

**ST. JOSEPH'S COLLEGE (AUTONOMOUS), BENGALURU-27**

M.Sc. CHEMISTRY: II SEMESTER

**SEMESTER EXAMINATION: APRIL - 2019**

**CH-8518 : SEPARATION TECHNIQUES**

- Note :** (i) The question paper has **five printed pages** and **three parts**. All parts are compulsory.  
(ii) Answer any **SIX** out of eight questions from part – A, Any **FOUR** out of six questions from part – B, and any **two** out of three questions from part – C.  
(iii) 2 Graph sheets are provided.

**Time : 2 ½ hrs**

**Max .Marks : 70**

**PART A**

**2 X 6 = 12**

1. An organic acid ( $K_a = 3 \times 10^{-5}$ ) has a distribution coefficient of 18.0 between water and methyl isobutyl ketone. If 100 ml of an aqueous solution of the acid buffered at pH 6.00 is extracted with 100 ml of methyl isobutyl ketone, calculate the fraction of the organic acid remaining in the aqueous phase.
2. In a gas chromatographic separation of alkanes the retention times are 1.21min for methane, 7.33 min for hexane 7.66 min for an unknown and 8.41min for heptane. Find the **Kovats index** for the unknown.
3. What does the notation -XN after the name of the resin indicates? (Eg: Dowex 1 x X4).
4. Write the nature of stationary phase and mobile phase in **HILIC**. What type of analytes can be separated by this method?
5. What are the three different types of buffers used in various stages of affinity chromatography? Explain why the spacer arm should not be too short or too long in the stationary phase of affinity chromatography.
6. Write the advantages of  $\text{CO}_2$  as the mobile phase in supercritical fluid chromatography.
7. With an example explain the principle of immobilized metal affinity chromatography.
8. Predict the order of elution of cis and trans isomers of 1,2- dimethoxy ethene in a mixture from a silica column. Justify your answer.

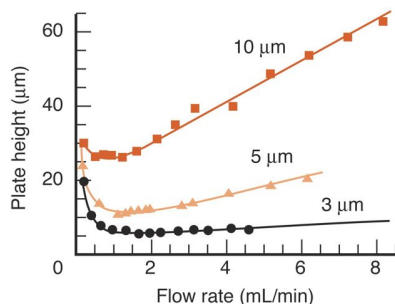
**PART B**

**12 x 4 = 48**

9. (a) (i) Write down the chemical equations for various equilibria involved in the extraction of Fe(III) into the ether layer from a 6M HCl aqueous solution. (ii) It is observed that if the HCl concentration is increased up to 6M, the extraction increases. Beyond 6M, the extraction decreases. Give the reason for this. (iii) Suggest an organic solvent for the extraction of Fe(III) at higher pH. (iv) Give an example for the extraction of an inorganic anion by ion-pair formation (v) How does the oxidation state of the cation of the salting-out agent influence the efficiency of the extraction? Why ammonium salts are generally preferred salt as salting out agents in solvent extraction?

(b) Two organic weak acids HA and HB have  $K_a$  values of  $2 \times 10^{-6}$  and  $2 \times 10^{-4}$  respectively. Their distribution coefficient between water and ether layer are 12.2 for HA and 8.3 for HB. Design a separation procedure for the extraction of ~ 99% of HA from the aqueous solution while retaining ~ 99% of HB in the aqueous solution. (7 + 5)

10. (a) Consider a chromatographic experiment in which two components with capacity factor  $k_1' = 4.00$  and  $k_2' = 5.00$  are injected into a column. The base width  $W$  and  $W_{1/2}$  are 1.26 and 0.74 for peak 1 and 1.52 and 0.90 for peak 2 respectively. The retention time of the less retained component  $t_{r1}$  is 10.0 min. Calculate (i)  $t_m$  (ii) retention time for component 2;  $t_{r2}$ . (iii)  $N$ , number of theoretical plates for peak 1 & 2 using  $W$  and  $W_{1/2}$  separately. (iv) the resolution between the two peaks.



(b) Consider the following experimental data collected for separations on three columns identical in all ways except for the size of the stationary phase particles (3, 5 or 10  $\mu\text{m}$ ).

- (i) Which column would show the best resolution for two similar solutes? What aspect(s) of the graph above allowed you to make this decision? (ii) Use the van Deemter equation to explain the differences in these three plots. (iii) What disadvantage in a chromatographic separation can result from a decrease in particle size? (7 + 5)

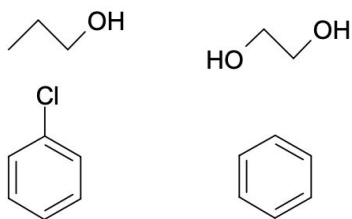
11. (a) Explain the functioning of the flame ionisation detector. Give the advantages and disadvantages of the detector.

(b) A mixture containing pentane, hexane, heptane, octane, and butanol are separated using a column with nonpolar stationary phase. Write the expected order of elution and give an explanation for your answer.

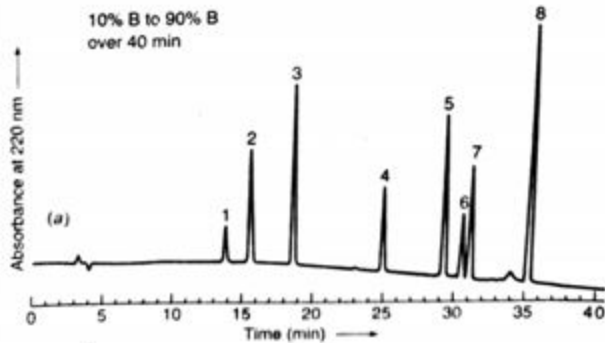
(c) Explain Purge and Trap method as used in Gas chromatography.

(d) A GC separation was conducted on a sample containing a pesticide analyte, compound X. This sample was treated with an internal standard of Q, giving a concentration of 15.0 ppm. A 1.0  $\mu\text{L}$  injection onto the GC gave an FID response of 1012 for Q and 3411 for X. A 1.0  $\mu\text{L}$  standard solution of 30.0 ppm X with 15.0 ppm of Q was injected giving a response of 899 and 2791 respectively. What is the concentration of X in the sample? (4 x 3)

- 12.(a) Predict the elution order of the following solutes in reversed phase HPLC. Give the reason/s for your conclusion.



(b) A mixture of 8 compounds were separated in HPLC with a broad gradient of 10% acetonitrile to 90% acetonitrile in 40 minutes. The chromatogram is given below. With necessary calculations from the data available in the chromatogram, will you suggest a gradient elution or isocratic elution for this separation? Draw a solvent gradient (time from injection Vs % of acetonitrile) on the given graph sheet. The dwell time is 5 minutes. From the graph suggest the condition for the second run. (4+8)



With necessary calculations from the data available in the chromatogram, will you suggest a gradient elution or isocratic elution for this separation? Draw a solvent gradient (time from injection Vs % of acetonitrile) on the given graph sheet. The dwell time is 5 minutes. From the graph suggest the condition for the second run. (4+8)

13. (a) Poly styrene standards of known molecular mass gave the following calibration data in a size exclusion column. Find the formula mass (FM) of the unknown which eluted with a retention time of 13 minutes.

FM	Retention time	FM	Retention time
$8.50 \times 10^6$	9.28	$6.60 \times 10^4$	12.74
$3.04 \times 10^6$	10.07	$2.85 \times 10^4$	13.38
$1.03 \times 10^6$	10.88	$9.20 \times 10^3$	14.20
$3.30 \times 10^5$	11.67	$3.25 \times 10^3$	14.96
$1.56 \times 10^5$	12.14	$5.80 \times 10^2$	16.04

(b) Lithium ion in an aqueous solution is exchanged for the sodium ion in the cation exchange polystyrene resin. Write an expression for the selectivity coefficient. Explain why the selectivity of alkali metal ion increases with the extent of crosslinking of the resin.

(c) Explain suppressed ion anion chromatography. (4 x 3)

14. (a) Consider a random mixture containing 87.0 weight% diamond (density 3.51 g/ml) and 13.0 weight % SiC (density 3.23 g/ml) with a uniform spherical particles of radius 0.100 mm. (i) Calculate the mass of a single particle of diamond and SiC each (ii) Calculate the number of diamonds and SiC in a sample of 1g of this mixture (iii) Calculate the relative sampling standard deviation in the number of particles of each type in 1g of the sample. (b) In a sampling of 4 trials, the relative standard deviation are given in brackets for each mass of the analyte taken for analysis. 0.54 g (6.33%) ; 1.78 g (3.21%) ; 4.22 g (2.00 %) ; 8.63 g (1.55 %). (i) Calculate the mean sampling constant  $K_s$ . (ii) What mass are required for a  $\pm 1\%$  sampling standard deviation?

- (c) Explain (i) solid phase extraction and (ii) preconcentration in sample preparation  
 (d) Name 3 types of interactions of chiral molecule in cyclodextrin stationary phase.  
 (6+3+3)

### Part C

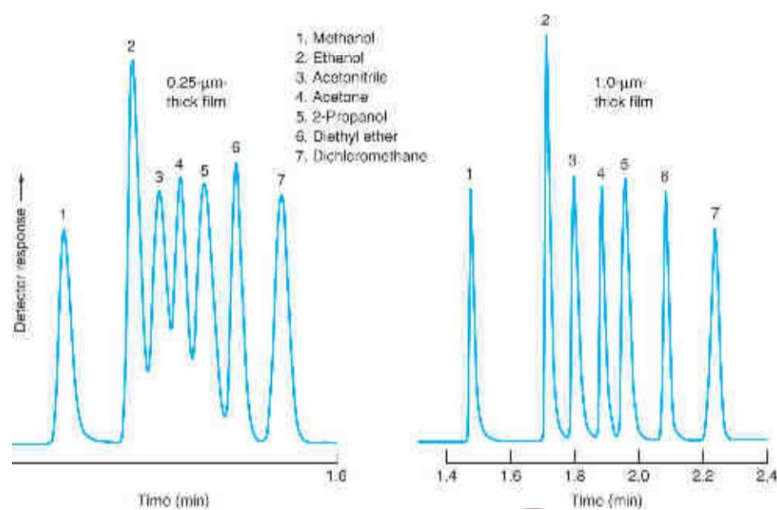
15. A chemist was trying to develop a separation of organic compounds by normal phase chromatography. He first tried separating chlorobenzene, nitrobenzene, toluene, and xylene on a phenyl bonded phase column using dichloromethane as the mobile phase and a fixed wavelength UV detector set at 254 nm. While chlorobenzene and nitrobenzene were adequately resolved on the column, toluene and xylene were found to coelute. He felt that, less polar carbon tetrachloride would improve the separation of toluene and xylene. To his surprise, when carbon tetrachloride was used as the mobile phase eluent, none of the solutes injected were detected under the same conditions. Puzzled, he analyzed the effluent from the hplc system by gas chromatography and discovered that the four solutes had in fact eluted from the column. What error of mobile phase selection did he make? Discuss all his decisions like selection of the stationary phase, mobile phases, detector etc. and comment whether his decisions are correct or wrong. Some details of the solvents are given below:

Carbon tetrachloride		Dichloromethane	
Molecular weight	153.82	Molecular weight	84.93
Boiling point	77°C	Boiling point	40°C
UV cut off	265 nm	UV cut off	233 nm
Viscosity	0.90cP	Viscosity	0.41cP
Dielectric constant	2.24	Dielectric constant	8.9

16. Alkaloids were analysed using TLC on silica gel G with three different solvents. Solvent 1: chloroform/acetone/diethylamine(5:4:1); Solvent 2: chloroform/diethylamine (9:1); Solvent 3: cyclohexane/chloroform/diethylamine (5:4:1). The  $R_f$  for different alkaloids are given in the table below. Which solvent would you choose to analyse
- (a) a sample seized by the police in a drug smuggling case and suspected to contain morphine, cocaine and aconitine?  
 (b) a mixture of reserpine and cocaine?  
 (c) a poison containing strychnine and reserpine? Justify your answer.  
 Also justify, why the other solvent is not suitable for the analysis of each mixture.

Alkaloid	solvent 1	solvent 2	solvent 3
Morphine	0.10	0.08	0.00
Quinine	0.19	0.26	0.07
Codeine	0.38	0.53	0.16
Cocaine	0.73	0.90	0.65
Strychnine	0.53	0.76	0.28
Aconitine	0.68	0.90	0.35
Reserpine	0.72	0.80	0.20

17. Two chromatograms show a group of solvents separated by GC on two capillary columns of different stationary phase thickness: 0.25  $\mu\text{m}$  and 1.0  $\mu\text{m}$  (left and right respectively). Both columns are 15m long, have internal diameter of 0.32mm and are coated with the same stationary phase. Temperature and carrier gas settings were also identical. Helium gas was used as the carrier gas in both cases with a linear velocity of 38cm/s and the oven temperature was 40°C.



stationary phase. Temperature and carrier gas settings were also identical. Helium gas was used as the carrier gas in both cases with a linear velocity of 38cm/s and the oven temperature was 40°C.

According to Van Deemter equation the  $C_s \propto d_p$ . From this relation one should expect that column with 0.25  $\mu\text{m}$  phase has higher efficiency. But the resolution is much better in the column with 1.0  $\mu\text{m}$  phase!

Explain why. Consider the peak

# 5, eluting at 2.0 min to do the necessary calculations.