FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY (APK)

MODULE: BIC2A01: BIOCHEMICAL TECHNIQUES AND ENZYMOLOGY

JULY SUPPLEMENTARY EXAMINATION (SSA)

DATE: July 2020 **TIME: 15:00-18:00**

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: 6 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 B [50]

Question 1 **[15]**

Multiple choice questions:

1. For gel filtration chromatography of proteins, which of the following is true?
a) Large or elongated proteins enter the pores of the beads.
b) First, the small proteins elute from the bottom of the column
c) Large or elongated proteins elute later from the bottom of the column.
d) None of the above answers is true.

2. Which of the following is not the application of gel filtration?
a) Purification
b) Relative molecular mass determination
c) Protein concentration
d) None of these

3. Which chromatographic technique depends on the highly specific interactions between pairs of biological materials such as enzyme-substrate?
a) Adsorption chromatography
b) Ion –exchange chromatography
c) Affinity chromatography
d) Gel-permeation chromatography

4. Reversed-phase chromatography uses a stationary phase which is …… in nature.
a) Hydrophilic
b) Hydrophobic
c) Both of above
d) None of above

5. Which is not generally used for separation of proteins, as proteins get denatured by it?
a) Ion –exchange chromatography
b) Reverse Phase Chromatography
c) Affinity chromatography
d) Gel-permeation chromatography

6. Which technique is much superior in terms of the speed, efficiency, sensitivity and ease of operation?
a) Adsorption Chromatography
b) High Performance Liquid Chromatography
c) Ion –exchange Chromatography
d) Gel-permeation Chromatography

7. Protein purification refers to the

a) Purification of proteins
b) Separation of proteins from other biomolecules
c) Separation of a particular protein from other contaminating proteins
d) all of these

8. Primary steps in protein purification includes
a) Homogenization
b) Differential centrifugation
c) All Of These
d) Solubilisation

9. The use of insulin hormone to purify its receptor is an example of
a) Ion exchange chromatography
b) Affinity chromatography
c) Gel filtration chromatography
d) Ligand mediated chromatography

10. In anion exchange chromatography,
a) the column contains negatively charged beads where positively charged proteins bind
b) the column contains positively charged beads where negatively charged proteins bind
c) the column contains both positive and negatively charged beads where proteins bind depending on their net charge
d) None of the above.

11. A combination of paper chromatography and electrophoresis involves
a) partition chromatography
b) electrical mobility of the ionic species
c) both (1) and (2)
d) none of these

12. Ion exchange chromatography is based on the
a) electrostatic attraction
b) electrical mobility of ionic species
c) adsorption chromatography
d) partition chromatography

13. In gas chromatography, the basis for separation of the components of the volatile material is the difference in
a) partition coefficients
b) conductivity
c) molecular weight
d) molarity

14. Salting out process involves:
a) precipitation of proteins using ammonium sulphate

b) precipitation of proteins using copper sulphate
c) precipitation of proteins using sodium chloride
d) none of these

15. Which of the following main component of mass spectroscopy deal with resolving the ions into their characteristic mass components according to their mass-to-charge ratio?

a) Ion Source
b) Analyzer
c) Detector System
d) Analyzer tube

QUESTION 2 **[5]**

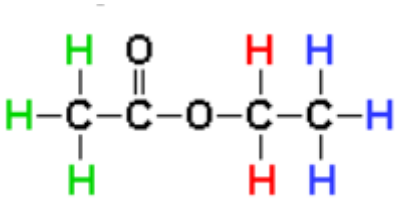
1. Assume that you are a Forensic Scientist.
   a) State the principle of gel electrophoresis. (1)
   b) Use a diagram of DNA collected from a crime scene to point out the criminal out of the 3 suspects. (4)

QUESTION 3 **[10]**

1. Write an assay on the advantages and disadvantages of HPLC in drug discovery. (10)

QUESTION 4 **[13]**

1. Given the following structure. Draw the NMR graph that would clearly represent the resonance of the structure. Only the highlighted ones. (8)



2. Draw the resonance of propanol using the ^{1}H-NMR. Show which H groups are represented on the graphs. Explain your answer (5)

QUESTION 5 **[7]**

1. Explain the differences between Affinity Chromatography and direct Elisa. (5)
2. What is the purpose of Ammonium sulphate in protein studies? (2)

SECTION B [50]

Question 1 [10]

True or False:

1. Substrate specificity is determined by chymotrypsin by the binding of the proper amino acid into a deep pocket on the enzyme.
2. The transition state of a catalyzed reaction is higher in energy than that of an uncatalyzed reaction
3. The amino acids involved in catalysis on lysozyme active site in the free enzyme are an aspartic acid with an unprotonated side chain and a glutamic acid with an protonated side chain.
4. Since proteins are limited in their ability to catalyze oxidation-reduction reactions, enzymes often employ coenzymes to assist with catalysis.
5. The enzyme "mutase" belongs to the "transferases" class of enzymes.
6. The concept of "induced fit" refers to the fact that substrate binding may induce a conformational change in the enzyme, which then brings catalytic groups into proper orientation.
7. The coenzyme of vitamin B2 (Riboflavin) is thiamine pyrophosphate
8. Preferential binding to the product is a catalytic mechanism in enzymatic catalysis
9. If the ΔG of the reaction $A \rightarrow B$ is – 40 kJ/mol, under standard conditions, the reaction will proceed spontaneously from left to right.
10. One of the enzymes involved in glycolysis, aldolase, requires Zn2+ for catalysis. Under conditions of zinc deficiency, when the enzyme may lack zinc, it would be referred to as the apoenzyme.

Question 2 [10]

1. How does the formation of the enzyme-substrate complex explain the reduction in the activation energy of chemical reactions (3)

2. How different are the curves of the graph of the variation in the speed of a reaction as function of substrate concentration and the graph of the variation in the speed of a reaction as function of temperature? (4)
3. In an enzymatic reaction, what is the effect of a substance with the same spatial conformation as the enzyme substrate? How is this type of substance recognized (3)

Question 3 [15]

1. Draw Cleland plots showing the different mechanisms by which multisubstrate enzyme reactions can occur. Give an example in each case.

Question 4 [15]

1. The following kinetic data are recorded for equal amounts of a preparation of an enzyme in the absence and presence of inhibitor 1 and inhibitor 2:

Substrate conc. [S] uM	**No inhibitor Vo (uM/s)**	**Inhibitor 1 Vo (uM/s)**	**Inhibitor 2 Vo (uM/s)**
1.2	0.22	0.21	0.2
2.1	0.29	0.26	0.24
3	0.32	0.3	0.28
6	0.4	0.36	0.32

a) Draw the Lineweaver-Burke plots and determine the Vmax and Km for the each of the three graphs. (14)
b) What type of inhibition can be observed from inhibitor 1 and inhibitor 2? Motivate your answer. (1)
