CHUKA UNIVERSITY

UNIVERSITY EXAMINATIONS

EXAMINATIN FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE (CHEMISTRY)

CHEM 241: SEPARATION TECHNIQUES

STREAMS: BSC (CHEM) **TIME: 2 HOURS**

DAY/DATE: MONDAY 15/4/2019 **11.30 A.M. – 1.30 P.M.**

INSTRUCTIONS: Answer question ONE and any other TWO questions

QUESTION ONE (20 MARKS)

(a) (i) In paper chromatographic separation of silver, lead and mercury, the solvent front was 18cm while fronts due to these elements were respectively 16, 12 and 16cms. Calculate R_f value of all metals [2 marks]

(ii) In a particular thin layer chromatographic separation, the R_f value of an unknown compound was 0.809. The fronts, due to compound A, B, C were 24,28, 30 cms and the solvent front was 34 cms. Identify the unknown compound [2 marks]

(b) (i) To decrease the plate height and yet increase the resolution, what courses of action are available? What penalties may accrue for each approach [3½ marks]

(ii) During the separation of carbohydrates using a bonded aminoalkyl functional group, an increase in the water concentration of the acetonitrile/water mobile phase decreases retention. Is the bonded phase acting in the "normal" or "reverse" mode? Give reason for your answer [½ mark]

(iii) Linear alkyl benzene sulpnonates are the major surfactants household detergents at the present time. Therefore the detection of such substances, and their separation based upon the alkyl chain length in environmental samples is desirable. Reverse phase technique using a methanol/water solvent resulted in only two major peaks and no peaks for individual alkyl members. Success is achieved if the ion-pair technique is used. Predict the effect of counterion size on retention considering ammonium, tetramethylammonium andtetrabutylammonium chlorides [2 marks]

(iv) Amphiprotic compounds such as the monofunctional amino acids are difficult to chromatograph

(I) What two different approaches could be used if ion exchange is selected? [2 marks]

(II) Reverse phase ion pair chromatography also offers two approaches. What are they [½ marks]

(v) By separation of adenosine mono, di, and triphosphate, nucleotides (AMP, ADP and ATP) was accomplished in a little over 3 min using 0.4M KH_2PO_4 (Plus 3% methanol) and a 15 cm by 2 cnm column packed with 10- μm particles of silica to which was bonded a 3- aminopropyl siloxane phase. The mobile phase viscosity was 1.4 cP. Flow rates was 100ml hr^{-1} at an inlet pressure of 2900psi. Suggest improvements (with reasons) in the operating procedure [1 mark]

(vi) Assume there is 1.000g of mixture to which is added 100mg internal standard measurement of the resultant chromatogram shows four components (including the internal standard) with areas (in arbitrary units) as follows

$A_1 = 27, A_{std} = 90, A_2 = 20, A_3 = 70$, and area sum = 197. Calculate the amount of component 3 present in the sample. Comment on your answer. [1 mark]

(c) (i) State two disadvantages which result from isothermal mode of operation of the gas chromatography [2 marks]

(ii) Briefly discuss the technique of programmed temperature gas chromatography [3 marks]

(iii) Explain why most of researchers prefer using a narrow bore column instead of the wider bore packed with same material while using HPLC for analysis [3½ marks]

(d) (i) Explain why organic modifiers such as methanol, ethanol etc. are added to supercritical fluid during analysis using supercritical fluid chromatography [2 marks]

(ii) Discuss the advantages of supercritical fluid extraction technique over organic solvents extraction methods [5 marks]

QUESTION TWO (20 MARKS)

(a) (i) Draw a well labeled pressure temperature diagram of CO_2 showing different regions [2 marks]

(ii) The table below provides a list of common chromatographically useful supercritical fluid mobile phases, along with pertinent physical data of each. Gives merits or demerits of each of them

Supercritical fluids	T_c °C	P_c (atm)	Dipole moment (Debye)
CO_2	31.3	72.9	0.00
NH_3	132.5	112.5	1.47
Pentane	196	33.3	0.00
SF_6	45.5	37.1	0.00
Xe	16.6	58.4	0.00

P_c=critical pressure

T_c = critical temperature [3½ marks]

(b) (i) Compare capillary electrophoresis and high performance liquid chromatography techniques [3 marks]

(ii) Discuss the advantages of capillaries into electrophoresis [3 marks]

(iii) Describe various factors which should be considered for selection of a buffer for capillary electrophoresis [4 marks]

(iv) In a hydrodynamic injection, a pressure difference of $2.5\,x\,10^3$ pa

$(a\,DP = 0.02\,atm)$

Was applied for 25 to a 75cm long capillary tube with an internal diameter of 50 μm. Work out the volume and length of sample which was injected, assuming

that the buffers viscosity was 10^{-3} $kg\,m^{-1}s^{-1}$ [2 marks]

(c) (i) Occasionally in size exclusion chromatography (SEC) values of $K>1$ are observed. Account for this phenomenon [1 mark]

(ii) Gelpermeation chromatography is to be used to separate a mixture of four polystyrene standards of molecular mass: 9200, 76000, $1.1x10^6$ and $3x10^6$ daltons. Three columns are available for this exercise. They are prepacked with gel with the following fractionation ranges for molecular weights

$A:70000¿4x10^5 daltons$

$B:10^5$ to $1.2x10^6$ daltons

$C:10^6$ to $4x10^6$ daltons

Suggest ways of separating these four polymers in a single operation if it is permitted to use two of the above columns end to end [1½ marks]

QUESTION THREE (20 MARKS)

(a) (i) Discuss the demerit of capillary electrochromatography [2½ marks]

(ii) A mixture of two compounds A and B migragtes from the origin to leave two spots with the following characteristics (migration distance x and spot diameter w)

$x_A=27\,mm$ $w_A=2.0\,mm$

$x_B=33\,mm$ $w_b=2.5\,mn$

The mobile phase front was 60mm from the starting line

(I) Calculate the retardation factor R_f, the efficiency N and the HETP H for each compound [4½ marks]

(II) Calculate the resolution factor between the two compounds A and B [1 mark]

(III) Establish the relationship between the selectivity factor and the R_f of the two compounds. Calculate its numerical value [5 marks]

(iii) An electrophoresis analysis in free solution (capillary electrochromatography) calls for the use of a capillary of 32 cm and with effective length of separation 24.5 cm. the applied voltage is 30kV. Under the conditions of the experiment the peak of a neutral marker appeared upon the electropherogram at 3 min

(I) Calculate the electrophoretic mobility of a compound whose migration time is 2.5 min. give the answer in precise units [4 marks]

(II) Calculate the diffusion coefficient under these conditions for this compound given that the calculated plate number is 80,000 [1 mark]

(b) A mixture of proteins is separated on a column with a stationary phase of carboxymethylated cellulose. The internal diameter of the column is 0.75 cm and its length is 20cm. the dead volume is 3 ml. the flow rate of the mobile phase is 1ml/min. the pH of the mobile phase is adjusted to 4.8. Three peaks appear upon the chromatogram corresponding to the elution volumes V_1, V_2 and V_3 at 12 mL, 18mL and 34mL respectively

Does this arise from an anionic cationic phase? Give reasons for your answer [2 marks]

QUESTION FOUR (20 MARKS)

(a) The method of internal normalization was chosen to determine the mass of a sample comprising a mixture of four esters of butanoic acids. To this end, a reference solution containing known % mass of these esters led to the following relative values of the response coefficients often butanoates of methyl (ME), of ethyl (EE) and of propyl (PE), all three in ratio with butyl-butanoate (BE)

$K_{ME/BE} = 0.919$ $K_{EE/BE} = 0.913,$ $K_{PE/BE} = 1.06$

From the chromatogram of the sample under analysis, reproduced below, and the information given in the table, find the mass composition of this mixture (ignore the first peak at 0.68 min)

(b) (i) Discuss the advantages of linking high performance liquid chromatography with mass spectrometry [2 marks]

(ii) What capabilities are required of the combination of high performance liquid chromatography and mass spectrometer? [3 marks]

(iii) What problems, if any, have to be addressed to allow the LC-MS combination to function, and function effectively? [2 marks]

(c) (i) Describe a convenient way of sampling volatile samples for GC analysis [1½ marks]

(ii) Methanol and ethanol are separated in a capillary GC column with retention times of 370 and 385 s respectively, and half widths ($W_{\frac{1}{2}}$) of 9.42 and 10.0s. An unretained butane peaks occurs at 10.0s. Calculate the separation factor and the resolution [3½ marks]

(d) Assume that in extraction from water into toluene analyte "A" has the distribution ratio D=10. A 20ml portion of an aqueous solution of A is extracted with toluene. Which of the following procedure will result in the most efficient removal of A from the aqueous phase into toluene

(I) One extraction with 40ml of toluene

(II) Two extractions with 20ml of toluene each

(III) Four extractions with 10ml of toluene each [3 marks]
