

**CORK INSTITUTE OF TECHNOLOGY**  
**INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ**

**Autumn Examination, 2013**

**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:** Autumn, 2013

**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)

**Answer all parts.**

### **Q1. Part A**

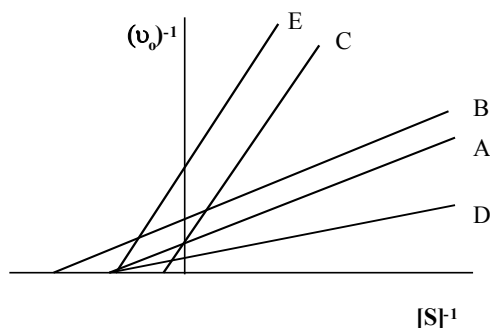
The kinetics of an enzyme was measured as a function of substrate concentration, in the presence and absence of an inhibitor ( $I_1$ ). The following results were obtained:

| [S] (M)              | $v_o$ ( $\mu\text{mol/l/min}$ ) |                                     |
|----------------------|---------------------------------|-------------------------------------|
|                      | <u>No inhibitor</u>             | <u>Inhibitor (<math>I_1</math>)</u> |
| $0.3 \times 10^{-5}$ | 10.4                            | 4.1                                 |
| $0.5 \times 10^{-5}$ | 14.5                            | 6.4                                 |
| $1.0 \times 10^{-5}$ | 22.5                            | 11.3                                |
| $3.0 \times 10^{-5}$ | 33.8                            | 22.6                                |
| $9.0 \times 10^{-5}$ | 40.5                            | 33.8                                |

- (a) Draw a Lineweaver-Burke plot of these results. (15 Marks)
  
- (b) What are the values of  $V_{\max}$  and  $K_m$  in (i) the absence and (ii) in the presence of the inhibitor? (10 Marks)
  
- (c) What type of inhibition is this? (5 Marks)

### **Part B**

In the following graph of an enzyme-catalysed reaction, Line A represents the Lineweaver-Burke plot for the reaction of a normal substrate in the absence of any inhibitor.



- (a) Which line would be expected in the presence of (i) an uncompetitive inhibitor, (ii) a noncompetitive inhibitor and (iii) a competitive inhibitor? In each case explain your chosen answer. (15 Marks)

- (b) Which line would be expected if the concentration of enzyme is doubled? Explain your chosen answer. (5 Marks)

## Section B (50 Marks)

Answer two questions.

**Q2.**

- (a) Explain, using a diagram, how enzymes affect the rate of a chemical reaction (10 Marks)
- (b) List five common features of enzyme active sites (5 marks)
- (c) Using a diagram, explain the difference between Fischers 'lock and key' model and Koshlands 'induced fit' model for enzyme-substrate (ES) binding. Which model, best describes the formation of the ES complex? Explain your choice. (10 Marks)

**Q3.**

- (a) For an enzyme reaction that follows Michaelis-Menten kinetics, define the following:  
a. Michaelis-Menten equation  
b.  $V_{max}$   
c.  $K_m$   
d.  $v_o$  (10 Marks)
- (b) To investigate enzyme reaction rates and identify enzyme kinetic data the Michaelis-Menten equation can be transformed into two other equations. Define these two other equations and graphically represent them. (10 Marks)
- (c) Using the information in Table 1 indicate which enzyme is the most efficient catalyst. Explain your answer. (5 Marks)

**Table 1: Turnover number, K<sub>cat</sub> for some enzymes**

| Enzyme                 | Substrate         | K <sub>cat</sub> (s <sup>-1</sup> ) |
|------------------------|-------------------|-------------------------------------|
| Catalase               | Hydrogen peroxide | 40,000,000                          |
| Carbonic anhydrase     | Bicarbonate       | 400,000                             |
| Acetylcholine esterase | Acetylcholine     | 14,000                              |
| Beta-lactamase         | Benzylpenicillin  | 2,000                               |
| Fumarase               | Fumarate          | 800                                 |

**Q4.**

For a multi-subunit enzyme showing cooperative binding of substrate:

- (a) Draw a graph of  $v_o$  versus substrate concentration

(5 Marks)

- (b) Explain the shape of the curve in terms of the enzyme's transition between T and R states

(10 Marks)

- (c) Define positive and negative allosterism.

(10 marks)