

Cork Institute of Technology

Bachelor of Science in Applied Biosciences – Stage 2

(SBIOS_7_Y2)

Autumn 2008

Bioanalytical Science 2

(Time: 3 Hours)

Answer Five questions.

Question 1 is compulsory.

TWO questions from Section B,

ONE from Section C and a fifth questions from
Section B or C

Examiners: Dr. R. Hourihane

Ms. A. Ward

Dr. C. Farrell

Prof. G. Walsh

Section A

Q1. Attempt any ten of the following. All carry equal marks.

- (i) Express ppb on (i) wt/wt basis (ii) wt/vol basis.
- (ii) Explain the significance of using internal standards in quantitative chromatography.
- (iii) How many theoretical plates produce a chromatography peak eluting at 12.83 min, with a width at half height of 8.7 s? If the column length is 15.8 cm what is the height equivalent to a theoretical plate?
- (iv) List three limitations of the pH glass electrode.
- (v) Distinguish between reference electrode and sensing electrode in potentiometry. Give one example of each.
- (vi) Illustrate a spectrophotometric titration where both the analyte and titrant absorb.
- (vii) Draw a simple diagram illustrating the principle of rocket immunoelectrophoresis.
- (viii) Define each of the following: (a) polyclonal antibody (b) monoclonal antibody.
- (ix) List TWO statistics that can be used to plot a control chart.
- (x) Define each of the following: (a) primary standard (b) secondary standard.
- (xi) Draw a diagram of the structure of an IgG molecule.
- (xii) Name the different types of centrifuge instruments available.

(xiii) A laboratory centrifuge operates at a rotational speed of 55,000rpm.

1. What is the magnitude of the centripetal acceleration on a red blood cell at a radial distance of 6.0cm from the centrifuges axis of rotation?
2. How does this acceleration compare to g?

(xiv) If a diffraction grating has 1500 grooves/mm, what wavelength (in nanometres) is it optimised for?

(xv) Name the 4 components that make up a spectrophotometer?

(xvi) What type of light source and what region of the electromagnetic spectrum are they optimised for:

1. laser
2. Deuterium lamp
3. Tungsten Filament
4. Heated Inert Solids

Section B

Q2. (a) (i) State the difference between transmittance, absorbance and molar absorptivity.

(ii) Which one is proportional to concentration?

(iii) Name and write the law which summaries the relationship referred to in (ii).

(6 marks)

(b) Apotransferrin has a molar absorptivity of $8.83 \times 10^4 \text{ cm}^{-1}\text{mol}^{-1}\text{dm}^3$ at 280 nm.

(i) Find the concentration of apotransferrin in water if the absorbance is 0.244 in 0.100cm cell.

(ii) If the molecular weight of apotransferrin is 81,000, express the concentration calculated in (i) above in g/dm^3 . Hence, or otherwise, calculate the absorptivity.

(6 marks)

(c) Complexation reagents/metal chelating agents are used in the spectroscopic determination of transmit metals.

(i) Explain the function of such reagents.

(2 marks)

(ii) Identify and explain in detail four properties of an ideal reagent.

(4 marks)

(iii) Give two examples of these reagents and the metal they are most likely to be used with.

(2 marks)

- Q3.** (a) Identify four classifications of electrons in a molecule. Which of these will undergo excitation as a result of absorption of ultraviolet / visible radiation? (6 marks)
- (b) The presence of an auxochrom in a molecule can effect the position and intensity of the absorption of a chromophore. Name and explain these spectral changes. Explain the underlined terms; give an example in each case. (10 marks)
- (c) Explain the term-conjugated chromophore. The presence of conjugation may result in a shift in λ_{max} of the chromophore to longer wavelength. Why? (4 marks)

Q4. Attempt three of the following:

- (a) List and describe briefly four interferences, which are associated with flame atomic absorption spectroscopy.
- (b) Write a note on solvent effects in fluorimetry.
- (c) Write a note explaining the term Thin Layer Chromatography (TLC).
In your discussion outline:
- (i) Whether the method is qualitative or quantitative?
 - (ii) How components are measured?
 - (iii) The mechanism by which the technique operates.
- (d) Write an informative note on Size Exclusion Chromatography. Include in your answer:
- (i) common mobile and stationary phases used
 - (ii) a description of the mechanism employed
 - (iii) an explanation of the terms exclusion limit and water regain value. Supporting diagrams may enhance your discussion.
- (e) Illustrate the graph obtained for the conductimetric titration of a strong acid e.g. HCl with a strong base e.g. NaOH. In your answer, account for the shape of the graph in terms of ions which are responsible for conductivity before, at and after the end point of the titration. (20 marks)

Q5. (a) Flame atomic emission spectrometry and atomic absorption spectrometry are related atomic spectroscopic techniques. List three similarities and two differences between the methods. (6 marks)

(b) A sample was analysed for sodium content using flame atomic emission spectrometry. An emission value of 755 was recorded. A series of standards were prepared and their emission values determined, according to literature methods. See table below. As can be seen from the data, the sample emission is outside the range of the standards emission.

The sample was thus diluted by taking 10 cm³ of the original solution and making up to the mark in 50 cm³ volumetric flask with water. This diluted sample was analysed, its emission value is shown on the data table.

Conc. / ppm	Emission
1	25
2.5	75
5	185
10	375
15	575
Sample	755
Diluted sample	255

(i) Plot the appropriate calibration curve.

(ii) Determine the concentration of sodium in the original sample. (10 marks)

(c) The standards listed in the table were prepared by dilution of 150 ppm stock solution.

What volume of this stock solution is required to prepare:

(i) 100 cm³ of the 2.5ppm standard solution

(ii) 25 cm³ of the 15ppm standard solution. (4 marks)

Section C

- Q6.** (a) Define each of the following performance characteristics required to achieve a reliable bioanalytical assay:
- (i) Precision
 - (ii) Accuracy
 - (iii) Specificity
 - (iv) Detection limit (8 marks)
- (b) Outline how you would assess the following:
- (i) Intra-assay precision
 - (ii) Inter-assay precision
 - (iii) Accuracy (9 marks)
- (c) List THREE common sources of errors associated with poor laboratory practice. (3 marks)
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- Q7.** (a) Draw a simple diagram illustrating the principle of immunodiffusion. (8 marks)
- (b) Outline the principle of a non-competitive sandwich ELISA for Ferritin. (12 marks)
- Use a diagram to illustrate your answer.