CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Semester 1 Examinations 20010/11

Module Title: Applied Enzymology

Module Code: **BIOL7001**

School: **Science**

Programme Title: Bachelor of Science in Applied Biosciences and Biotechnology
Bachelor of Science (Honours) in Herbal Science

Programme Code: SBIBI_7_Y3
SHERB_8_Y3

External Examiner(s): **Dr. Don Faller**

Internal Examiner(s): **Dr Siobhán O'Sullivan**

Instructions: **Answer 3 questions**

Duration: **2 hours**

Sitting: **Winter 2010**

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.

Q1.
The relationship between initial rate (v_0) measurements and substrate concentration [S] is given by the Michaelis-Menten equation:

$$v_0 = Vmax\,[S] / Km + [S]$$

where V_{max} and K_m are the kinetic parameters. You are studying an enzyme that obeys this equation for which the K_m is known to be 0.2mM.

(i) An assay at [S] =2mM gives a rate of 100 international units (1 IU= 1 micromole product per minute). Calculate the V_{max} of this enzyme. (10 marks)
(ii) Given that 1nM is the enzyme concentration used, calculate the turnover number, k_{cat}. (10 marks)
(iii) What [S] would give a rate of 45 IU? (10 marks)
(iv) Enzymes can be classified into different groups based on the reactions they catalyse. List the groups and for each group give an example of a reaction that such an enzyme catalyses. (20 marks)

Q2.
(i) Complete the following purification table (25 marks):

Step	**Total activity (U)**	**Total protein (mg)**	**Specific activity (U/mg)**	**Purification factor**	**Yield (%)**
Extract	4,201		2.1	1	100
Ammonium Sulphate precipitation	3,010	1,500			
Electrodialysis	2,500			5.95	60
DEAE-cellulose anion exchange		100	18.5		
Sephadex G-200 Gel filtration	1,550		31		
Affinity	1,400	10		67	33

(ii) Illustration and explain the basis of anion and cation exchange chromoatography. (20 marks)
(iii) How can the isoelectric point of a protein help us in deciding whether to use anion or cation exchange chromatography? (5 marks)

Q3

An enzyme was found to elute at a volume of 13.7 ml from a sephadex G-200 gel filtration column. When treated with β-mercaptoethanol two peaks were found to elute at volumes of 14.8 and 16.2 ml, respectively. This column was calibrated with standard proteins which eluted at the following elution volumes;

Protein	M_r (kDa)	Elution Volume (ml)
Chymytrypsinogen 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
β-Galactosidase	540	13.3
Thyroglobulin	660	12

(i) Draw a graph to illustrate the relationship between Mr and elution volume (20 marks)
(ii) What conclusions may you draw from this experiment about this enzyme? (20 marks)
(iii) Briefly outline the basis of gel filtration chromatography and suggest a situation where the results obtained from such an experiment might not be valid.(10 marks)

Q4. ((i)Discuss in detail and illustrate using examples where possible, **four** mechanisms of how cells can regulate enzyme activity. (40 marks)
(ii)For an enzyme reaction that follows Michaelis-Menten kinetics, define the following terms: V_{max}, K_m, υ_o, k_{cat} and k_{cat}/K_m. (10 marks)