FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY (APK)

MODULE: BIC2A01: BIOCHEMICAL TECHNIQUES AND ENZYMOLOGY

SPECIAL SSA EXAMINATION

EXAMINER 1 (Section A)	**Prof R Motadi**
EXAMINER 2 (Section B)	**Dr M Choene**
INTERNAL MODERATORS	Dr MBC Simelane
TIME 3 HOURS	MARKS 100

NUMBER OF PAGES: 7 PAGES

INSTRUCTIONS: **ANSWER ALL THE QUESTIONS.**
DO NOT USE RED INK.
PLEASE HAND IN YOUR QUESTION PAPER WITH YOUR EXAM BOOK.

REQUIREMENTS: **ANSWER ALL THE QUESTIONS IN YOUR EXAM BOOKS PROVIDED**
ANSWER SECTION A (TECHNIQUES) AND SECTION B (ENZYMOLOGY) IN TWO SEPARATE EXAM BOOKS

Additional Information:

pKa Values

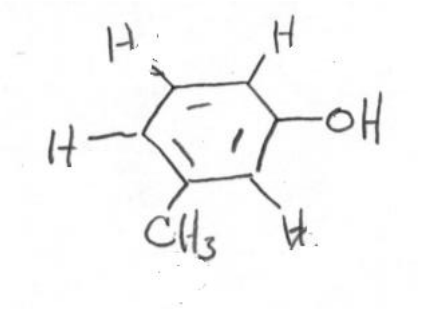
Carboxyl group	: 2.2
Amino group	: 9.4
Side Chains	: Tyr (10.46); Cys (8.37); Lys (10.54); Arg (12.48); His (6.04); Asp (3.90); Glu (4.07)

SECTION A [50]

QUESTION 1 [15]

As a Drug discovery technician at a pharmaceutical company that produce Insulin specifically raised from pig. You are asked to purify insulin for pharmaceutical purposes that will yield 99.5% purity. Which technique will you chose? Discuss using the principle of the technique the procedure you will follow.

QUESTION 2 [10]



a) Provide the C-NMR and H-NMR spectrum of the compound having a molecular formula of C7H8O that is consistent with the following above structure. [8]
b) State the advantages of X-ray Crystallogram over NMR. [2]

QUESTION 3 [10]

a) Differentiate between Paper and Thin layer chromatography. [3]
b) If you are given 4 amino acids (Ser -weak polar, Met Strong polar, phy neutral and pro-semi-neutral). If your mobile phase is polar draw the migration of the amino acid. [5]
c) What is Rf value and what does it imply or indicate? [2]

QUESTION 4 [10]

Draw all possible spectrum of 1-propanol from Mass spectrophotometry.

QUESTION 5 [5]

a) Give a brief explanation of how the tertiary structure of a protein is formed through folding.

SECTION B [50]

QUESTION 1 [10]

Multiple choice

1. The following is an example of a:

a) ping pong bi-substrate reaction

b) group transfer reaction

c) secondary reaction

d) random bi-substrate reaction

e) ordered bi-substrate reaction

2. Coenzymes:

a) The non-protein part of enzymes

b) Usually vitamin derivatives

c) Termed prosthetic group if bonded tight to their enzymes

d) All of the above are correct

3. In an enzyme catalyzed reaction, __ provides information on __ and __ provides information on __.

a) K_M, chemical step, V_{MAX}, substrate binding.

b) K_D, substrate binding, k_{CAT}, chemical step.

c) K_M, substrate binding, V_{MAX}, chemical step.

d) k_{CAT}, substrate V_{MAX}, chemical step.

4. Which of the following is true of enzymes?

I. They increase the rate of reaction by stabilizing the transition state

II. They raise activation energy to shift the equilibrium to favor the products

III. They lower activation energy by altering the products of a reaction

a) I only

b) I & III

c) I & II

d) III only

5. Which of the following statements is false with respect to an enzyme's ability to catalyze a reaction?

a) An enzyme provides a reaction surface and a suitable environment for the reaction to take place

b) An enzyme binds reactants such that they are positioned correctly and can attain their transition-state configurations

c) An enzyme allows the reaction to go through a less stable transition state than would normally be the case

d) An enzyme can weaken bonds in reactants through the binding process

6. Which of the following statements is true of competitive inhibitors?

a) Km is unchanged

b) Vmax is unchanged

c) Cannot be overcome by increasing substrate concentration

d) Binds to a site other than the active site

7. For serine proteases, the first step of the mechanism in which the serine side chain attacks the scissile peptide bond is best described as an example of….

a) general acid catalysis

b) electrophilic catalysis

c) electrostatic catalysis

d) covalent catalysis

8. When the rate of a reaction does not depend on the concentration of reactant molecules, the order of reaction is:

a) 1

b) Zero

c) 2

d) 3

9. Enzyme activity can be regulated by the following mechanism:

a) allosteric control

b) covalent modification

c) feedback inhibition

d) all of the above

10. To overcome the difficulties of accurately determining the Km with a Michaelis-Menten plot, data can be reordered into a _____

a) Direct linear

b) Scatchard

c) Hill

d) Direct linear and Hill

QUESTION 2 [13]

1. The activity of enzymes involved in regulation/rate-limiting steps and other essential processes can be controlled in various ways. Discuss these mechanisms by using the specific given examples to illustrate each of your answers:

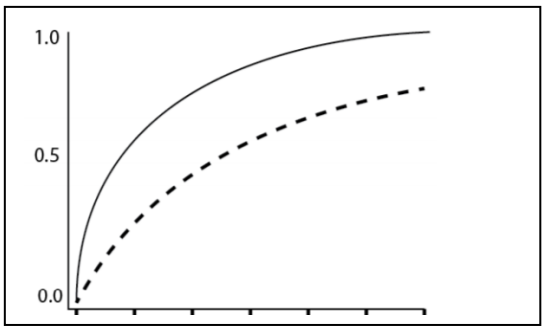
   a) Inactive precursors/zymogens, e.g. trypsin [3]

   b) Covalent modification and allosteric regulation, e.g. glycogen phosphorylase [10]

QUESTION 3 [21]

1. The lineweaver-Burk plot is a linearization of the Michaelis Menten equation. Derive the Lineweaver-Burk Relationship starting from Michaelis Menten equation. [4]

2. You are studying two enzymes X (solid line) and Y (dotted line) that utilize the same substrate and collect the following data:



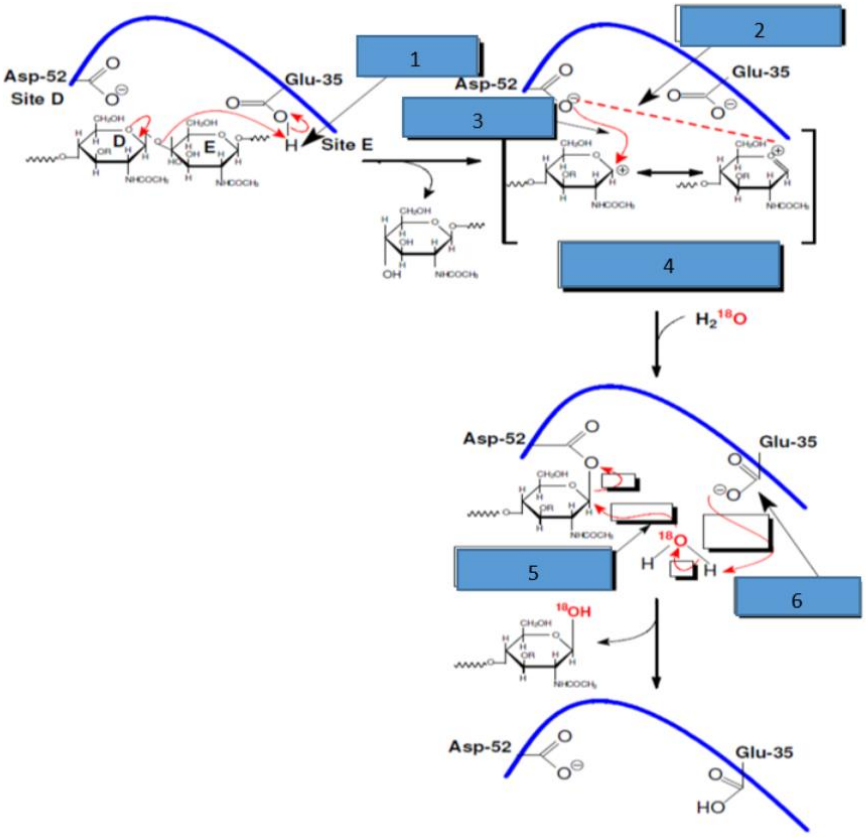
   a) Estimate the Km of enzyme X [1]
   b) Which enzyme has a higher affinity for the enzyme? [2]

3. The following kinetic data are recorded for equal amounts of a preparation of an enzyme in the absence and presence of an inhibitor:

Substrate conc. [S] uM	**No inhibitor Vo (uM/s)**	**Inhibitor Vo (uM/s)**
7	0.6	0.4
11	0.83	0.6
17	0.94	0.69
22	0.97	0.75
28	1.05	0.9

a) Draw the Lineweaver-Burke plots and determine the Vmax and Km for both graphs. [10]
b) What type of inhibition can be observed? Motivate your answer. [2]
c) Where on the enzyme molecule, relative to the active site, does the inhibitor likely bind? Explain. [1]
d) What effect would increasing substrate concentration have on the above inhibition, if any? [1]

QUESTION 4 **[6]**



a) Complete the boxes numbered 1-6 based on lysozyme mechanism. [6]