

# Cork Institute of Technology

## Bachelor of Science in Applied Biosciences and Biotechnology - Award

(NFQ – Level 7)

Autumn 2007

### Bioanalytical Science

(Time: 3 Hours)

Answer a total of FIVE questions.  
Answer TWO questions from Section A  
Answer THREE questions from Section B

Examiners: Prof. R. Fitzgerald  
Ms. C. Devaney  
Dr. L. Goold  
Ms. A. Ward

### Section A

- Q1. (a) Describe the nature of the stationary phase as well as the underlying principles of separation in (i) ion exchange chromatography and (ii) size exclusion chromatography. (10 marks)
- (b) A chromatography separation yielded the following data for a given set of experimental conditions:-

**Column Length:** 20 cm. **Phase Ratio** ( $V_s/V_m$ ): 0.25

**Retention Times:-** non retained component = **30 seconds**  
Component A = **1.5 minutes**  
Component B = **2.0 minutes**

**Peak Base Widths:** Component A = **10 seconds**  
Component B = **15 seconds**

Calculate the following:

- (i) The capacity factor,  $k^1$ , for Component A.
- (ii) The partition co-efficient,  $K_d$ , for component B.
- (iii) The resolution,  $R$ , between A and B.
- (iv) The length of column required to give a resolution value of 1.50.

(10 marks)

- Q2.(a) Construct a labelled block diagram of a High Performance Liquid Chromatography (HPLC) instrument and use it to **briefly describe** the function of each component. (8 marks)
- (b) Describe reverse phase HPLC analysis under the following headings:- (i) polarity of mobile phase and stationary phase giving typical examples of each. (ii) order of elution of components of varying polarity and (iii) polarity changes to mobile phase required to increase elution power. (6 marks)
- (c) Briefly explain the processes of isocratic and gradient elution modes of HPLC analysis. Indicate a type of sample mixture that would require the gradient mode for successful separation. Explain your answer. (6 marks)
- Q3.(a) Explain the principles of operation of a flame ionisation detector in a gas chromatographic instrument. (10 marks)
- (b) Discuss the basis for quantitation in gas chromatographic analysis and in particular refer to the internal standard method of quantitation. Explain why the internal standard method of quantitation is particularly suited to gas chromatographic analysis. (10 marks)

## Section B

- Q4.(a) Outline, using a detailed diagram for illustration, the principle of a non- competitive sandwich Enzyme Linked Immunosorbent Assay (ELISA). (14 marks)
- (b) Write a brief note on (i) enzyme and (ii) fluorescent labels in use in immunoassay systems. (6 marks)
- Q5. Write notes on Polyacrylamide Gel Electrophoresis (PAGE).  
In your answer outline the important parameters to be considered in the experimental design of this analytical system. (20 marks)

Q6.(a) Outline the important parameters required in the design of an immunoassay validation experiment. (10 marks)

(b) Write notes on **TWO** of the following:

(i) Internal Quality Control

(ii) Random & Systematic error

(iii) External Quality Assessment schemes (10 marks)

Q7. (a) (i) Tritium, Carbon14 and Iodine 125 are radioactive isotopes that are used as “labels” in immunoassay. Write the decay equation for TWO of the above radioactive isotopes. (6 marks)

(ii) Distinguish between **internal** and **external** “labels”. (2 marks)

(b) With reference to centrifugation, explain the following terms:

- Relative Centrifugal Force

- Sedimentation Coefficient (4 marks)

(c) Describe, with the aid of a diagram, the operation of a Scintillation Counter.

(6 marks)

State one advantage of this detector. (2 marks)