CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Semester 1 Examinations 2011/12

Module Title: Applied Enzymology

Module Code: BIOL7001

School: Science

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

Programme Code: SBIBI-7-Y3
SHERB-8-Y3
SPHBI_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 2011

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 <u>all</u> parts.

a) Write a note on enzyme active sites.

b) 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.

c) List the five main strategies used by living organisms to regulate enzyme activity.

d) What is the equilibrium constant (K_{eq}) of a reaction? A large K_{eq} (>100) indicates a reaction that tends to completion – true or false?

e) Define each of the following terms: holoenzyme, apoenzyme, cofactor, prosthetic group.

f) Distinguish between reversible and irreversible inhibitors of enzyme reactions.

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

h) For a multisubunit enzyme showing **cooperative** binding of substrate:
   a. Draw a graph of υ_o versus substrate concentration
   b. Explain the shape of the curve in terms of the enzyme's transition between T and R states
   c. On this 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.

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 0.12 absorbance units per minute was obtained using 1 ng of enzyme and 250 nmol of NAD^+ in a 3 ml reaction mixture. From the V_{max} calculate the following:

a. the units of **activity** in μmoles per minute (10 marks)

b. the **specific activity** of the enzyme (10 marks)

c. the percentage of NAD^+ converted to NADH per minute (3 marks)

From your answer to part (c), what is the time period during which V_{max} can be reliably measured? (2 marks)

Q.3 The kinetics of an enzyme was measured as a function of substrate concentration, in the presence and absence of 1 mM inhibitor. The following results were obtained:

[S] (μM)	υ_o (μmol/**l/min)**	
	<u>No inhibitor</u>	<u>1 mM Inhibitor</u>
3	10.4	4.1
5	14.5	6.4
10	22.5	11.3
30	33.8	22.6
90	40.5	33.8

a. Draw a Lineweaver-Burke plot of these results. (8 marks)

b. What are the values of V_{max} and K_m in (i) the absence and (ii) in the presence of the inhibitor? (8 marks)

c. What type of inhibition is this? (4 marks)

d. What is the Ki value of the inhibitor? (5 marks)

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