

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

<p>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.</p>
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Section A (50 marks)

Q1. (*compulsory*) Answer all 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 v_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 , v_o , k_{cat} and k_{cat}/K_m .

Section B (50 marks)

Answer any two questions.

Q.2 The extinction coefficient (ϵ) 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_{\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:

- the units of **activity** in $\mu\text{moles per minute}$ (10 marks)
- the **specific activity** of the enzyme (10 marks)
- 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)	v_o ($\mu\text{mol/l/min}$)	
	<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

- Draw a Lineweaver-Burke plot of these results. (8 marks)
- What are the values of V_{\max} and K_m in (i) the absence and (ii) in the presence of the inhibitor? (8 marks)
- What type of inhibition is this? (4 marks)
- What is the K_i value of the inhibitor? (5 marks)

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