Damietta University
Faculty of Science
Chemistry Department

Final Sheet of Chromatography (404 c) for "4th year Biochemistry Students"

Student Name: ***Student No.:***

1) Answer the following questions either true or false with correction:

1. The R_f of valine is smaller than glutamic acid. ( )
2. Ammonium persulfate is the source of free radicles in photochemical polymerization of PAGE ( )
3. Repulsion forces between the like charged groups cause the protein particles to aggregate ( )

2)Write the role of the following in SDS-PAGE:

1. Glycerol.
2. Brilliant blue dye.
3. SDS

3)Write the full name of :

1. SDS- PAGE
2. TEMED

4) In what direction will pepsin migrate in an electric field at these pH =1, pH =2 and pH =7? (PI= 2).

5) One directional filter paper chromatography of leucine, alanine, lysine and tryptophan was carried out in a solvent system containing n-butanol, water and acetic acid. Predict the relative mobilities of the amino acids in the system described, and explain why?

6) Proteins are large molecules found in all living organisms, serve a variety of functions in metabolism, such as catalysis, transport, storage, control of growth and immune protection. Every amino acid has an amino group, a carboxyl and a distinctive side chain.

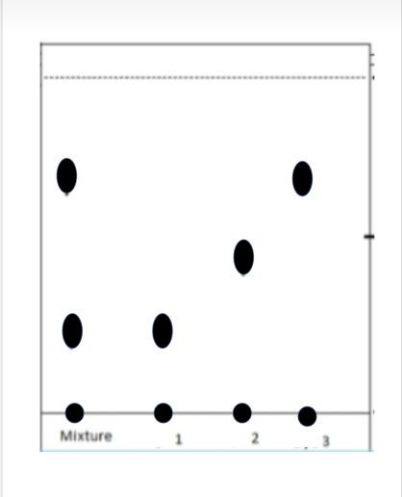
- The three standard amino acids that you will separate by T.L.C are **1, 2** and **3**
- Unknown sample number is mixture.

1) Determine the R_f values for all spots seen on the amino acid chromatogram

2) Identify the amino acid (s) contained in the unknown solution that you tested

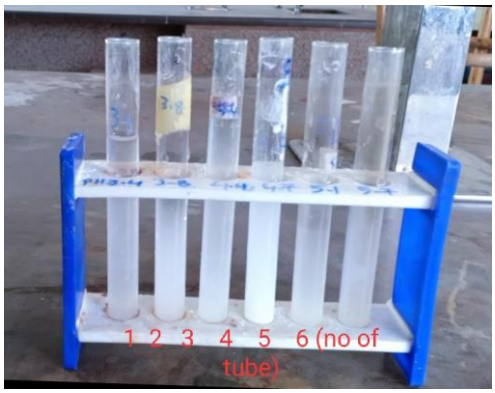
then **comment on your results.**

3) What are advantages of using T.L.C. over Paper chromatography?

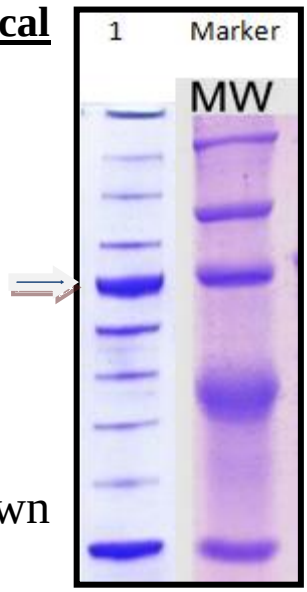


7) You are provided with dialysis experiment; please show the system is true or false? Then comment.

8) You are provided with iso-electric point of protein (albumin) experiment; please indicate its iso-electric point? Then comment.



9) **<u>You are provided with SDS-PAGE vertical electrophoresis.</u>**



<u>Lane 1</u> is a mixture of proteins separated.

<u>Lane 2</u> is a marker of proteins with molecular weights

(180, 60,520, 320, 460 KDa)

Determine the molecular weight of the unknown sample with the arrow, then comment.