

Cork Institute of Technology
Bachelor of Science in Applied Biosciences & Biotechnology - Award
(SBIBI_7_Y3)
Autumn 2008
Biochemistry
(Time: 3 Hours)

Section A – Compulsory, attempt all 12 parts

Section B – Answer TWO questions only

Section C – Answer TWO questions only

Examiners: Dr. Jim O'Mahony

Prof. Gary Walsh

Use a separate answer book for each section

Section A
Attempt all questions in this section
(3 marks each)

- Q1. (a) Write a brief note on enzyme structure.
- (b) Describe 2 ways to determine K_m (at least one must involve an equation).
- (c) "Recording enzyme activity measurements is usually based on gathering indirect data".
State briefly what this means.
- (d) From the perspective of Enzyme nomenclature list the 6 major classes of enzymes.
- (e) Using a named example describe the principle of "coupled assays".
- (f) Explain briefly why spectrophotometry is used so extensively for enzyme analysis.
- (g) Outline what you understand by the terms "sensitivity" and "specificity" in terms of enzyme assays.
- (h) Describe one technique for immobilising enzymes.

- (i) Briefly, outline what you understand by the term “High throughput screening”
- (j) Outline the main hazards that may be likely to damage an enzyme during cell disruption.
- (k) Using a named example, outline the techniques needed for cloning genes.
- (l) What types of changes may occur to a biological molecule during manufacture?

Section B

Answer 2 questions

(16 marks each)

- Q2. Describe in detail the steps involved in gathering data from enzyme experiments to prepare a graph in relation to the following:
- (a) V_0 vs S_0 (8 marks)
- How could the data gathered for this graph be transformed and used for:
- (b) a Lineweaver Burke plot (8 marks)
- Q3. Write a detailed account on reversible inhibition under the following 2 headings:
- (a) mode of action (6 marks)
 - (b) applications (6 marks)
 - (c) Briefly outline how you could determine the impact of a named inhibitor on the enzyme β -galactosidase. (4 marks)
- Q4. (a) Discuss a simple screening strategy that could be used to identify novel protease enzymes from bacteria. (10 marks)
- (b) Outline industrial applications where these enzymes may be beneficial. (6 marks)

Section C

Answer 2 questions

(16 marks each)

Q5. “The physical properties of an enzyme largely influence its purification strategy”. Explain if you agree with this statement.

(16 marks)

Q6. Describe the principles and applications of protein engineering in relation to biopharmaceutical products.

(16 marks)

Q7. (a) From the following table calculate the (i) specific activity, (ii) fold purification & (iii) % yield for each purification step.

(3 marks each)

Starting material	Protein (mg)	Enzyme (units)	<i>Specific activity</i>	<i>Fold purified</i>	<i>% yield</i>
30% (NH ₄) ₂ SO ₄	480	4044	?	?	?
Gel filtration	201	3881	?	?	?
Affinity Chromatography	35	2871	?	?	?

(b) In an experiment we measure the initial rate of an enzyme reaction, v , with various concentrations of substrate, $[S]$. The concentration of enzyme is $7.3 \mu\text{M}$. We plot $1/v$ vs. $1/[S]$ and observe a straight line in which the y-intercept is 0.0189_s and the slope is $9.25_s \mu\text{M}$. What are the K_M value and the V_{\max} for this enzyme reaction? What is the turnover number for this enzyme? $33 \mu\text{M}$ of an inhibitor, E , is added and we observe that the y-intercept is 0.0455_s and the slope is $22.27 s \mu\text{M}$. What kind of inhibition is this?

(7 marks)