ST. JOSEPH'S COLLEGE (AUTONOMOUS), BENGALURU-27
M.Sc. CHEMISTRY: III SEMESTER
Special Supplementary Examination, JUNE 2019

CH-9216 : SEPARATION AND ELECTROCHEMICAL TECHNIQUES

Note : (i) The question paper has **four printed pages** and **three parts**. All parts are compulsory.

(ii) **Graph sheet will be provided on request.**

(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.

Supplementary candidates only.

Attach the question paper with the answer booklet

Time : 2 ½ hrs **Max .Marks : 70**

PART A **6 x 2 =12**

1. Analyte A may be extracted from an aqueous solution into CCl_4 for which the K_D is 80. If 50 ml of aqueous solution containing 2.00 x 10^{-2} mmol of A is extracted with 25 mL of CCl_4, what will be the amount of A remaining in the aqueous phase?
2. Calculate the approximate radius of a column which will separate a pair of analytes in a 20 mg sample with same resolution given by 2 mg of the same sample in 0.5 cm radius column. The stationary phase and the chromatographic process are the same in both cases.
3. Calculate the resolution of two chromatographic peaks which are separated by 3σ, assuming both peaks are perfect Gaussian.
4. Sketch one chromatogram each for the separation of following analytes in (a)polar stationary phase (b) non polar stationary phase. The analytes are #1) n-heptane #2) tetrahydro furan #3) 2-butanone and #4) n-propanol.
5. Explain the importance of spacer arm in affinity chromatography. Mention three buffers used in affinity chromatography.
6. Separation by capillary isoelectric focusing is based on differences in equilibrium properties of the analytes rather than on differences of rates of migration- Substantiate.
7. Draw the excitation and response signal in the case of square wave voltammetry.
8. What is meant by half wave potential? What is its significance?

PART B **4 x 12 = 48**

9. a) Two weak organic acids HA (K_a= 1 x 10^{-6} and K_D= 12.2) and HB (K_a= 3 x 10^{-4} and K_D= 8.3) are in an aqueous solution. Suggest a method to separate ~99% HA from aqueous solution retaining ~99% HB in aqueous solution.

   b) A gas chromatographic method for the separation of a mixture of cyclohexane, t-butanol and benzene on a 10 m capillary column gave the following data:

Parameter	Compound A	Compound B	Compound C
t_R	3 min 20 s	3 min 30 s	3 min 45 s
$W_{1/2}$	4.6 s	5.1 s	6.2 s
W_b	8 s	9 s	11 s

Calculate the (i) number of theoretical plates for Compound A using $W_{1/2}$ and W_b (ii) the average HETP in mm using the results from the previous calculations (iii) resolution between Compound A / Compound B peaks and Compound B/ Compound C peaks.

(6+6)

10. (a) Answer the following with respect to gas chromatography:
(i) What is the advantage of temperature programming in gas chromatography?

(ii) What is the advantage of an open tubular column over a packed column? Does a narrower or wider open tubular column provide higher resolution?

(iii) When would you use split and splitless injection?

(iv) To which kind of analytes do the flame ionisation detectors and electron capture detector respond?

(v) Explain headspace analysis. (5 x 2 = 10)

b) Explain hydrophilic interaction chromatography. (2)

11. (a) Alkyl amines, such as ethanamine (pK_a = 9.50) and butanamine (pK_a = 10.64), are difficult to separate by HPLC using silica based C18 columns. The amine groups are relatively polar and the pKa values are given for their conjugate acids.
i) Indicate why separations are difficult with standard silica-based C18 columns.

ii) Indicate an alternative way of using a type of liquid chromatography to separate them - Mention the type of column and the type of eluent needed (including pH) for successful separation.

iii) Write the elution order of three analytes A,B and C in normal phase as well as in reverse phase columns. The solute polarity is given as A>B>C.

iv) What change in the mobile phase will bring the peaks closer in each case of the problem (iii)?

v) There is a leftover of a water /acetonitrile 80:20 mixture. How will you prepare 1 liter of water/acetonitrile 65:35?

vi) Predict the elution order of ortho, meta and para methyl anilines in a silica column (normal phase chromatography) (6 x 2 = 12)

12. (a) The following gel permeation chromatography was performed and the data are provided: The data within the brackets are their molecular weight and Ve. bovine serum albumin dimmer (MM=134,000Da, Ve=14.9 mL), bovine serum albumin (MM = 67,000Da, Ve =17.3 mL), ovalbumin (MM= 43,000Da Ve=18.8 mL), ribonuclease (MM=13,700 Ve=22.5 mL). Estimate the molecular mass for two different proteins with elution volume 16.5 mL and 20.3 mL.

(b) Write the Van Deemter equation and explain the factors affecting each terms. Plot HETP Vs linear velocity for each term and show the optimum velocity to achieve the least HETP.

(c) Explain (i) Supercritical Fluid Extraction (ii) Microwave Assisted Solvent Extraction

(d) A mixture contains 1% KCl particles and 99% KNO_3 particles. If 10^4 particles are taken, what is the expected number of the KCl particles? What is the standard deviation if the experiment is repeated many times? (4+4+2+2)

13. a) Write the structure of silica glass membrane indicating singly charged ions within the structure, write the symbolic representation and explain the hygroscopicity of glass electrode.

b) Explain with relevant schematic diagram the process of separation of anions based on capillary isotachophoresis. (6 +6)

14. a) With the help of relevant plots explain how do you carry out an amperometric titration and get the equivalence point if the solute as well as the reagent is electroactive.

b) With relevant expressions discuss how do you extract qualitative information from voltammetry?

c) Describe the construction of LaF_3 electrode and explain the mechanism of potential development and how it can be employed to measure fluoride ion concentrations.

(4+4+4)

Part C **2 x 5 =10**

15. A researcher was trying to develop a separation of organic compounds present in industrial sewage by normal phase adsorption chromatography. She first tried separating chlorobenzene, nitrobenzene, toluene, and xylene on a silica 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 silica column, toluene and xylene were found to coelute. Being an expert at liquid chromatography, she realized a less polar mobile phase solvent would improve the separation of toluene and xylene. When carbon tetrachloride was used as the mobile

phase eluent, none of the solutes injected were detected under the same conditions. Puzzled, she 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 she make? Discuss all her decisions. Explain the statements are correct or wrong.

Carbon tetrachloride		Dichloromethane	
Molecular weight	153.82	Molecular weight	84.93
Boiling point	77^0C	Boiling point	40^0C
Refractive Index	1.457	Refractive Index	1.421
UV cut off	265nm	UV cut off	233 nm
Viscosity	0.90cP	Viscosity	0.41cP
Dielectric constant	2.24	Dielectric constant	8.9

16. In a chromatographic equipment, a solution containing 0.0837M of analyte X and 0.0666M Internal Standard give peak area of A_x= 423 and A_{IS}= 347. To analyze an unknown sample, 10.0 mL of 0.146 M IS was added to 10.0 mL of X, and the mixture was diluted to 25.0 mL. This mixture gave a chromatography spectrum with area A_x= 553 and A_{IS}= 582. Calculate the concentration of the analyte X.

17. a) Balaji caries out a polarographic reduction of Zn^{+2}, Cu^{+2}, Cd^{+2} and Pb^{+2} in potassium nitrate using DME by stripping voltammetric method using DME as the working electrode. He applies a an anodic deposition potential under unstirred condition at the rate of 15 mV s^{-1} for 120 seconds in order to pre-concentrate the ions at the surface of the working electrode. After allowing it to equilibrate for 10 seconds under stirred condition, he applies cathodic potential at a sweep rate of 50 mV s^{-1} with a pulse amplitude of 50 mV s^{-1}. Unfortunately Balaji was unable to see any stripping peaks. Identify any four mistakes committed by him which would justify that this experiment was not at all carried out according to the correct protocol.

    b) What are the constituents required to fabricate a crystalline membrane ion selective electrode for the determination of Copper. (2+3)