

CHUKA



UNIVERSITY

UNIVERSITY EXAMINATIONS

**EXAMINATION FOR THE AWARD OF DEGREE OF BACHELOR OF
SCIENCE IN CHEMISTRY**

CHEM 241: SEPARATION TECHNIQUES

STREAMS: BSC. CHEM

TIME: 2 HOURS

DAY/DATE: WEDNESDAY 23/04/2025

2.30 P.M. – 4.30 P.M.

INSTRUCTIONS

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		$\text{J K}^{-1} \text{mol}^{-1}$
		8.314 47	10^{-2}	$\text{dm}^3 \text{bar K}^{-1} \text{mol}^{-1}$
		8.205 74	10^{-2}	$\text{dm}^3 \text{atm K}^{-1} \text{mol}^{-1}$
		6.236 37	10	$\text{dm}^3 \text{Torr K}^{-1} \text{mol}^{-1}$
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	$\epsilon_0 = 1/c^2 \mu_0$	8.854 19	10^{-12}	$\text{J}^{-1} \text{C}^2 \text{m}^{-1}$
	$4\pi\epsilon_0$	1.112 65	10^{-10}	$\text{J}^{-1} \text{C}^2 \text{m}^{-1}$
Vacuum permeability	μ_0	4π	10^{-7}	$\text{J s}^2 \text{C}^{-2} \text{m}^{-1} (= \text{T}^2 \text{J}^{-1} \text{m}^3)$
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	$a_0 = 4\pi\epsilon_0\hbar^2/m_e e^2$	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	$\sigma = 2\pi^5 k^4/15h^3 c^2$	5.670 51	10^{-8}	$\text{W m}^{-2} \text{K}^{-4}$
Rydberg constant	$R = m_e e^4/8h^3 c \epsilon_0^2$	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}	$\text{N m}^2 \text{kg}^{-2}$

*Exact value

QUESTION ONE (30 MARKS)

- a) i) To decrease the plate height and yet increase the resolution what courses of action are available? What penalties may accrue for each approach, explain. (3 marks)
- ii) Distinguish the following methods in chromatography; Calibration method, internal standard and standard addition method, with appropriate examples. (3 marks)
- iii) Discuss the applications of Kovats retention indices in industries, with appropriate examples. (3 marks)
- (b) i) The following data were obtained for four compounds separated on a 20-m capillary column.

Compound	t_r (min)	w (min)
A	8.04	0.15
B	8.26	0.15
C	8.43	0.16

I. Calculate the number of theoretical plates for each compound and the average number of theoretical plates for the column. (2 marks)

II. Calculate the average height of a theoretical plate. (1 mark)

III. Explain why it is possible for each compound to have a different number of theoretical plates. (1 mark)

ii) Write short notes on Eddy diffusion, longitudinal diffusion, and mass transfer in chromatography. (3 marks)

iii) Combined standard solution was prepared to contain $25.0\mu\text{m}$ caffeine and $5.0\mu\text{m}$ of an appropriately chosen internal standard. Analysis of this combined standard gave signals for the caffeine and internal standard of 0.155 and 0.233(arbitrary units), respectively. A 1.00ml aliquot of internal standard solution was added to a 10.0ml sample to yield a $10.0\mu\text{m}$ solution of the internal standard in the diluted sample. Analysis of the spiked sample yields signals for the analyte and internal standard of 0.274 and 0.198, respectively

I. What considerations go into choosing an appropriate internal standard? (1.5 marks)

II. Is the analytical technique in this case more sensitive to caffeine or the internal standard? (1 mark)

III. Report the concentration of caffeine in units of ppm. (2 marks)

iv) In a separation of a mixture of hydrocarbons the following adjusted retention times were measured; 2.23 min for propane, 5.71 min for isobutene, and 6.67 min for butane. Determine the Kovat's retention index for each of these hydrocarbons. (1.5 marks)

c) i) Explain the ether extraction of iron (III) from strong hydrochloric acid solution. (3 marks)

ii) Describe the equilibrium process involved in the solvent extraction of metal chelates using suitable examples. (2 marks)

iii) The concentration of pesticide residues on agricultural products, such as oranges, may be determined by GC-MS. Pesticide residues are extracted from the sample using methylene chloride,

and the concentrations of the extracted pesticides are concentrated by evaporating the methylene chloride to a smaller volume. Calibration is accomplished using anthracene-d₁₀ as an internal standard. In a study to determine the parts per billion of heptachlor epoxide on oranges, a 50.0-g sample of orange rinds was chopped and extracted with 50.00 mL of methylene chloride. After removing any insoluble material by filtration, the methylene chloride was reduced in volume, spiked with a known amount of the internal standard, and diluted to 10 mL in a volumetric flask. Analysis of the sample gives a peak–area ratio ($A_{\text{anal}}/A_{\text{int stan}}$) of 0.108. A series of calibration standards, each containing the same amount of anthracene-d₁₀ as the sample, give the following results.

ppb Heptachlor Epoxide	$A_{\text{anal}}/A_{\text{int stan}}$
20.0	0.065
60.0	0.153
200.0	0.637
500.0	1.554
1000.0	3.198

Report the concentration of heptachlor epoxide residue (in nanograms per gram) on the oranges. (3 marks)

QUESTION TWO (20 MARKS)

a) i) The separation of hydrocarbons was done on a column 1.5m length with a 2.3mm bore, packed with n-C₈ alkane bonded to porous silica. Column temperature; 25°C, detector; flame ionization, carrier gas; nitrogen at 25ml min⁻¹. Retention characteristics of selected peaks are:

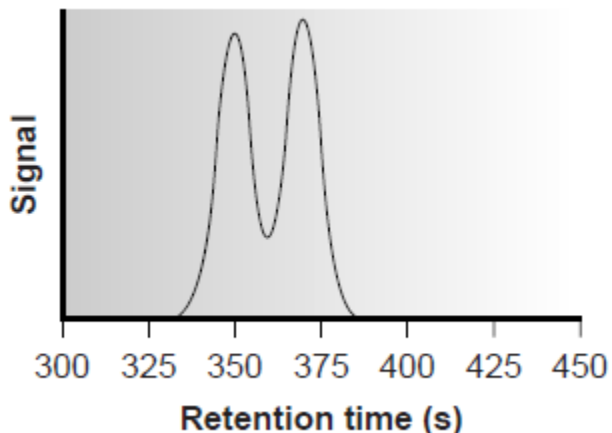
Compound	peak	tR, min	Wb, min
methane	1	0.39	
propane	4	0.86	
n-butane	7	1.75	
Trans-butene-2	10	3.12	
Cis-butene-2	11	3.43	0.31
n-pentane	13	4.31	

2-methyl pentane	17	9.55	0.71
------------------	----	------	------

- I. Calculate the Kovats retention indices for all the compounds. (2.5 marks)
- II. Estimate the position of n-hexane on the chromatogram. (1 mark)
- III. What is the effective plate number for 2-methylpentane. (1.5 marks)
- IV. What is the resolution between trans-butene-2 and cis-butene-2. (0.5 mark)
- V. Calculate k^1 (retention factor) for cis-butene-2 and for 2-methylpentane. (1 mark)
- VI. To achieve a resolution of 1.5 for the separation of peaks 10 and 11, how many plates would be required? (1.5 marks)
- VII. Suggest three ways in which the additional plates could be achieved for part (VI). Provide a firm number or basis for each suggested alteration in operating procedure. (1.5 marks)
- VIII. What is the velocity of the carrier gas through the column? (1 mark)
- ii) Differentiate between Craig method of multiple extraction and continuous countercurrent extraction. (4 marks)
- b) i) Discuss different modes of injection in capillary electrophoresis and electrochromatography. (3 marks)
- ii) Explain how temperature programming improve separations in gas chromatography. (2.5 marks)

QUESTION THREE (20 marks)

- (a) i) Compare derivatization in gas chromatography and liquid chromatography, with examples. (3 marks)
- ii) Explain how solid phase extraction differs from solid phase micro extraction, in liquid chromatography. (2 marks)
- iii) Derive the distribution ratio of benzoic acid between organic phase and aqueous phase, taking into account benzoic acid partially forms a dimer in the organic phase. (2 marks)
- b) i) The distribution ratio between 3 M HCl and tri-*n*-butylphosphate for PdCl₂ is 2.3. Determine the percent PdCl₂ that will be extracted from 25.0 mL of a $7.0 \times 10^{-4}M$ solution into 10.0 mL tri-*n*-butylphosphate. (1 mark)
- ii) Using the partial chromatogram shown here, determine the resolution between the two solute bands. (2 marks)



iii) Diet soft drinks contain appreciable quantities of aspartame, benzoic acid, and caffeine. What is the expected order of elution for these compounds in a capillary zone electrophoresis separation using a pH 9.4 buffer solution, given that aspartame has p^{K_a} values of 2.964 and 7.37, benzoic acid's p^{K_a} is 4.2, and the p^{K_a} for caffeine is less than 0? (2 marks)

c) i) An intimate mixture of an H^+ form strong-acid type cation exchanger and an OH^- form strong-base type anion exchanger is called a mixed-bed resin. If you slowly percolate some tap water through a bed of such resin and measure its conductivity, what will you expect the specific conductance of the effluent to be? (1 marks)

ii) A series of polyvinylpyridine standards of different molecular weight were analyzed by size-exclusion chromatography, yielding the following results.

Formula Weight	Retention Volume (mL)
600,000	6.42
100,000	7.98
20,000	9.30
3,000	10.94

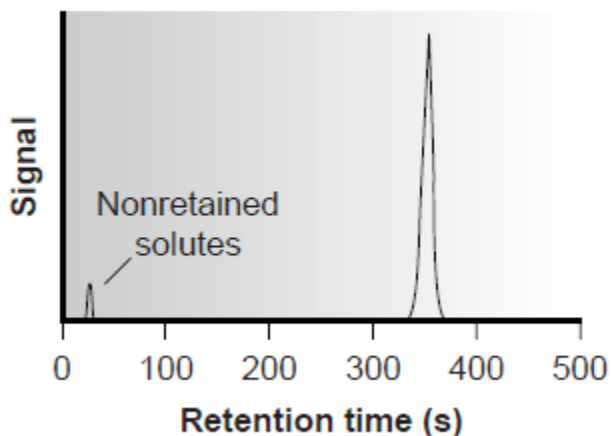
When a preparation of polyvinylpyridine of unknown formula weight was analyzed the retention volume was found to be 8.45. Report the average formula weight for the preparation. (3 marks)

iii) Ethidium bromide is still the most common DNA intercalating fluorescence tag. But there is increasingly a move to other tags. Explain. (1 mark)

iv) Bohman and colleagues described a reverse-phase HPLC method for the quantitative analysis of vitamin A in food using the method of standard additions. In a typical example, a 10.067-g sample of cereal is placed in a 250-mL Erlenmeyer flask along with 1 g of sodium ascorbate, 40 mL of ethanol, and 10 mL of 50% w/v KOH. After refluxing for 30 min, 60 mL of ethanol is added, and the solution is cooled to room temperature. Vitamin A is extracted using three 100-mL portions of hexane. The combined portions of hexane are evaporated, and the residue containing vitamin A is transferred to a 5-mL volumetric flask and diluted to volume with methanol. A standard addition is prepared in a similar manner using a 10.093-g sample of the cereal and spiking it with 0.0200 mg of vitamin A. Injecting the sample and standard addition into the HPLC gives peak areas of 6.77×10^3 and 1.32×10^4 , respectively. Report the vitamin A content of the sample in milligrams/100 g cereal. (3 marks)

QUESTION FOUR (20 marks)

(a) i) Using the chromatogram shown here, which was obtained on a 2-m column, determine values for t_r , w , t_r^1 , k^1 , N , and H . (3 marks)



ii) Compare supercritical fluid chromatography, high performance liquid chromatography and gas chromatography. (3 marks)

iii) Outline the advantages of size exclusion chromatography in the analysis of synthetic polymers or biopolymers. (2 marks)

(b) i) Describe how the following situations could occur. In one case, two compounds are well resolved ($R > 1.5$) despite the separation being characterized by only a selectivity of 1.02. In another, two compounds are unresolved ($R < 1.5$) in spite of having a selectivity of 1.8 between them. (2 marks)

ii) Compare Wall-Coated Open Tubular (WCOT), Support-Coated Open Tubular (SCOT), and Porous Layer Open Tubular (PLOT) columns. (3 marks)

iii) The analysis of NO_3^- in aquarium water was carried out by CZE using IO_4^- as an internal standard. A standard solution of 15.0-ppm NO_3^- and 10.0-ppm IO_4^- gives peak heights (arbitrary units) of 95.0 and 100.1, respectively. A sample of water from an aquarium is diluted 1:100, and sufficient internal standard added to make its concentration 10.0 ppm. Analysis gives signals of 29.2 and 105.8 for NO_3^- and IO_4^- , respectively. Report the parts per million of NO_3^- in the sample of aquarium water. (2 marks)

c) i) Ion chromatography is unique among HPLC techniques in using a number of electro dialysis techniques to generate and manipulate the eluent. Explain. (2.5 marks)

ii) Zhou and colleagues determined the %w/w H_2O in methanol by GC, using a capillary column coated with a nonpolar stationary phase and a thermal conductivity detector. A series of calibration standards gave the following results.

% w/w H_2O	Peak Height (arb. units)
0.00	1.15
0.0145	2.74
0.0472	6.33
0.0951	11.58
0.1757	20.43
0.2901	32.97

I. Determine the %w/w H_2O in a sample giving a peak height of 8.63. (1.5 marks)

II. The %w/w H_2O in a freeze-dried antibiotic is determined in the following manner. A 0.175-g sample is placed in a vial along with 4.489 g of methanol. Water in the vial extracts into the methanol. Analysis of the sample gave a peak height of 13.66. Determine the %w/w H_2O in the antibiotic. (1 mark)

.....