

Cork Institute of Technology

Bachelor of Science (Honours) in BioSciences (ACCS) - Award

(NFQ Level 8)

Summer 2007

Bioanalytical Science

(Time: 3 Hours)

Answer one question from each of Section A, B, C and D. Each question carries equal marks.

Examiners: Dr. B. Fogarty
Prof. R. Fitzgerald

Use separate answer books for each section and mark the question attempted

Section A

- Q1. (a) Name the main components of a typical HPLC system and illustrate your answer with a system schematic. (5 marks)
- (b) Explain the principle of size exclusion chromatography. (5 marks)
- (c) The following data was generated from the separation of a mixture of proteins using reverse-phase HPLC.

<u>Retention time</u>	<u>tR (mins)</u>	<u>Peak width</u>
Solvent front	3.1	-
Protein A	5.4	0.41
Protein B	13.3	1.07
Protein C	14.1	1.16
Protein D	21.6	1.72

Calculate:

- i) The number of plates (N) for Proteins A and B.
- ii) The capacity factor (k) for Protein B.
- iii) The resolution (R) and selectivity factor (α) for Proteins B and C. (15 marks)

- Q2. (a) Describe three pumping systems commonly used in HPLC. (9 marks)
- (b) What is the difference between isocratic and gradient elution in HPLC? (4 marks)
- (c) A series of standards were prepared for the analysis of creatinine in a urine sample using HPLC. The stock solution was prepared by weighing out 0.050g of the creatinine which was dissolved in 100 mls of distilled water.

i) What was the concentration of the stock solution in mg/L?

ii) A working solution of 50 mg/L was prepared from the stock solution. A series of standards were prepared by pipetting the following volumes of the 50mg/L working solution into separate 25 ml volumetric flasks and making up to the mark with water. What was the concentration (mg/L) of each creatinine standard prepared?

Standard	Volume working solution used (mls)	Peak area
1	12.5	26090
2	10	19988
3	7.5	14899
4	5	10285
5	2.5	5143

iii) Use the concentrations calculated and the peak area results provided to construct a calibration curve of creatinine concentration (mg/L) versus peak area. The peak area for the creatinine in a urine sample was determined to be 13798. The urine sample was diluted by a factor of 10 before analysis. Using the calibration curve, determine the concentration of creatinine in the urine sample. (12 marks)

Section B

- Q3. (a) Explain the significance of column temperature and temperature programming in Gas Chromatography. (5 marks)
- (b) Describe the use of capillary (open tubular) columns in gas chromatography. Use diagrams to illustrate your answer. (10 marks)
- (c) Explain the principle of flame ionisation detection. (10 marks)
- Q4. (a) What are the considerations and interface options for coupling Gas Chromatography online to Mass Spectrometry. (5 marks)
- (b) Describe the principle of electrospray ionisation for mass spectrometry. (10 marks)
- (c) What is the main function of a mass analyser? Describe how a quadrupole mass analyser works. (10 marks)

Section C

- Q5. (a) Silica gel, alumina and cellulose are three sorbents commonly used in thin layer chromatography. Briefly describe the characteristics of each sorbent material. (10 marks)
- (b) Discuss the considerations for the selection of mobile phase and solvents for thin layer chromatography. (5 marks)
- (c) Describe the use of ascending and multiple developments in thin layer chromatography. (10 marks)
- Q6. (a) Discuss the principles of solid phase extraction (SPE). (10 marks)
- (b) Describe the 5 main steps in a typical solid phase extraction procedure. Illustrate your answer with diagrams. (5 marks)
- (c) What are the advantages of solid phase microextraction (SPME) over other approaches to sample preparation? Explain the term headspace sampling. (10 marks)

Section D

- Q7. (a) What considerations should be taken into account when designing biosensors? (10 marks)
- (b) Molecular recognition is commonly used in biosensor devices. Briefly discuss the characteristics of molecular recognition systems with respect to specificity; binding constants and the chemical basis of binding. (5 marks)
- (c) Describe any **two** of the following groups that exhibit recognition properties. (10 marks)
- Monoclonal antibodies
 - Lectins
 - DNA
 - Enzymes
- Q8. (a) Describe the use of fluorescence probes in immunoassays. (7.5 marks)
- (b) What is time-resolved fluorescence? (2.5 marks)
- (c) Explain the principle of the DELFIA assay. (15 marks)