Stirring help in entry of water inside the sac and small molecules to getting out of out of the sac)



7- every 15 min, pipette 1.5 ml from the solvent (outside) and examine each fraction for the presence of starch or maltose.

8- At the end of experiment (90 min):
cut the dialysis sac carefully pipette 1.5 ml from solution inside, examine them for starch and maltose.

Test for starch: Change of the color of iodine during separation

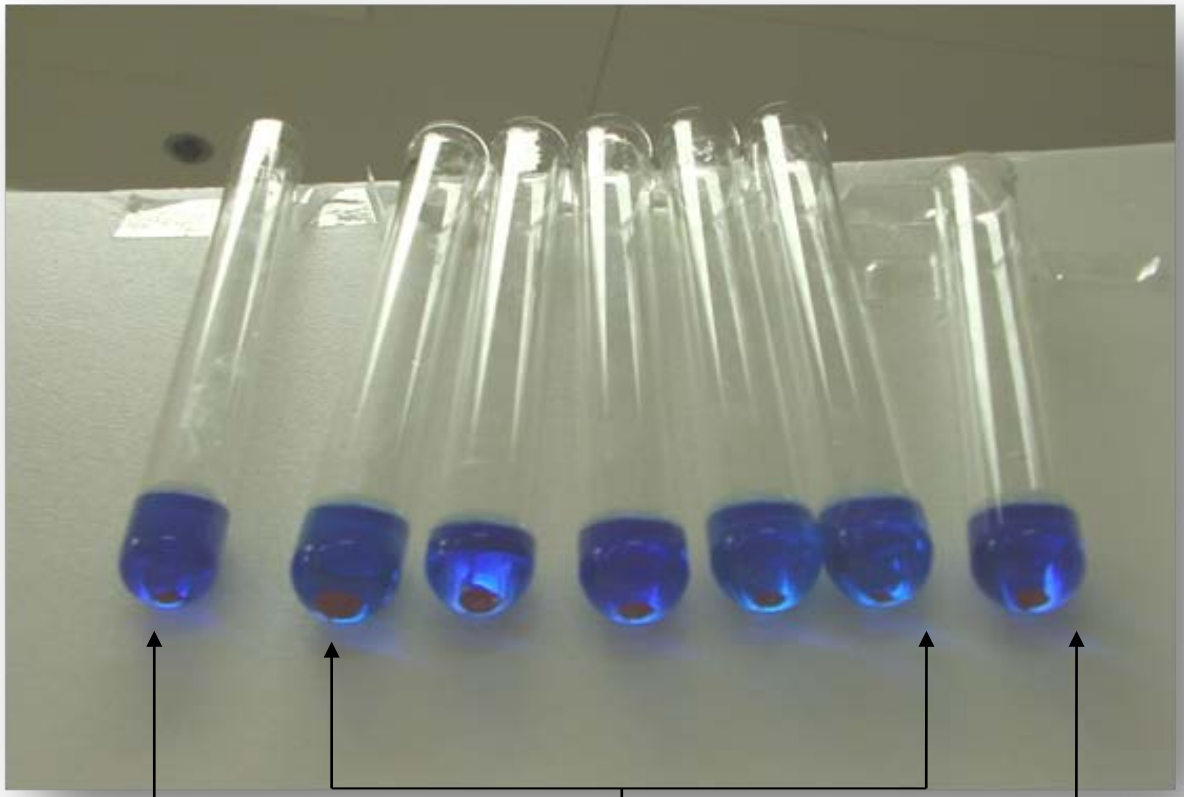


75 min
(inside)
yellow color
7 S

15 to 75 min
(outside)
yellow color
2S- 6S

0 min
(inside)
blue color
1S

Test for maltose: Formation of red precipitate during the separation



75 min (inside)
red ppt
7 M

15 to 75 min (outside)
Red ppt
2 M to 6 M

0 min (inside)
red ppt
1M

Result sheet

- Make a table of time intervals (min), and observe result for each fraction with iodine and Fehling's tests.

- Note observation in table for Fehling's test as follows:

  (+) for a few ppt,

  (++) for more ppt,

  (+++) for much more ppt and so on.

Tabel : 1

Time (min)	Inside tubing		Comment
	I_2 test	Fehling's test	
0			

Tabel : 2

Time (min)	Outside tubing		Comment
	I_2 test	Fehling's test	
15			
30			
45			
60			
75			
90			

Tabel : 3

Time (min)	Inside tubing		Comment	Outside tubing		Comment
	I_2 test	Fehling's test		I_2 test	Fehling's test	
90						