

# Cork Institute of Technology

## Bachelor of Science Applied Biosciences & Biotechnology - Award

(NFQ Level 7)

Spring 2007

### Biochemistry

(Time: 3 Hours)

Section A – Compulsory, attempt all 12 parts

Section B – Answer TWO questions only

Section C – Answer TWO questions only

**Use a separate answer book for each section**

Examiners:

Dr. Jim O'Mahony

Prof. R. J. Fitzgerald

### Section A

**Attempt all questions in this section**

(3 marks each)

- Q1. (a) Write a short note on the active site of an enzyme.
- (b) Draw a graph which clearly shows the relationship that exists between  $[E]$ ,  $[ES]$ ,  $[S]$  and  $[E_{total}]$  during a typical enzyme catalysed reaction.
- (c) Outline 2 ways of estimating  $K_m$  from experimental data.
- (d) What classes do the following enzymes belong?
- (i) racemase, (ii) phosphotransferase (iii) Pyruvate carboxylase
- (e) Write a brief account on the usefulness of “coupled assays”.
- (f) Write a short account on the limitations of the Michaelis Menten equation and outline how linearised methods show greater accuracy.
- (g) Outline the usefulness of the ELISA to a modern biochemistry laboratory.
- (h) Write a brief account of entrapment as a means of immobilising enzymes.
- (i) What types of approaches may be taken to lyse biological cells prior to protein purification strategies?

- (j) Briefly, what needs to be considered in formulating an enzyme for sale?
- (k) Outline briefly the experimental steps used for cloning insulin.
- (l) What types of changes may occur to a biological molecule during manufacture?

## Section B

### Answer 2 questions

(16 marks each)

- Q2. Write a detailed account of enzyme inhibition under the following headings:
- (i) Binding sites for reversible inhibitors (4 marks)
  - (ii) Lineweaver Burke plots (4 marks)
  - (iii) Applications for enzyme inhibitors (6 marks)
  - (iv) Devising an experiment which distinguishes reversible from irreversible inhibitors. (2 marks)
- Q3. (a) Clearly outline the experimental steps required to determine the effect of substrate concentration on enzyme activity for a conventional enzyme. (8 marks)
- (b) What kinetic values can be attained from this graph? (2 marks)
- (c) Why are these useful? (3 marks)
- (d) In an experiment we measure the initial rate of an enzyme reaction,  $v$ , with various concentrations of substrate,  $[S]$ . The concentration of enzyme is  $5 \mu\text{M}$ . We plot  $1/v$  vs.  $1/[S]$  and observe a straight line in which the y-intercept is  $0.04_s$  and the slope is  $28_s \mu\text{M}$ . What are the  $K_M$  and the  $V_{\max}$  values for this enzyme reaction? What is the turnover number for this enzyme? (3 marks)

- Q4. (a) Sketch a rough graph for a typical allosteric enzyme and explain its significance relative to a non-allosteric enzyme. (4 marks)
- (b) Describe the main features of an allosteric enzyme. (4 marks)
- (c) Using protein kinase A as an example show how allosteric effectors can influence enzyme activity. (8 marks)

## Section C

### Answer 2 questions

(16 marks each)

- Q5. Using suitable examples, write an essay on the selection criteria required for developing a new diagnostic assay for a biochemistry laboratory. (16 marks)
- Q6. “Drug design is largely dependant upon High Throughput Screening approaches”. Discuss this statement in detail using examples and suitable flowcharts to support your answer (16 marks)
- Q7. (a) Using appropriate diagrams to support your answer, write an essay on the use of gel filtration as a means of purifying a protein. (10 marks)
- (b) Complete the following table in your answer book showing clearly the equations used to generate your answers (6 marks)

Your Steps	Total Recovery		Specific Activity	Fold - Purified	% Yield
	Protein (mg)	Enzyme (units)			
Initial Starting Material	511	4200	8.2	-	-
40% (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation	181	3991			
DEAE flow through, pH 7.0	67.6	3787			
Isoelectric Focusing (pH 9.7)	3.8	2949			