

**NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY**  
 FACULTY OF APPLIED SCIENCES  
 BACHELOR OF SCIENCE HONOURS DEGREE EXAMINATIONS  
 DEPARTMENT OF APPLIED BIOLOGY AND BIOCHEMISTRY  
**THEORY : INTRODUCTION TO ENZYMOLOGY AND IMMUNOLOGY SBB 2104**

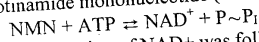
DECEMBER 2004  
 3 Hours (100 marks)

**INSTRUCTIONS**

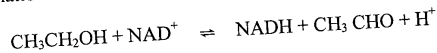
Answer **Four (4)** Questions. Each question carries 25 marks. Where a question contains subdivisions, the mark value for each subdivision is given in brackets. Illustrate your answer where appropriate with large, clearly labeled diagrams.

**SECTION A**

1. The nicotinamide mononucleotide (NMN) adnyl transferase catalyzes the reaction.



The rate of formation of NAD<sup>+</sup> was followed by coupling the above reaction with that catalyzed by alcohol dehydrogenase.



The formation of NADH was measured spectrophotometrically at 340nm. At pH 4.95 the results, after correcting for the blank values in the parallel control experiments were the following:

[S] x 10 <sup>-3</sup> M OF NMN	Y mole NADH formed in 3 min with 1mg of enzyme
0.138	0.148
0.220	0.171
0.291	0.234
0.560	0.324
0.766	0.390

Determine the apparent values of K<sub>m</sub> and V<sub>max</sub> using:

- (a) the direct plot; (25 marks)  
 (b) the Lineweaver – Burk plot;  
 (c) the Eadie – Hofstee plot.
- 2.(a) “Chemically reactive amino acid side chains and cofactors act in concert in enzymatic catalysis”. (10 marks)  
 Discuss this statement giving **one** example of an enzyme that illustrates this statement.

- (b) In a series of experiments, tubes containing varying concentrations of substrates were incubated with 1.0 µg enzyme (MW 40 000) in a final volume of 4.0 ml. One milliliter aliquots from the reaction mixture were withdrawn and assayed for product formed. The maximum velocity as determined by the Lineweaver-Burk plot was found to be 2.8 µmoles/min/ml reaction mixture. Calculate the turnover number of the enzyme. (6 marks)

- (c) Sketch diagnostic plots for sequential (single displacement) and ping-pong (double displacement) mechanisms for a bi-substrate enzyme-catalyzed reaction system. (6 marks)
- (d) What do you understand by the terms positive co-operativity and negative co-operativity? (3 marks)

3.(a) The table below shows the Michaelis constants for chymotrypsin.

Substrate	$K_m$ (M)
N-Formyltyrosinamide	$1.2 \times 10^{-2}$
N-Benzoyltyrosinamide	$2.0 \times 10^{-3}$
N-Acetyltyrosinamide	$3.2 \times 10^{-2}$
Glycyltyrosinamide	$1.2 \times 10^{-1}$

- (i) Determine the relative specificity of chymotrypsin for these substrates. Explain your answer. (5 marks)
- (b) Consider a reaction given by  $A + B \xrightleftharpoons[k_{-1}]{k_1} 2C$  (2 marks)
- (i) Write rate equations for both the forward and reverse reactions. (3 marks)
- (ii) What is the reaction order for each reactant considering both the forward and reverse reactions? (1 mark)
- (iii) What is the order of the overall reaction? (2 marks)
- (iv) What are the units of the rate constant and the rate of the reaction? (2 marks)
- (c) Distinguish between the following terms used in Enzymology (2 marks)
- (i)  $K_s$  and  $k_{cat}$  (2 marks)
- (ii)  $[S]_{0.5}$  and  $K_m$  (2 marks)
- (iii) coenzyme and cofactor (3 marks)
- (d) (i) Sketch the Hill plot and label fully. (3 marks)
- (ii) What information can be extracted from the Hill plot? (3 marks)

#### SECTION B

4. Write an essay on the major classes of antibodies found in man and clearly show how their structures are related to their function(s). (25 marks)
- 5.(a) Define or describe the following immunological terms: (5 marks)
- (i) antibody
- (ii) plasma cell
- (iii) epitope
- (iv) hapten
- (v) allergen
- (b) If a pure protein is injected into a rabbit and antibodies raised to a high titre, the antibodies produced will be a mixed population with various binding constants. Why would a single protein produce many different antibodies? (8 marks)
- (c) Name an antibody which is involved in an allergic response. How would you test an individual for the presence of this antibody for a particular allergen. (6 marks)
- (d) Briefly discuss conditions which favour the establishment of tolerance. (6 marks)

- (a) Write short notes on what role the Fc region of an antibody such as IgG or IgE plays in the immune response. (12 marks)
- (b) Explain why  $F(ab)_2$  and not Fab can form a precipitate with corresponding antigens. (5 marks)
- (c) Describe briefly how  $CD4^+$  cells are activated. Why is this important to both cell mediated immunity and humoral immunity? (10 marks)

**END OF EXAMINATION**