# CHUKA UNIVERSITY

**UNIVERSITY EXAMINATIONS**

**EXAMINATION FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE**

**CHEM 241: CHEMICAL SEPARATION TECHNIQUES**

**STREAMS: BSC**
**TIME: 2 HOURS**

**DAY/DATE: MONDAY 05/07/2021** **5.00 P.M. – 7.00 P.M.**

**INSTRUCTIONS:**

- **Answer question ONE and any other TWO questions.**

**QUESTION ONE (30 MARKS)**

1. (a) (i) Why is an alkylphorus acid is preferred over carboxylic acid for the extraction of a metal ion.

($2\frac{1}{2}$ marks)

(ii) The extraction of uranyl nitrate in a solvating extractant is enhanced by the addition of equal amounts of $LINO_3$, $NaNO_3$ and $C_sNO_3$ to the aqueous phase. Arrange these alkali metal nitrates in their capability to enhance the extraction. Give reasons also.

($1\frac{1}{2}$ marks)

(iii) Why must a species be electrically neutral to take part in an interphase partitions. (1 mark)

(iv) An acidic solute, $HA$, has a $Ka\ of\ 1.00 \times 10^{-5}$ and a $K_D$ between water and hexane

of 3.0. Calculate the extraction efficiency if 50.0 $ml$ sample of a 0.025 $M$ aqueous solution of $HA$, buffered to a pH of 3.0 was extracted with 50 ml of hexane.

(2 marks)

(v) A liquid –liquid extraction of the divalent metal ion, $M^{2+¿¿}$, uses the scheme outlined in figure below. The partition coefficients for the ligand $K_D HL$ and for the metal-ligand complex $K_D, C$ are $1.0 \times 10^4$ and $7.0 \times 10^4$ respectively. The Ligand's acid dissociation constant, $Ka\, is\, 5.0 \times 10^{-5}$ and the formalation constant for the metal-Ligan complex $\beta_2$ is $2.5 \times 10^{16} M$ aqueous solution of $M^{2+¿¿}$, buffered to a pH of 1.0, was extracted with 10.0 ml of an organic solvent that is 0.1 mm in the chelating agent? What is the extraction efficiency if 100ml of a $1.0 \times 10^{-6} M$

(b) Describe the procedure you will have to follow to determine experimentally a suitable solvent for recrystallization of a crude solid organic compound if no information is given.

(5 marks)

(c) (i) Describe the three principal factors in the van deemter equation for band broadening.

(4 marks)

(ii) In a chromatographic separation, the mobile phase velocity is $4.0\,cm/s$. For a length of stationary phase of 25 m.

(I) Calculate dead (void) time and the retention time of component with the capacity factor of 1.3 and 9.0.
(4 marks)

(II) If a component in question 1 (ii) has a retention time of 3782 seconds. Calculate its capacity factor.

(III) If the mobile phase volume is 5.3 times that of the stationary phase. Calculate the value of distribution coefficient for the component.
(1 mark)

(iii) (I) Calculate the value of the number of theoretical plates and the height equivalent theoretical plate for a separation in which a peak has a retention time of 923 and the full width at half maximum of 3.5s. The length of the stationary phase is 10m.
(2 marks)

(II) Calculate the number of theoretical plates a separation system would need to resolve ¿ Resolution = 1.5) two peaks whose retention times were 824s and 829s.
(2 marks)

(iv) Comment on the usefulness of a table of boiling points in predicting the elution order of compounds separated by gas chromatoghraphy.
(2 marks)

**QUESTION TWO (20 MARKS)**

Fig 1: Representative chromatograms of the some samples taken with columns that have different resolutions

2. (a) (i) Explain the appearance of new peaks in the chromatograms of fig 1 above as the resolution increases, using Giddings and Davis theory of peak overlap.

(ii) Discuss the general elution problem in gas chromatography. (3 marks)

(iii) Suggest the solution to the general elution problem for gas chromatography. (1 mark)

(iv) Nitrogen gas exhibits a fairly low range of useful flow rates as a carrier. Hydrogen exhibits the widest range of useful flow rates among common carrier gases. Postulate on the relative advantages and disadvnatges of using $H_2$ versus $N_2$ to explain why $N_2$ is more commonly used than $H_2$

($1\frac{1}{2}$ marks)

(v) State and explain the type of GC detector you would choose for analyzing urine samples from patients taking low levels of thiazide diuretics.

($1\frac{1}{2}$ marks)

2. (a) Comment on the following statements

(i) Maximal dead volume is a desired characteristic of pulse dampers of flow smootheners used in liquid chromatography.

(1 mark)

(ii) Quard column used in liquid chromatography allows particles that cause precipitation upon contact with stationary or mobile phase.

($\frac{1}{2}$ mark)

c. (i) Dicarboxylic acids $HO_2C(CH_2¿_nCO_2H)$ can not be analyzed easily directly by GC. However by converting them to diesters $CH_3O_2C(CH_2)nCO_2CH_3¿$ good GC separation and detection is possible. What is the major effect of this derivatization that leads to improvement

(1 mark)

(ii) What is the purpose of flow restrictor in supercritical fluid chromatography. ($\frac{1}{2}$ mark)

(iii) Explain why supercritical fluid chromatography is particularly good for preparative separations. (1 mark)

(iv) List a change to the mobile phase that will decrease retention in anion exchange chromatography if the stationary phase is the strong anion exchanger type. (1 mark)

(v) A biochemist analyzing triglycerides by normal phase HPLC wants to perform a gradient using the eluents and methylene chloride. State with reason which of the solvent. It's percentage should be increased in the gradient run. (1 mark)

(d) Alkylamines, such as those listed below are difficult to separate by HPLC using silica based $C_{18}$ columns. The amine groups are relatively polar and the $PK_a$ are given for their conjugate acids

(i) Indicate why separations are difficult with standard silica-based C18 columns. (2 marks)

(ii) Indicate an alternative way of using a type of liquid chromatography to separate them and also indicate the type of column and the type of element needed (including pH) for successful separation

**QUESTION THREE (20 MARKS)**

3. (a) A researcher is analyzing sediments that have been contaminated by various solvent. The major interest is particularly in quantifying halogenated solvents using $GC \times GC$ With flame ionization detection on sediment extracts. The first column is non-polar while the second column is polar. The following 2D chromatogram is produced where stars show location of halogenated solvents and circles show locations of non-halogenated solvents

(i) Assuming the peaks had widths shown by the circles of stars, was 2DGC needed to separate out all observed ($r_s, a\,1\,D$ separation using either column)? ($\frac{1}{2}$ mark)

(ii) What can be said of the polarity of the halogenated compounds vs that of the non-halogenated compounds? ¿ mark)

(iii) Suggest a way in which the halogenated compounds would be well resolved and quantified using only 1DGC with different detector. (1 mark)

(b) The gas chromatogram shown below was for the analysis of $1\,\mu$L of liquid sample (reaction products) injected using a split ratio of 1:4 (one part in 4 injected into the column), with separation on a $0.25\,mm \times 15\,m$ open tubular with $0.2\,\mu m$ film thickness (normally a high efficiency column)

(i) Indicate a problem in how the GC was run in connection with the quality of the chromatogram. ($1\frac{1}{2}$ marks)

(ii) Indicate a change in conditions which could result in an improved chromatogram. (1 mark)

(c) An HPLC separation is performed with a 4.6 mm (diameter) by 150 mm length amino column (polar stationary phase) with $5\mu m$ diameter packaging material using 60% ethyl acetate/ 40% methanol as eluent. To speed up the separation the chemist wants to switch to 3.5 $\mu$m diameter packaging in a 100 mm column. Assuming the flow rate, mobile phase composition and $\beta$ (volume ratio) are the same, answer the following questions.

(i) Using calculations, estimate the percent change (including direction) in the pressure. (1 mark)

(ii) Using calculations, estimate the percent change (including direction in $N$ the number of theoretical plates) (1 mark)

(iii) Explain why is it that after the switch, lower than calculated values of the number of theoretical plates ($N$) are observed for early eluting peaks. (1 mark)

(d) (i) Discuss why affinity chromatography can be used to separate proteins. (1 mark)

(ii) Briefly discuss how would you determine the quaternary of antibody (structure shown below) using a combination of gel filtration (size exclusion) chromatography and SDS. PAGE polyacrylamide gel electrophoresis) (2 marks)

(iii) Discuss the main difference between high-performance thin layer chromatography and the classical thin layer chromatography in connection with process steps. ($3\frac{1}{2}$ marks)

(iv) Explain the basic principle of reverse plane chromatography and its applicability to paper chromatography and thin layer chromatography. (1 mark)

(v) Explain reverse phase paper chromatography and thin layer chromatography and in what respect are these different from normal paper chromatography and thin layer chromatography. (2 marks)

(vi) Enumerate reasons for lack of modern developments in paper chromatography whereas in case of thin layer chromatography plate concept has been adopted and it has been further developed as HPTLC. (2 marks)

**QUESTION FOUR (20 MARKS)**

4. (a) (i) Account for importance of suppressor in the ion chromatography. (6 marks)

(ii) Describe the separation mechanism in ion-pair chromatography. (5 marks)

(b) (i) What are the strengths of capillary electrophoresis compared to other separate techniques? (2 marks)

(ii) List advantages and disadvantages of micellar electro kinetic chromatography. (3 marks)

(iii) Imagine that you need to separate a mixture that contains the components given in the table below. You decide to separate the components by capillary zone

electrophoresis in an untreated capillary with the injection done at the positive end of the capillary and the detector is at the negative end what is the elution order of the component?
Explain why the components come out in this order. (4 marks)

| Analyte | Charge | Radius |
|---|---|---|
| 1 | +1 | 1.0 |
| 2 | -2 | 5.0 |
| 3 | 0 | 10.0 |
| 4 | -1 | 3.0 |
| 5 | 0 | 25.0 |
| 6 | +1 | 3.0 |
| 7 | +3 | 2.5 |

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