

CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Semester 1 Examinations 2009/2010

Bioanalytical Science V

Module Code: BIOT7002

School: Science

Programme Title: Bachelor of Science in Applied Biosciences and Biotechnology

Programme Code: SBIBI_7_Y3

External Examiner(s): Prof. G. Walsh

Internal Examiner(s): Dr. L. Goold

Ms. A. Ward

Instructions: Attempt 2 questions from Section A and 2 questions from Section B

Duration: 2 hours

Sitting: Winter 2009

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. Discuss (a) ion-exchange chromatography and (b) size exclusion chromatography under the following headings:- (i) nature and physical state(s) of the stationary phase (ii) interaction of components with the stationary phase and (iii) basis for separation of components.

(20 marks)

Q2. A mixture of fatty acids were derivatised and then analysed by gas chromatographic analysis. The fatty acid derivatives were introduced to a gas chromatographic instrument using the split injection technique, separated in a capillary column which was operated under temperature programmed mode and subsequently detected by a flame ionisation detector.

(a) Give reasons why, in general, derivatisation may be necessary prior to gas chromatographic analysis and briefly give details of a suitable derivatisation procedure used in the mixture of fatty acids.

(4 marks)

(b) Briefly describe what happens in the split injection technique and explain why it is necessary when using a capillary column.

(4 marks)

(c) Describe the manner in which the stationary phase is contained in the capillary column and explain why a capillary column is more efficient at separating than a packed column.

(3 marks)

(d) Explain temperature programming and discuss, in general, the merits of this mode compared with isothermal analysis.

(4 marks)

(e) Describe, with the aid of a suitable diagram, the principles of operation of a flame ionisation detector.

(5 marks)

- Q3. (a) Construct a labelled block diagram of a High Performance Liquid Chromatography (HPLC) instrument and use it to **briefly** describe the function of each component. (8 marks)
- (b) Briefly explain the processes of isocratic and gradient elution modes of HPLC analysis. Indicate a type of sample mixture that would require the gradient mode for successful separation. Explain your answer. (6 marks)
- (c) Distinguish between pre-column and post-column derivatisation approaches in HPLC analysis and compare the advantages and disadvantages of each approach (6 marks)

SECTION B

- Q4. (a) Describe with the aid of a diagram the principle of a reagent excess non-competitive immunoassay system. (8 marks)
- (b) Outline the main optimisation parameters required for the above assay. (6 marks)
- (c) Write a brief note on non-isotopic labels currently in use in immunoassay systems. (6 marks)
- Q5. (a) Describe, using a diagram for illustration the principle of ONE of the following immunoassay systems:
- (i) Homogeneous EMIT
 - (ii) Particle agglutination immunoassay (8 marks)
- (b) Write a brief outline of the main immunoassay validation parameters (7 marks)
- (c) Outline how you would assess the precision of an immunoassay system in a validation study (5 marks)

- Q6. (a) Outline the important experimental considerations in designing a Polyacrylamide Gel Electrophoresis (PAGE) system. (8 marks)
- (b) Describe the current methods in use for the detection and quantitation of proteins post-electrophoresis (7 marks)
- (c) Write a brief note on centrifugation as an important separation technique in the bioanalytical laboratory (5 marks)