CHUKA UNIVERSITY

UNIVERSITY EXAMINATIONS

EXAMINATION FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE AND BACHELOR OF EDUCATION (SCIENCE)

CHEM 241: SEPARATION TECHNIQUES

SREAMS: BED SCI &BSC **TIME: 2 HOURS**

DAY/DATE: TUESDAY 11/04/2023 **11.30 A.M. – 1.30 P.M.**

INSTRUCTIONS

- **Answer question one and any other two questions**
- **Useful data are provided**

General data and fundamental constants

Quantity	Symbol	Value	Power of ten	Units
Speed of light	c	2.997 925 58*	10^{8}	$m\ s^{-1}$
Elementary charge	e	1.602 176	10^{-19}	C
Faraday's constant	$F = N_A e$	9.648 53	10^{4}	$C\ mol^{-1}$
Boltzmann's constant	k	1.380 65	10^{-23}	$J\ K^{-1}$
Gas constant	$R = N_A k$	8.314 47		$J\ K^{-1}\ mol^{-1}$
		8.314 47	10^{-2}	$dm^3\ bar\ K^{-1}\ mol^{-1}$
		8.205 74	10^{-2}	$dm^3\ atm\ K^{-1}\ mol^{-1}$
		6.236 37	10	$dm^3\ Torr\ K^{-1}\ mol^{-1}$
Planck's constant	h	6.626 08	10^{-34}	J s
	$\hbar = h/2\pi$	1.054 57	10^{-34}	J s
Avogadro's constant	N_A	6.022 14	10^{23}	mol^{-1}
Atomic mass constant	m_u	1.660 54	10^{-27}	kg
Mass				
electron	m_e	9.109 38	10^{-31}	kg
proton	m_p	1.672 62	10^{-27}	kg
neutron	m_n	1.674 93	10^{-27}	kg
Vacuum permittivity	$\varepsilon_0 = 1/c^2\mu_0$	8.854 19	10^{-12}	$J^{-1}\ C^2\ m^{-1}$
	$4\pi\varepsilon_0$	1.112 65	10^{-10}	$J^{-1}\ C^2\ m^{-1}$
Vacuum permeability	μ_0	4π	10^{-7}	$J\ s^2\ C^{-2}\ m^{-1}\ (= T^2\ J^{-1}\ m^3)$
Magneton				
Bohr	$\mu_B = e\hbar/2m_e$	9.274 01	10^{-24}	$J\ T^{-1}$
nuclear	$\mu_N = e\hbar/2m_p$	5.050 78	10^{-27}	$J\ T^{-1}$
g value	g_e	2.002 32		
Bohr radius	$a_0 = 4\pi\varepsilon_0\hbar^2/m_e e^2$	5.291 77	10^{-11}	m
Fine-structure constant	$\alpha = \mu_0 e^2 c/2h$	7.297 35	10^{-3}	
	α^{-1}	1.370 36	10^{2}	
Second radiation constant	$c_2 = hc/k$	1.438 78	10^{-2}	m K
Stefan–Boltzmann constant	$\sigma = 2\pi^5 k^4/15h^3c^2$	5.670 51	10^{-8}	$W\ m^{-2}\ K^{-4}$
Rydberg constant	$R = m_e e^4/8h^3 c\varepsilon_0^2$	1.097 37	10^{5}	cm^{-1}
Standard acceleration of free fall	g	9.806 65*		$m\ s^{-2}$
Gravitational constant	G	6.673	10^{-11}	$N\ m^2\ kg^{-2}$

Exact value

QUESTION ONE (30 MARKS)

a) (i) Describe the principle of solvent extraction. (2 marks)

(ii) Outline the disadvantages of solvent extraction (1 mark)

(iii) Why must a species be electrically neutral to take part in an interphase partition? (1.5 marks)

(iv) Why would the species involved in interphase partition have different concentrations in the two phases if the chemical potential are equal? (1 mark)

(v) Why is an extraction more efficient if the extractant liquid is used in several fractions instead of all at once? (1.5 marks)

b) You wish to extract I_2 from 100ml of aqueous phase solution. Take K_d of I_2 for extraction with CCl_4 is 85.

(i) What fraction of I_2 remains in the aqueous phase after a single extraction with 100ml of CCl_4 (1 mark)

(ii) What fraction of I2 remains in the aqueous phase after 10 extractions with 10ml of CCl_4 (total CCl_4 volume of 100ml)? (1 mark)

(iii) Using five extractions, what is the minimum volume of CCl_4 required to achieve a 99.9% extraction of the I_2 (2 marks)

c) (i) How is it that moving one of the phases in the partition convert a difference in partition coefficient to a difference in velocity (1.5 marks)

(ii) What is the capacity factor and how is it related to the partition coefficient? (1 mark)

(iii) What is a height equivalent theoretical plate and how is it related to band broadening? (1.5 marks)

(iv) Describe the three principal factors in the Van Deemter equation for band broadening. (4 marks)

d) (i) In a chromatographic separation, the mobile phase velocity is 4.0cm/s.For a length of stationary phase of 25m calculate void time and the retention of component with capacity factor values of 1.3 and 9.0 (2.5 marks)

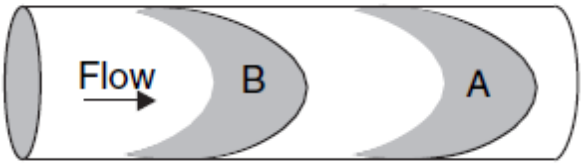
(ii) Explain the usefulness of a table of boiling points in predicting the elution order of compounds separated by gas chromatography (1.5 marks)

(iii) Why is peak area better related to analyte amount than peak height for the flame detector? (1 mark)

(iv) Why is an internal standard so often used with gas chromatography (0.5 marks)

e) (i) Occasionally in SEC value of $k>1$ are observed. How might this phenomenon be accounted for? (1 mark)

(ii) Imagine that following a capillary isoelectric focusing experiment, two polypeptides A and B are immobilized in one section of the capillary as indicated in the figure. The isoelectric point of B is superior to that of A, complete the figure indicating how the pH would increase at the positive end of the capillary while showing the orientation of the corresponding electric field. (3 marks)



(iii) Write short notes on paper chromatography. (2.5 marks)

QUESTION TWO (20 MARKS)

a) Substances A and B have retention times of 16.40 and 17.63min, respectively, on a 30.0-cm column. An unretained species passes through the column in 1.30 min. The peak widths (at base) for A and B are 1.11 and 1.21 min, respectively.

Calculate;

(i) The column resolution (1 mark)

(ii) The average number of plates in the column (1.5 marks)

(iii) The plate height, (1 mark)

(iv) The length of column required to achieve a resolution of 1.5 (2 marks)

(v) The time required to elute substance B on the column that gives an R_s value of 1.5 (1.5 marks)

(vi) The plate height required for a resolution of 1.5 on the original 30-cm column and in the original time. (1.5 marks)

b) (i)

The relative areas for the five gas chromatographic peaks obtained in the separation of five steroids are given next. Also shown are the relative responses of the detector to the five compounds. Calculate the percentage of each component in the mixture.

Compound	Peak Area, Relative	Detector Response, Relative
Dehydroepiandrosterone	27.6	0.70
Estradiol	32.4	0.72
Estrone	47.1	0.75
Testosterone	40.6	0.73
Estriol	27.3	0.78

(2.5 marks)

(ii) Explain the characteristics of the ideal detector for GC (4 marks)

iii)

What is meant by temperature programming in GC? Why is it frequently used?

(2 marks)

iv)

Discuss why the combination of GC and mass spectrometry is so powerful.

(2 marks)

v)

What properties should the stationary-phase liquid for GC possess? (1 mark)

QUESTION THREE (20 MARKS)

i) Define

(i) ion-pairing chromatography (1 mark)

(ii) Ion chromatography (1 mark)

ii) Assume that in extraction from water into toluene, analyte A has the distribution ratio $D = 10$. A 20-mL portion of an aqueous solution of A is extracted with toluene. Which of the following procedures will result in the most efficient removal of A from the aqueous phase into toluene?

(a) One extraction with 40mL of toluene;

(b) Two extractions with 20mL of toluene each;

(c) Four extractions with 10mL of toluene each? (2 marks)

iii)

A chromatogram of a two-component mixture on a 25-cm packed LC column is shown in the following figure. The flow rate was 0.40 mL/min.

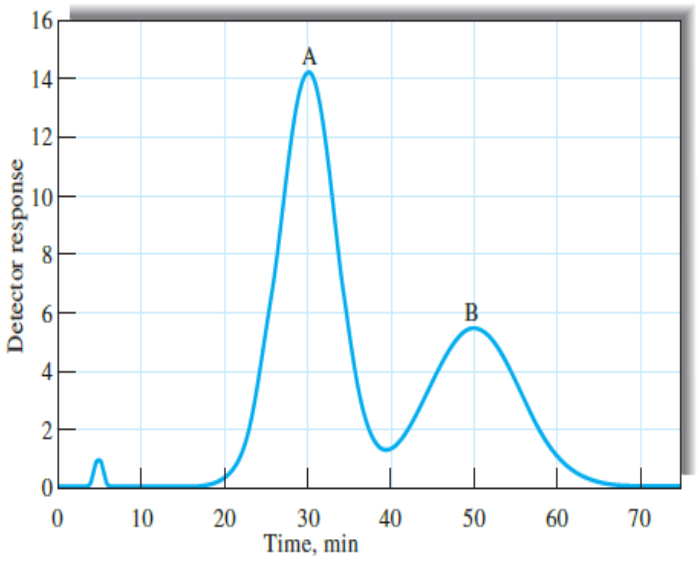
(a) Find the times that components A and B spend in the stationary phase.

(b) Find the retention times for A and B.

(c) Determine the retention factors for the two components.

(d) Find the full widths of each peak and the full width at half-maximum values.

(e) Find the resolution of the two peaks.



(f) Find the average number of plates for the column.

(g) Find the average plate height.

(h) What column length would be needed to achieve a resolution of 1.75?

(i) What time would be required to achieve the resolution in part (h)?

(j) Assume that the column length is fixed at 25 cm and the packing material is fixed. What measures could you take to increase the resolution to achieve baseline separation?

(k) Are there any measures you could use to achieve a better separation in a shorter time with the same column as in part (j)?

a)1.5 marks b)1 mark c)1 mark d)1 mark e)1 mark f)2 marks g)1 mark h)2 marks i) 1 mark j)1 mark k) 2 marks

QUESTION FOUR (20 MARKS)

a**)** What is the effect of pH on the separation of amino acids by electrophoresis? Why? (2 marks)

b) What is the principle of separation by capillary zone electrophoresis? (2 marks)

c) A certain inorganic cation has a electrophoretic mobility of $5.13 \times 10 cm^2\ s^{-1}\ V^{-1}$. This same ion has a diffusion coefficient of $9.1\times\ 10^{-6}\ cm^2\ s^{-1}$. If this ion is separated by capillary zone electrophoresis with a 50-cm capillary, what is the expected plate count, *N*, at applied voltages of

(i) 5 kV? (1 mark)
(ii) 10 kV? (1 mark)
(iii) 20 kV? (1 mark)
(iv) 30 kV? (1 mark)

d) The cationic analyte of Problem c was separated by capillary zone electrophoresis in a 50-cm capillary at 20 kV. Under the separation conditions, the electroosmotic flow rate was 0.65 mms^{-1} toward the cathode. If the detector were placed 40 cm from the injection end of the capillary, how long does it take in minutes for the analyte cation to reach the detector after the field is applied? (2 marks)

e) In an interesting paper, Zheng and coworkers (J. Zheng, L. T. Taylor, J. D. Pinkston, and M. L. Mangels, J. Chromatogr. A, 2005, 1082, 220, DOI: 10.1016/j.chroma.2005.04.086) discuss the elution of polar and ionic compounds in SFC.

(i) Why are highly polar or ionic compounds usually not eluted in SFC? (1 mark)

(ii) What types of mobile-phase additives have been used to improve the elution of highly polar or ionic compounds? (1 mark)

(iii) Why is ion-pairing SFC not often used? (1 mark)

(iv) Why are ammonium salts sometimes added as mobile-phase modifiers in SFC? (1 mark)

(v) The authors describe an SFC system that uses mass spectrometry (MS) as a detector. Discuss the interfacing of an SFC unit to a mass spectrometer. Compare the compatibility of SFC with MS to that of HPLC and GC with MS. (1 mark)

(vi) The authors studied the effect of column outlet pressure on the elution of sodium 4-dodecylbenzene sulfonate on three different stationary phases with five mobile-phase additives. What effect was observed, and what was the explanation for the effect? (1 mark)

(vii) What elution mechanisms were considered by the authors? (1 mark)

(viii) Which mobile-phase additive gave the fastest elution of the sulfonate salts? Which provided the longest retention times? (2 marks)

(ix) Did a silica column give results similar to or different from a cyano bonded-phase column? (1 mark)

PERIODIC TABLE OF THE ELEMENTS

Metals
Nonmetals
Metalloids

	IA 1	IIA 2	IIIB 3	IVB 4	VB 5	VIB 6	VIIB 7	VIIIB 8	VIIIB 9	VIIIB 10	IB 11	IIB 12	IIIA 13	IVA 14	VA 15	VIA 16	VIIA 17	0 18
1	1 H 1.008																1 H 1.008	2 He 4.0026
2	3 Li 6.941	4 Be 9.0122											5 B 10.81	6 C 12.011	7 N 14.007	8 O 15.999	9 F 18.9984	10 Ne 20.1797
3	11 Na 22.9898	12 Mg 24.3050											13 Al 26.9815	14 Si 28.085	15 P 30.9738	16 S 32.06	17 Cl 35.453	18 Ar 39.948
4	19 K 39.0983	20 Ca 40.078	21 Sc 44.9559	22 Ti 47.867	23 V 50.9415	24 Cr 51.9961	25 Mn 54.9380	26 Fe 55.845	27 Co 58.9332	28 Ni 58.6934	29 Cu 63.546	30 Zn 65.38	31 Ga 69.723	32 Ge 72.63	33 As 74.9216	34 Se 78.96	35 Br 79.904	36 Kr 83.798
5	37 Rb 85.4678	38 Sr 87.62	39 Y 88.9058	40 Zr 91.224	41 Nb 92.9064	42 Mo 95.96	43 Tc (98)	44 Ru 101.07	45 Rh 102.9055	46 Pd 106.42	47 Ag 107.8682	48 Cd 112.411	49 In 114.818	50 Sn 118.710	51 Sb 121.760	52 Te 127.60	53 I 126.9045	54 Xe 131.293
6	55 Cs 132.9055	56 Ba 137.327	57 La 138.9055 *	72 Hf 178.49	73 Ta 180.9479	74 W 183.84	75 Re 186.207	76 Os 190.23	77 Ir 192.217	78 Pt 195.084	79 Au 196.9666	80 Hg 200.59	81 Tl 204.38	82 Pb 207.2	83 Bi 208.9804	84 Po (209)	85 At (210)	86 Rn (222)
7	87 Fr (223)	88 Ra (226)	89 Ac (227) **	104 Rf (265)	105 Db (268)	106 Sg (271)	107 Bh (270)	108 Hs (277)	109 Mt (276)	110 Ds (281)	111 Rg (280)	112 Cn (285)	113 Uut (284)	114 Fl (289)	115 Uup (288)	116 Lv (293)	117 Uus (294)	118 Uuo (294)

*Lanthanide Series

58 Ce 140.116	59 Pr 140.9076	60 Nd 144.242	61 Pm (145)	62 Sm 150.36	63 Eu 151.964	64 Gd 157.25	65 Tb 158.9254	66 Dy 162.500	67 Ho 164.9303	68 Er 167.259	69 Tm 168.9342	70 Yb 173.054	71 Lu 174.9668

** Actinide Series

90 Th 232.0381	91 Pa 231.0359	92 U 238.0289	93 Np (237)	94 Pu (244)	95 Am (243)	96 Cm (247)	97 Bk (247)	98 Cf (251)	99 Es (252)	100 Fm (257)	101 Md (258)	102 No (259)	103 Lr (262)

Note: Atomic masses are 2009 IUPAC values (up to four decimal places). More accurate values for some elements are given in the table inside the back cover.