CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Autumn Examinations 2010

Module Title: Applied Enzymology

Module Code:	BIOL7001
School:	Science
Programme Title:	Bachelor of Science in Applied Biosciences and Biotechnology Bachelor of Science in Herbal Science
Programme Code:	SBIBI_7_Y3 SHERB_8_Y2
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:	Autumn 2010
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) Draw a graph showing how the energy distribution of a population of molecules changes with increasing temperature.

b) For an enzyme that follows Michaelis Menten kinetics, draw graphs showing the relationship between (a) initial velocity and enzyme concentration and (b) initial velocity and substrate concentration.

c) 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.

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) Briefly describe 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, draw a graph of υ_o versus substrate concentration.

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)

d. the **turnover number** of the enzyme. (2 marks)

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

Q.3 Discuss in detail the advantages and disadvantages of three different methods for monitoring the rate of an enzyme-catalyzed reaction. Illustrate your answer with practical examples. (25 marks)

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