
UNIVERSITI SAINS MALAYSIA

Second Semester Examination
Academic Session 2007/2008

April 2008

KAT 244 – Separation Methods
[Kaedah Pemisahan]

Duration : 3 hours
[Masa : 3 jam]

Please check that this examination paper consists of **TWELVE** printed pages before you begin the examination.

Instructions:-

Answer any **FIVE** (5) questions.

Answer each question on a new page.

You may answer either in Bahasa Malaysia or in English.

If a candidate answers more than five questions, only the answers to the first five questions in the answer sheet will be graded.

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1. (a) Describe two principal solvent extraction systems used for metal ions. Give examples of each. (7 marks)
- (b) How does solid-phase extraction differ from solvent extraction? (5 marks)
- (c) The following data were obtained by gas chromatography (GC) on a 40 cm packed column:

Compound	t_R , min	w_h , min
Air	1.9	-
Methylcyclohexane	10.0	0.76
Methylcyclohexene	10.9	0.82
Toluene	13.4	1.06

Note: w_h = the width of the peak measured at a height of one-half of the peak height.
 t_R = retention time.

Calculate;

- (i) an average number of plates from the data.
 (ii) the standard deviation for the average in (i).
 (iii) an average plate height for the column. (8 marks)
2. (a) Ninety-six percent of a solute is removed from 100 mL of an aqueous solution by extraction with two 50-mL portions of an organic solvent. What is the distribution ratio of the solute? (6 marks)
- (b) For a normal-phase separation, predict the order of elution of n-hexane, n-hexanol and benzene. Give your explanation. (4 marks)
- (c) Compare the advantages of high performance liquid chromatography (HPLC) and gas chromatography (GC). What are the similar characteristics for both methods? (10 marks)

3. (a) For a solute with a distribution ratio of 25.0, show by calculation which is more effective, extraction of 10 mL of an aqueous solution with 10 mL organic solvent or extraction with two separate 5.0-mL portions of organic solvent.

(5 marks)

- (b) The mobile phases used by GC, supercritical fluid chromatography, and HPLC are gases, supercritical fluids, and liquids respectively. The properties of gases, supercritical fluids, and liquids are shown in the following table:

	Gas (at STP)	Supercritical Fluid	Liquid
Density, g cm^{-3}	$(0.6 - 2) \times 10^{-3}$	0.2 - 0.5	0.6 - 1.6
Diffusion coefficient, $\text{cm}^2 \text{s}^{-1}$	$(1 - 4) \times 10^{-1}$	$10^{-3} - 10^{-4}$	$(0.2 - 2) \times 10^{-5}$
Viscosity, $\text{g cm}^{-1} \text{s}^{-1}$	$(1 - 3) \times 10^{-4}$	$(1 - 3) \times 10^{-4}$	$(0.2 - 2) \times 10^{-2}$

Based on the information in the above table, what conclusion can you make regarding the properties of supercritical fluids as compared to the properties of gases and liquids.

Please relate these properties to the separation time, and the band broadening in each method.

(6 marks)

- (c) Describe the principles of capillary electrophoresis. What are its advantages?

(5 marks)

- (d) In what order would $\text{CH}_3\text{CH}_2\text{OH}$, CH_3CHO , and $\text{CH}_3\text{CO}_2\text{H}$ be eluted from an alumina column using n-hexane as the eluent? Give your reasons.

(4 marks)

4. The following data are for a liquid chromatographic column:

Length of packing	24.7 cm
Flow rate	$0.313 \text{ mL min}^{-1}$
Mobile phase volume, V_m	1.37 mL
Stationary phase volume, V_s	0.164 mL

A chromatogram of a mixture of species A, B, C, and D provided the following data:

	Retention Time, min	Bandwidth of Peak (w_b), min
Nonretained	3.1	-
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.6	1.72

- (a) Calculate;
- the number of plates, N , from each peak.
 - the mean and the standard deviation for N .
 - the plate height for the column.
 - the retention factor.
- (8 marks)
- (b) From the data above, calculate for species B and C;
- the resolution.
 - the separation factor.
 - the length of the column necessary to separate the two species with a resolution of 1.5.
- (12 marks)
5. (a) Three compounds, A, B, and C, exhibit retention factors on a column having only 500 plates of $k_A = 1.40$, $k_B = 1.85$, and $k_C = 2.65$. Can they be separated with a minimum resolution of 1.05?
- (8 marks)
- (b) Eighty percent of a metal chelate is extracted when equal volumes of aqueous and organic phases are used. What will be the percent extracted if the volume of the organic phase is doubled?
- (5 marks)
- (c) To what types of compounds is supercritical fluid chromatography particularly applicable?
- (4 marks)
- (d) Describe the basis of separation in electrophoresis.
- (3 marks)

- 5 -

6. (a) Describe the principles of the following gas chromatography detectors:
- thermal conductivity,
 - flame ionization, and
 - electron capture.

Compare the above mentioned detectors with respect to sensitivity and types of compounds that can be detected.

(12 marks)

- (b) The concentration of polyaromatic hydrocarbons (PAHs) in soil can be determined by first extracting the PAHs with methylene chloride. The extract is then diluted, if necessary, and the PAHs are separated by HPLC using a UV/Vis or fluorescence detector. Calibration is achieved by using one or more external standards. In a typical analysis, a 2.013 g sample of dried soil is extracted with 20.00 mL of methylene chloride. After filtering to remove the soil, a 1 mL portion of the extract is removed and diluted to 10 mL with acetonitrile. Injecting 5 μL of the diluted extract into an HPLC gives a signal of 0.217 (arbitrary units) for the PAH fluoranthene. When 5 μL of a 20.0 ppm fluoranthene standard is analysed using the same conditions, a signal of 0.258 is measured. Calculate the parts per million of fluoranthene in the soil.

(8 marks)

7. (a) When a driver is arrested for suspicion of driving under the influence of alcohol (ethanol or EtOH), the blood alcohol content is determined to see if it exceeds the legal limit. In cases where there is an accident, injury, or death, the blood alcohol is usually determined directly, by analyzing a blood sample by gas chromatography.

A 5.00 mL blood sample from a suspect is spiked with 0.500 mL of aqueous 1% propanol (PrOH) internal standard. A 10 μL portion of the mixture is injected into the GC, and the peak areas are recorded. Standards are treated in the same way. The following results were obtained:

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- 6 -

%(w/v) EtOH	Peak Area EtOH	Peak Area PrOH
0.020	114	457
0.050	278	449
0.100	561	471
0.150	845	453
0.200	1070	447
Unknown	782	455

Plot a suitable calibration curve (or use your scientific calculator) to determine the blood alcohol concentration in the unknown sample.

(6 marks)

- (b) What is the multi-solvent selection method in HPLC? What two factors are optimized in solvent selection?

(4 marks)

- (c) Describe the principles of size exclusion chromatography. What is the exclusion limit?

(5 marks)

- (d) Describe the factors that affect the selectivity of ion exchange resins.

(5 marks)

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1. (a) Terangkan dua sistem pengekstrakan pelarut utama bagi ion logam. Berikan contoh masing-masing. (7 markah)
- (b) Bagaimanakah pengekstrakan fasa pepejal berbeza daripada pengekstrakan pelarut? (5 markah)
- (c) Data berikut diperolehi daripada kromatografi gas (GC) dengan turus padatan 40 cm:

Sebatian	t_R , min	w_h , min
Udara	1.9	-
Metilsikloheksana	10.0	0.76
Metilsikloheksena	10.9	0.82
Toluena	13.4	1.06

Nota: w_h = kelebaran puncak yang diukur pada separuh ketinggian puncak.
 t_R = masa penahanan

Kira;

- (i) purata bilangan plat daripada data.
 (ii) sisihan piawai bagi purata dalam (i).
 (iii) purata tinggi plat bagi turus.

(8 marks)

2. (a) Sebanyak 96% suatu zat terlarut telah dapat dipisahkan daripada 100 mL larutan akueus dengan dua kali pengekstrakan dengan dua bahagian 50 mL pelarut organik. Berapakah nisbah taburan zat terlarut ini? (6 markah)
- (b) Bagi pemisahan fasa normal, ramalkan turutan elusi n-heksana, n-heksanol dan benzena. (4 markah)
- (c) Bandingkan kelebihan-kelebihan kromatografi cecair keupayaan tinggi (HPLC) dengan kelebihan-kelebihan kromatografi gas. Apakah ciri-ciri yang sama bagi kedua-dua kaedah? (10 markah)

- 9 -

3. (a) Bagi suatu zat terlarut yang mempunyai nisbah taburan 25.0, tunjukkan dengan pengiraan yang mana lebih berkesan, pengekstrakan 10 mL larutan akueus dengan 10 mL pelarut organik atau dua pengekstrakan berasingan dengan 5.0 mL bahagian pelarut organik.

(5 markah)

- (b) Fasa bergerak yang digunakan oleh kromatografi gas, kromatografi bendalir lampau genting dan kromatografi cecair kepayaan tinggi adalah masing-masing gas, bendalir lampau genting dan cecair. Sifat-sifat gas, bendalir lampau genting dan cecair ditunjukkan dalam jadual berikut:

	Gas (pada STP)	Bendalir Lampau Genting	Cecair
Ketumpatan, g cm^{-3}	$(0.6 - 2) \times 10^{-3}$	0.2 - 0.5	0.6 - 1.6
Pekali Pembauran, $\text{cm}^2 \text{s}^{-1}$	$(1 - 4) \times 10^{-1}$	$10^{-3} - 10^{-4}$	$(0.2 - 2) \times 10^{-5}$
Kelikatan, $\text{g cm}^{-1} \text{s}^{-1}$	$(1 - 3) \times 10^{-4}$	$(1 - 3) \times 10^{-4}$	$(0.2 - 2) \times 10^{-2}$

Berdasarkan maklumat dalam jadual di atas, apakah kesimpulan yang diperolehi tentang sifat bendalir lampau genting berbanding dengan sifat gas dan cecair.

Sila kaitkan sifat-sifat ini kepada masa pemisahan dan perlebaran jalur dalam setiap kaedah.

(6 markah)

- (c) Terangkan prinsip elektroforesis rerambut. Apakah kelebihanannya?

(5 markah)

- (d) Apakah urutan $\text{CH}_3\text{CH}_2\text{OH}$, CH_3CHO , dan $\text{CH}_3\text{CO}_2\text{H}$ yang dielusi daripada turus alumina menggunakan n-heksana sebagai pengelusi? Berikan alasan kamu.

(4 markah)

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4. Data berikut adalah bagi suatu turus kromatografi cecair:

Panjang padatan	24.7 cm
Kadar alir	0.313 mL min ⁻¹
Isipadu fasa bergerak, V_m	1.37 mL
Isipadu fasa pegun, V_s	0.164 mL

Kromatogram bagi suatu campuran spesies A, B, C dan D memberikan data seperti berikut:

	Masa Penahanan, min	Lebar Jalur Puncak (w_b), min
Tidak ditahan	3.1	-
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.6	1.72

- (a) Kira;
- bilangan plat, N , daripada setiap puncak.
 - purata dan sisihan piawai bagi N .
 - tinggi plat bagi turus.
 - faktor penahanan.
- (8 markah)
- (b) Daripada data di atas, kira bagi spesies B dan C;
- Bezajelas (resolusi).
 - faktor pemisahan.
 - panjang turus yang diperlukan untuk memisahkan kedua-dua spesies dengan resolusi 1.5.
- (12 markah)
5. (a) Faktor penahanan k_A , k_B dan k_C bagi tiga sebatian A, B dan C adalah masing-masing 1.40, 1.85 dan 2.65 dalam suatu turus yang mempunyai 500 plat. Adakah sebatian-sebatian ini dapat dipisahkan dengan satu resolusi minimum 1.05?
- (8 markah)
- (b) Sebanyak 80% suatu logam kelat dapat diekstrak apabila isipadu fasa akueus dan fasa organik adalah sama. Berapakah peratus pengekstrakan apabila isipadu fasa organik digandakan (2 kali isipadu asal)?
- (5 markah)

- 11 -

- (c) Apakah jenis-jenis sebatian yang sesuai dianalisis menggunakan kromatografi bendalir lampau genting?
(4 markah)
- (d) Terangkan prinsip pemisahan dalam elektroforesis.
(3 markah)
6. (a) Terangkan prinsip pengesanan kromatografi gas berikut:
- (i) Kekonduksian terma.
 - (ii) Pengionan nyala.
 - (iii) Penangkapan elektron.
- Bandingkan pengesanan-pengesanan di atas daripada segi kepekaan dan jenis-jenis sebatian yang dapat dikesan.
(12 markah)
- (b) Kepekatan hidrokarbon poliaromatik (PAH) dalam tanah dapat ditentukan dengan mengekstrak PAH dengan metilena klorida. Kemudian ekstrak itu dicairkan, jika perlu, dan PAH dipisahkan menggunakan HPLC dengan pengesanan ultralembayung-nampak atau pendarfluor. Tentukan diperolehi dengan menggunakan satu atau lebih piawai luaran. Dalam suatu analisis tipikal, sebanyak 2.013 g sampel tanah kering diekstrak dengan 20.00 mL metilena klorida. Selepas penurasan untuk mengasingkan tanah, sebanyak 1 mL bahagian ekstrak diasingkan dan dicairkan kepada 10 mL dengan asetonitril. Penyuntikan 5 μ L ekstrak yang telah dicairkan tadi ke dalam HPLC memberi isyarat 0.217 (unit arbitrari) bagi PAH fluorantena. Apabila 5 μ L 20.0 ppm piawai fluorantena dianalisis menggunakan keadaan yang sama, isyarat 0.258 disukat. Kira kepekatan fluorantena di dalam sampel tanah dalam unit bahagian per juta.
(8 markah)

7. (a) Apabila seseorang pemandu ditangkap kerana disyaki memandu di bawah pengaruh alkohol (etanol atau EtOH), kandungan alkohol darah ditentukan untuk mengetahui samada kandungannya melebihi had yang dibenarkan. Dalam kes yang mana kemalangan, kecederaan atau kematian, kandungan alkohol darah ditentukan terus dengan menganalisis sampel darah menggunakan kromatografi gas.

Sebanyak 5.00 mL sampel darah daripada suspek dipaku (spiked) dengan piawai dalaman 0.500 mL propanol (PrOH) akueus 1%. Sebanyak 10 μ L bahagian campuran disuntik ke dalam GC dan luas puncak direkodkan. Piawai dilakukan dengan cara yang sama. Keputusan berikut diperolehi:

% (w/v) EtOH	Luas Puncak EtOH	Luas Puncak PrOH
0.020	114	457
0.050	278	449
0.100	561	471
0.150	845	453
0.200	1070	447
Anu	782	455

Plot kelok tentukuran yang sesuai (atau gunakan mesinkira saintifik kamu) untuk menentukan kepekatan alkohol darah dalam sampel anu.

(6 markah)

- (b) Apakah kaedah pemilihan berbilang-pelarut dalam HPLC? Apakah dua faktor yang dioptimumkan dalam pemilihan pelarut?

(4 markah)

- (c) Terangkan prinsip kromatografi penyisihan saiz. Apakah had penyisihan?

(5 markah)

- (d) Terangkan faktor-faktor yang mempengaruhi kepilihan resin pertukaran ion.

(5 markah)