## *FACULTY OF SCIENCE*

**DEPARTMENT OF BIOCHEMISTRY (APK)**

**MODULE:** BIC2A01: BIOCHEMICAL TECHNIQUES AND ENZYMOLOGY

***JUNE EXAMINATION***

**DATE: 25 May 2019** **TIME: 08:00-11:00**

EXAMINER 1 (Section A) **Dr L Sitole**
EXAMINER 2 (Section B) **Dr M Choene**
INTERNAL MODERATORS

Dr JT James

TIME 3 HOURS MARKS 125

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**NUMBER OF PAGES: 5 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**

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**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 [60]

## Question 1 [25]

1. What is the net charge of each one of these amino acids glycine, serine, aspartic acid, glutamine and arginine at the following pH values? [10]

a) pH 2.01 b) pH 3.96 c) pH 5.68 d) 10.76

2. A mixture of lysine, glycine, alanine, isoleucine and glutamic acid are separated by ionic exchange chromatography. What is the order of elution of these amino acids if you use gradient buffer system from pH 10 to pH 2? (Explain your answer) [7]

a) With a cation exchange resin?
b) With an anion exchange resin?

3. Draw the structure of the following peptide GWYQR. Indicate the ionic form of the peptide at the following pH: [8]

a) pH 2.0 b) pH 7.0 c) pH 10.5

## QUESTION 2 [11]

4. Consider the following peptide:

ALKMPEYISTDQSNWHHR

Indicate the fragments generated after the following digestions: [11]

a) Trypsin b) Pepsin c) Protease V8 d) Cyanogen bromide

## QUESTION 3 [6]

5. A student analyses bovine serum albumin (BSA) with a denaturing polyacrylamide gel electrophoresis (SDS-PAGE). During the experiment, the student forgets to add β-mercaptoethanol to the sample. When comparing his sample to those of his classmates he realizes that the molecular mass of his BSA sample determined by SDS-PAGE is 57kDa, while all the other students (those that added β-mercaptoethanol) found a molecular mass of 68kDa. Explain the difference. [2]

6. Why do we often use ammonium sulphate precipitation in initial purification steps of proteins? [2]

7. An enzyme (MW 24kDa, pI 5.5) is contaminated with two other proteins, one with a similar molecular mass and a pI of 7.0 while the other has a molecular mass of 100kDa and a pI of 5.4. Suggest a procedure to purify the contaminated enzyme. [2]

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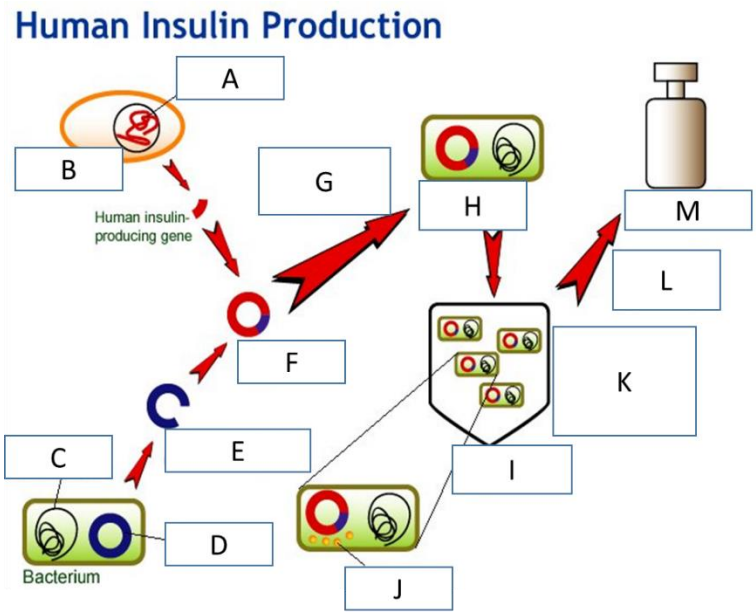
**QUESTION 4** **[18]**

8. Name two advantages of using recombinant DNA technology for protein purification [2]

9. Label the following diagram for human insulin production [13]




10. Give three components of Mass Spectrometry [3]

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## SECTION B [65]

## Question 1 [10]

1. Match each of the terms in the left-hand column with a description from the right hand column:

| | |
|---|---|
| a) Zymogen | 1. Use ATP to join two molecules together |
| b) ATCase | 2. Transfer functional groups e.g. methyl |
| c) Ligases | 3. Atomic rearrangement within a molecule |
| d) Transferases | 4. Also called a proenzyme, is an inactive precursor of an enzyme |
| e) Isoenzymes | 5. Catalyze the cleavage of C-C, C-N and C-O bonds |
| f) Metalloenzymes | 6. The more-active state of an allosteric enzyme |
| g) R state | 7. Enzymes whose metal ions are intrinsically part of the molecule |
| h) T state | 8. Enzymes with similar enzymatic activities |
| i) Isomerases | 9. The less-active state of an allosteric enzyme |
| j) Lyases | 10. Allosterically inhibited by CTP |

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## Question 2 [18]

1. Under which class of enzymes does chymotrypsin belong? [1]
2. What role do the enzymes mentioned in (1) play in the eukaryotic body? Where are the synthesized? [2]
3. The enzymes mentioned in (1) are usually synthesized as proenzymes. Why is this important? [2]
4. Which three amino acids play an important role in Chymotrypsin's catalytic mechanism? Describe in detail their role and type of catalytic mechanism they employ [12]
5. Which region on this enzyme is the binding site for the aromatic side chains of its specific substrates? [1]

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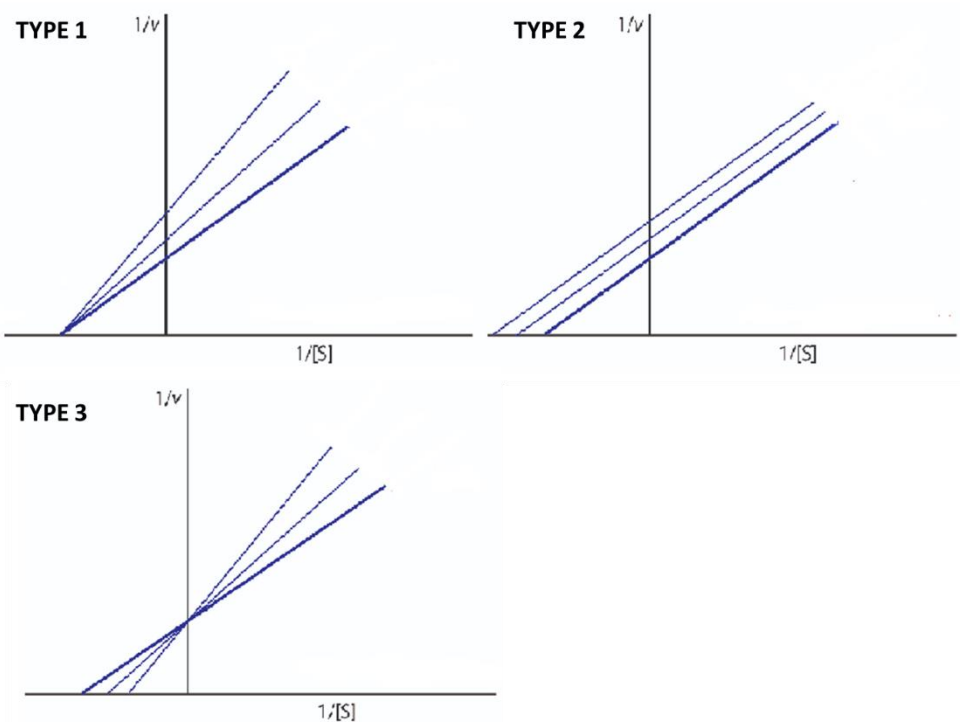
## Question 3 [18]

1. Below are three types of enzyme inhibitions.




a) Name the type of inhibition involved in each, and state the effect of the inhibitor on the measured values of Km and Vmax in the presence of the inhibitor. [9]
b) Which one of the three inhibitions can be overcome by increasing substrate concentration? [1]
c) Give the practical applications of enzyme inhibitors in health sciences and treatment of disease. [8]

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## Question 4 [7]

1. A single celled organism was recently discovered. The extracts of the organism catalyzed the hydrolysis of ATP, showing a Michaelis-Menten kinetics with a Km of $2.4 \times 10^{-5}$ M and a Vmax of 75µmoles/min.

    a) Give the Michaelis-Menten equation [1]
    b) Calculate the velocity of the enzymatic reaction at the following ATP concentrations: (i) $S = 0.8 \times 10^{-6}$ M; (ii) S = 0.05M [2]
    c) Using GTP as a substrate the kinetic parameters were a Km of $6.2 \times 10^{-3}$ M and a Vmax of 310 µmoles/min. Would you consider GTP or ATP a "better" substrate? Explain your answer. [4]

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## Question 5 [12]

1. How is an enzyme allosterically regulated? Describe the allosteric regulation of enzymes with examples. [12]

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