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)	υ_o (n**mol/l/min)**
6.25×10^{-6}	15.00
7.50×10^{-5}	56.25
1.00×10^{-4}	60.00
1.00×10^{-3}	74.90
1.00×10^{-2}	75.00

(a) estimate the value of V_{max} and $K_{m,}$ (20 marks)

(b) what would υ_o be at [S] = 2.5×10^{-5} M and [S] = 5.0×10^{-5} M? (10 marks)

(c) what would υ_o be at [S] = 5.0×10^{-5} M, if the enzyme concentration was doubled? (5 marks)

(d) the υ_o values given in the table were determined by measuring [product] that had accumulated over a 10 minute period. Verify that υ_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_{cat}
v. K_{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 β-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)