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)

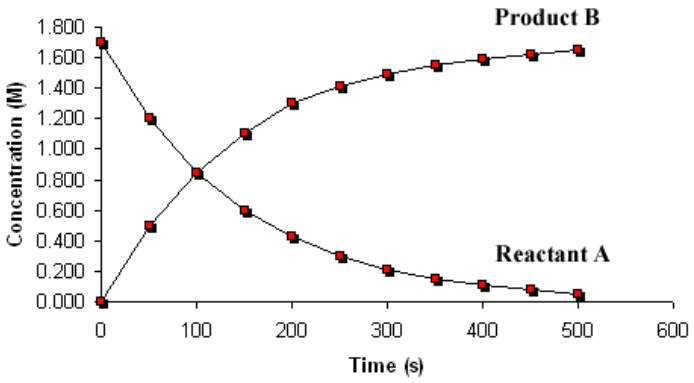
Q1. *(compulsory)* Answer ten parts (5 marks each)

(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 υ_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, υ_o, k_{cat} and k_{cat}/K_m.

(k) An enzyme that follows Michaelis-Menten kinetics has a K_m of 1 μM. The initial velocity is 0.1 μM/min at a substrate concentration of 100 μM. What is the initial velocity when [S] is equal to (a) 1 mM, (b) 1μM and (c) 2 μM?

Section B (50 marks)

Answer any <u>two</u> questions.

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

a. the units of **activity** (μmoles/ml/min) (10 Marks)
b. the **specific activity** of the enzyme (μmoles/min/mg of enzyme (5 Marks)
c. the percentage of 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)