

Cork Institute of Technology
Higher Certificate in Science in Applied Biology - Award

(NFQ – Level 6)

Summer 2006

Bioanalytical Science 2

(Time: 3 Hours)

Instructions

Answer FIVE questions. Question 1 is compulsory. TWO questions from Section B, ONE from Question C and a fifth question from Section B or C.

All questions carry equal marks.

LOG TABLES required.

Examiners: Dr. R. Hourihane
Ms. A. Ward
Dr. J. G. Murphy
Prof. R. Fitzgerald

Section A

Q1. Attempt all of the following:

- (i) Identify the molecular process which is induced on the absorption of:
 - (a) UV/Visible radiation
 - (b) Infrared radiation
 - (c) Microwave radiation
- (ii) Give Beer's Law and state in words the relationship it illustrates.
- (iii) Distinguish between a chromophore, chromogen and an auxochrom in relation to UV/Visible spectroscopy. Give an example in each case.
- (iv) List three deviations from Beer's Law, which is most significant.
- (v) Illustrate a conductimetric titration graph for a strong acid (HCl) versus a strong base (NaOH). Show how the end point of the titration is estimated.
- (vi) Calculate ϵ_{max} for ethanoic acid (CH_3COOH) given the following data.

ϵ_{max} / $\text{Scm}^2 \text{mol}^{-1}$	Material
426	HCl
126	NaCl
91	CH_3COONa

- (vii) Name the Law which is illustrated by the calculation in (vi) above.
- (viii) List three properties of an ion selective electrode (ISE), give an example.
- (ix) Identify the four types of ISE.
- (x) When determining the concentration of an unknown solution using electrode potentials, what graph must be plotted.
- (xi) Define each of following:
 - (i) Antigenic Determinant or Epitope
 - (ii) Antigen Binding Site or Paratope
- (xii) Draw a simple diagram outlining the principle of immunoaffinity chromatography.
- (xiii) List the five main classes of immunoglobulins.
- (xiv) List three methods of identification and quantitation of proteins post electrophoresis.
- (xv) Define what is meant by a primary and secondary immune response. (20 marks)

Section B

Q2. (a) Draw a labelled energy level diagram detailing the process of molecular fluorescence. (4 marks)

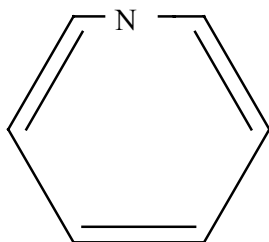
(b) The O₀0 transition gives rise to a band in both the fluorescence excitation and emission spectrum. Its wavelength may not in fact be identical in both spectra. A small displacement to longer wavelength is generally observed in the emission spectrum. Explain why this occurs. Diagram required. (5 marks)

(c) (i) In order for a molecule to exhibit fluorescence it must contain certain structural features. Identify and explain at least two of these features. (4 marks)

(ii) In fluorescence determination there are some practical considerations which must be adhered to. Identify and explain at least three of these. (4 marks)

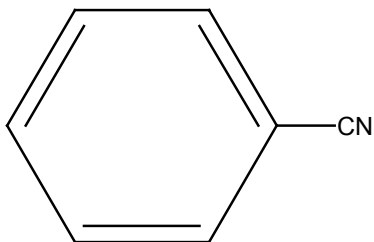
(iii) Which of the following would you expect to fluoresce and briefly state why the selected compound(s) should fluoresce?

(a)



(b) $\text{CH}_3 - \text{CH}_2 - \text{CH}_3$

(c)



(3 marks)

- Q3. (a) (i) Write a note outlining the method of adsorption chromatography. A diagram is required.
- (ii) Name three other classification/types of interaction of solute with the stationary phase. (7 marks)
- (b) (i) What is the column efficiency required to give complete separation of two components with retention times of 25 and 26s?
Assume that the width of both peaks are the same.
- (ii) If the column length is 150m, what is the height equivalent to a theoretical plate (H.E.T.P)? (6 marks)
- (c) (i) Name three reasons why an initially sharp band of solute broadens as it moves through a chromatography column.
- (ii) Explain the dependence of each mechanism on flow rate.
- (iii) Give an equation which quantifies these broadening mechanisms. (7 marks)
- Q4. (a) Write a note comparing and contrasting flame atomic emission spectroscopy and atomic absorption spectroscopy under the following headings:
- (i) Source of excitation
- (ii) Sample type/s
- (iii) Purpose of the flame and flame type
- (iv) Quantitation (8 marks)
- (b) Two blood samples were analysed for sodium content by low temperature flame emission spectroscopy. To this end a series of sodium standards were prepared in concentrations ranging from 5 to 30 ppm from an appropriate stock solution. When the results of the

analysis were inspected, it was seen that one of the samples was outside the range of the standards.

This was dealt with by taking 5ml of this solution and diluting it to 25ml. Its emission value was obtained. The data is contained in the table below.

Na Std./ppm	Emission Reading
5	28
10	69
15	110
20	153
25	195
30	236
Blood sample 1 emission reading (103)	
Blood sample 2 emission reading (250)	
Diluted Blood sample 2 emission reading (162)	

- (i) Plot the appropriate calibration curve.
- (ii) Determine the concentrations of Na^+ the two blood samples. (8 marks)
- (c) What volume of 260ppm sodium stock solution is required to prepare:
 - (i) 20ml of 10ppm standard solution
 - (ii) 25ml of 30ppm standard solution. (4 marks)
- Q5. (a) Describe the operation of Reflection gratings as used in Monochromators. (4 marks)
- (b) Draw a diagram of the optical arrangement of a monochromator you have studied.
List the function of each component. (5 marks)
- (c) Draw a diagram illustrating the optical arrangement of an interferometer. (5 marks)
- (d) State two advantages of Fourier transform IR spectrophotometers. (2 marks)
- (e) Outline the principle of operation of a typical desktop centrifuge. (4 marks)

Section C

- Q6. (a) Outline, using a diagram for illustration, the principle of a non-competitive sandwich enzyme linked immunosorbent assay (ELISA). (10 marks)
- (b) Give a brief overview of one immunoanalytical technique which involves a precipitation reaction between an antibody and an antigen. (10 marks)
- Q7. (a) Describe the principle of separation of SDS-polyacrylamide gel electrophoresis. (6 marks)
- (b) Outline how you would assess the reliability of an analytical method under the following headings.
- (i) Precision (5 marks)
- (ii) Accuracy (5 marks)
- (c) List two statistics that could be used to plot a control chart for analytical data. (4 marks)