

# Cork Institute of Technology

Higher Certificate in Science in Applied Biology – Award  
(National Certificate in Science in Applied Biology – Award)  
(NFQ – Level 6)

Summer 2005

## Biochemistry

(Time: 3 Hours)

Answer Section A (compulsory) and TWO questions from each of Sections B and C.

Examiners: Dr. H. Tarrant  
Dr. T. Beresford

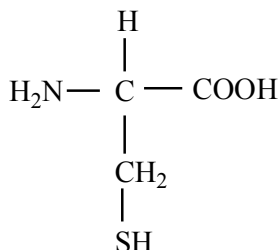
Use separate answer books for each section and mark the questions attempted.

### Section A

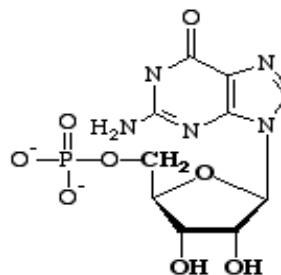
**Q1. Compulsory**, answer **12** parts only. (24 marks)

(a) The properties and biological activities of biomolecules are largely determined by their functional groups. Identify each of the functional groups in the following two molecules:

i. Cysteine (an amino acid)



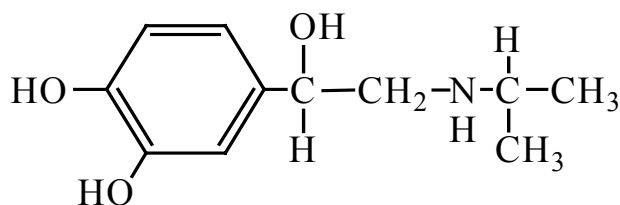
ii. Guanosine monophosphate (a nucleotide)



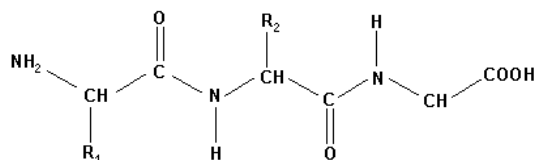
(b) A well-designed assay will incorporate one or more **standards** and a **reagent blank**. Define each of these terms.

(c) Distinguish between **systematic** and **random** experimental error.

(d) The D isomer of the drug isoproterenol is used to treat mild asthma, while the L isomer is 80 times less effective. Why would the two stereoisomers have such different biological activity? Identify the chiral (asymmetric) carbon in isoproterenol:



- (e) What is an allosteric enzyme? With the aid of diagrams, explain the terms **negative allosterism** and **positive allosterism**.
- (f) Name two structural features that affect the melting point of a fatty acid.
- (g) Integral membrane proteins are involved in transport of molecules across the cell membrane. Define the terms **passive** and **active** transport, and distinguish between **symport** and **antiport** mechanisms.
- (h) Define the Beer Lambert Law. The  $A_{340}$  of a solution of NADH was found to be 0.29. What was the concentration of this solution? ( $\epsilon_{\text{NADH}} = 6220 \text{ M}^{-1} \text{ cm}^{-1}$ ).
- (i) Although oxygen does not participate directly in the citric acid cycle, the cycle operates only when  $\text{O}_2$  is present. Why?
- (j) Calculate the number of millilitres of 5M  $\text{H}_2\text{SO}_4$  required to prepare 1250 ml of a 0.03 M  $\text{H}_2\text{SO}_4$  solution.
- (k) The concentration of glucose in human blood plasma is held at about 5 mM. The concentration of free glucose inside muscle cells is much lower. Why is the concentration so low in the cell? What happens to the glucose upon entry into the cell?
- (l) In the following diagram of a dipeptide (i) which carbons are the  $\alpha$ -carbons? (ii) What do the R groups represent? (iii) Why is there no free rotation around the  $\text{C}=\text{O}$  to N peptide bond?



- (m) Describe how you would prepare 100 ml of a 50 mM phosphate buffer from a 1 M stock solution.
- (n) Describe a non-destructive method for generating a protein elution profile after column chromatography.

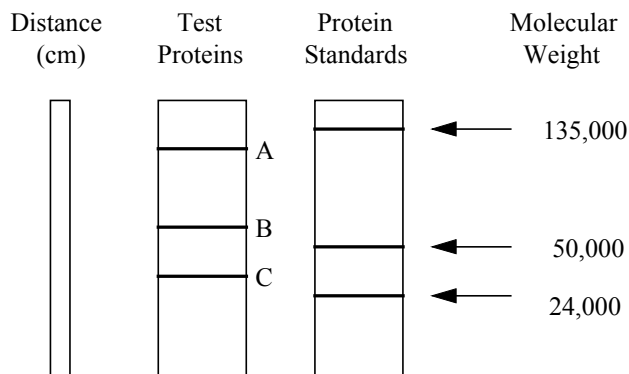
## Section B

(Analytical Biochemistry - 38 marks)

Answer any **two** of the following questions (Q2, Q3 or Q4).

- Q2.** (a) Explain why a weak acid or base can effectively buffer pH, while a strong acid or base cannot. Use titration curves to illustrate your answer. [5 marks]
- (b) List the different factors to be considered when choosing a buffer for use. [5 marks]
- (c) Describe in detail the preparation of 0.8 L of a 0.05 M phosphate buffer, pH 7.4, from  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  (fw, 138) and  $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$  (fw, 268.07) salts. ( $\text{pK}_a = 7.2$ ) [9 marks]

- Q3.** (a) List four factors that will cause protein denaturation. [2 marks]
- (b) Describe how the amino acid sequence of a protein may be determined. [8 marks]
- (c) Proteins of different molecular weight may be separated by SDS-PAGE. After electrophoresis the protein bands can be visualised by staining. Results from such an experiment are shown below, together with data from the electrophoresis of standard proteins of known molecular weight:



Draw a graph of log molecular weight versus distance (cm) to determine the molecular weights of proteins A, B, and C. [9 marks]

- Q4.** Write brief informative notes on three of the following;
- (i) DNA sequencing using the Sanger (di-deoxy) method
  - (ii) Affinity chromatography
  - (iii) Bioluminescence and chemiluminescence
  - (iv) Fixed time or continuous monitoring methods of enzyme measurement [19 marks]

## Section C

(Structural and Metabolic Biochemistry - 38 marks)

Answer any **two** of the following questions (Q5, Q6, Q7 or Q8).

**Q5. (a)** Oxidative phosphorylation is the final stage in the energy-yielding metabolism of aerobic organisms. Write an essay on this process making use of diagrams to illustrate your answer. [13 marks]

(b) In normal mitochondria, the transfer of electrons is tightly coupled to the synthesis of ATP. Some molecules can uncouple these two events and the uncoupler 2,4-dinitrophenol was once prescribed as a weight-reducing drug. Such uncouplers are no longer used as deaths occurred following their use.

(i) Explain, in principle, how 2,4-dinitrophenol could cause weight-loss.[3 marks]

(ii) Explain why ingestion of uncouplers can cause death. [3 marks]

**Q6. (a)** Although lactose exists as two anomeric forms, no anomeric forms of sucrose exist. Explain why, and name a chemical test that will distinguish between lactose and sucrose.

[5 marks]

(b) Both cellulose and starch consist of (1→4)-linked D-glucose units. Despite this, a person on a diet of starch will gain weight whereas a person on a diet of cellulose (grass) will starve. With the aid of diagrams, explain this fact. [5 marks]

(c) The enzyme invertase (sucrase) catalyses the hydrolysis of sucrose into glucose and galactose. The following data was obtained in a study of the effect of pH on sucrase activity:

pH	Sucrase Activity ( $\mu\text{mol/ml/min}$ )
2.5	0.58
3.5	0.60
4.5	0.66
5.5	0.34
6.5	0.23
7.5	0.20

Plot a graph of pH versus sucrase activity and determine the pH optimum of the enzyme.

[6 marks]

What chemical test might have been used to determine the concentration of reducing sugars produced in this reaction? [3 marks]

- Q7.** (a) Summarise the essential features of the genetic code. Is the code universal? [3 marks]
- (b) Explain how the Meselson-Stahl experiment proved that DNA undergoes semi-conservative replication in *E. coli*. [6 marks]
- (c) Write a short essay describing the process of replication using diagrams to illustrate your answer. [10 marks]

- Q8.** (a) Given an enzyme that obeys Michaelis-Menton kinetics, draw the following graphs:
- (i) initial reaction rate ( $v_o$ ) versus substrate concentration
- (ii) initial reaction rate ( $v_o$ ) versus enzyme concentration. [4 marks]
- (b) Draw a Lineweaver-Burk graph of  $v_o$  versus  $[S]$  for a typical enzyme reaction (i) in the absence of an inhibitor, (ii) in the presence of a competitive inhibitor and (iii) in the presence of a non-competitive inhibitor. [3 marks]
- (c) The kinetics of an enzyme are measured as a function of substrate concentration in the presence and absence of an inhibitor.

[S] ( $\mu\text{M}$ )	Rate of Reaction ( $\mu\text{mol/ml/min}$ )	
	No inhibitor	Inhibitor
3	10.4	4.1
5	14.5	6.4
10	22.5	11.3
30	33.8	22.6
90	40.5	33.8

Use a Lineweaver-Burk plot to determine the values of  $K_m$  and  $V_{max}$  in the absence and presence of the inhibitor. [10 marks]

What type of inhibition is this? [2 marks]