CHUKA UNIVERSITY

UNIVERSITY EXAMINATIONS

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

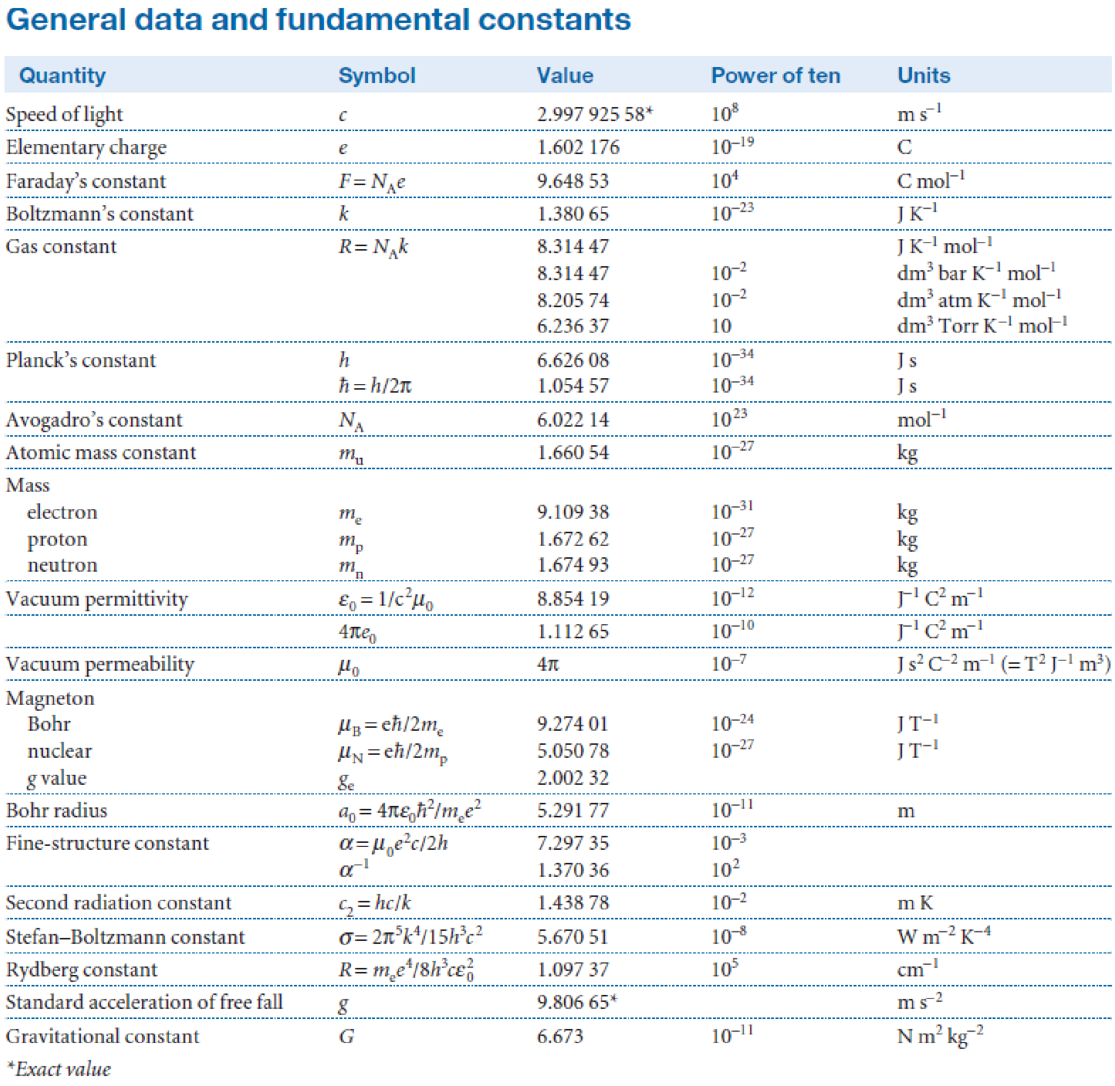
CHEM 241: SEPARATION TECHNIQUES

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

DAY/DATE: MONDAY 08/04/2024 **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}	$\mathrm{m\ s^{-1}}$
Elementary charge	e	1.602 176	10^{-19}	C
Faraday's constant	$F=N_Ae$	9.648 53	10^{4}	$\mathrm{C\ mol^{-1}}$
Boltzmann's constant	k	1.380 65	10^{-23}	$\mathrm{J\ K^{-1}}$
Gas constant	$R=N_Ak$	8.314 47 8.314 47 8.205 74 6.236 37	 10^{-2} 10^{-2} 10	$\mathrm{J\ K^{-1}\ mol^{-1}}$ $\mathrm{dm^3\ bar\ K^{-1}\ mol^{-1}}$ $\mathrm{dm^3\ atm\ K^{-1}\ mol^{-1}}$ $\mathrm{dm^3\ Torr\ K^{-1}\ mol^{-1}}$
Planck's constant	h $\hbar=h/2\pi$	6.626 08 1.054 57	10^{-34} 10^{-34}	J s J s
Avogadro's constant	N_A	6.022 14	10^{23}	$\mathrm{mol^{-1}}$
Atomic mass constant	m_u	1.660 54	10^{-27}	kg
Mass electron proton neutron	 m_e m_p m_n	 9.109 38 1.672 62 1.674 93	 10^{-31} 10^{-27} 10^{-27}	 kg kg kg
Vacuum permittivity	$\varepsilon_0=1/c^2\mu_0$	8.854 19	10^{-12}	$\mathrm{J^{-1}\ C^2\ m^{-1}}$
	$4\pi\varepsilon_0$	1.112 65	10^{-10}	$\mathrm{J^{-1}\ C^2\ m^{-1}}$
Vacuum permeability	μ_0	4π	10^{-7}	$\mathrm{J\ s^2\ C^{-2}\ m^{-1}}$ $(=\mathrm{T^2\ J^{-1}\ m^3})$
Magneton Bohr nuclear g value	 $\mu_B=e\hbar/2m_e$ $\mu_N=e\hbar/2m_p$ g_e	 9.274 01 5.050 78 2.002 32	 10^{-24} 10^{-27}	 $\mathrm{J\ T^{-1}}$ $\mathrm{J\ T^{-1}}$
Bohr radius	$a_0=4\pi\varepsilon_0\hbar^2/m_ee^2$	5.291 77	10^{-11}	m
Fine-structure constant	$\alpha=\mu_0e^2c/2h$ α^{-1}	7.297 35 1.370 36	10^{-3} 10^{2}	
Second radiation constant	$c_2=hc/k$	1.438 78	10^{-2}	m K
Stefan–Boltzmann constant	$\sigma=2\pi^5k^4/15h^3c^2$	5.670 51	10^{-8}	$\mathrm{W\ m^{-2}\ K^{-4}}$
Rydberg constant	$R=m_ee^4/8h^3c\varepsilon_0^2$	1.097 37	10^{5}	$\mathrm{cm^{-1}}$
Standard acceleration of free fall	g	9.806 65*		$\mathrm{m\ s^{-2}}$
Gravitational constant	G	6.673	10^{-11}	$\mathrm{N\ m^2\ kg^{-2}}$

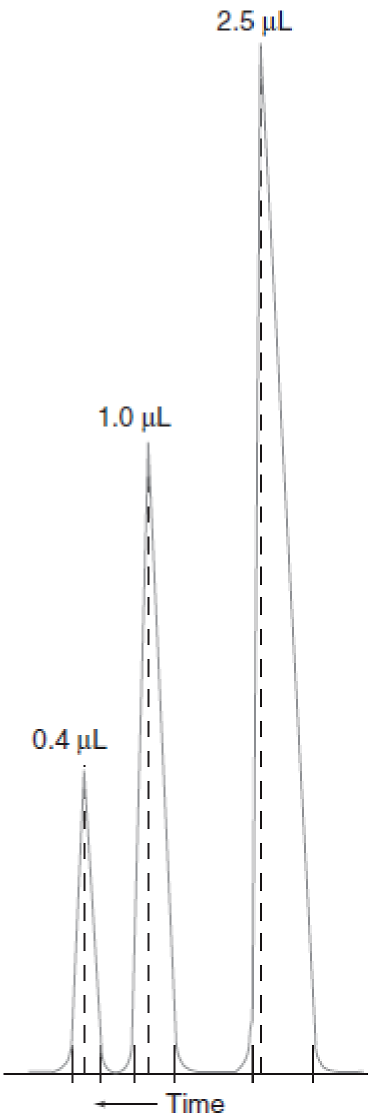
**Exact value*

QUESSTION ONE (30MARKS)

(a)

i. A mixture of benzene, toluene, and methane was injected into a gas chromatograph. Methane gave a sharp spike 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. (2marks)

ii. The three chromatograms shown below were obtained with 2.5, 1.0, and 0.4 µL of ethyl acetate injected on the same column under the same conditions. Explain why the peak becomes less symmetric with increasing sample size. (2marks)



iii. The relative retention for two compounds in gas chromatography is 1.068 on a column with a plate height of 0.520 mm. The capacity factor for compound 1 is 5.16.

I. Find the separation factor (γ) for the two compounds. (2 marks)

II. What length of column will separate the compounds with a resolution of 1.00? (2 marks)

III. The retention time (t_m)for air is 2.00 min. If the number of plates is the same for both compounds, find $t_r$and $W_{1/2}$for each peak. (1 mark)

IV. If the ratio of stationary phase to mobile phase is 0.30, find the partition coefficient for compound 1 (1 mark)

(b)

i. Two solutes have a separation factor of γ=1.06. How many plates are required to give a resolution of 1.0? of 2.0? If the plate height is 0.20 mm, how long must the column be for a resolution of 1.0? (1 mark)

ii. A band from a column eluted at a rate of 1.35 mL/min has a width at half-height of 16.3 s. The sample was applied as a sharp plug with a volume of 0.30 mL, and the detector volume is 0.20 mL. Find the variances introduced by injection and detection. What would be the width at half-height if broadening occurred only on the column? (2 marks)

iii. Water samples are likely to contain trace amounts of other organic compounds, many of which will extract into pentane along with the trihalomethanes. A short, packed column, such as the one used in this method, generally does not do a particularly good job of resolving chromatographic peaks. Why do we not need to worry about these other compounds? (1 mark)

iv. Indicate the order in which the following compoundswould be eluted from an HPLC column containing areversed-phase packing:

   I. Benzene, diethyl ether, *n*-hexane. (½ mark)
   II. Acetone, dichloroethane, acetamide. (½ mark)

v. Explain the major difference between isocratic elution and gradient elution and state the types of compoundsare these two elution methods most suited for.

(1 mark)

(c)

i. Marriott and Carpenter report the following data for five replicate injections of a mixture that contains 1% v/v methyl isobutyl ketone and 1% v/v *p*-xylene in dichloromethane [Marriott, P. J.; Carpenter, P. D. J. Chem. Educ. 1996, 73, 96–99]. Assume that p-xylene (peak 2) is the analyte, and that methyl isobutyl ketone (peak 1) is the internal standard. Determine the 95% confidence interval for a single-point standardization with and without using the internal standard. (4 marks)

injection	peak	peak area (arb. units)
I	1	48075
	2	78112
II	1	85829
	2	135404
III	1	84136
	2	132332
IV	1	71681
	2	112889
V	1	58054
	2	91287

ii. One advantage of an HPLC analysis is that a loop injector often eliminates the need for an internal standard. Why is an internal standard used in this analysis? What assumption(s) must we make when using the internal standard? (3marks)

(d)

i. Compare capillary electrophoresis with GC and HPLC. (3 marks)

ii. Methanol, which elutes at 4.69 min, is included as a neutral species to indicate the electroosmotic flow. When using standard solutions of each vitamin, capillary zone electrophoresis (CZE) peaks are found at 3.41 min, 4.69 min, 6.31 min, and 8.31 min. Examine the structures and pKa information in Figure 12.7.11 and identify the order in which the four B vitamins elute. (2 marks)

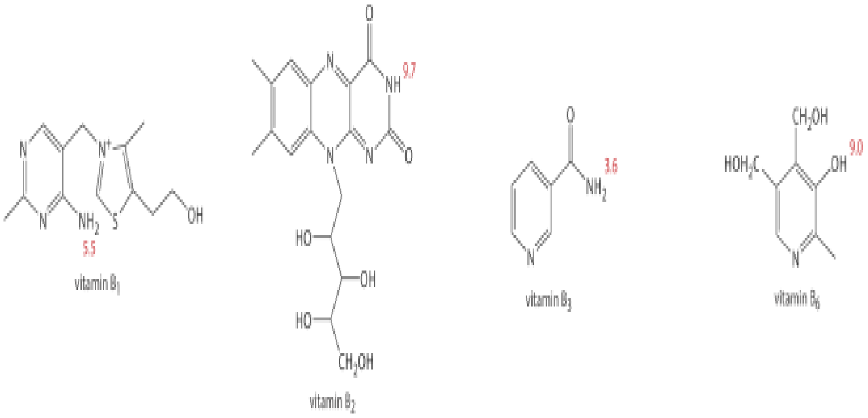


Figure 12.7.11 . Structures of the four water soluble B vitamins in their predominate forms at a pH of 9; pK_a values are shown in red.

iii. For quantitative work for determination of vitamin B complex using micellarelectrokinetic capillary chromatography an internal standard of o-ethoxybenzamide is added to all samples and standards. Why is an internal standard necessary? (1mark)

iv. The order of elution when using micellarelectrokinetic capillary chromatography (MEKC) is vitamin B (5.58 min), vitamin B (6.59 min), vitamin B (8.81 min), and vitamin B (11.21 min). What conclusions can you make about the solubility of the B vitamins in the sodium dodecylsulfate micelles? The micelles elute at 17.7 min. (1mark)

QUESTION TWO (20 MARKS)

a)

i. Describe the effect of pressure on supercritical fluid chromatograms. (2marks)

ii. For supercritical carbon dioxide, predict the effect that the following changes will have on the elution time in an SFC experiment:

(I) Increasing the flow rate (at constant temperature and pressure) (1mark)

(II) Increasing the pressure (at constant temperature and flow rate) (1mark)

(III) Increasing the temperature (at constant pressure and flow rate) (1mark)

iii. For SFE, differentiate between

(I) on-line and off-line processes. (1mark)

(II) Static and dynamic extractions. (1mark)

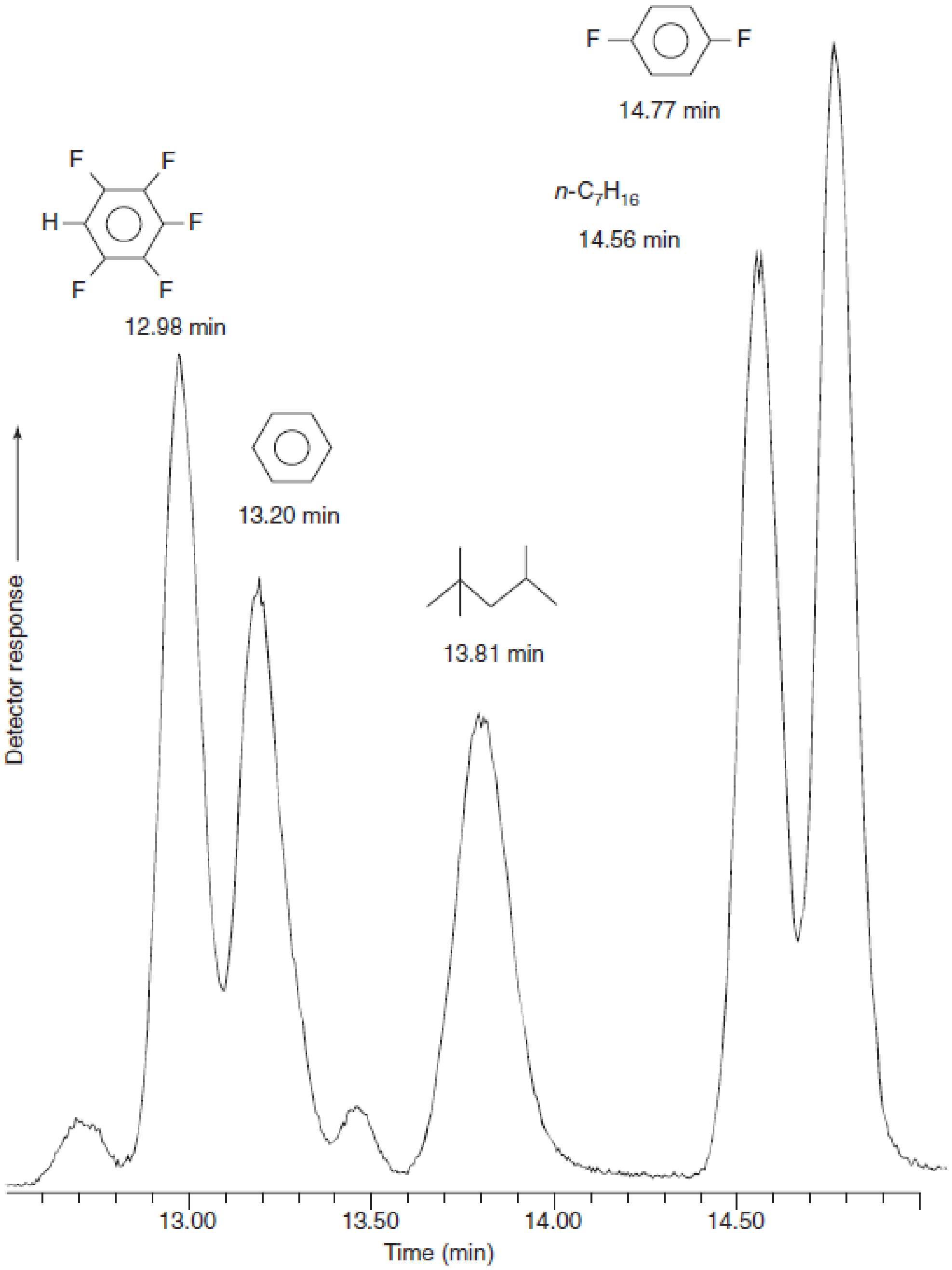
b)

i. Describe the fundamental difference between ion-exchange and size-exclusion chromatography. (2marks)

ii. Describe the differences between single-column and suppressor-column ion chromatography. (1mark)

iii. Mass spectrometry is an extremely versatile detection system for GC. However, interfacing an HPLC system to a mass spectrometer is a much more difficult task. Describe the major reasons why it is more difficult to combine HPLC with mass spectrometry than it is to combine GC with mass spectrometry. (3marks)

c) Consider the peaks for pentafluorobenzene and benzene in the chromatogram shown below. The elution time for unretained solute is 1.06 min. The open tubular column is 30.0 m in length and 0.530 mm in diameter, with a layer of stationary phase thick 3.0 μm on the inner wall.



i. Find the adjusted retention times and capacity factors for both compounds.

(1mark)

ii. Find the relative retention, α. (1mark)

iii. Find the separation factor, γ. (1mark)

iv. MeasuringW ½ on the chromatogram, find the number of plates, N_1andN_2, and the plate height for these two compounds. (1mark)

v. Measuring the width, *w*, at the baseline on the chromatogram, find the number of plates for these two compounds. (1mark)

vi. Use your answer to part **(v)** to find the resolution between the two peaks. (1mark)

vii. Using the number of plates $N=\sqrt{(N_1N_2)}$, with values from part **(v)**] and the observed separation factor, calculate what the resolution should be and compare your answer with the measured resolution in part **(vi)** (1mark)

QUESTION THREE (20 MARKS)

(a)

i. Although temperature does not have nearly the effect on HPLC separations that it has on GC separations, it nonetheless can play an important role. Discuss how and why temperature might or might not influence the following separations:

(a) A reversed-phase chromatographic separation of a steroid mixture. (1mark)

(b) An adsorption chromatographic separation of a mixture of closely related isomers. (1mark)

ii. Explain what determines the elution order in sedimentation Field-Flow Fractionation (FFF) (1marks)

iii. Three large proteins are ionized at the pH at which an electrical Field-Flow Fractionation (FFF) separation is carried out. If the ions are designated A^{2+}, B^{+}, and C^{3+}, predict the order of elution. (1marks)

(b)(i) Explain what would be the effect of the following on the plate height of a column.

(I) increasing the mass of the stationary phase relative to the packing mass. (½ mark)

(II) Decreasing the rate of sample injection. (½ mark)

(III) Increasing the injection port temperature. (½ mark)

(IV) Increasing the flow rate. (½ mark)

(V) Reducing the particle size of the packing. (½ mark)

(VI) Decreasing the column temperature. (½ mark)

(ii) Explain why gas chromatographic stationary phases often are bonded and cross-linked. (1mark)

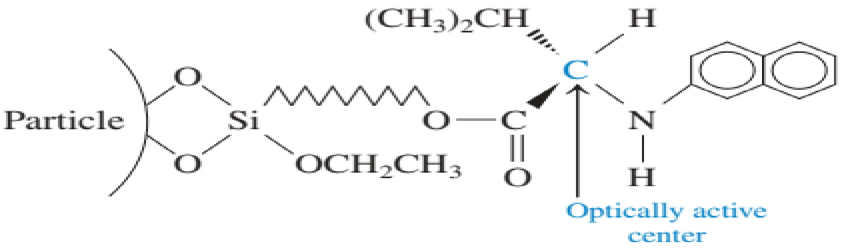
(c)

i. A known mixture of compounds A and B gave the following HPLC results:

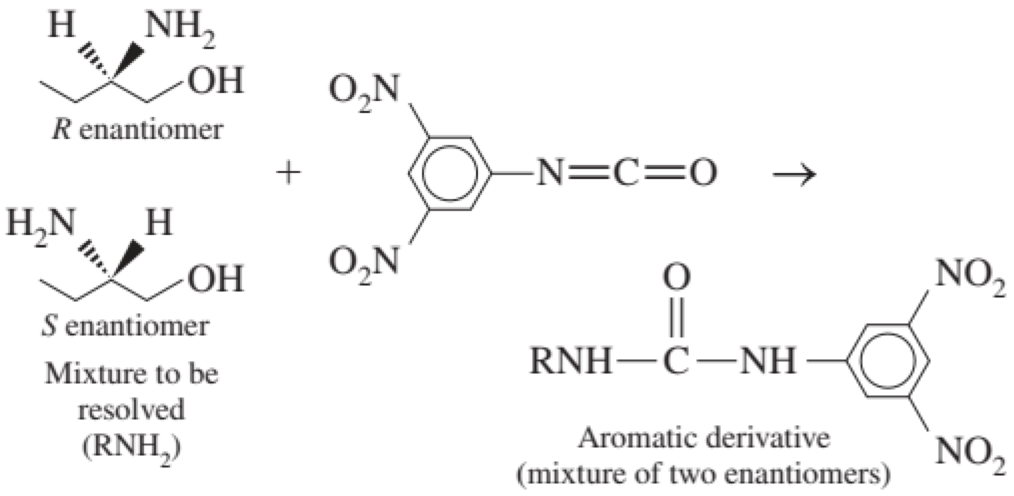
Compound	Concentration (mg/mL in mixture)	Peak area (arbitrary units)
A	1.03	10.86
B	1.16	4.37

A solution was prepared by mixing 12.49 mg of B plus 10.00 mL of unknown containing just A and diluting to 25.00 mL. Peak areas of 5.97 and 6.38 were observed for A and B, respectively. Find the concentration of A (mg/mL) in the unknown. (2marks)

ii. A bonded stationary phase for the separation of optical isomers has the structure



To resolve the enantiomers of amines, alcohols, or thiols, the compounds are first derivatized with a nitroaromatic group that increases their interaction with the bonded phase and makes them observable with a spectrophotometric detector

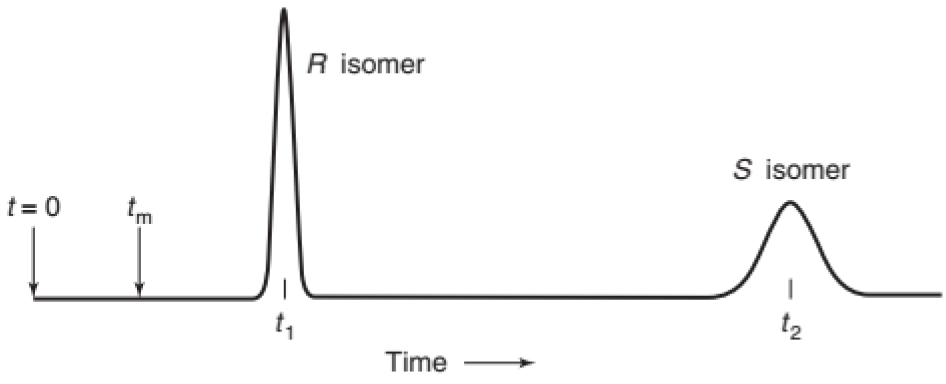


When the mixture is eluted with 20 vol% 2-propanol in hexane, the *R* enantiomer is eluted before the *S* enantiomer, with the following chromatographic parameters:

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = 7.7 \qquad \text{Relative retention } (\alpha) = 4.53$$

$$k' \text{ for } R \text{ isomer} = 1.35 \qquad t_m = 1.00 \text{ min}$$

where is the average width of the two Gaussian peaks at their base



I) Find t_1, t_2, and w_{av}, with units of minutes. (1.5marks)

II) The width of a peak at half-height is w ½ (Figure 23-9). If the plate number for each peak is the same, find for each peak. (1mark)

III) The area of a Gaussian peak is 1.064 x peak height w ½ , where w ½ is the width at half-height in Figure 23-9. Given that the areas under the two bands should be equal, find the relative peak heights (heirght$_R$/height$_S$). (1.5marks)

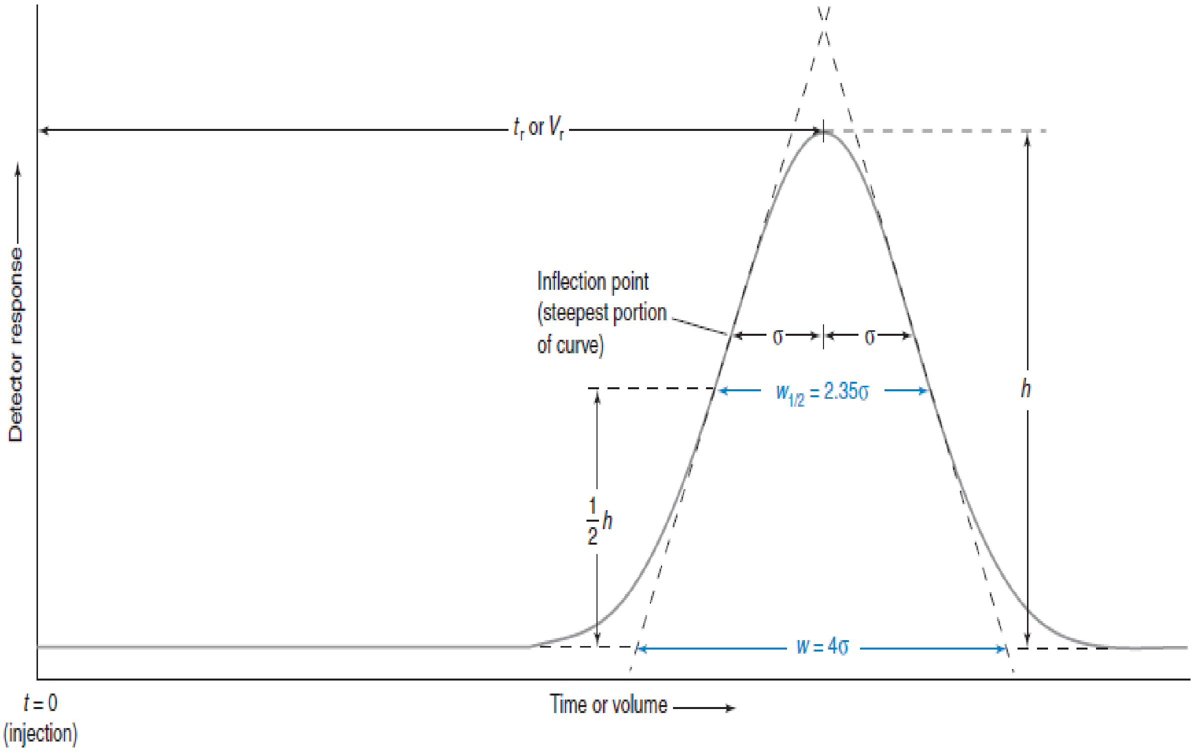


Figure 23-9 Idealized Gaussian chromatogram showing how *w* and $w_{1/2}$ are measured. The value of *w* is obtained by extrapolating the tangents to the inflection points down to the baseline.

iii. A GC column was operated under the following conditions: column: 1.10 m x 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

(I) the average flow rate in the column. (1mark)

(II) The corrected retention volumes for air and the three esters. (1mark)

(III) The specific retention volumes for the three components. (1mark)

(IV) The distribution constants for each of the esters. (1mark)

(V) A corrected retention volume and retention time for methyl n-hexanoate. (1mark)

(iv) Explain the effect of stationary-phase film thickness on gas chromatograms. (1mark)

QUESTION FOUR (20MARKS)

a)

(i) Describe the basis of separation in capillary zone electrophoresis(CZE) and contrast with that in chromatography. (4 mark)

(ii) Describe what two fundamental injection modes are used in capillary electrophoresis (CE) and the advantage and disadvantage of each. (2 marks)

(iii) Describe how you would plumb a single 10-port valve to inject the same sample into two different columns, each connected to a separate pump and detector.(1 mark)

(iv) In a 50 cm capillary operated at +25 kV, pressure injection is used to introduce a dilute aqueous sample. How much sample is introduced by a 5 psi, 2 s injection? In the same capillary, a sample containing chloride and iodate is electrokinetically introduced at +2 kV for 5 s. If equal amounts of the two ions were introduced, what was their concentration ratio in the original sample? (2 marks)

v)Alkali metal ions can be determined volumetrically by passing a solution of them through a cation exchange column in the hydrogen form. They displace an equivalent amount of hydrogen ions that appear in the effluent and can be titrated. How many millimoles of potassium ion are contained in a liter of solution if the effluent obtained from a 5.00-mL aliquot run through a cation exchange column requires 26.7mL of 0.0506 M NaOH for titration? (3 marks)

b)

i. Describe the equilibrium processes involved in the solvent extraction of metal chelates. (2 marks)

ii. Discuss the effect of the PH and of the reagent concentration on the solvent extraction of metal chelates. (3marks)

iii. An acidic solute HA,has a ka of 1.0 x $10^{-5}$and kd between water and hexane of 3.0. calculate the extraction efficiency if we extract a 50ml sample of 0.025M aqueous solution of HA, 3.00, with 50ml of hexane. Repeat for PH levels of 5.0 and 7.0. (1.5marks)

iv. using the table below, explain how we can separate the metal ions in an aqueous of Cu^{2+}, Cd^{2+} , and $Ni^{2+}$by extracting with an equal volume of dithizone in CCl_4 . (1.5 marks)

Table: minimum PH for extracting 99% of an aqueous metal ion using 4.om M dithizone in CCl_4 Vaq=Vorg

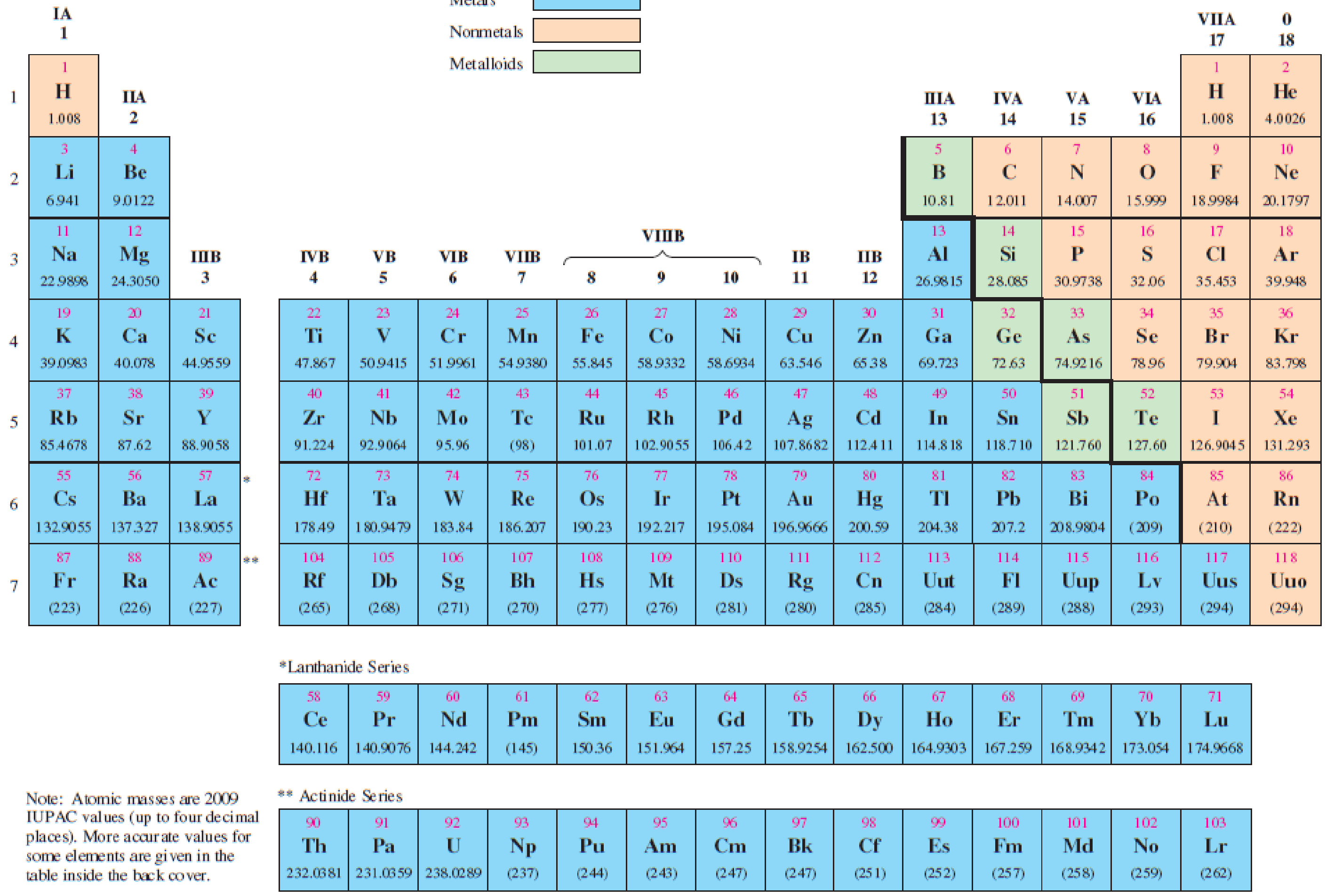
Metal ion	Hg^{2+}	Ag^{+}	Cu^{2+}	Bi^{3+}	Zn^{2+}	Cd^{2+}	Co^{2+}	Pb^{2+}	Ni^{2+}	Ti^{2+}
Minimum pH	-8.7	-1.7	-0.8	0.9	2.3	3.6	3.6	4.1	6.0	8.7

PERIODIC TABLE OF THE ELEMENTS

Metals 🟦

Nonmetals 🟧

Metalloids 🟩



	IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIIIB			IB	IIB	IIIA	IVA	VA	VIA	VIIA	0
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	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.