

CHEM 841: ADVANCED SEPARATION TECHNIQUE

Instruction: Answer ALL the questions.

Useful data are provided

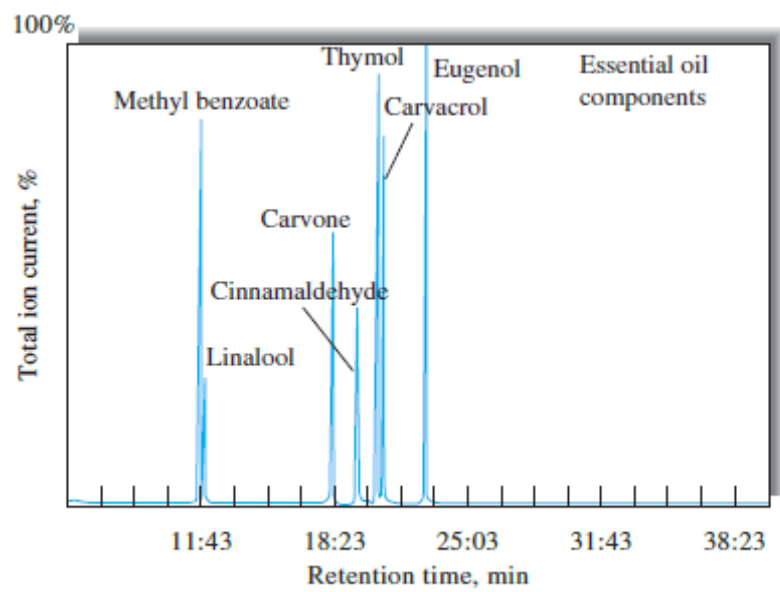
General data and fundamental constants

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		$\text{J K}^{-1} \text{mol}^{-1}$
		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}	$\text{J s}^2 \text{C}^{-2} \text{m}^{-1} (= \text{T}^2 \text{J}^{-1} \text{m}^3)$
Magnetron				
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

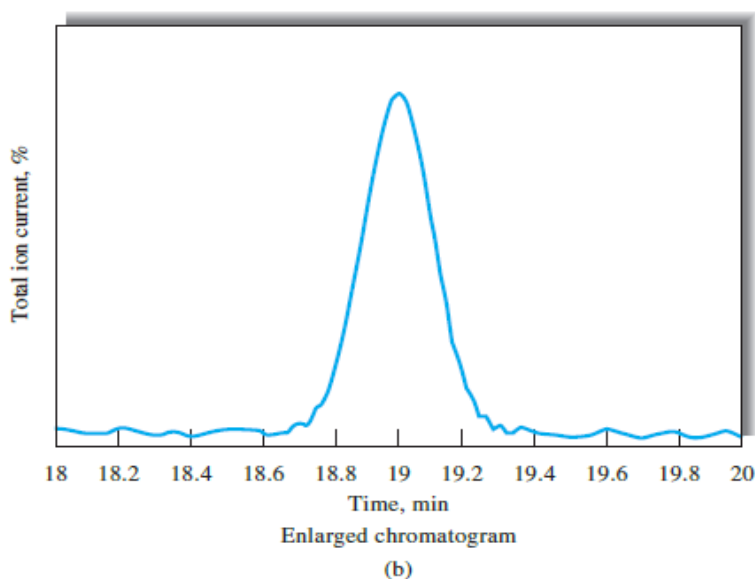
QUESTION ONE (20 MARKS)

- (A) Cinnamaldehyde is the component responsible for cinnamon flavor. It is also a potent antimicrobial compound present in essential oils. The GC response of an artificial mixture containing six essential oil components and methyl benzoate as an internal standard is shown in part (a) of the figure.



(a)

- (a) An idealized enlargement of the region near the cinnamaldehyde peak is given in part (b) of the figure. Determine the retention time for cinnamaldehyde.



- (b) From part (b) of the figure, determine the number of theoretical plates for the column.
- (c) The fused-silica column was $0.25 \text{ mm} \times 30 \text{ cm}$ with a $0.25\text{-}\mu\text{m}$ film. Determine the height equivalent to a theoretical plate from the data in parts (a) and (b).
- (d) Quantitative data were obtained by using methyl benzoate as the internal standard. The following results were found for calibration curves of cinnamaldehyde, eugenol, and thymol. The values under each component represent the peak area of the component divided by the peak area of the internal standard.

Concentration, mg sample/ 200 μL	Cinnamaldehyde	Eugenol	Thymol
0.50	—	0.4	—
0.65	—	—	1.8
0.75	1.0	0.8	—
1.10	—	1.2	—
1.25	2.0	—	—
1.30	—	—	3.0
1.50	—	1.5	—
1.90	3.1	2.0	4.6
2.50	4.0	—	5.8

Determine the calibration curve equations for each component. Include the R^2 values.

- (e) From the data in part (d), determine which of the components has the highest calibration curve sensitivity? Which has the lowest?
- (f) A sample containing the three essential oils in part (d) gave the peak areas relative to the internal standard area: cinnamaldehyde, 2.6; eugenol, 0.9; thymol, 3.8. Determine the concentrations of each of the oils in the sample and the standard deviations in concentration.

- (g) A study was made of the decomposition of cinnamaldehyde in cinnamon oil. The oil was heated for various times at different temperatures. The following data were obtained:

Temp, °C	Time, min	Cinnamaldehyde, %
25, initial		90.9
40	20	87.7
—	40	88.2
—	60	87.9
60	20	72.2
—	40	63.1
—	60	69.1
100	20	66.1
—	40	57.6
—	60	63.1
140	20	64.4
—	40	53.7
—	60	57.1
180	20	62.3
—	40	63.1
—	60	52.2
200	20	63.1
—	40	64.5
—	60	63.3
210	20	74.9
—	40	73.4
—	60	77.4

Determine whether temperature has a statistical effect on the decomposition of cinnamaldehyde using analysis of variance (ANOVA). (For how to perform ANOVA, see S. R. Crouch and F. J. Holler, *Applications of Microsoft® Excel in Analytical Chemistry*, 3rd ed., Chap. 3, Belmont, CA: Cengage Learning, 2017.) In the same way, determine if time of heating has an effect.

- (h) Using the data in part (g), assume that decomposition begins at 60°C and test the hypothesis that there is no effect of temperature or time.

(12 marks)

(B)

One method for quantitative determination of the concentration of constituents in a sample analyzed by GC is the area-normalization method. Here, complete elution of all of the sample constituents is necessary. The area of each peak is then measured and corrected for differences in detector response to the different eluates. This correction involves dividing the area by an empirically determined correction factor. The concentration of the analyte is found from the ratio of its corrected area to the total corrected area of all peaks. For a chromatogram containing three peaks, the relative areas were found to be 16.4, 45.2, and 30.2 in the order of increasing retention time. Calculate the percentage of each compound if the relative detector responses were 0.60, 0.78, and 0.88, respectively.

(2 marks)

(C)

A GC column was operated under the following conditions:

column: 1.10 m \times 2.0 mm, packed with Chromosorb P; mass of stationary liquid added, 1.40 g; density of liquid, 1.02 g/mL

pressures: inlet, 26.1 psi above room; room, 748 torr

measured outlet flow rate: 25.3 mL/min

temperature: room, 21.2°C; column, 102.0°C

retention times: air, 18.0 s; methyl acetate, 1.98 min; methyl propionate, 4.16 min; methyl *n*-butyrate, 7.93 min

peak widths of esters at base: 0.19, 0.39, and 0.79, respectively

Calculate

- (a) the average flow rate in the column.
- (b) the corrected retention volumes for air and the three esters.
- (c) the specific retention volumes for the three components.
- (d) the distribution constants for each of the esters.
- (e) a corrected retention volume and retention time for methyl *n*-hexanoate.

(6 marks)

QUESTION TWO (20 Marks)

(A)

An HPLC method was developed for the separation and determination of ibuprofen in rat plasma samples as part of a study of the time course of the drug in laboratory animals. Several standards were chromatographed and the following results obtained:

Ibuprofen Concentration, µg/mL	Relative Peak Area
0.5	5.0
1.0	10.1
2.0	17.2
3.0	19.8
6.0	39.7
8.0	57.3
10.0	66.9
15.0	95.3

Next, a 10 mg/kg sample of ibuprofen was administered orally to a laboratory rat. Blood samples were drawn at various times after administration of the drug and subjected to HPLC analysis. The following results were obtained:

Time, h	Peak Area
0	0
0.5	91.3
1.0	80.2
1.5	52.1
2.0	38.5
3.0	24.2
4.0	21.2
6.0	18.5
8.0	15.2

Find the concentration of ibuprofen in the blood plasma for each of the times given above and plot the concentration versus time. On a percentage basis, during what half-hour period (first, second, third, etc.) is most of the ibuprofen lost?

(4 marks)

(B)

Assume for simplicity that the HPLC plate height, H , can be given by Equation

$$H = \frac{B}{u} + C_S u + C_M u = \frac{B}{u} + C u$$

where $C = C_S + C_M$.

- (a) By using calculus to find the minimum H , show that the optimal velocity u_{opt} can be expressed as

$$u_{\text{opt}} = \sqrt{\frac{B}{C}}$$

- (b) Show that this leads to a minimum plate height H_{min} given by

$$H_{\text{min}} = 2\sqrt{BC}$$

- (c) Under some conditions for chromatography, C_S is negligible compared to C_M . For packed HPLC columns, C_M is given by

$$C_M = \frac{\omega d_p^2}{D_M}$$

where ω is a dimensionless constant, d_p is the particle size of the column packing, and D_M is the diffusion coefficient in the mobile phase. The B coefficient can be expressed as

$$B = 2\gamma D_M$$

where γ is also a dimensionless constant. Express u_{opt} and H_{min} in terms of D_M , d_p , and the dimensionless constants γ and ω .

- (d) If the dimensionless constants are on the order of unity, show that u_{opt} and H_{min} can be expressed as

$$u_{\text{opt}} \approx \frac{D_M}{d_p} \quad \text{and} \quad H_{\text{min}} \approx d_p$$

- (e) Under the preceding conditions, how could the plate height be reduced by one-third? What would happen to the optimal velocity under these conditions? What would happen to the number of theoretical plates N for the same length column?
- (f) For the conditions in part (e), how could you maintain the same number of theoretical plates while reducing the plate height by one-third?
- (g) The preceding discussion assumes that all band broadening occurs within the column. Name two sources of extracolumn band broadening that might also contribute to the overall width of HPLC peaks.

(8 marks)

(c)

Gel permeation chromatography is to be used to separate a mixture of four polystyrene standards of molecular mass: 9 200, 76 000, 1.1×10^6 and 3×10^6 daltons. Three columns are available for this exercise. They are prepacked with gel with the following fractionation ranges for molecular weights:

A : 70 000 to 4×10^5 daltons

B : 10^5 to 1.2×10^6 daltons

C : 10^6 to 4×10^6 daltons

How might these four polymers be separated in a single operation if it is permitted to use two of the above columns end to end?

(4 marks)

(D)

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

1. Calculate the electrophoretic mobility, μ_{EP} , of a compound whose migration time is 2.5 min. Give the answer in precise units.
2. Calculate the diffusion coefficient under these conditions for this compound, remembering that the calculated plate number, $N = 80\,000$.

(4 marks)

QUESTION THREE (20 MARKS)

(A)

Answer the following questions with respect to SFC.

- (a) Why are highly polar or ionic compounds usually not eluted in SFC?
- (b) What types of mobile-phase additives have been used to improve the elution of highly polar or ionic compounds?
- (c) Why is ion-pairing SFC not often used?
- (d) Why are ammonium salts sometimes added as mobile-phase modifiers in SFC?
- (e) 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.
- (f) 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?
- (g) What elution mechanisms were considered by the authors?
- (h) Which mobile-phase additive gave the fastest elution of the sulfonate salts? Which provided the longest retention times?
- (i) Did a silica column give results similar to or different from a cyano bonded-phase column?

(7 marks)

(B) Discuss separation mechanisms in field-flow fractionation

(3 marks)

(C)

Doxorubicin (DOX) is a widely used anthracycline that has been effective in treatments of leukemia and breast cancer in humans (A. B. Anderson, C. M. Ciriaks, K. M. Fuller, and E. A. Ariaga, *Anal. Chem.*, 2003, 75, 8, DOI: 10.1021/ac020426r). Unfortunately, side effects, such as liver toxicity and drug resistance, have been reported. In a recent study, Anderson et al. used laser-induced fluorescence (LIF) as a detection mode for CE to investigate metabolites of DOX in single cells and subcellular fractions. The following are results similar to those obtained by Anderson et al. for quantifying doxorubicin by LIF. The CE peak areas were measured as a function of the DOX concentration to construct a calibration curve.

DOX Concentration, nM	Peak Area
0.10	0.10
1.00	0.80
5.00	4.52
10.00	8.32
20.00	15.7
30.00	26.2
50.00	41.5

- Find the equation for the calibration curve and the standard deviations of the slope and intercept. Find the R^2 value.
- Rearrange the equation found in part (a) to express concentration in terms of the measured area.
- The limit of detection (LOD) for DOX was found to be 3×10^{-11} M. If the injection volume was 100 pL, what was the LOD in moles?
- Two samples of unknown DOX concentration were injected and peak areas of 11.3 and 6.97 obtained. What were the concentrations and their standard deviations?
- Under certain conditions, the DOX peak required 300 s to reach the LIF detector. What time would be required if the applied voltage were doubled? What time would be required if the capillary length were doubled at the same applied voltage?
- The capillary used in part (e) under normal conditions had a plate count of 100,000. What would N be if the capillary length were doubled at the same applied voltage? What would N be if the applied voltage were doubled at the original capillary length?
- For a 40.6-cm-long capillary of inside diameter 50 μm , what would the plate height be for a capillary with $N = 100,000$?
- For the same capillary as in part (g), what is the variance σ^2 of a typical peak?

(10 marks)

PERIODIC TABLE OF THE ELEMENTS

IA 1		IIA 2		VIII										VIIA 17		0 18	
Metals		Nonmetals		Metalloids													
1	1 H 1.008															1 H 1.008	2 He 4.0026
2	3 Li 6.941	4 Be 9.0122														9 F 18.9984	10 Ne 20.1797
3	11 Na 22.9898	12 Mg 24.3050														17 Cl 35.453	18 Ar 39.948
4	19 K 39.0983	20 Ca 40.078	21 Sc 44.9559													35 Br 79.904	36 Kr 83.798
5	37 Rb 85.4678	38 Sr 87.62	39 Y 88.9058													53 I 126.9045	54 Xe 131.293
6	55 Cs 132.9055	56 Ba 137.327	57 La 138.9055													85 At (210)	86 Rn (222)
7	87 Fr (223)	88 Ra (226)	89 Ac (227)													117 Uus (294)	118 Uuo (294)
				IVB 4	VB 5	VIB 6	VII 7	VIII 8	VIII 9	VIII 10	VIII 11	VIII 12		IVA 14	VA 15	VIA 16	
				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		32 Ge 72.63	33 As 74.9216	34 Se 78.96	
				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		50 Sn 118.710	51 Sb 121.760	52 Te 127.60	
				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		82 Pb 207.2	83 Bi 208.9804	84 Po (209)	
				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)		114 Fl (289)	115 Uup (288)	116 Lv (293)	

*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
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** 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)
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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.