

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: SBIOS_7_Y2
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 all 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 initial velocity (a) enzyme concentration and (b) 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 v_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 , 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:

- a. the units of **activity** in $\mu\text{moles per minute}$ (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)