Ahmedabad University

School of Arts and Sciences

BIO209 Introductory Biochemistry

Mid Semester Examination 2023 (Monsoon Semester)

Total Marks = 50 **Time – 2 hours**

Date: 18-09-2023 (Exam date)
No. of printed pages: 5 (Pages of question paper)

- **Open book exam- Use of physical copy of coursepack is permitted.**
- **Use of scientific calculator is permitted.**
- **Use of electronic devices such as mobile phones, laptops etc. is prohibited.**

1. The bicarbonate buffer system is important in maintaining a constant H_3O^+ concentration in the blood. The system is a process we could consider as two chemical reactions:
   Reaction 1: CO_2 (aq) + H_2O (1) $\rightarrow H_2CO_3$(aq)

   Reaction 2: H_2CO_3(aq) + H_2O(l) $\rightarrow HCO_3^-$(aq) + H_3O^+(aq)

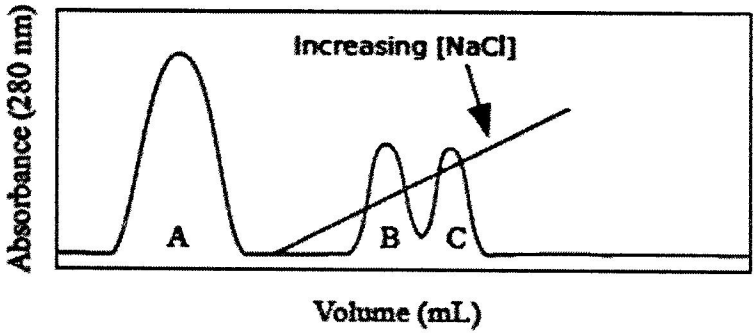
   Consider what would happen after additional H_3O^+ enters our blood from excessive exercise or a meal too high in acidic foods.
   a) Which direction should each of these equilibria shift? Describe the resulting change in concentration of each aqueous or gas-phase species in these equations. (2.5 marks)
   b) How does breathing faster and more deeply help to remove excess H_3O^+ from the bloodstream? (2.5 marks)

2. The following questions pertain to the bicarbonate buffer system in the blood plasma:
   a) What is the HCO_3^-/CO_2 ratio and the concentration of each buffer component present at pH 7.4? (2 marks)
   b) What would the pH be if 10^{-2} M H^+ is added under conditions where the increased [CO_2] cannot be released? (1.5 marks)
   c) What would the pH be if 10^{-2} M H^+ is added and the excess CO_2 eliminated? (Thus, the original [CO2] is kept) (1.5 marks)

   NOTE: use the following equation:
   - $pH = 6.1 + \log_{10}[HCO_3^-]/[CO_2]$.

- The total pool of $HCO_3^- + CO_2$, in the blood plasma is $2.52\ 10^{-2}$ M.

3. a) Consider the following cation exchange chromatogram run at pH 6.0. A mixture of proteins was applied to the column in a low [NaCl] buffer. Proteins retained by the cation exchange column were eluted by gradually increasing the NaCl concentration within the buffer. What can be concluded regarding the pI and relative charge of the protein(s) in each chromatogram peak? (3 marks)



b) A mixture of the following dipeptides was applied to a cation exchange column.

Ala-Cys His-Asp Tyr-Glu Leu-Arg Val-Lys

i. Which dipeptide(s) would bind at pH 8.0 (1 mark)
ii. Would the result differ at pH 4.5? Explain your reasoning. (1 mark)

4. For the purification table below, calculate the specific activity, % yield, and fold purification for the empty cells. (2 marks)

Step	Total Protein (mg)	Mb (μ mol)	Specific Activity (μ mol Mb/mg total Protein)	% Yield	Fold Purification
1. Crude extract	1550	0.75		100	1
2. DEAE-cellulose chromatography	550	0.35			
3. Affinity chromatography	5.0	0.28			

a) Which step-DEAE or affinity chromatography-causes the greatest loss of Mb? (1 mark)
b) Which step causes the greater purification of Mb? (1 mark)
c) If you wanted to use only one purification step, which technique would you choose? (1 mark)

5. You are a top-notch biochemist who has isolated a polypeptide from deadly spider venom. Unfortunately, your lab is poor, and you cannot afford an amino acid sequencer. However, you are capable of going "old-school" and you obtain the following data: (1) Amino acid composition: $(Lys)_2$, Gly, Met, Phe, His, Ser, Glu;

(2)Treatment with chymotrypsin yielded a dipeptide and a hexapeptide; (3)Treatment with CNBr had no effect; (4) Treatment with trypsin yielded a tripeptide, a tetrapeptide, and free Met; (5) Treatment with dabsyl chloride yields dabsyl-Ser; (6) Treatment with S. protease yielded a pentapeptide and tripeptide with no charge at pH 5. What is the sequence of the polypeptide? You need to clearly explain the logic of how you arrived at the sequence. (5 marks)

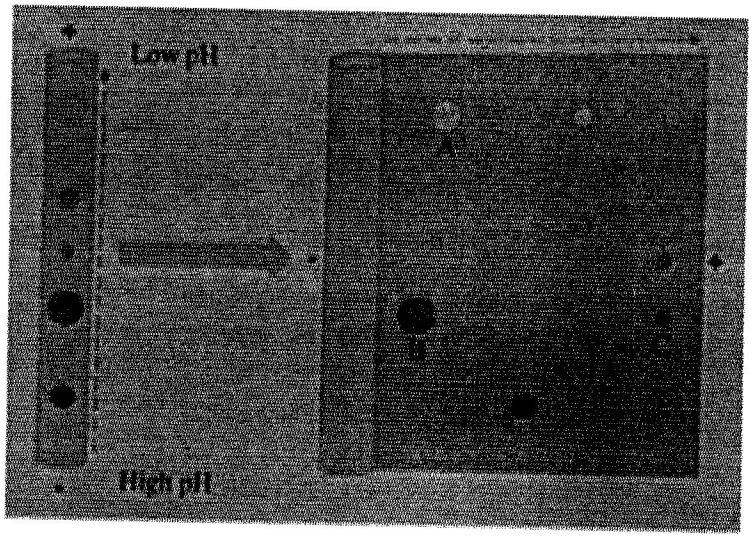
6. a) A protein has a molecular weight of 200 kD. On treatment with urea, two bands of 100kD and 50kD are obtained in SDS PAGE. After treatment with 2-mercaptoethanol, three bands of 40, 60 kD and 50kD are obtained. What information about the quaternary structure of the protein can be obtained? (2 marks)
b) Consider the following multiple sequence alignment of proteins isolated from various ghoulish abominations:

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Vampire 1	A	D	T	L	P	L	I	W	R	S	G	Q	M	R	G	T	N	L	D	P	L	E	Q	G	K
Vampire 2	F	N	G	L	V	L	L	W	G	T	G	Q	M	R	G	T	N	L	D	D	K	L	L	G	P
Vampire 3	S	R	S	L	V	L	I	W	F	S	G	Q	M	R	G	T	N	L	D	Q	Y	E	Q	R	N
Zombie 1	S	A	P	L	A	L	I	W	S	N	A	C	I	R	G	T	N	L	D	G	S	A	Q	P	T
Zombie 2	F	D	I	L	A	L	L	W	P	D	A	C	I	R	G	T	N	L	D	A	G	Q	L	D	F
Zombie 3	T	G	G	L	A	L	I	W	A	S	A	C	I	R	G	T	N	L	D	D	Y	D	L	F	A

i. Give four examples of amino acids that are rigorously conserved in the enzymes throughout the alignment (give the amino acid and its numeric position). (2 marks)
ii. Give two examples of positions where the amino acid group is functionally conserved. (1 mark)

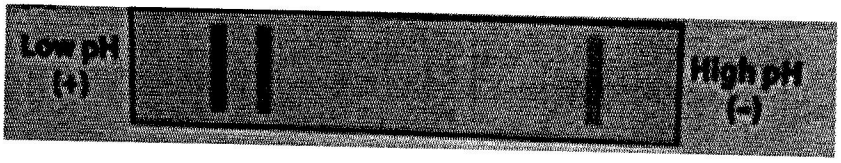
7. You need a buffer with a pH of 3.30 for a certain experiment. You decide to prepare this buffer using citric acid, $H_3C_6H_5O_7$, (K_a= 7.4 x 10^{-4}) and monosodium citrate, $NaH_2C_6H_5O_7$.
a) If the concentration of citric acid in this buffer is 0.620 M, what should be the concentration of the monosodium citrate?
b) How many grams of citric acid (192.12 g/mol) and monosodium citrate (214.10 g/mol) should be used to make 75.0 mL of this buffer?

8. a) The results of a 2-D electrophoresis experiment are shown below. Isoelectric focusing was performed, followed by SDS-PAGE. From these results, explain the differences in proteins A, B, and C. (3 marks)

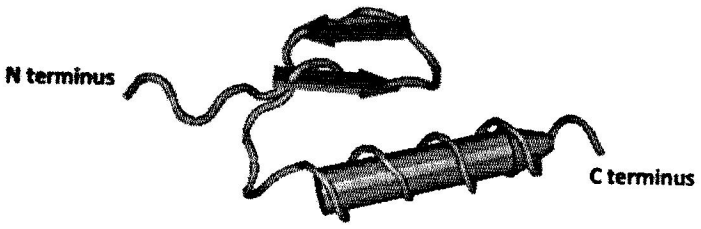


b) In an isoelectric focusing experiment, identify the peptides in the picture of the pH strip. (This is not a multiple-choice question, identify each band in the picture as i, ii, iii or iv). (2 marks)

i. R-L-V-A
ii. E-E-E-E
iii. D-V-L-D
iv. K-L-K-H



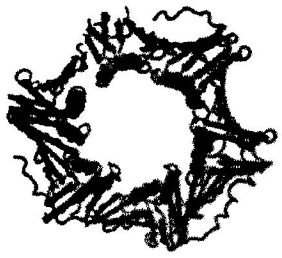
9. a) Below is a model of the 3D structure of a small protein. (This is a zinc finger domain, which binds to DNA, a type of protein structure found in many transcription factors.)



i. Describe its tertiary structure. (1 mark)
ii. On the image, label distinct elements of secondary structure such as beta sheets, alpha helices and beta turns. (2 marks)
iii. Circle the end of the protein which was translated first. (1 mark)

b) The protein represented below is composed of 3 identical subunits (one pink, one blue, one brown). (This is a protein that encircles the DNA and assists in DNA replication.) How could you describe this protein's quaternary structure? (1 mark)

i. Monomer
ii. Dimer
iii. Trimer
iv. Quatramer
v. Pentamer
vi. Hexamer



10. A student titrated a 400 mL solution of an unknown amino acid with 1.0 M sodium hydroxide and drew the below titration curve. From this titration curve they found that the amino acid had three functional groups with the pKa of the functional groups displayed on the graph. To do this titration they added in a total of 75 mL of 1.0 M sodium hydroxide. They also calculated that they added a total of 21 mL of sodium hydroxide to get to pK1 (indicated by arrow) and a total of 37 mL of sodium hydroxide to get to pK2(indicated by arrow). What was the initial concentration of the amino acid solution in millimolar (mM)? (5 marks)

