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UNIVERSITY OF RUHUNA – FACULTY OF ALLIED HEALTH SCIENCES
DEPARTMENT OF PHARMACY
FOURTH BPHARM PART I EXAMINATION – JUNE 2022
PH 4123 PHARMACEUTICAL ANALYSIS (SEQ)
TIME: TWO HOURS

INSTRUCTIONS

- Answer **all** questions in parts **A, B C** and **D**.
- No paper should be removed from the examination hall.
- Do not use any correction fluid.
- Use illustrations where necessary.

Part A

01

1.1

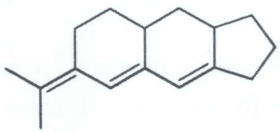
1.1.1 What is Beer's law? Write down the mathematical equation and label the symbols indicating units. (***15 marks***)

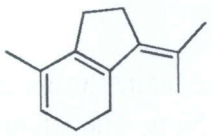
1.1.2 If the concentration of a solution is reduced by half, how will this affect the absorbance of a solution? (***10 marks***)

1.2

1.2.1 Explain the effect of conjugation in UV-vis absorption. (***05 marks***)

1.2.2 Use Woodward-Fieser rules to estimate the expected λ_{max} for the following two compounds. (***20 marks***)

1.2.2.1. 

1.2.2.2. 

1.3 The Jablonski diagram below shows some of the photophysical processes that occur after absorption of a photon by a molecule.

1.3.1 Identify each of the labelled processes **A-E** from the following list:

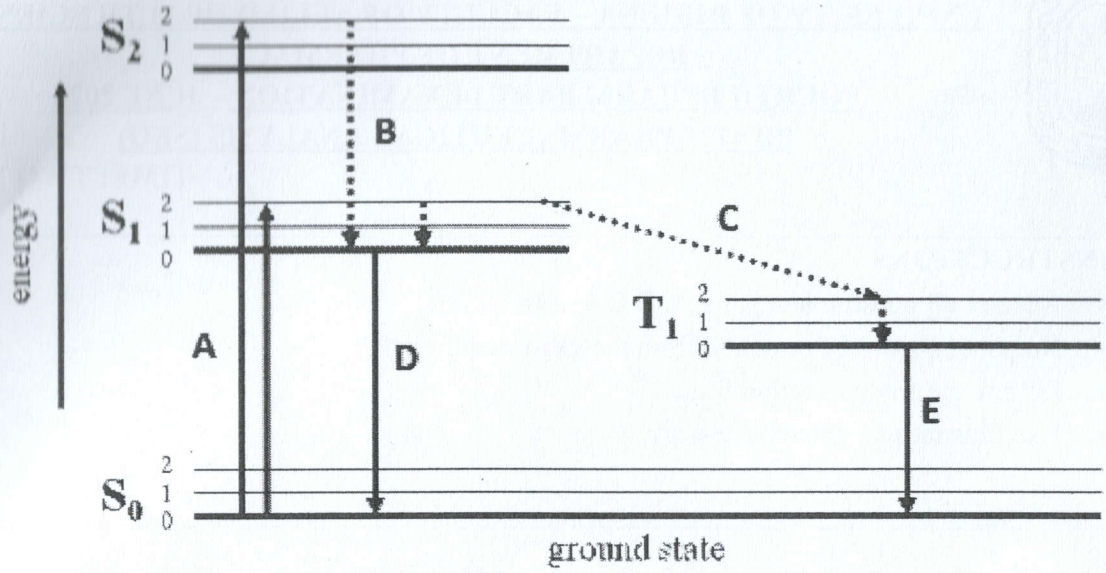
(a) Fluorescence (b) Phosphorescence (c) Internal Conversion

(d) Intersystem Crossing (e) Excitation. (***20 marks***)

1.3.2 Which state has a longer lifetime, S_1 or T_1? (***05 marks***)

1.3.3 Which of the processes involve absorption or emission of radiation? (***06 marks***)

1.3.4 Which of the processes involve the flipping of electron spin? (***04 marks***)



Jablonski diagram

1.4 Qualaquin (quinine sulphate) is an antimalarial drug used to treat malaria. During the determination of quinine sulphate in a solution of unknown concentration the following data were recorded:

Sample	Fluorescence intensity (I_F) @ 455 nm
0.5 M H_2SO_4 (blank)	28, 29, 31, 32
0.4 ppm standard	428, 430, 432
"Unknown"	330, 332, 328

1.4.1 Calculate the concentration of quinine sulphate in the "Unknown" solution. ***(15 marks)***

Note: The relationship between intensity of the fluorescence (I_F) and the concentration in solution is given by the equation: $\mathbf{I_F = k\,c}$ where I_F is the fluorescence intensity, k is a constant that depends on the sensitivity of the photomultiplier tube, the quantum yield of the fluorescence, incident power, the molar absorptivity, and the path length of the cell.

02.

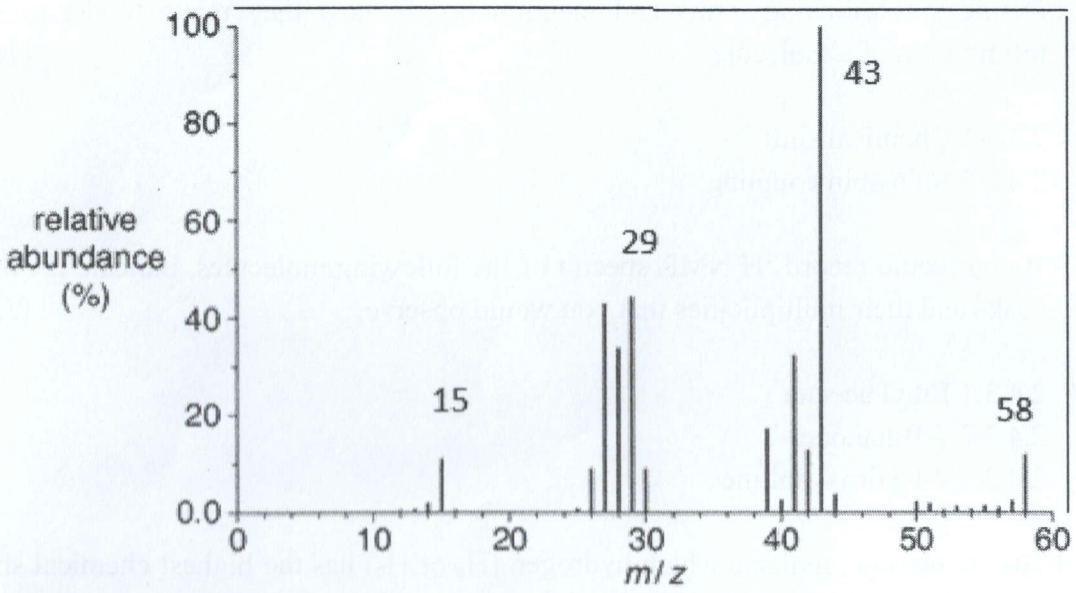
2.1

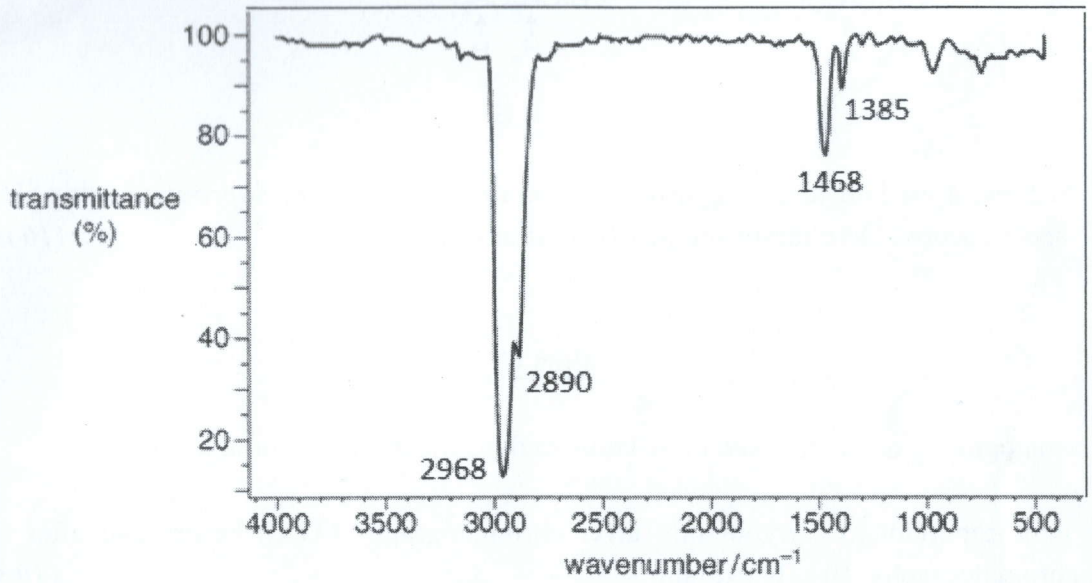
2.1.1 What are the normal modes of vibration of a polyatomic molecule? ***(10 marks)***

2.1.2 Some modern breathalysers use IR spectroscopy to detect ethanol in the breath of drunk drivers. Suggest two characteristic IR absorption bands with approximate values that could be used to identify the presence of ethanol vapour. ***(10 marks)***

2.2 Explain why the mass spectrum of 2-chloro-2-methyl propane shows molecular ion peaks at m/z values of 92 and 94 in the ratio of 3:1. (***05 marks***)

2.3 The ^1H NMR spectrum of an unknown organic compound has two signals (a quartet and a triplet) while its mass and IR spectra appear as follows.





2.3.1 Identify the fragment ions that gives rise to the peaks at m/z 15, 29, 43 and explain the peak at m/z 58. (***10 marks***)

2.3.2 Assign all key peaks in the IR spectrum to specific bonds of the unknown. (***10 marks***)

2.3.3 Propose a structure for the unknown and draw structural isomers of it. (***05 marks***)

Part B

2.4 Nuclear Magnetic Resonance (NMR) Spectroscopy is a useful technique in structure elucidation of organic molecules.

2.4.1 What are the NMR active nuclei? Give five examples. ***(05 marks)***

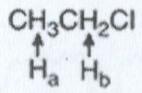
2.4.2 Define the following terms and mention briefly how they relate to derive structural information of a molecule. ***(10 marks)***

2.4.2.1 Chemical shift
2.4.2.2 Spin-spin coupling

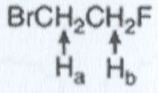
2.4.3 If you would record ^1H NMR spectra of the following molecules, indicate the number of peaks and their multiplicities that you would observe. ***(15 marks)***

2.4.3.1 Ethyl acetate
2.4.3.2 *2*-Butanone
2.4.3.3 *4*-Hydroxyaniline

2.4.4 Giving reasons, indicate which hydrogen (H_a or H_b) has the highest chemical shift in the molecules **A** and **B**. ***(10 marks)***



A



B

2.4.5 Suggest a method to distinguish *cyclopropane* and *chlorocyclopropane* using ^1H NMR spectroscopy. Give reasons to justify your answer. ***(10 marks)***

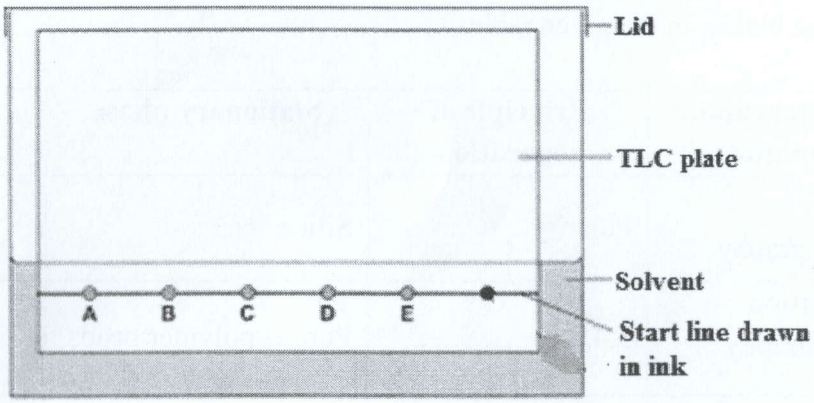
Part C

03.

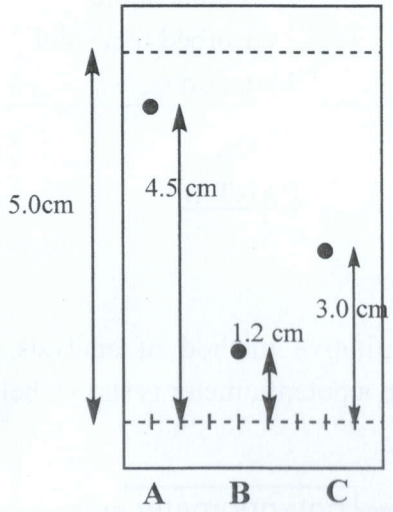
3.1 Chromatography is widely used in isolation and purification of natural products.

3.1.1 It is important to carryout thin layer chromatography (TLC) before and after column chromatography. Briefly explain. ***(10 marks)***

3.1.1 A student has set the following TLC apparatus for an analysis. Identify three errors in this apparatus and briefly explain consequences of these errors. ***(15 marks)***



3.2 Another student has set a TLC apparatus correctly using a TLC plate made up of silica. A mixture of hexane and dichloromethane was used as the developing solvent in the analysis of compounds **A**, **B** and **C**. A sketch of the resulting TLC plate visualized under UV light is shown below.



3.2.1 Calculate the R_f values of **A**, **B**, and **C**. Comment on polarities of **A**, **B**, and **C**.
(***20 marks***)

3.2.2 If he performed HPLC for a mixture of **A**, **B** and **C** using C_{18} reversed phase column and acetonitrile: water as the mobile phase, what would be the expected elution order of **A**, **B** and **C**?
(***10 marks***)

3.3 Name five types of detectors used in gas chromatography (GC). (***15 marks***)

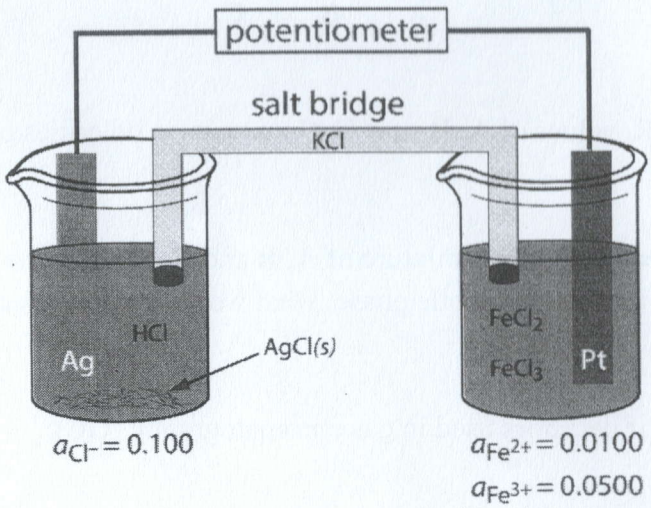
3.4 Fill the blanks in the given table. (*30 marks*)

Chromatographic technique	Principle of separation	Stationary phase	Mobile phase
Thin layer Chromatography	Polarity	Silica	3.4.1.....
Size exclusion chromatography	3.4.2.....	Porous polymer beads	3.4.3. .....
Affinity chromatography	3.4.4.....	3.4.5.....	Buffer system
Ion exchange chromatography	3.4.6.....	3.4.7.....	Aqueous solution of salts
Gas chromatography	3.4.8..... 3.4.9.....	Viscous liquid adsorbed to a solid support	3.4.10.....

PART D

04.

4.1 Potentiometry is a useful quantitative method of analysis. A schematic diagram of an electrochemical cell connected to a potentiometer is shown below:



Answer the following questions pertaining to potentiometric analysis.

4.1.1 Discuss briefly the principle behind the potentiometric analysis that makes it advantageous. (*10 marks*)

4.1.2 What are the anodic, the cathodic, and the overall reactions responsible for the potential of the given electrochemical cell? ***(10 marks)***

4.1.3 In a potentiometric electrochemical cell one of the two half-cells provides a fixed reference potential and the potential of the other half-cell responds the analyte's concentration. Identify the reference and indicator electrodes of the given cell. ***(06 marks)***

4.1.4 Give the reason(s) for separating the electrodes using a salt bridge. ***(10 marks)***

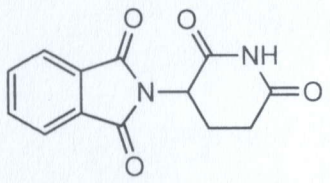
4.1.5 Write down the short hand notation for the given cell. ***(08 marks)***

4.1.6 What is the potential of the given electrochemical cell? Standard electrode potential of Fe^{2+}/Fe^{3+} couple is 0.771 V and that of AgCl/Ag couple is 0.2223 V. ***(16 marks)***

4.2 Polarimetry is a sensitive, nondestructive technique for measuring the optical activity of chiral inorganic and organic compounds.

4.2.1 What are the common usages and research advantages of polarimetry in pharmaceutical analysis? ***(10 marks)***

4.2.2 Thalidomide is a drug that is mainly used for treating leprosy and melanomas. Sketch the molecule and mark the chiral centre(s) with an asterisk (*). ***(05 marks)***



Thalidomide

4.2.3 Specific rotation is a property of a chiral compound. What is the formula for specific rotation? Name all the symbols in it. ***(05 marks)***

4.2.4 The observed rotation of a 2.0 g of compound in 50 mL solution in a polarimeter tube of 20 cm length was measured to be + 13.4°. Find the specific rotation of the compound. ***(10 marks)***

4.2.5 (S)-(+)-Monosodium glutamate (MSG) is a flavour enhancer used in many foods. If (S)-(+)-MSG has a specific rotation of +24°, what are the specific rotations of (R)-(-)-MSG and a racemic mixture of MSG. ***(10 marks)***

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