Your Roll No

B.Sc. Prog./III   **J**

AC–301   Separation Methods in Analytical Chemistry

(NC – Admissions of 2005 & onwards)

Time   3 Hours   *Maximum Marks   75*

*(Write your Roll No on the top immediately
on receipt of this question paper)*

Attempt **FIVE** *questions in all
Question No* **1** *is compulsory
All questions carry equal marks*

1   (a) Describe in detail the steps you would take in order to pack an anion exchange column You are provided with the dry beads of the exchanger in chloride cycle and the packed column is required in hydroxyl cycle

   (b) 100 ml 0 150 M acetic acid are shaken with 10 g of alumina until equilibrium is attained The absorbe is filtered off and 50 0 mL aliquot require 20 mL of 0 112 M NaOH to reach the phenolphalein end point Calculate the specific adsorp (mg/g) of acetic acid on alumina

                                        P T O

(c) Solute A has a partition coefficient of 3 between toluene and water, with three times as much in the toluene phase Suppose that 100 mL of a 0 010M aqueous solution of A is extracted with toluene What fraction of A remains in the aqueous phase (i) if one extraction with 500 mL is performed or (ii) if five extractions with 100 mL are performed ? Which extraction is more efficient ?

2   (a) Explain how gel filtration can be used for desalting a protein sample obtained after ammonium sulphate precipitation

   (b) An experiment was carried out to monitor the enzyme activity of a protease The protease of molecular weight 56,000 Da was used to digest a membrane protein of molecular weight 65,000 Da into two protein fragments of 43,000 and 22,000 Da Gel filtration chromatography was used to monitor the reaction and could detect the presence of four major protein fractions Identify the four proteins fractions and the order i which they elute

3   (a) Define isoelectric pH of a compound

   (b) What is the principle underlyi Electrophoresis ? Enumerate the factors that aft the mobility of a protein during electrophore

   (c) Describe how isoelectric foing is used for separation of proteins

(d) Does a single band at the isoelectric point mean you have obtained a single pure protein ? Explain

4 (a) What are the main types of gas chromatography ? How does temperature programming improve separations ?

   (b) Describe the factors that affect the selectivity of ion exchange resins

   (c) Enumerate the experimental factors that determine the success of adsorption chromatographic separation

5 (a) Define a theoretical plate and give an expression that relates HETP (plate height) to peak width and retention time

   (b) Define an adsorption isotherm When does an S adsorption isotherm occur ?

   (c) In a normal-phase partition column chromatography, a solute was found to have a retention time of 58 2 minutes, while an unretained species had a retention time of 2 10 minutes when the mobile phase was 50% by volume chloroform and 50% n-hexare Calculate k' for the solute

6 (a) Why is the chief equilibrium process in paper chromatography considered to be partition ? What are the two solvents involved ? Which is the

stationary one and which is the mobile one ? Where does the stationary liquid phase come from ?

(b) What type of distribution processes can be used in a thin-layer form to separate mixtures of solutes? Why should the developing chamber be saturated with the vapor of the eluent, especially when using mixed solvents, before putting the thin-layer plate in it ?

(c) 0 00396 g of bromine (Br_2) is dissolved in 50 0 mL of water This solution is shaken with 10 0 mL of carbon tetrachloride Calculate the number of milligrams of bromine present in each layer at equilibrium K_D for bromine (CCl_4/H_2O) is 27 00 at 25°C

7 (a) All HPLC systems include pumps to force the mobile phase through the column Enumerate the desirable characteristics of a pump for use in HPLC

(b) Describe the different methods by which a column chromatogram can be developed

(c) A mixture of benzene, toluene, and methane was injected into a gas chromatogaphic column Methane gave a sharp peak in 42 s, whereas benzene required 251 s and toluene was eluted in 333 s Find the adjusted retention time and capacity factor for each solute and the relative retention

(300)****