

CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Semester 1 Examinations 2014/15

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. Gillian Gardiner
Internal Examiner(s): Dr. Fiona O Halloran

Instructions: Answer Section A (compulsory) and TWO questions from Section B.

Duration: 2 Hours

Sitting: Winter 2014

Requirements for this examination: Scientific calculator, graph paper

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 – compulsory

Answer **eight** of the following (each question carries five marks)

Q1.

- (a) Explain, using a diagram, how enzymes affect activation energy levels in biochemical reactions
- (b) An enzyme that follows Michaelis-Menten kinetics has a K_m of 0.25mM. At substrate concentration of 100 μ M the initial velocity is 0.2 μ mol/min. What is the initial velocity when substrate concentration is equal to (a) 1 mM and (b) 250 μ M?
- (c) Explain, using a Lineweaver-Burke plot, the effect an uncompetitive inhibitor has on the kinetics of an enzyme.
- (d) How does high cellular concentrations of ATP affect the activity of the glycolytic enzyme phosphofructokinase (PFK)?
- (e) Describe two disadvantages of enzyme immobilization technology.
- (f) What is the difference between a 'fixed-time' enzyme assay and a 'kinetic' enzyme assay?
- (g) The K_{cat} values for enzyme A and enzyme B were determined to be 200 s^{-1} and 20,000 s^{-1} respectively. Which is the most efficient enzyme? Explain your answer.
- (h) Describe the principle of the Glucose Hexokinase enzyme assay
- (i) Name the three amino acids that constitute the catalytic triad within the active site of serine proteases and give one example of a serine protease enzyme that you have studied.
- (j) The initial velocities were measured for the reaction of α -chymotrypsin (enzyme) with tyrosine benzyl ester (substrate) at six different substrate concentrations. Use the data below to make a reasonable estimate of the V_{max} and K_m value for this substrate:

[S] (mM)	0.00125	0.01	0.04	0.10	2.0	10
v_o (mM/min)	14	35	56	66	69	70

(40 Marks)

Section B. Answer two questions

Q2. Methyl- β ,D-galactopyranoside (MGP) is a known competitive inhibitor of the enzyme beta-galactosidase.

- (a) Using a Lineweaver-Burke plot describe the effects of this inhibitor on the kinetics of the enzyme.

(20 Marks)

- (b) What is the difference between a reversible enzyme inhibitor and an irreversible enzyme inhibitor, name one example of each that you have studied

(5 Marks)

- (c) For an enzyme that follows Michaelis-Menten kinetics, use graphs to explain the relationship between initial velocity and (i) enzyme concentration and (ii) substrate concentration.

(5 Marks)

Q3.

- (a) Differentiate between the terms enzyme activity and specific enzyme activity

(5 Marks)

- (b) Using an example you have studied, explain how reporter enzymes work

(15 Marks)

- (c) An enzyme with a K_m of 2.4×10^{-4} M was assayed at the following substrate concentrations:

- (i) 2×10^{-7} M
- (ii) 6.3×10^{-5} M
- (iii) 1×10^{-4} M
- (iv) 0.05 M

If the velocity observed at 0.05 M was 128 nmoles / l / min, calculate the initial velocities (v_o) at each of the substrate concentrations.

(10 Marks)

Q4. 'Enzyme Immobilization is a convenient and economical process that facilitates large-scale production and minimizes down-stream purification processes'

Discuss this statement by defining the principle of enzyme immobilization, listing the advantages of the technique, giving examples of commercial applications and describing one method used to immobilize an enzyme

(30 Marks)