

**CORK INSTITUTE OF TECHNOLOGY**  
**INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ**

**Semester 1 Examinations 2008/09**

**Module Title:     Applied Enzymology**

**Module Code:**                **BIOL7001**

**School:**                        Science

**Programme Title:**        Bachelor of Science in Applied Biosciences and Biotechnology  
Bachelor of Science (Honours) in Herbal Science

**Programme Code:**        SBIBI\_7\_Y3  
SHERB\_8\_Y3

**External Examiner(s):**    Dr. Don Faller  
**Internal Examiner(s):**    Dr. Heloise Tarrant

**Instructions:**                Answer Section A (compulsory) and TWO questions from Section B.

**Duration:**                    2 hours

**Sitting:**                        Winter 2008

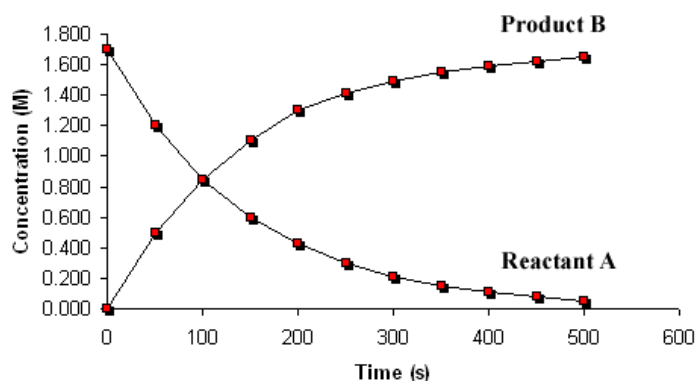
**Requirements for this examination:**     Scientific calculator

**Note to Candidates:** Please check the Programme Title and the Module Title to ensure that you have received the correct examination paper.  
If in doubt please contact an Invigilator.

## Section A (50 marks)

**Q1. (compulsory)** Answer ten parts (5 marks each)

- (a) Define each of the following terms: holoenzyme, apoenzyme, cofactor, prosthetic group.
- (b) For an enzyme that follows Michaelis Menten kinetics, draw graphs showing the relationship between initial velocity (a) enzyme concentration and (b) substrate concentration.
- (c) Write a brief note on enzyme active sites.
- (d) Distinguish between the “instantaneous rate” and the “average rate” of a reaction. On the graph below, show how you would measure each.



- (e) The Michaelis-Menten and Briggs-Haldane models of enzyme reactions make a number of assumptions, designed to keep kinetic measurements and calculations as simple as possible. List these assumptions.
- (f) List the five main strategies used by living organisms to regulate enzyme activity.
- (g) Distinguish between reversible and irreversible inhibitors of enzyme reactions.
- (h) For a multisubunit enzyme showing cooperative binding of substrate;
  - i. Draw a graph of  $v_o$  versus substrate concentration.
  - ii. On your graph indicate the changes you might expect to see in the presence of an allosteric activator and an allosteric inhibitor.
- (i) What advantage, if any, does the cooperative binding of substrate present to the cell?
- (j) For an enzyme reaction that follows Michaelis-Menten kinetics, define the following terms:  $V_{max}$ ,  $K_m$ ,  $v_o$ ,  $k_{cat}$  and  $k_{cat}/K_m$ .
- (k) An enzyme that follows Michaelis-Menten kinetics has a  $K_m$  of 1  $\mu\text{M}$ . The initial velocity is 0.1  $\mu\text{M}/\text{min}$  at a substrate concentration of 100  $\mu\text{M}$ . What is the initial velocity when  $[S]$  is equal to (a) 1 mM, (b) 1  $\mu\text{M}$  and (c) 2  $\mu\text{M}$ ?

## Section B (50 marks)

Answer any two questions.

**Q.2** The extinction coefficient ( $\epsilon$ ) of NADH is  $6.22 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ , at 340 nm. In an experiment to examine the kinetics of a purified dehydrogenase\*, a  $V_{\text{max}}$  of 0.13 absorbance units per minute was obtained using 1 ng of enzyme and 300 nmol of  $\text{NAD}^+$  in a 3 ml reaction mixture. From the  $V_{\text{max}}$  calculate the following:

- a. the units of **activity** ( $\mu\text{moles/ml/min}$ ) (10 Marks)
- b. the **specific activity** of the enzyme ( $\mu\text{moles/min/mg}$  of enzyme) (5 Marks)
- c. the percentage of  $\text{NAD}^+$  converted to NADH per minute (5 Marks)
- d. the **turnover number** of the enzyme. (5 Marks)

\* *Molecular weight 150,000, one catalytic centre per enzyme molecule.*

**Q.3** Discuss the principles of enzyme assay design, illustrating your answer with graphs and practical examples. (25 Marks)

**Q.4** Write an essay describing the methods used, and the benefits arising from, enzyme immobilisation. (25 Marks)