UNIVERSITY OF RUHUNA
BACHELOR OF SCIENCE SPECIAL DEGREELEVEL I (Semester I)
EXAMINATIONS – JULY/AUGUST-2017

SUBJECT: CHEMISTRY
COURSE UNIT: CHE 4342 (Advanced Biochemistry) **TIME: Two (02)** hours

Answer **all** questions

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01. Answer **both** parts.

(a) The Michaelis-Menten model proposed, which is the simplest one that accounts for the kinetic properties of many enzymes, is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

(i) What is the equilibrium dissociation constant for ES complex (K_d)?
(ii) Under what condition K_m can be taken as a measure of the dissociation constant (K_d) for the ES?
(iii) Starting from Michealis –Menton equation show that at low substrate concentration, the velocity of the reaction is proportional to the substrate concentration and the enzyme concentration with the proportionality constant which is equal to specificity constant
(iv) A, B and C are three different enzymes. Using the information given below, determine which enzyme(s) is (are) closest to the diffusion limit in its (their) catalytic efficiency? Justify your answer.

Enzyme	K_m	k_{cat} (s^{-1})
A	0.3 mM	500
B	1.0 μM	2
C	20 nM	85

[50 marks]

(b) The conversion of dissolved carbon dioxide in blood to HCO_3^- and H_3O^+ is catalyzed by the enzyme carbonic anhydrase.

$$CO_2 + 2\,H_2O \xrightarrow{\textit{carbonic anhydrase}} HCO_3^- + H_3O^+$$

This reaction obeys Michaelis-Menten kinetics with all elementary steps as follows:

$$CO_2 + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E.CO_2$$

$$E.CO_2 + 2\,H_2O \xrightarrow{k_2'} HCO_3^- + H_3O^+ + E$$

Using the above elementary steps for the reaction mechanism:

(i) Write an expression for rate of the reaction d[P]/dt where P= HCO_3^-

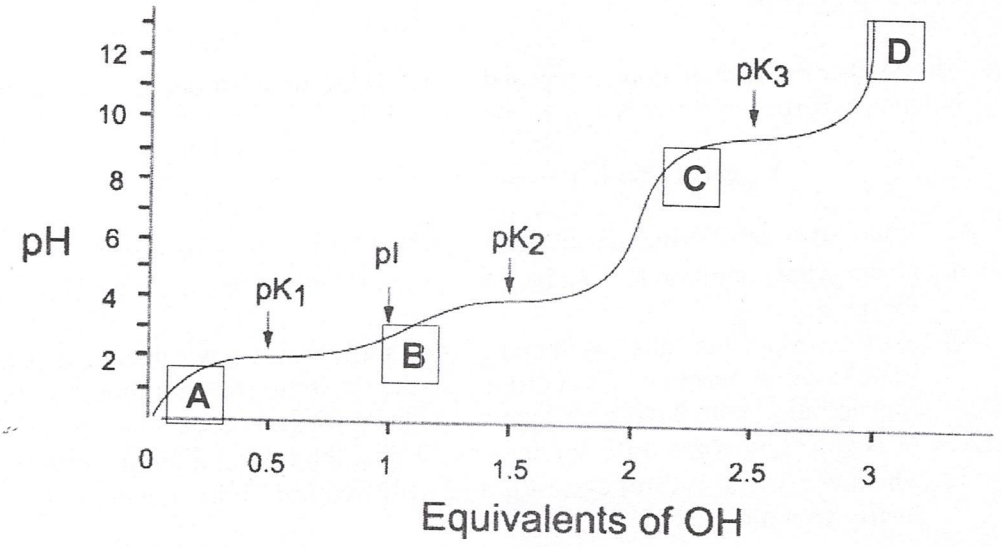
[10 marks]

(ii) Derive an expression for the initial rate in terms of the total enzyme concentration [E_0], the concentration of carbon dioxide [CO_2] and the constants K_m and k_2 ($k_2 = k_2'[H_2O]^2$).

[40 marks]

02. Answer **all** parts.

(a) The titration curve of Aspartic acid (Asp), which has three dissociable hydrogens, is given below. The values of pK_1, pK_2 and pK_3 of Asp are 2.1, 3.9, and 9.8 respectively and pK_2 is of the side chain of Asp.



(i) Draw the structures of predominant forms of Asp at the points **A**, **B**, **C**, and **D** as shown in the above graph.

[12 marks]

(ii) Give the form of equilibrium occurs at pK_2 and pK_3 of Asp

[06 marks]

(iii) Using the given pK values determine the isoelectric point of Asp.

[06 marks]

(b) The isoelectric point (p*I*) is used as a tool in the separation of mixtures of peptides.

(i) Give three specific properties of peptides at their isoelectric point.

[06 marks]

(ii) Describe briefly how the isoelectric point can be used to separate a mixture of two peptides.

[12 marks]

(c) Amino acids can be classified as nonpolar, polar, and polar-charged. Briefly describe the location of above types of amino acids found in a three-dimensional structure of:

(i) water soluble globular protein.

[12 marks]

(ii) water insoluble membrane protein.

[06 marks]

(d) The structures of proteins are mainly stabilized by large number of weak non-covalent interactions which are of four types.

(i) What are the four types of interactions that exist in a protein?

[05 marks]

(ii) Three types of helices can be identified in proteins depending on the type of hydrogen bonding. State these helical types and give their specific features, such as type of hydrogen bonding, residues per turn, and pitch of the helix.

[15 marks]

(e) Give a short account on the Ramachandran plot by explaining specific regions and giving the definitions of *phi* and *psi* angles.

[20 marks]

03. Answer **all** parts.

(a) What is the major challenge faced in the synthesis of peptides? Describe briefly the steps that can be taken to overcome this challenge.

[20 marks]

(b) An analysis of the polypeptide, **P**, isolated from the bacteria *Chretientus negativii*, gave the following results (i) to (iv): Using these results, giving reasons, determine the amino acid sequence of the polypeptide **P**.

(i) acid hydrolysis gave: Ala_2 , Val, Lys_2 , Arg, Gly, Asp, Met, Pro, Trp.
(ii) carboxypeptidase gave Lys and fluorodinitrobenzene (FDNB) gave Val.
(iii) cyanogen bromide treatment generated two polypeptides, A and B:
peptide A: (Gly, Arg, Trp, Asp, Lys, Ala); which yielded Gly with FDNB.
peptide B: (Ala , Lys, Val, Met, Pro)

(iv) trypsine digestion: yielded three peptides, **C**, **D**, and **E**:
peptide C: (Lys, Trp, Ala); which yielded Trp with FDNB.
peptide D: (Ala , Val, Lys, Pro)
peptide E: (Met, Asp, Gly, Arg); which yielded Met with FDNB.

[30 marks]

(c) Answer the following questions which are based on the chemistry of nucleic acids.

(i) Give the trivial names of the following compounds:
(I) 6-amino purine. (II) 2-amino-6-oxy purine.
(II) 2,4-dioxy pyrimidine. (IV) 2-oxy-4-amino pyrimidine.

[10 marks]

(ii) *Pseudouridine* (ψU), an unusual nucleoside, is present as a constituent of the transfer RNAs. Its β-glycosidic linkage occurs between the C1 of ribose and C5 of uracil. The uracil moiety at physiological pH has one oxy group in the *keto* form and the other in the *enol* form. Draw the structures of:

(I) uridine (1-ribosyl uracil)
(II) pesudouridine (5-ribosyl uracil)

[20 marks]

(iii) 5-Bromouracil (BrU) exists in three tautomeric forms that have different base pairing properties. It is a common mutagen used to treat certain types of skin cancer. The mutagenicity arises because of the bromine atom increases the population of the enol form of 5-bromouracil which mimics cytosine and hence forms hydrogen bonding to guanosine instead of adenosine.

(I) Draw the structures of the 2 possible enol tautomers for 5-BrU.
(II) Illustrate how the keto tautomer of 5-BrU forms a hydrogen bond pair with adenine.
(III) Illustrate how one of the tautomers in part (I) forms complementary H-bonds with guanine.

[20 marks]

04. Answer **all** parts.

(a) (i) Reversible phosphorylation is a control mechanism used throughout metabolism. What are the general names of the enzymes involved in reversible phosphorylation and what general reactions do they catalyze?

(ii) Provide a reasonable systematic name for an enzyme that catalyzes the following reaction:

fructose + ATP —> fructose-1 phosphate + ADP

[20 marks]

(b) (i) Draw the complete structures and write the names of the reactants and products for the reactions catalyzed by the following three enzymes from the glycolysis pathway:

(I) phosphoglucose isomerase.
(II) aldolase.
(III) pyruvate kinase.

[30 marks]

(ii) Give the mechanism of phosphoglucose isomerase catalyzed reaction.

[30 marks]

(c) Arachidonic acid is a C 20 fatty acid with double bonds at carbons 5, 8, 11, 14. When one mole of arachidonic acid is oxidized to CO_2 and H_2O by β-oxidation and the Krebs cycle, how many moles of NADH, $FADH_2$, ATP, and CO_2 are produced?

[20 marks]

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