

**CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ**

Semester 1 Examinations 2011/12

Module Title: Bioanalytical Science V

Module Code: BIOT7002

School: Science and Informatics

Programme Title: BSc in Applied Biosciences and Biotechnology
BSc (Hons) in Pharmaceutical Biotechnology

Programme Code: SBIBI_7_Y3
SPHBI_8_Y3

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Internal Examiner(s): Dr L. Goold
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Instructions: Attempt 2 questions from Section A and 2 questions from Section B.

Duration: 2 hours

Sitting: Winter 2011

Requirements for this examination:

Note to Candidates: Please check the Programme Title and the Module Title to ensure that you have received the correct examination paper.
If in doubt please contact an Invigilator.

SECTION A

- Q1. (a) Define the term 'partition co-efficient, K_d ' as it applies to a chromatographic process. Indicate how order of elution of components in a chromatographic process is linked to the partition co-efficient values of the components. List three changes that could be made in a chromatographic experiment in order to change the K_d values of the components.

(6 marks)

- (b) Discuss the mechanism of separation in size exclusion (gel permeation) chromatography.

(8 marks)

- (c) Predict the changes, if any, to the theoretical plate value, N , and the retention time, t_r , of a component in each of the following situations:-

(i) The length of a column is doubled in a chromatographic experiment. The flow rate of the mobile phase and the stationary phase in the column are both maintained.

(ii) The particle size of the stationary phase in a chromatographic process is doubled. The flow rate of the mobile phase and the column length are both maintained.

In the case of both (i) and (ii) suggest how the mobile phase flow rate would be maintained.

(6 marks)

- Q2. The gas chromatographic analysis of sample mixture was carried out using temperature programming and a capillary column containing a non polar stationary phase. The sample was introduced to the column by the split injection technique. The detector used was a flame ionisation detector. The chromatogram displayed a number of peaks. Two peaks were identified as compound A (retention time, $t_r=1.5$ minutes and base width, $W=30$ seconds) and compound B (retention time, $t_r=1.75$ minutes and base width, $W=45$ seconds)

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Q2. (cont.)...

- (a) Explain the process of temperature programming in gas chromatographic analysis. Contrast the process with the process of isothermal analysis and briefly explain the advantage of using the temperature programming mode.

(4 marks)

- (b) Describe the general features of a capillary column and compare it to a packed column. Explain why the split injection is required when using a capillary column.

(6 marks)

- (c) Outline the principles of operation of a flame ionisation detector.

(6 marks)

- (d) Predict the order of elution of components based on their boiling points and polarity.(note:- the stationary phase is non-polar)

(2 marks)

- (e) Calculate the resolution value, R , between A and B.

(2 marks)

Q3. 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 cm^3 volumetric flasks by the addition of 1.0, 2.0, 3.0, 4.0 and 5.0 cm^3 of stock standard solution of caffeine (75.0 mg dm^{-3}) followed by dilution to the mark with solvent. 2.0 cm^3 of the soft drink were diluted to 25.0 cm^3 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 (cm^3 of 75.0 mg dm^{-3} stock used)	Caffeine Peak Area (area counts $\times 10^{-3}$)
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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Q3. (cont.)...

- (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 solvent mixture 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 composition of the mobile phase mixture that would decrease the retention time of caffeine and explain your answer.
(5 marks)
- (c) Calculate the mg dm^{-3} concentration of caffeine in each of the prepared standard solutions. Construct a calibration plot and use it to determine the mg dm^{-3} concentration of caffeine in the original soft drink.
(7 marks)

SECTION B

Q4. (a) Define each of the following terms;

- | | | |
|-------|----------------------------------|-----------|
| (i) | Centrifugal Force | (2 marks) |
| (ii) | Relative Centrifugal Force (RCF) | (2 marks) |
| (iii) | Revolutions per minute (RPM) | (2 marks) |

(b) Briefly outline each of the following types of centrifugal separation technique:

- | | | |
|------|---------------------------------|-----------|
| (i) | Differential Centrifugation | (4 marks) |
| (ii) | Density Gradient Centrifugation | (4 marks) |

(c) List the main cell disruption techniques used for cell fractionation (6 marks)

Q5. (a) Describe using a diagram for illustration the principle of ONE of the following immunoassay techniques:

- (i) Enzyme Multiplied Immunoassay Technique (EMIT)
- (ii) Reagent limited competitive Enzyme Linked Immunosorbent Assay (ELISA)

(6 marks)

(b) Distinguish between homogeneous and heterogeneous immunoassay systems

(4 marks)

(c) Write an overview of Internal Quality Control (IQC) in immunoassays under the following headings:

- (i) IQC statistics (3 marks)
- (ii) Quality control charts & acceptance criteria (3 marks)

(d) Define each of the following:

- (i) Calibration Standard (2 marks)
- (ii) International Standard (2 marks)

Q6. (a) Outline the important parameters to be considered in the design of a Polyacrylamide Gel Electrophoresis system. (6 marks)

(b) Write a brief overview of analysis methods for proteins after electrophoresis.

(4 marks)

(c) Describe the main method validation parameters required to complete a typical immunoassay validation protocol

(6 marks)

(d) Outline the critical success factors to be considered before immunoassay method development and optimization.

(4 marks)