

Registration Number:

Date & session:



**ST. JOSEPH'S UNIVERSITY, BENGALURU-27**

**M.Sc. (CHEMISTRY) – 2<sup>nd</sup> SEMESTER  
SEMESTER EXAMINATION: APRIL 2024**

(Examination conducted in May/June 2024)

**CH 8522: SEPARATION TECHNIQUES**

**(For current batch students only)**

**Time: 2 Hours**

**Max Marks: 50**

**This paper contains four printed pages and three parts**

**PART-A**

**Answer any EIGHT of the following:**

**(8 x 2 = 16)**

1. Draw the structure of dimethyl glyoxime and mention its significance in metal ion extraction.
2. What is the difference between isocratic elution and gradient elution?
3. Name any two chiral stationary phases used in chiral liquid chromatography.
4. How do gas-liquid and gas-solid chromatography differ?
5. Why is CO<sub>2</sub> a preferred mobile phase for super critical fluid chromatography?
6. What is the basis of separation in TLC?
7. How does eluent strength of solvents affect elution?
8. What is the role of (i) matrix (ii) spacer arm in affinity chromatography?
9. Write van Deemter equation for plate height.
10. Briefly explain 2D TLC.

**PART-B**

**Answer any TWO of the following:**

**(2 x 12 = 24)**

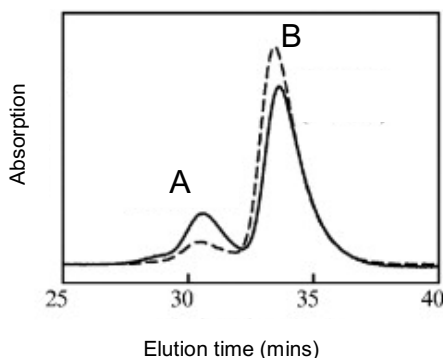
11. a) Explain how does pH affect the extraction of a compound that undergoes only dissociation in water with a pK<sub>a</sub> value, 5.  
b) An analyte A may be extracted from an aqueous solution into chloroform for which the K<sub>D</sub> is 80. If 50 ml of aqueous solution containing 2.00 x 10<sup>-2</sup> mmol of A is extracted with 25 ml of chloroform, what will be the amount of A extracted into the organic phase?  
c) What is bonded stationary phase in HPLC? Write the chemical reaction involved in the preparation of bonded stationary phase.  
d) Explain the principle of gel filtration chromatography. Mention its application. (3+3+3+3)
12. a) Calculate the number of theoretical plates obtained for a column of length 20 cm, as the stationary phase particle size varies from 6, 4 and 1 μm. Comment on the resolution of the chromatograms obtained in the above cases, assuming that other parameters remain constant for the column.

- b) An unknown liquid sample is analyzed using paper chromatography using solvent X as the mobile phase. One spot is observed after the paper is developed and visualized. The same unknown sample is re-analyzed using solvent Y as the mobile phase. This time, three spots are observed after the paper is developed and visualized. Is the unknown sample a pure substance or a mixture? Explain your answer, including a possible reason for the different observations in the two experiments.
- c) Explain ion pair chromatography. (4+4+4)
13. a) Name the appropriate detector to be used in gas chromatography for the following samples/purposes:
- for halogenated organic compounds.
  - to obtain element specific information.
  - for quantitative analysis of an organic compound whose concentration is in the range of fg / mL ( $\text{fg} = 10^{-15} \text{ g}$ ) in a sample mixture.
- b) Explain the significance of on-column injection. Write any two differences between on-column injection and a split injection.
- c) Describe the principle and working of Ni- Immobilized Metal Affinity Chromatography (Ni-IMAC). (3+3+6)

### PART-C

**Answer any TWO of the following: (2x 5= 10)**

14. (a) The acidic solution containing trimethylamine (A), diethylamine (B) and methylamine (C) ( $\text{pK}_a$  of cations 9.8, 10.8 and 10.6 respectively) was loaded on a cation exchange column. Write the order of their elution (in increasing order of retention time) with a gradient of increasing pH > 7. Justify your answer.
- (b) A pure protein sample of 20 kDa molecular weight was passed through a gel filtration chromatographic column. The chromatogram given below, obtained using a UV detector (both dashed line and solid line corresponds to UV absorption of the protein) had two peaks, A and B. The molecular weight of the fractions collected from A and B peaks determined by further biophysical analysis were found to be 40 kDa and 80 kDa.

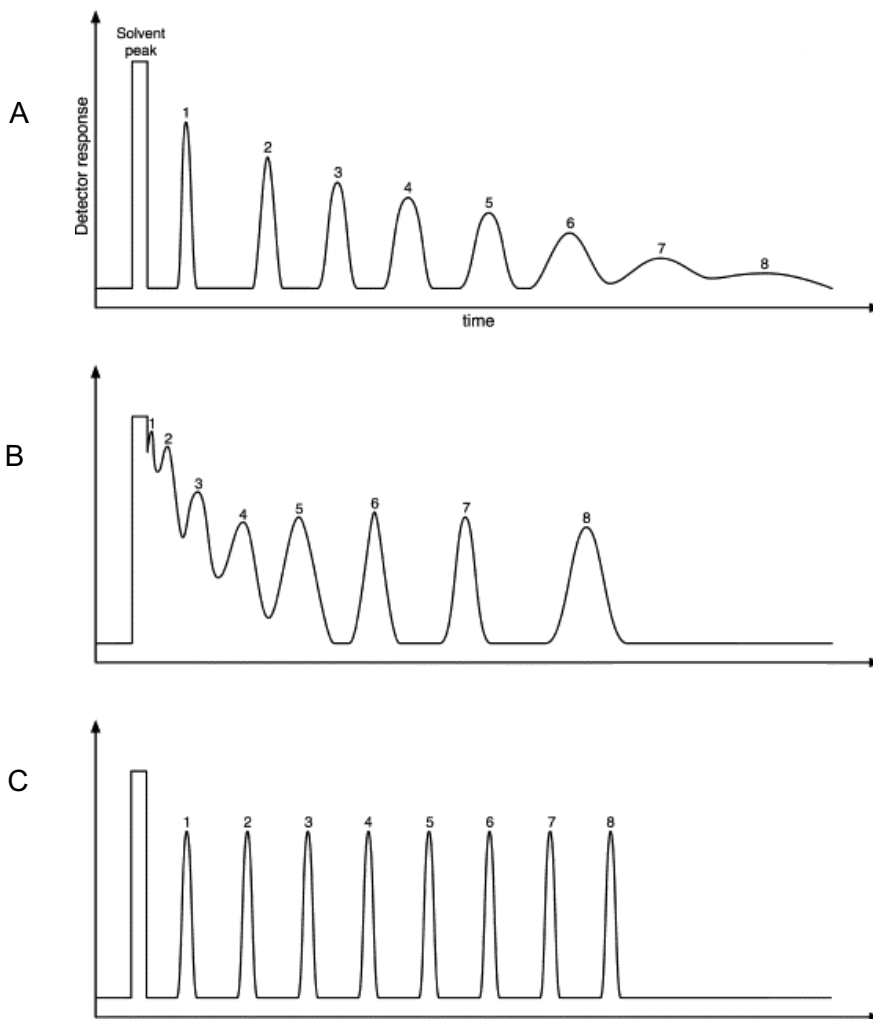


P.T.O

i) Why did the pure protein sample give two peaks that do not match with the molecular weight of the protein?

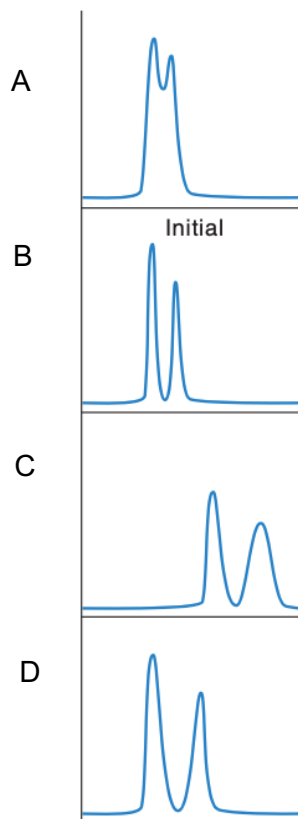
ii) Assign the molecular weights obtained from biophysical experiments to the peaks A and B. (3+2)

15. An analyst performed gas chromatographic analysis of a sample containing 8 compounds using an open tubular column with a non-polar stationary phase at 60° C. Analyze the problem with the chromatogram (A). The analyst performed two more analysis using the same sample with certain modifications in the procedure and obtained chromatograms B and C. What modification did he do to obtain chromatograms B and C? Explain.



P.T.O.

16. The liquid chromatographic separation of two compounds gave the chromatogram, A. In order to improve resolution, the analyst performed three different LC separations where he increased only one of the three different parameters,  $N$  (number of theoretical plates),  $k'$  (capacity factor) and  $\alpha$  (separation factor) in each separation and obtained the chromatograms B, C and D respectively.



- i) Name which parameter is increased in each of the chromatograms B, C and D.
- ii) Suggest any possible way the analyst tried to increase any two of the parameters.

(3+2)

.....end of questions.....