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Sr. No. of Question Paper : 1564-F C Roll No.....

Unique Paper Code : 217467

Name of the Course : B.Sc. (P)

Name of the Paper : Analytical Chemistry – 4, Separation Methods – II  
(ACPT-404)

Semester : IV

Duration : 3 Hours

Maximum Marks : 75

**Instructions for Candidates**

1. Write your Roll No. on the top immediately on receipt of this question paper.
2. Answer any five questions.

1. (a) In a chromatography experiment, a solution containing 0.0837 M 'X' and 0.066 M 'S' gave peak areas of  $A_X = 423$  and  $A_S = 347$ . (Areas are measured in arbitrary units by the instrument's computer). To analyze the unknown, 10.0 mL of 0.146 M S were added to 10.0 mL of unknown, and the mixture was diluted to 25.0 mL in a volumetric flask. This mixture gave a chromatogram with peaks areas  $A_X = 553$  and  $A_S = 582$ . Find the concentration of X in the unknown.  
  
(b) Occasionally in gel chromatography values of  $K_d > 1$  are observed. How might this phenomenon be accounted for?  
  
(c) How is an adsorbent activated?  
  
(d) Define distribution ratio, D, in partition chromatography. What happens if the numerical value of D is very small or too large?  
  
(e) 100 ml of 0.150 M acetic acid are shaken with 10 g of alumina until equilibrium is attained. The adsorbent is filtered off and 50.0mL aliquot requires

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65.0 mL of 0.112 M NaOH to reach the phenolphthalein end point. Calculate the specific adsorption (mg/g) of acetic acid on alumina. (4+1+2+3+5)

2. (a) Why is programmed temperature chromatography useful ?
  - (b) Describe the relative response factor method for analyzing gas chromatograms.
  - (c) What do you understand by the term *dead space* or *gas hold up* of a column ? How this gas hold is up measured for a GLC column ?
  - (d) In gas chromatography for separation of xylene, benzene and toluene the areas under respective peaks were 53.2, 31.0 and 14.5 square centimeters. Calculate the percent composition of the sample. (3+5+2+5)
3. (a) Describe two solids used as support in liquid-liquid partition chromatography.
  - (b) Enumerate the experimental factors that determine the success of adsorption chromatographic separation.
  - (c) Define an adsorption isotherm. When does an L adsorption isotherm occur ?
  - (d) Explain how each of the following variables affect the retention volume of a solute in partition chromatography :
    - (i) The temperature at which the column is operated.
    - (ii) Solubility of the solute in stationary phase.
    - (iii) The density of packing of the column assuming the total volume of the packed column is constant. (4+5+3+3)

4. (a) A gel filtration column is prepared using Sephadex gel packing which excludes molecules whose molecular weights are larger than 500,000. The volume within the gel particles which is available for accepting solutes is 220 mL. The void volume is 60 mL.
- (i) What is the expected retention volume ( $V_R$ ) for a protein of molecular weight 700,000. Explain.
  - (ii) What is the expected  $V_R$  for a protein of molecular weight 137,000 which can freely penetrate all the pores of the gel? Explain.
- (b) An analyte eluted from a chromatographic column as a Gaussian peak with retention time of 7 min 45 sec and a peak base width of 30 sec. Calculate
- (i) The number of theoretical plates in the column
  - (ii) The plate height if the column was 7.5 cm long.
- (c) Describe any two methods used for measurement of peak areas in Gas Liquid chromatography. (5+5+5)
5. (a) What is the unique property of biomolecules that is exploited in affinity chromatography?
- (b) What are the characteristics that an ideal affinity chromatography matrix must possess?
- (c) Give a diagrammatic representation of purification of an enzyme by affinity chromatography. (5+5+5)
6. Write short notes on any **three** of the following :
- (a) Thermal conductivity detector

(b) Supports for reverse phase chromatography

(c) Isoelectric focusing

(d) SDS-PAGE

(e) Reciprocating pumps

(f) Gradient mixers

(5×3)