

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

(NFQ – Level 6)

Autumn 2006

Bioanalytical Science 2

(Time: 3 Hours)

Instructions

Answer FIVE questions.

Question 1 is compulsory. TWO questions
from Section B and ONE question from Section
C.

A fifth question from either B or C.

Please use separate answer books for each
section.

Log tables required.

Examiners: Dr. R. Hourihane

Dr. J.G. Murphy

Ms. A. Ward

Prof. R. Fitzgerald

Section A

Q1. Attempt 10 of the following

All carry equal marks.

- (i) Distinguish between absorbance and absorptivity. Give the units where appropriate.
- (ii) Give the wavelength range for the near ultra violet and vacuum ultra violet regions.
- (iii) What is meant by the term ‘atomisation in atomic spectroscopy’? Give an example.
- (iv) Explain the term ‘resonance absorption’ with reference to atomic spectroscopy.
- (v) What is molecular fluorescence? A simple diagram may aid the definition.
- (vi) What is the inner filter effect? What affect does it have on absorption?
- (vii) Distinguish between retention time and volume. Give the symbol for each.
- (viii) List three types of band broadening in chromatography. How are each dependant on flow rate?

- (ix) Explain the term liquid junction potential in electrochemical cells? How may it be minimized?
 - (x) Distinguish between reference electrode and indicator electrode in potentiometry.
 - (xi) Draw a simple diagram of an IgG molecule.
 - (xii) What is cellulose acetate electrophoresis?
 - (xiii) Draw a diagram illustrating the principle of the Ouchterlony immunodiffusion assay.
 - (xiv) Define the following terms:
 - (i) primary immune response
 - (ii) secondary immune response
- (20 marks)

Section B

- Q2. (a) In chloroform solution, acetone ($\text{CH}_3\text{C}=\text{O}$) has an ultraviolet absorption band involving the carbonyl group, centered at 275nm. What is meant when we say this band represents an $n - \pi$ transition?
- What other transition may also be possible, why may it be difficult to access it?
(Show the transition by illustrating the movement of electrons within the molecule).
- (6 marks)
- (b) What is a conjugated chromophore?
- What effect, if any, will conjugation have on the position and intensity of the absorption?
Illustrate with an example.
- (7 marks)
- (c) A sample in a 10mm cell is determined with a spectrometer to transmit 75% of light at a certain wavelength. If the absorptivity of this substance at this wavelength is $3.00\text{cm}^{-1}\text{g}^{-1}\text{L}$, what is the concentration of the substance?
- If the molecular mass of the substance is 105g/mol, what is the concentration of the material mol/L?
- (7 marks)

Q3. Attempt three of the following.

- (i) Identify three methods of background correction in atomic spectrometry. Discuss two in detail.
- (ii) Write a detailed note on the method of size exclusion chromatography. A diagram to illustrate the technique is required.
- (iii) Illustrate a typical conductometric titration for a weak acid and a strong base.

Show which ions are present at various stages of the titration. How may the endpoint of the titration be estimated from the graph?

- (iv) List three types of glass membrane ion selective electrode. Discuss one in detail. Mention any limitations of use.
- (v) List and explain three characteristics of an ideal complexation reagent used in UV spectrometry. (20 marks)

Q4. (a) An excited molecule can return to the ground state by a combination of several mechanistic steps of which only two involve release of a photon. The other deactivation steps are radiationless processes.

- (i) Identify the two radiative processes referred to above.
- (ii) List at least two of the non radiative processes, referred to. Explain one of these processes in detail. An energy level diagram is required. (8 marks)

(b) Lipid oxidation is one of the major causes of quality deterioration in meat. Several methods have been developed to assess lipid oxidation products (LOP). One such method is a fluorometric method which is widely used because of its simplicity and speed. To this end, meat samples were prepared and analysed in triplicate according to literature methods [1]. Samples and standards were analysed fluorometrically at 570nm excitation and 550nm emission. A series of standards were prepared from an 1000ppm appropriate stock solution by serial dilution. The results and data are contained in the following table.

As can be seen from the table, the sample readings are outside the range of standard concentrations. The sample was diluted by 50%. The diluted sample was within range and the analysis was concluded successfully.

[1] Poultry Science 1998, 475-480

Fluorescence Intensity (FI)	Conc/ppm
115	0.01
240	0.02
352	0.03
501	0.04
610	0.05
980	LOP Sample (undiluted)
520	Diluted LOP sample (1)
523	Diluted LOP sample (2)
525	Diluted LOP sample (3)

- (i) Calculate the concentration of LOP in diluted sample.
- (ii) Calculate the concentration of LOP in the original sample.
- (iii) Explain the term serial dilution as mentioned in text. Suggest how this may be applied to the 1000 ppm stock in order to prepare the standard solutions.

(12 marks)

- Q5. (a) As the concentration of a sample in a sample cell increases, how do (i) the transmittance and (ii) the absorbance of the sample vary? Use diagrams to support your answer. (6 marks)
- (b) What are the basic elements that form a modern spectrophotometer? Provide a block diagram. Briefly describe the function of each element. (5 marks)
- (c) Write a brief note on a photodiode. Comment on the suitability of this detector for the detection of u.v. – visible radiation. (5 marks)
- (d) Outline the principle of operation of a typical desktop centrifuge with specific reference to the physical principles involved. Use diagrams to support your answer. (4 marks)

Section C

- Q6. (a) Define each of the following:
- (i) monoclonal antibody (3 marks)
 - (ii) polyclonal antibody (3 marks)
- (b) Describe with the aid of a diagram the principle of immunoaffinity chromatography as a purification technique in polyclonal antibody production. (8 marks)
- (c) Outline the main differences between a heterogeneous and homogenous immunoassay system. (6 marks)
- Q7. (a) Describe the method of immunoelectrophoresis. (8 marks)
- (b) Outline how you would assess the precision and accuracy of an analytical method for an internal quality control testing scheme. (8 marks)
- (c) List three sources of error in the laboratory that can contribute to unreliable results. (4 marks)