

CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Semester 1 Examinations 2015/16

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. Brendan O Donnell
Internal Examiner(s): Dr. Fiona O Halloran

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

Duration: 2 Hours

Sitting: Winter 2015

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) Using an example you have studied briefly describe two strategies that are available to a cell to regulate enzyme activity.
- (b) Draw and explain the representative Lineweaver-Burke plots for enzyme inhibitors that display (i) competitive inhibition and (ii) noncompetitive inhibition.
- (c) Explain why pH affects the activity of an enzyme.
- (d) The initial velocity calculated for an enzyme catalyzed reaction was $0.25 \mu\text{mol/min}$ at a substrate concentration of 0.1mM . What is the initial velocity when substrate concentration is equal to (a) 0.01M and (b) $200 \mu\text{M}$? ($K_m = 0.25\text{mM}$).
- (e) Describe two ways to protect the biological activity of enzymes during enzyme extraction procedures.
- (f) What is the principle of enzyme immobilization technology? List two advantages of this technology.
- (g) Differentiate between a 'fixed-time' enzyme assay and a 'kinetic' enzyme assay.
- (h) In relation to enzyme activity differentiate between the terms 'thermostable' and 'thermosensitive'. Use graphs to support your answer.
- (i) Define the Arrhenius equation.
- (j) Using Cleland plots explain the difference between *random sequential* and *ordered sequential* enzyme mechanisms.

(40 Marks)

Section B. Answer two questions

Q2.

- (a) Define the following terms: Activation energy (E_a), Michaelis constant (K_m), Catalytic constant (k_{cat}), Equilibrium constant (K_{eq}).

(10 Marks)

- (b) In a kinetic enzyme assay the following data was generated under defined conditions of temperature and pH. Using the data in Table 1 determine the *initial rate* of the reaction, the *average rate* of the reaction and the *instantaneous rate* of the reaction at 8 mins.

Table 1: Absorbance data recorded at 1 minute time intervals.

Time (min)	Absorbance (@420nm
1	0.09
2	0.198
3	0.30
4	0.41
5	0.509
6	0.598
7	0.674
8	0.735
9	0.781
10	0.811
11	0.829
12	0.843
13	0.851
14	0.855
15	0.855

(15 Marks)

- (c) An enzyme following Michaelis-Menten kinetics (with a K_m of 2.5mM) yields 100nmol product per minute at saturating substrate concentration. A non-competitive inhibitor lowers the activity to 50 nmol/min. Given that the concentration of the inhibitor is 0.1mM, calculate the K_i for the inhibitor.

(5 Marks)

Q3. Phosphofructokinase (PFK) is an important regulatory enzyme in the glycolytic pathway.

(a) Describe the reaction that this enzyme catalyses and explain why this reaction is an important metabolic control point.

(5 Marks)

(b) Using graphs describe how the activity of PFK is allosterically regulated

(15 Marks)

(c) Explain how energy balance and blood sugar levels influence the activity of PFK.

(10 Marks)

Q4.

(a) List three distinct mechanisms that enzymes use to catalyse chemical reactions

(6 Marks)

(b) Describe the role that zinc plays in the catalytic mechanism of carbonic anhydrase.

(20 Marks)

(c) 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.

(4 Marks)