CORK INSTITUTE OF TECHNOLOGY
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ

Semester 1 Examinations 2010/11

Module Title: Bio Analytical Science III

Module Code: CHEA6003

School: Science

Programme Title: Bachelor of Science (Honours) in Applied Bio Sciences – Year 2
Bachelor of Science (Honours) in Nutrition & Health Science – Year 2
Bachelor of Science (Honours) in Pharmaceutical Biotechnology – Year 2

Programme Code: **SBIOS_7_Y2**
SNHSE_8_Y2
SPHBI_8_Y2

External Examiner(s): **Dr. C. Lennon**
Internal Examiner(s): **Dr. Rosamund Hourihane, Dr. M. Lehane, Ms. E. Norris**

Instructions: **Attempt Three questions.**
Section A, Question1., is compulsory.
Attempt any two questions from Section B
Show all calculations and rough work on the answer script

Duration: 2 Hours

Sitting: Winter 2010

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.

Attempt 8 of the following 12 parts. All carry equal marks. (5 marks each)

(i) Gravimetry, spectrometry and chromatography are examples of different analytical methods. In one sentence each, describe, how these methods operate to obtain a result. Rank the methods in ascending order in terms of selectivity, speed and cost.

(ii) In a UV/Vis analysis you are asked to prepare a 40% w/v buffer solution of HCl to alter the pH of the analyte solution so as to stabilise the compound of interest. Determine the molarity of the 40% solution of HCl which has a density of 1.20g/ml. (note: Density = mass/volume).

(iii)

%T	**CONCENTRATION µg/dm^3**
27	0.030
43	0.025
57	0.020
78	0.015
92	0.010

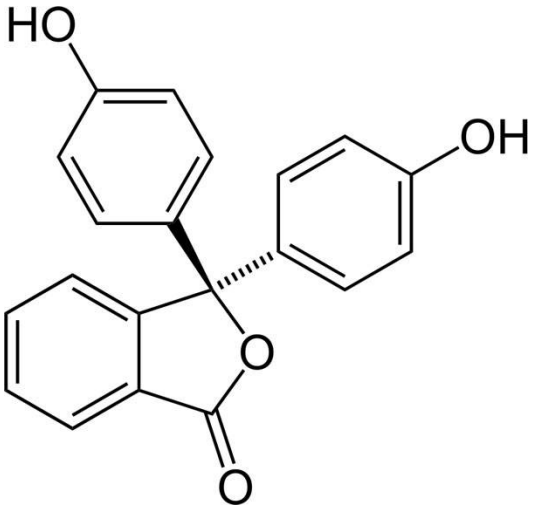
Convert the above data into absorbance values; explain why the %T increases with decreasing concentration.

(iv) For the analysis of a food dye by UV spectrophotometry **describe how you would prepare the following solutions**:

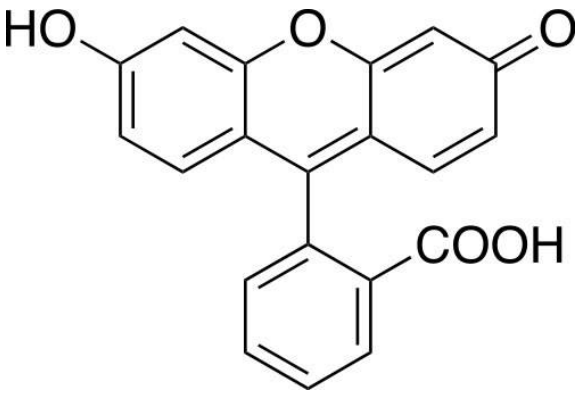
(a) 250 cm^3 of a solution of 0.050g dm^{-3} from the 0.500 gdm^{-3} stock solution provided.

(b) Using a series of 50 cm^3 volumetric flasks, solutions of the following concentrations: 0.010, 0.015, 0.020, 0.025 and 0.030 from the 0.050gdm^{-3} solution you made up in part (a).

(v) Consider the two molecules given below which compound would you expect to give the greatest fluorescence quantum yield? Explain your answer.



COMPOUND A



COMPOUND B

(vi**)** Pure hexane has a negligible UV absorbance above a wavelength of 200nm and is therefore a good solvent for UV/Vis spectroscopy.

A solution was prepared by dissolving 25.8mg of benzene (C_6H_6 molecular mass = 78.11gmol^{-1}) in hexane and diluting to 250 ml. An absorption peak was observed at 256nm and the absorbance value was 0.266 in a 1cm cell. ***Calculate the molar absorptivity*** of the benzene at this wavelength. ***Explain the term molar absorptivity***

(vii) Give a description of the electromagnetic spectrum. Include detail about properties of different wavelength regions. What is the wavelength range for the visible part of the electromagnetic spectrum?

(viii) Describe a laboratory experiment that illustrates either (i) characteristics and behaviour of a light detector, or (ii) characteristics of light sources suitable for use in spectroscopy.

(ix) Draw a fully labelled diagram that shows the functional layout of double beam spectrophotometer and its main components. Explain why a double beam spectrophotometer is used.

(x) In relation to spectroscopy, what is the function of a monochromator? Draw a diagram which illustrates the optical layout of a Czerny-Turner monochromator. Write down the grating equation. Explain why groove spacing on a grating is important.

(xi) Write down the grating equation and identify each variable. A reflection grating has a groove separation of 8.3×10^{-7} m. Calculate the groove spacing per millimeter. State giving a reason whether this grating would be suitable for use in an infrared spectrophotometer working in the region of 1500 nm.

(xii) Give a simple explanation of Raman Spectroscopy. What information does Raman spectroscopy provide about the sample being tested? Suggest an appropriate light source for Raman spectroscopy.

(40 marks)

Section B

Attempt three of the following

Q2. (i) What is conductance? List two quantities upon which it depends.

(3marks)

(ii) The concentration of a solution of hydrochloric acid, HCl, was determined by conductometric titration. To this end, 20cm^3 of the diluted HCl solution, (25% concentration of the original acid solution), was titrated against a solution of sodium hydroxide, 0.1$moldm^{-3}$. The data collected from the titration are listed in the following table.

Conductivity/ mS cm^{-1}	Volume of NaOH / cm^3
0.894	0
0.840	0.5
0.768	1.0
0.708	1.5
0.647	2.0
0.478	3.0
0.423	4.0
0.230	6.0
0.298	7.0
0.367	8.0
0.490	10.0
0.515	10.5
0.542	11.0
0.573	11.5
0.601	12.0

(a) Plot the appropriate graph. (5 marks)

(b) Illustrate clearly on the graph the end point of the titration, describing briefly how it was determined. (2 marks)

(c) Write an equation to describe the titration. (1mark)

(d) Calculate the concentration of the original HCl solution in

(i) $moldm^{-3}$ (ii) gdm^{-3}.

Atomic masses H = 1.008$gmol^{-1}$

Cl = 35.453$gmol^{-1}$ (5marks)

(e) What ions are responsible for the conductivity values recorded (i) before, (ii) at and (iii) after the end point of the titration.

(6 marks)

(ii) (a) What is an ion selective electrode, ISE?

(b) Name four types of ISE.

(c) List four properties of ISE's. (8marks)

Q3. (i) Electronic transitions rely on the absorption of Ultraviolet,(UV), or Visible, (Vis), radiation by specific groups known as chromophores within a molecule.

(a) Explain the underlined terms, giving examples and diagrams where appropriate (4 marks)

(b) Give the wavelength range for both the UV and Vis regions of the electromagnetic spectrum (2marks)

(c) Explain why absorption by simple chromophores may be difficult to measure? (2marks)

(d) Explain too, why the absorption by conjugated chromophores occur at longer wavelength than that of a simple chromophore?

(3marks)

(ii) Beer's Law can be applied to measurements in both the UV and Vis regions of the electromagnetic spectrum. However, there are a number of conditions, which must applied.

(a) List three of these conditions. (3marks)

(b) Failure to apply these conditions correctly results in deviations from Beer's Law. Identify and describe briefly, two such deviations which are related to the solution concentration and two which are connected to the instrument. (8marks)

(iii) (a) List two structural features a molecule must possess in order to be successfully measured by flourimetery. (2 marks)

(b) Fluorescence, is an extremely sensitive technique, limited to solutions of very low concentration. Both characteristics present problems, when conducting experimental determinations, not normally encountered with solutions of high concentration.

List four such problems, giving possible remedies/solutions which may be applied to minimise their effect. (6 marks)

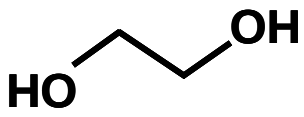
Q4.

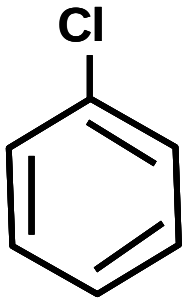
a) Discuss how a multi component sample is separated in column chromatography, making reference to the equilibrium of the analyte between stationary phase and mobile phase and the height equivalent to a theoretical plate (HETP); the mechanism of adsorption (the types of attraction between the analyte and the mobile and stationary phases); normal and reverse phase separations; and mobile phase selection. (10 marks)

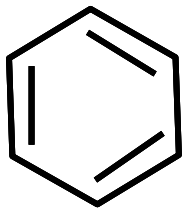
b) (i) According the Van Deemter Equation, HEPT = A +B/μ + Cμ, there are three factors that are a source of band broadening, discuss each term and suggest ways of minimising these sources of band broadening. (3 marks)

(ii) Predict the elution order of the following compounds on a normal phase column and explain the rationale for your selection. (2 marks)

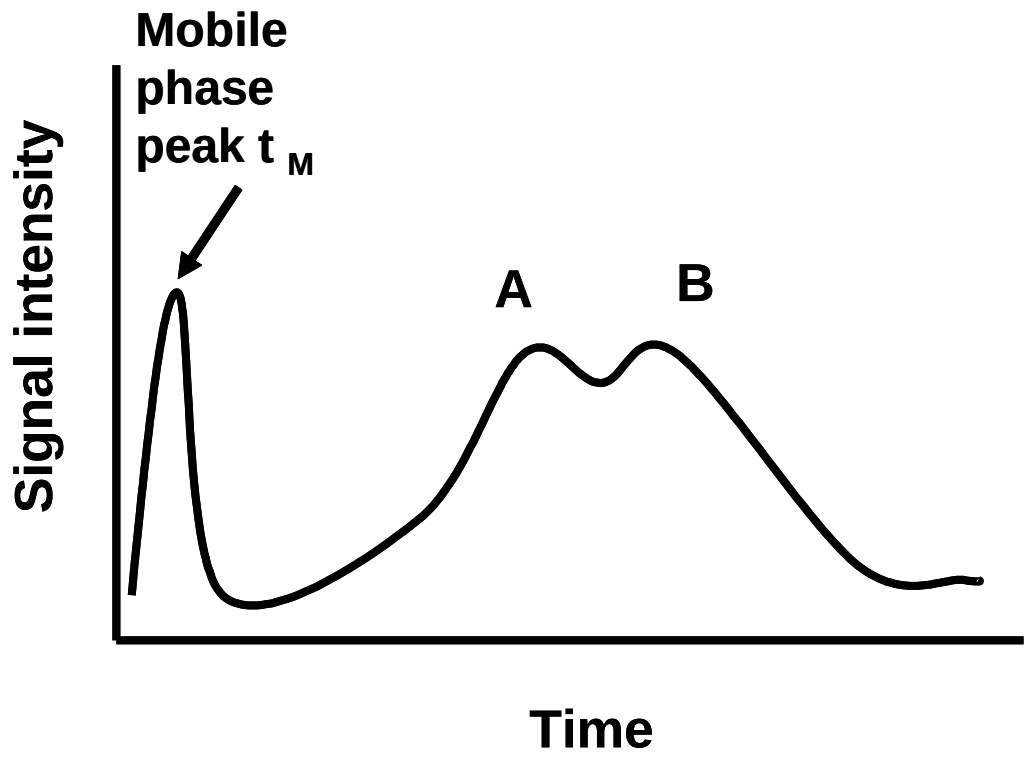
A. 

B. 

C. 

D. 

(iii) Consider the chromatogram below and suggest ways to improve the resolution (refer to the factors that affect separation) and define resolution (3 marks).



(iv) Describe the reasons for tailing and fronting in chromatography and briefly suggest strategies for reducing these problems (2 marks).

c) Write a brief note on ion exchange chromatography making reference to stationary phases, mobile phases, the basic principle of ion exchange and the applications of the technique (10 marks)