

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

**Autumn Examinations 2011**

**Module Title:**     **Applied Enzymology**

**Module Code:**         BIOL7001

**School:**                 Science

**Programme Title:**     Bachelor of Science in Applied Biosciences and Biotechnology  
Bachelor of Science in Herbal Science

**Programme Code:**     SBIBI\_7\_Y3  
SHERB\_8\_Y3

**External Examiner(s):** Dr. Don Faller  
**Internal Examiner(s):** Dr. Heloise Tarrant

**Instructions:**             Answer THREE questions.

**Duration:**                2 hours

**Sitting:**                  Autumn 2011

**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.

**Q.1** Given the following data for an enzyme-catalysed reaction;

[S] (M)	$v_o$ (nmol/l/min)
$6.25 \times 10^{-6}$	15.00
$7.50 \times 10^{-5}$	56.25
$1.00 \times 10^{-4}$	60.00
$1.00 \times 10^{-3}$	74.90
$1.00 \times 10^{-2}$	75.00

- (a) estimate the value of  $V_{max}$  and  $K_m$ , (20 marks)
- (b) what would  $v_o$  be at  $[S] = 2.5 \times 10^{-5}$  M and  $[S] = 5.0 \times 10^{-5}$  M? (10 marks)
- (c) what would  $v_o$  be at  $[S] = 5.0 \times 10^{-5}$  M, if the enzyme concentration was doubled? (5 marks)
- (d) the  $v_o$  values given in the table were determined by measuring [product] that had accumulated over a 10 minute period. Verify that  $v_o$  represents a true initial velocity. (10 marks)

**Q.2** Complete the following purification table: (25 marks)

Procedure	Total Activity (U)	Total Protein (mg)	Specific Activity (U/mg)	Purification Factor	Yield (%)
Extract	4200		2.1	1	100
Ammonium Sulphate Precipitation	3011	1505			
Electrodialysis	2500			5.95	60
DEAE-cellulose anion exchange chromatography		100	18.5		
Sephadex G-200 gel filtration	1550		31		
Affinity chromatography	1401	10		67	33

- (b) Write explanatory notes on three of the protein purification techniques used in the procedure above. (25 marks)

**Q.3** (a) For an enzyme reaction that follows Michaelis-Menten kinetics, define the following terms:

- i.  $V_{\max}$
  - ii.  $K_m$
  - iii.  $V_o$
  - iv.  $K_{\text{cat}}$
  - v.  $K_{\text{cat}}/K_m$
- (25 marks)

- (b) Discuss the different mechanisms used to regulate enzyme activity *in vivo*. (25 marks)

**Q.4** Sephadex G-200 gel filtration chromatography was performed on an enzyme solution. The column was calibrated with a set of standard proteins, and the elution volumes are summarised in the following table:

Protein	Mr (kDa)	Elution Volume (ml)
Chemotrypsinogen A	24	18.2
Bovine Serum Albumin	66	16
Creatine Kinase	80	16.4
Lactate Dehydrogenase	140	16
Aldolase	160	15.3
Catalase	230	14.5
Leucine Aminopeptidase	300	14
b-Galactosidase	540	13.3
Thyroglobulin	660	12

- (a) Draw a graph of  $\log_{10} M_r$  (molecular weight) versus  $V_e$  (elution volume), and comment. (15 marks)
- (b) If the enzyme eluted at a volume of 13.7 ml, what is its molecular weight? (15 marks)
- (c) When treated with  $\beta$ -mercaptoethanol, the enzyme solution eluted in two peaks at volumes of 14.8 ml and 16.2 ml. What extra information does this give us? (20 marks)