

Cork Institute of Technology

Bachelor of Science in Applied Biosciences –Stage 2

(SBIOS_7_Y2)

Summer 2008

Bioanalytical Science 2

(Time: 3 Hours)

Answer **Five** questions.

Question 1 (Section A) is compulsory.

Answer **TWO** questions from Section B, and

ONE from Section C and a **fifth** question from Section B or C.

Please use separate answer books for each Section.

Examiners: Dr. R. Hourihane

Ms. A. Ward

Mr. C. O' Farrell

Prof. G. Walsh

Section A

Attempt any ten of the following

(20 marks)

- Q1.** (i) List four methods of background corrections used in atomic spectroscopy.
- (ii) Illustrate a typical conductimetric titration graph for a strong acid versus a strong base. Show how the end point of the titration may be estimated.
- (iii) List three types of band broadening in chromatography. Show the dependence on flow rate in each case.
- (iv) State Beer's Law. Give the equation and give the units for each term.
- (v) Distinguish between chromophore, chromogen and auxochrom in relation to UV/visible spectroscopy. Give examples in each case.
- (vi) Define each of the following terms: (a) Epitope (b) Paratope (c) Antigen.
- (vii) List the five main classes of immunoglobulin.
- (viii) Draw a simple diagram illustrating the principle of an Ouchterlony Immunodiffusion assay.
- (ix) Define what is meant by the primary and secondary immune responses.
- (x) Draw a diagram of the structure of an IgG molecule.

- (xi) Name the different types of centrifuge instruments available?
- (xii) A laboratory centrifuge operates at a rotational speed of 5,500rpm.
- (i) What is the magnitude of the centripetal acceleration on a red blood cell at a radial distance of 6.50cm from the centrifuges axis of rotation?
 - (ii) How does this acceleration compare to g?
- (xiii) If a diffraction grating has 1100 grooves/mm, what wavelength (in nanometres) is it optimised for?
- (xiv) Name the 4 components that make up a spectrophotometer?
- (xv) Name the components and sketch a typical setup for a monochromator?
- (xvi) What type of light source and what region of the electromagnetic spectrum are they optimised for:
- (i) laser
 - (ii) Deuterium lamp
 - (iii) Tungsten Filament
 - (iv) Heated Inert Solids

Section B

- Q2.** (a) Chromatography is one of the most powerful tools for separating and measuring components in a complex mixture.
- (i) Explain the following chromatographic terms:
Mobile phase, stationary phase, partition, eluent, eluate, elution.
- (ii) The ability of a column to resolve components of a mixture is dependant upon a number of variables. List and explain briefly three of these variables. (8 marks)
- (b) Chromatography may be classified by the type of interaction of the solute with the stationary phase.
- (i) Write a brief note explaining size exclusion chromatography.
A diagram may aid your description.
- (ii) List the other classifications. (6 marks)
- (c) The table of data below contains the relative peak areas for five gas chromatographic peaks obtained in the analysis of a mixture of alcohols.
The relative responses of the detector for the five alcohols are also listed.

Calculate the percentage of each alcohol in the mixture.

<i>Alcohol</i>	<i>Relative Peak Areas</i>	<i>Relative Detector Response</i>
1	27.6	0.70
2	32.4	0.72
3	47.1	0.75
4	40.6	0.73
5	27.3	0.78

(6 marks)

- Q3.** (a) One of the most important steps in the analytical process is obtaining and dissolving the sample.
- List and explain briefly the steps involved in the sampling process.
 - Explain what is meant by interference, how may it be dealt with? (8 marks)
- (b) Below are listed a number of analytical methods. Write on one line of explanation for each method.
- Spectrometry
 - Atomic spectroscopy
 - Chromatography
 - Gravimetric analysis
- Rank these methods in order of increasing sensitivity. (6 marks)
- (c) What volume of concentrated sulphuric acid (H_2SO_4 94% w/w solution) is required to prepare 1 litre of 0.1 mol/dm^3 solution? Density of $\text{H}_2\text{SO}_4 = 1.831 \text{ g/cm}^3$. (6 marks)

Q4. Attempt three of the following:

- Two non radiative processes compete with molecular fluorescence; these are internal conversion and intersystem crossing. Describe briefly both processes.
An energy level diagram is required.
- Describe the method of flame atomic emission spectrometry (FAES).
In your description include:
 - typical atomisation temperatures
 - source of excitation
 - sample type
 - quantitative applications
- Draw a simple energy level diagram containing the molecular orbitals σ , σ^* , π , π^* and n. Identify the common /useful transitions which occur as a result of absorption of ultra violet or visible radiation. Why are the other transitions less useful?
Identify molecules which undergo useful transitions.
- List and discuss briefly the four classifications of ion selective electrodes (ISE).
- A solution containing 5.00 mg of sodium in 250 ml of water was observed to transmit 50% of the incident light compared to the appropriate blank.
 - What is the absorbance of the solution at this wavelength?
 - What would the transmittance value be for a solution of sodium which is twice as concentrated?

Q5.

(a) Describe the method of atomic absorption spectrometry. In your description:

- (i) Identify sample types which are most appropriate to this method
- (ii) Explain what is meant by nebulization
- (iii) Identify processes which occur in the flame

(8 marks)

(b)

<i>Absorbance</i>	<i>[Fe] / mg/L</i>
0.009	1.00
0.028	3.00
0.048	5.00
0.067	7.00
0.096	10.00
0.900	Atmospheric water sample
0.081	Diluted sample

A sample of atmospheric water was analysed for iron content using atomic absorption spectroscopy. To this end, a series of standards were prepared from a 100 ppm iron stock solution. Their absorbance values were measured at 510 nm. The water sample was treated according to literature methods and its absorbance value was also determined.

The data for both sample and standard is contained in the data table above.

As can be seen from the data, the absorbance value for the water sample is outside the range of the standards. The water sample was diluted to 10% of its original concentration, and reanalysed.

The absorbance of the diluted sample is shown on the data table too.

- (i) Plot the appropriate calibration curve
- (ii) Determine the concentration of iron in the original water sample. (8 marks)

(c) The standards listed in the table were prepared from 100 ppm iron stock solution.

What volume of this stock is required to prepare:

- (i) 100 cm³ of the 7 ppm standard solution
- (ii) 25 cm³ of the 1 ppm standard solution (4 marks)

Section C

Q6. (a) Draw a diagram illustrating the principle of immunoaffinity chromatography.

(8 marks)

(b) Using a detailed diagram for illustration, outline the principle of ONE of the following:

(i) Non- Competitive sandwich ELISA for ferritin

(ii) Competitive ELISA for T4

(12 marks)

Q7. (a) List the four performance characteristics which should be evaluated to achieve a reliable bioanalytical assay.

(4 marks)

(b) Outline how to assess the accuracy of an assay by using a recovery experiment.

(8 marks)

(c) Write a short note on internal quality control and the use of control charts.

(8 marks)