

Cork Institute of Technology
Bachelor of Science Applied Biosciences & Biotechnology -
Award

(NFQ Level 7)

Spring 2007

Bioanalytical Science

(Time: 3 Hours)

Answer a total of FIVE questions.

Answer TWO questions from Section A

Answer THREE questions from Section B

Examiners: Prof. R. Fitzgerald

Ms. C. Devaney

Dr. L. Goold

Ms. A. Ward

Section A

- Q1. (a) Describe the function of a detector in a gas chromatographic instrument. List and explain the important properties that need to be considered when choosing a particular detector for gas chromatographic analysis. Give a brief description of the mode of operation of **either** a thermal conductivity **or** a flame ionisation detector. (10 marks)
- (b) Distinguish between isothermal and temperature programmed modes of operation of a gas chromatographic instrument. Indicate the type of sample that would require the latter mode (i.e temperature programmed mode) for efficient analysis and explain your answer. (6 marks)
- (c) Explain how numerical values for the following can be obtained from a chromatogram:- (i) Resolution, R and (ii) Column Theoretical Plate Value, N (Note:- Indicate clearly the meaning of any terms used in any equations given). (4 marks)

PTO

- Q2. The following experimental procedure was carried out in order to determine the concentration of caffeine in a soft drink by High Performance Liquid Chromatography (HPLC):- Standard solutions of caffeine were prepared in separate 25.0 cm³ volumetric flasks by the addition of 1.0, 2.0, 3.0, 4.0 and 5.0 cm³ of stock standard solution of caffeine (75.0 mgdm⁻³) followed by dilution to the mark with solvent. 2.0 cm³ of the soft drink were diluted to 25.0 cm³ and the resulting solution and the prepared standards were analysed using reverse phase mode of HPLC analysis. The caffeine eluted after about 2 minutes and the following caffeine peak areas were obtained from the chromatograms of the analysed solutions:-

Standard Solution Analysed (cm³ of 75.0 mgdm⁻³ stock used)	Caffeine Peak Area (area counts x10⁻³)
1.0	12.3
2.0	25.2
3.0	35.9
4.0	49.0
5.0	61.8

Soft Drink Solution Analysed	42.5
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- (a) Sketch a labelled block diagram of a HPLC instrument and use the diagram to briefly explain how the analysis of any solution containing caffeine is performed. (8 marks)
- (b) Name a typical mobile phase and a stationary phase used in reverse phase HPLC analysis and in each case state whether it is polar or non-polar. Suggest a change to the mobile phase that would decrease the retention time of caffeine and explain your answer. (5 marks)
- (c) Construct a calibration plot for the above analysis data and use it to determine the concentration of caffeine in the original soft drink. (7 marks)

PTO

- Q3. (a) Describe the fundamental modes of vibration of the CO₂ molecule. For each mode of vibration, indicate whether the mode is infra-red active or infra-red inactive and explain why. (6 marks)
- (b) Sketch a labelled block diagram of a Fourier Transform Infra-Red Spectrophotometer and briefly describe its mode of operation. (Note:- a detailed explanation of the principles of operation of an interferometer is not necessary) (8 marks)
- (c) Identify the 4 main regions of an infra-red spectrum (4000 cm⁻¹-400 cm⁻¹) where correlation between structural features(i.e bond types) in a molecule and transmittance peaks are made and give the significant types of bond vibrations associated with each region. (6 marks)

Section B

- Q4. (a) Distinguish between the following main classifications of immunoassay:
- (i) Heterogeneous enzyme immunoassay (5 marks)
 - (ii) Homogeneous enzyme immunoassay (5 marks)
- (b) Describe, using a diagram for illustration, the principle of an Enzyme Multiplied Immunoassay Technique (EMIT) (10 marks)
- Q5. Write notes on **TWO** of the following:
- (a) Internal quality control & external quality assessment schemes in bioanalytical methods. (10 marks)
 - (b) Optimisation and validation parameters in immunoassay development. (10 marks)
 - (c) Assessment of accuracy of an analytical method (10 marks)

- Q6. (a) Write a brief account of non-isotopic labels in use in immunoassays. (10 marks)
- (b) List the important experimental considerations in the design of Polyacrylamide Gel electrophoresis system. (10 marks)
- Q7. (a) “ The gain in signal measurement over the potential loss of immunoreactivity is sufficient to make iodine-125 the isotope of choice for immunoassay systems”.
- (i) Comment **BRIEFLY** on this statement. (3 marks)
- (ii) Give the decay equation for iodine-125. (3 marks)
- (iii) Describe, with the aid of a diagram, the detector (Scintillation counter) used to measure the signal referred to in the above statement. (8 marks)
- (b) By considering the centripetal force on a particle in a liquid spinning in a centrifuge and Stokes equation, arrive at a definition of the **sedimentation coefficient**. (6 marks)