UNIVERSITY OF RUHUNA
BACHELOR OF SCIENCE SPECIAL DEGEREE LEVEL I (SEMESTER II) EXAMINATIONS- DECEMBER- 2021

SUBJECT: Chemistry
COURSE UNIT: CHE4242 (Advanced Analytical Chemistry II)

TIME: Two (02) hours

Answer **all** questions.

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01. Answer all parts

(a) A research has been conducted to determine the As and Cd concentrations of locally grown rice varieties using the electrothermal atomic absorption (EAAS) spectroscopy. As and Cd were determined at 228.8 nm and 193.7 nm wavelength and with 4 mA and 10 mA of lamp current respectively.

(i) List the main differences between EAAS and flame atomic absorption spectrometry (FAAS).

(12 marks)

(ii) What is meant by the "lamp" referred in the text (underlined) and what is the function of it.

(12 marks)

(iii) Explain how samples could have been prepared for the above analysis.

(12 marks)

(iv) Briefly explain the procedure to determine the concentrations of As or Cd of a given sample. (sample preparation steps and instrumentation details are not necessary)

(12 marks)

(b) In a similar study another group of researchers used Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) technique to determine concentrations of same elements of locally grown rice.

(i) List the key steps behind ICP-MS technique.

(18 marks)

(ii) State the main difference in principle of ICP-OES and ICP-MS.

(12 marks)

(c) Select which of the above two methods in part (a) and (b) (ie. EAAS and ICP-MS) you prefer in conducting this elemental analysis. Critically explain the reasons for your choice by arguing on the pros and cons of each method.

(22 marks)

02. Answer all parts

(a) Write down the best procedure for separating the following mixtures:

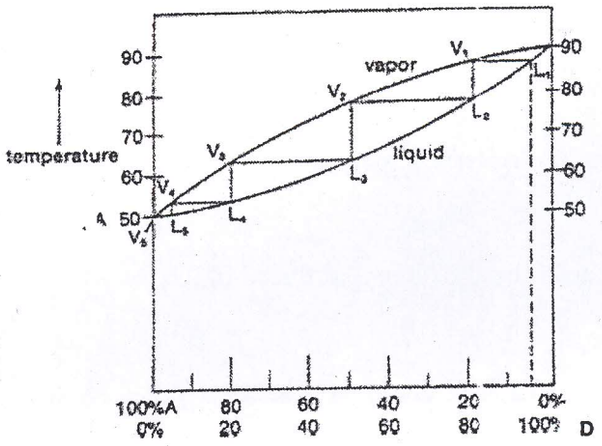
(i) Two miscible organic liquids that have over 50 °C degrees difference in their boiling points.

(05 marks)

(ii) Two miscible organic liquids which have only a few degrees difference in their boiling points.

(05 marks)

(b) Referring to the boiling point-composition diagrame given below for the mixture of A and D.



(i) What is the boiling point and composition of liquid L_1?

(05 marks)

(ii) What is the composition of its vapour?

(05 marks)

(iii) If the vapour is recondensed into a liquid, what would be its boiling point and composition?

(05 marks)

(iv) How many theoretical plates are required to get a distillate with 80% of A if the liquid L_1 is subjected to distillation?

(05 marks)

(c) (i) Define the term "distribution ratio" pertaining to solvent extraction.

(05 marks)

(iii) Relevant equilibrium reactions for the extraction of hexafluoroacetylacetone (HA) in an aqueous solution by trioctylphospine oxide ((B) into hexane are given below.

$$HA(aq) \rightleftharpoons HA(org)$$

$$HA(aq) + B(org) \rightleftharpoons HAB(org)$$
$$HA(aq) + 2B(org) \rightleftharpoons HAB_2(org)$$

(I) Write down the equations for the distribution ratio D_0 of uncomplexed HA from the aqueous phase to hexane and the equilibrium constants K_{ad1} and K_{ad2} for the formation of the adducts, HAB and HAB_2.

(15 marks)

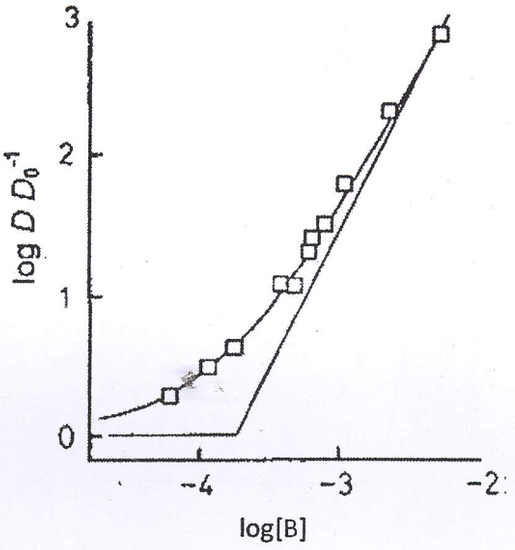
(II) Hence show that

$$DD_0^{-1} = 1 + K_{ad1}[B]_{org} + K_{ad2}\,[B]^2_{org}$$

Where D is the distribution ratio of HA from aqueous phase to hexane in the presence of adduct former B.

(20 marks)

(III) Following graph shows the relative distribution, log (D/D_0), HA as a function of the concentration of the adduct former B into hexane from 0.1M $NaClO_4$ at pH = 2.



If the fitted curve for the above curve is $D/D_0 = 1 + 10^{4.22}[B]_{org} + 10^{7.51}\,[B]^2_{org}$, determine the values of K_{ad1} and K_{ad2}.

(IV) Which adduct would predominate in the given concentration range?

(15 marks)

(d) Explain why supercritical fluids were found to be highly efficient extraction media?

(15 marks)

03. Answer all parts.

(a) Briefly describe the following which are related to Circular Dichroism (CD) spectroscopy.

(i) Linearly polarized light

(ii) Circularly polarized light

(iii) Circular dichroism

(24 marks)

(b) CD spectroscopy is a widely used analytical tool in conformational analysis of biological macromolecules.

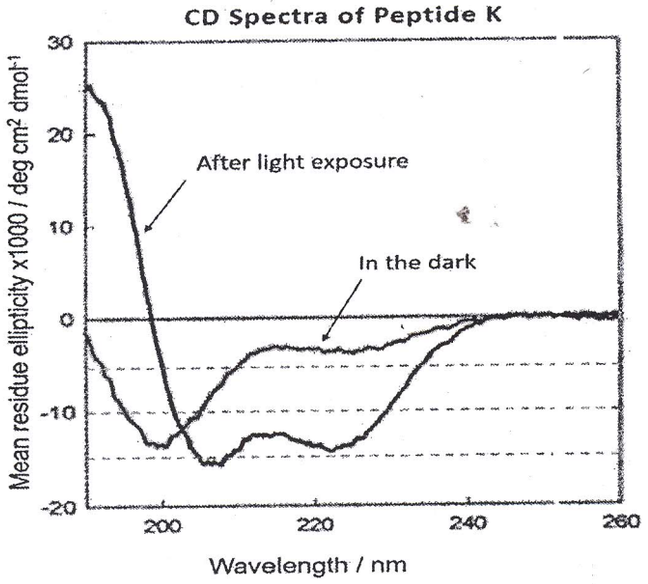
(i) What is the basic requirement of a molecule to be fulfilled to show a CD spectrum?

(08 marks)

(ii) List four advantages of the use of CD spectroscopy in conformational analysis of proteins/peptides compared to other techniques.

(08 marks)

(c) A bifunctional iodoacetamide derivative of azobenzene cross links a pair of Cys residues in the Peptide **K**. The azobenzene group adopts a *trans* conformation in the dark while it is forced to adopt a *cis* conformation in the exposure of visible light resulting a conformational change in Peptide **K**. The following CD spectra were obtained for Peptide **K** in the dark and after light exposure.



(i) Mention the specific bands in a CD spectrum which are responsible in the identification of α-helix, β-sheet and random coil conformations of a protein.

(14 marks)

(ii) Giving reasons, derive the conformation of Peptide **K** in the dark and in the presence of visible light.

(12 marks)

(iii) Estimate the helical content of Peptide **K** in both states separately assuming that the mean residue ellipticity is 32,000 for peptides having 100% helical content.

(14 marks)

(d) Near-IR (NIR) spectroscopy is used in quantitative analysis of solid and liquid samples containing OH, NH and CH bonds.

(i) List **five** types of samples which can be quantitatively analyzed using NIR spectroscopy. (05 marks)

(ii) IR spectroscopy is less straight forward in quantitative analysis compared to UV-visible method. Giving reasons justify this statement. (15 marks)

04. Answer all parts.

(a) Fluorescence and phosphorescence are two common molecular *luminescence* methods.

(i) Define the term fluorescence.

(10 marks)

(ii) State the main difference between fluorescence & phosphorescence with regard to the lifetime.

(05 marks)

(iii) Explain the reasons for (ii) using a Jablonski energy level diagram.

(10 marks)

(b) Indicate whether the following statements are **true or false**.

- If it is true, briefly **explain** the statement with reasons.
- If it is false, **correct the statement** and **give an explanation** with reasons.

(You can include diagrams in your explanations)

(i) A fluorophore is a molecule with the ability to absorb light at a particular wavelength and then emit radiation at a lower wavelength.

(15 marks)

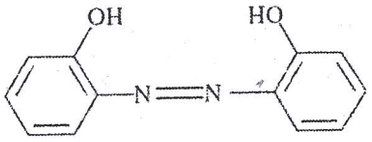
(ii) Phosphorimetry usually conducted at liquid N_2 temperature.

(10 marks)

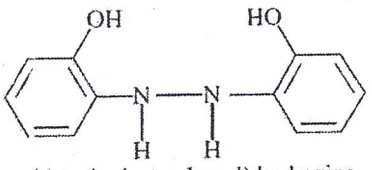
(iii) External radiation sources are not used in chemiluminescence methods.

(10 marks)

(iv) o,o'-dihydroxyazobenzene (compound A) shows lower fluorescence quantum yield than bis-(o-hydroxyphenyl) hydrazine (compound B). [Structures of the two compounds are given below.]



A. *o,o'*-dihydroxyazobenzene



B. *bis*(*o*-hydroxyphenyl) hydrazine

(15 marks)

(c) Fluorescence excitation and emission spectra of oxidized synthetic melanin (top) and oxidized natural melanin (bottom) are shown below. Fluorescence intensity is shown in arbitrary units.

(i) Explain how emission and excitation spectra are generated.

(08 marks)

(ii) Identify approximate excitation and emission peaks.

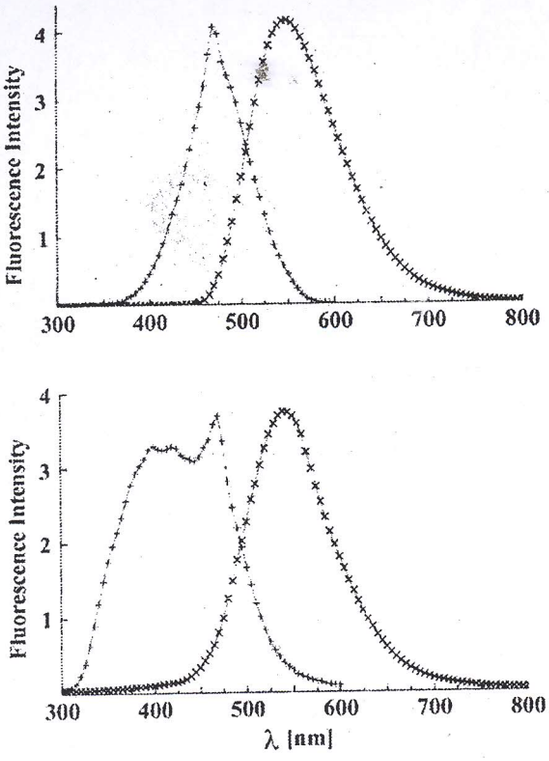
(08 marks)

(iii) Why excitation maxima are different from emission maxima? Explain.

(04 marks)

(iv) Identify the difference in top and bottom spectra and give a possible reason.

(05 marks)



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