UNIVERSITY OF SWAZILAND
FINAL EXAMINATION 2012/13

TITLES OF PAPER: SEPARATION METHODS & ENVIRONMENTAL ANALYTICAL TECHNIQUES

COURSE CODES : C611 & ERM642

TIME ALLOWED : THREE(3) HOURS

INSTRUCTIONS : ANSWER ANY FOUR(4) QUESTIONS. EACH QUESTION CARRIES 25 MARKS.

A PERIODIC TABLE AND OTHER USEFUL DATA HAVE BEEN PROVIDED WITH THIS PAPER

REQUIRED: GRAPH PAPER

DO NOT OPEN THIS QUESTION PAPER UNTL PERMISSION TO DO SO HAS BEEN GRANTED BY THE CHIEF INVIGILATOR

Question 1(25 marks)

(a) For the extraction of a weak acid, HB into an organic phase, the acid being monomeric and its anion being insoluble in the organic phase, employ the basic equilibria involved to:

(i) Obtain the expression for the distribution ratio, D, in terms of K_a, K_{DHB} and $[H_3O^+]$. (4)

(ii) Show how a linearized form of the expression for D above can be used in evaluating the values of K_a and K_{DHB} graphically. (3)

(b) (i) The distribution coefficient, K_D, of an organic compound between water and an organic solvent is18.0. If 100 mL of an aqueous solution of the compound, buffered at pH 6.00 is extracted three times with 50 mL of the organic solvent, calculate the percentage remaining in the aqueous phase. ($K_a = 2.0 \times 10^{-6}$). (7)

(ii) What will be the value of D at pH 4? (3)

(iii) Comment on the results of (i) and (ii) above. (1)

(e) A certain metal ion M^{n+} is extracted by a chelating agent. The concentration of the chelating agent is 0.010M and the following data are obtained:

pH	1	2	3	4	5
D	10^{-8}	10^{-4}	1	10^{4}	10^{8}

From the plot of log D vs. pH obtain the values n and K (collection of constants) (7)

Question 2 (25 marks)

(a) (i). State the expression that relates the net retention volume, V_n, and the specific retention volume, V_g and define the other parameters in it. (2)

(ii) What are the factors that influence the value of V_g in a solvent and what assumption is made in this respect? (3)

(iii) Assuming an ideal behaviour, how is the net volume affected by an increase in the temperature and volatility of the solutes? (3)

(b). A 5.00-μL sample containing aniline ($C_6H_5NH_2$) and anisole ($C_6H_5OCH_3$) together with other substances was injected into a GC. The heights for the peaks of these two solutes in the resulting chromatogram were 4.22 (aniline) and 7.60 (anisole) chart divisions. Another 5.00-μL sample was injected together with 0.25 μL of pure aniline (all in the same syringe), producing aniline and anisole peak heights of 8.73 and 7.60 chart divisions. Calculate the concentration, in volume %, of the two components under the following assumptions:

(i) The detector responds equally to both compounds. (5)

(ii) The detector response (on a volume basis) is 1.35 times more for anisole than for aniline (4)

© (i) Give the two expressions for the resolution, R_s of two adjacent peaks in a chromatogram. Account for the factors that influence its value. (4)

(ii) Using a 2.0 m column, what height of a theoretical plate is needed to achieve a resolution of 1.0? (Given that $\alpha = 1.05$, and k' = 0.5). (4)

Question 3 (25 marks)

(a) Explain the term 'band broadening' in GC analysis. (1)

(b) With reference to the van Deemter equation, account for the contribution by each of the factors responsible for band broadening and column efficiency in terms of HETP. (8)

©. By making reference to the Van Deemter equation, predict the effect (increase, no effect, cannot determine), on the plate height, H, in each of the following conditions, with only one parameter varied at a time:

(i) Decreasing the particle size.
(ii) Increasing the column temperature.
(iii) Increasing the thickness of the liquid coating material.
(iv) Increasing the linear gas flow rate. (2)

(d). The analysis of n-hexane was carried out by injecting 2-μL samples unto a 3.00m GC column. The following table contains the data obtained:

Sample	1	2	3	4	5	6	7	8
Flow Rate (mL/s)	2.00	1.51	1.20	1.05	0.84	0.67	0.53	0.43
Retention time, t_s (s)	329.4	382.2	430.2	457.2	517.2	589.8	678.6	761.4
Peak Width (s)	21.0	23.4	25.8	28.2	32.4	40.8	48.6	57.0

(i) Prepare a van Deemter plot (i.e. plot HETP vs. flow rate). (8)
(ii) Determine the optimum flow rate (2)
(iii) Calculate N and H (HETP), at the optimum flow rate. (4)

Question 4(25 marks)

(a) For the HPLC technique, distinguish between:
(i) Analytical and guard columns.
(ii) Normal phase chromatography and reverse phase chromatography.
(iii) Isocratic elution and gradient elution. (6)

(b) Give four of the basic requirements for a HPLC pump. (4)

© Describe two of the different kinds of pumps commonly employed in HPLC. What are the advantages and disadvantages of each? (9)

(d) During the HPLC determination of the caffeine in an analgesic tablet, a 10 - μL injection loop was used in preparing the caffeine standards. The following data were obtained:

[Standard], (ppm)	50.0	100.0	150.0	200.0	250.0
Signal(arbitrary units)	8354	16925	25218	33584	42002

A single analgesic tablet was placed in a small beaker, dissolved with 10.0 mL of methanol and the contents , including the binder, were transferred into a 25 – mL volumetric flask and diluted to level with methanol. The sample was then filtered and a 2.50 mL aliquot was transferred into another 25 – mL volumetric flask, diluted to level with methanol and analyzed similarly as the standards. A signal of 21469 was obtained for the caffeine. Estimate the amount of caffeine (in mg), in the analgesic tablet. (6)

Question 5(25 marks)

(a) Define the following terms and discuss the factors that influence their values:
  (i) Electrophoretic mobility.
  (ii) Electroosmotic flow velocity. (6)

(b) Give a brief account of the principles of capillary zone electrophoresis(CZE). What is its main limitation and how is it overcome by the micelar electrokinetic chromatography(MEKC)? (7)

© Discuss the efficiency and solute resolution of capillary electrophoresis, indicating the parameters that influence them. (6)

(d) CZE was employed for the analysis of NO_3^- in aquarium water, using IO_4^- as an internal standard. Standard solutions of 30.0 ppm NO_3^- and 20.0 ppm of IO_4^- gave peak heights (arbitrary units), of 190.0 and 200.2 respectively. A 2.50 mL water sample from an aquarium was transferred into a 250.0 mL volumetric flask and then diluted to volume after adding sufficient internal standard to make its concentration 10.00 ppm. Analysis gave signals of 29.2 and 105.8 NO_3^- and IO_4^-, respectively. Estimate the concentration of NO_3^- in the aquarium sample in ppm. (6)

Question 6(25 marks)

(a) What are the advantages of supercritical fluid chromatography over GC and HPLC? (4)

(b) Discuss the principles of size exclusion chromatography. What are the likely sources of error during the application of this technique for analysis? (6)

© Distinguish gel - filtration from gel - permeation exclusion chromatography. (2)

(d) During a gel – permeation experiment, a 2.5 x 50 – cm Sephadex G – 200 column was used, giving the following elution data:

Compound	Molecular Wt.(M.W.)	Retention volume (mL)
Sucrose	342	242
Glucagon	5,500	233
Cytochrome C	11,000	214
Chymotripsinogen	24,000	188
Bovine serum albumin	80,000	149
Aldolase	153,000	127
α – Conaractin	486,000	92
α – Crystallin	825,000	78
Blue dextran	2,000,000	75
Unknown	?	119

(i) Plot an appropriate graph from the data.
(ii) Determine the M.W. of the unknown from your plot.
(iii) Why is the retention volume nearly independent of the M.W. at both very low and very high M.W's?
(iv) Estimate the retention volume for an enzyme with M.W. of 39,000.
(v) Calculate N(the number of theoretical plates), for two peaks having $R_s = 1$ and retention volumes of 200 – mL and 205 – mL. (13)

General data and fundamental constants

Quantity	Symbol	Value
Speed of light†	c	$2.997\,924\,58 \times 10^{8}$ m s^{-1}
Elementary charge	e	$1.602\,177 \times 10^{-19}$ C
Faraday constant	$F = eN_A$	9.6485×10^{4} C mol^{-1}
Boltzmann constant	k	$1.380\,66 \times 10^{-23}$ J K^{-1}
Gas constant	$R = kN_A$	$8.314\,51$ J K^{-1} mol^{-1}
		$8.205\,78 \times 10^{-2}$ dm^{3} atm K^{-1} mol^{-1}
		62.364 L Torr K^{-1} mol^{-1}
Planck constant	h	$6.626\,08 \times 10^{-34}$ J s
	$\hbar = h/2\pi$	$1.054\,57 \times 10^{-34}$ J s
Avogadro constant	N_A	$6.022\,14 \times 10^{23}$ mol^{-1}
Atomic mass unit	u	$1.660\,54 \times 10^{-27}$ kg
Mass of		
electron	m_e	$9.109\,39 \times 10^{-31}$ kg
proton	m_p	$1.672\,62 \times 10^{-27}$ kg
neutron	m_n	$1.674\,93 \times 10^{-27}$ kg
Vacuum permeability†	μ_0	$4\pi \times 10^{-7}$ J s^{2} C^{-2} m^{-1}
		$4\pi \times 10^{-7}$ T^{2} J^{-1} m^{3}
Vacuum permittivity	$\varepsilon_0 = 1/c^2\mu_0$	$8.854\,19 \times 10^{-12}$ J^{-1} C^{2} m^{-1}
	$4\pi\varepsilon_0$	$1.112\,65 \times 10^{-10}$ J^{-1} C^{2} m^{-1}
Bohr magneton	$\mu_B = e\hbar/2m_e$	$9.274\,02 \times 10^{-24}$ J T^{-1}
Nuclear magneton	$\mu_N = e\hbar/2m_p$	$5.050\,79 \times 10^{-27}$ J T^{-1}
Electron g value	g	$2.002\,32$
Bohr radius	$a_0 = 4\pi\varepsilon_0\hbar^2/m_e e$	$5.291\,77 \times 10^{-11}$ m
Rydberg constant	$R_\infty = m_e e^4/8h^3c$	$1.097\,37 \times 10^{5}$ cm^{-1}
Fine structure constant	$\alpha = \mu_0 e^2 c/2h$	$7.297\,35 \times 10^{-3}$
Gravitational constant	G	$6.672\,59 \times 10^{-11}$ N m^{2} kg^{-2}
Standard acceleration of free fall†	g	$9.806\,65$ m s^{-2}

† Exact (defined) values

Prefixes

f	p	n	μ	m	c	d	k	M	G
femto	pico	nano	micro	milli	centi	deci	kilo	mega	giga
10^{-15}	10^{-12}	10^{-9}	10^{-6}	10^{-3}	10^{-2}	10^{-1}	10^{3}	10^{6}	10^{9}

PERIODIC TABLE OF ELEMENTS

GROUPS

PERIODS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	IA	IIA	IIIB	IVB	VB	VIB	VIIB		VIIIB		IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA
1	1.008 H 1																	4.003 He 2
2	6.941 Li 3	9.012 Be 4										Atomic mass → Symbol → Atomic No. →	10.811 B 5	12.011 C 6	14.007 N 7	15.999 O 8	18.998 F 9	20.180 Ne 10
3	22.990 Na 11	24.305 Mg 12				TRANSITION ELEMENTS							26.982 Al 13	28.086 Si 14	30.974 P 15	32.06 S 16	35.453 Cl 17	39.948 Ar 18
4	39.098 K 19	40.078 Ca 20	44.956 Sc 21	47.88 Ti 22	50.942 V 23	51.996 Cr 24	54.938 Mn 25	55.847 Fe 26	58.933 Co 27	58.69 Ni 28	63.546 Cu 29	65.39 Zn 30	69.723 Ga 31	72.61 Ge 32	74.922 As 33	78.96 Se 34	79.904 Br 35	83.80 Kr 36
5	85.468 Rb 37	87.62 Sr 38	88.906 Y 39	91.224 Zr 40	92.906 Nb 41	95.94 Mo 42	98.907 Tc 43	101.07 Ru 44	102.91 Rh 45	106.42 Pd 46	107.87 Ag 47	112.41 Cd 48	114.82 In 49	118.71 Sn 50	121.75 Sb 51	127.60 Te 52	126.90 I 53	131.29 Xe 54
6	132.91 Cs 55	137.33 Ba 56	138.91 *La 57	178.49 Hf 72	180.95 Ta 73	183.85 W 74	186.21 Re 75	190.2 Os 76	192.22 Ir 77	195.08 Pt 78	196.97 Au 79	200.59 Hg 80	204.38 Tl 81	207.2 Pb 82	208.98 Bi 83	(209) Po 84	(210) At 85	(222) Rn 86
7	223 Fr 87	226.03 Ra 88	(227) **Ac 89	(261) Rf 104	(262) Ha 105	(263) Unh 106	(262) Uns 107	(265) Uno 108	(266) Une 109	(267) Uun 110								

*Lanthanide Series	140.12 Ce 58	140.91 Pr 59	144.24 Nd 60	(145) Pm 61	150.36 Sm 62	151.96 Eu 63	157.25 Gd 64	158.93 Tb 65	162.50 Dy 66	164.93 Ho 67	167.26 Er 68	168.93 Tm 69	173.04 Yb 70	174.97 Lu 71
**Actinide Series	232.04 Th 90	231.04 Pa 91	238.03 U 92	237.05 Np 93	(244) Pu 94	(243) Am 95	(247) Cm 96	(247) Bk 97	(251) Cf 98	(252) Es 99	(257) Fm 100	(258) Md 101	(259) No 102	(260) Lr 103