
UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2011/2012 Academic Session

June 2012

KAT 244 – Separations Methods
[Kaedah Pemisahan]

Duration : 3 hours
[Masa : 3 jam]

Please check that this examination paper consists of FOURTEEN pages of printed material before you begin the examination.

Instructions:

Answer any **FIVE** (5) questions. If a candidate answers more than five questions only the first five questions in the answer sheet will be graded.

Answer each question on a new page.

You may answer the questions either in Bahasa Malaysia or in English.

In the event of any discrepancies, the English version shall be used.

Answer any **FIVE** (5) questions

1. (a) One gram of benzoic acid originally dissolved in 100 mL of water is to be equilibrated with 100 mL of ether. The distribution coefficient, K_D , is 100 and the dissociation constant, K_a , is 6.5×10^{-5} . Calculate the distribution ratio, D , if the aqueous layer is at pH 3, pH 5 and pH 7.
(10 marks)
- (b) Assume that 4 g of butyric acid is to be extracted from 500 mL of water with 500 mL of ether. The distribution coefficient for this system is 3.0 at 25 °C. Calculate the amount of butyric acid extracted
- If the ether is used in a single batch.
 - If the ether is used in two successive 250-mL portions.
 - If the ether been divided into five 100-mL portions.
- (10 marks)
2. (a) Calculate the distribution of a substance in each vessel after 3 transfers in a Craig apparatus for which $V_s = 2$ mL and $V_m = 4$ mL. The distribution coefficient is 3.0.
(Percentage of a substance in the first four vessels)
(6 marks)
- (b) A sample containing approximately 1 g of an organic substance A is dissolved in 100 mL of water. The aqueous solution is then shaken with 100 mL of ether. Analysis of the ether layer shows that 0.7 g of A is extracted.
- What is the value of $K_D = C_{et}/C_{aq}$ for this system?
 - How many additional extractions with 100- mL portions of ether would be required to extract 95% of the substance?
 - What fraction of A would have been extracted by four 25-mL portions of ether?
- (6 marks)
- (c) Formaldehyde has a distribution coefficient, $K_D = 0.111$ at 25°C.
- How many liters of water will be required to remove in one extraction, 95% of the formaldehyde from 1 L of ether containing 0.5 g, of formadehyde?
 - How much formaldehyde would remain in 50 mL of ether initially containing 5 g of formaldehyde after five successive extractions with 25-mL portions of water?
- (8 marks)

3. (a) What do the capacity factor, k' , and the selectivity factor, α , describe?
(4 marks)

- (b) Derive van Deemter equation.
i. Explain each term in this equation and why some are influenced by flow and some are not.
ii. Show graphically the effect of particle size on the van Deemter curve and explain why?
(6 marks)

- (c) An extract of drinking water shows two closely-eluting GC Electron Capture Detector (ECD) peaks which are believed to be methyl trichloroacetate and methyl dichloroacetate. The column used is a 2 m x 4 mm glass column packed with 3% SP-1000 on 100/200 mesh Supelcoport. The stationary phase weight is 2.54 g, and it has a density of 0.699 g/mL. The inlet column pressure was 22 psig, the outlet flow rate was 60 mL/min, and the column temperature was isothermal at 65°C.

Retention Time (min)	Peak Width (min)	Identity
0.40	-	Unretained solvent
8.85	0.63	methyl trichloroacetate?
9.55	0.71	methyl dichloroacetate?

- i. Calculate k' for each of the suspected esters
ii. Calculate the average number of theoretical plates
iii. Describe how you would determine the true identity of the two suspected esters. You only have access to a GC with a flame ionization detector FID and an ECD.
(10 marks)

4. You have a surface water sample that you suspect to be contaminated with pentabromobenzoic acid (C_6Br_5COOH). This aromatic compound is considered to be very toxic, so you are interested in trace quantities. One of your concerns is interference from naturally-occurring organic matter which is present at concentrations that are 3 or more orders of magnitude greater than the analyte. Propose how you would analyze such a sample for pentabromobenzoic acid. Explain the reasons for your choice of methods and procedures. Consider the following points; extraction and/or concentration, sample cleanup, derivatization, separation and quantification, and identification.
(20 marks)

5. (a) The following data apply to a column for liquid chromatography. The length of column is 35.7 cm, the volume of the mobile phase and stationary phase is 1.6 mL and 0.134 mL respectively. The column was run at a flow rate of 0.5 mL/min. A mixture of 4 components (A,B,C and D) provided the following data:

	Retention time (min)	Width of Peak Base (min)
-	non-retained, 2.1	-
A.	5.4	0.51
B.	10.3	1.03
C.	12.1	1.25
D.	20.6	1.62

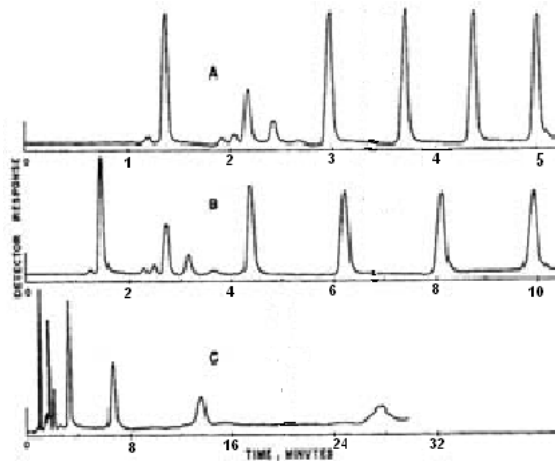
- i. Using peak C, calculate the number of plates (N) and the plate height (H) for the column.
- ii. For peak D, calculate the capacity factor (k') and the partition coefficient (K).
- iii. For peaks B and C, calculate the resolution (R) and the relative retention.

(10 marks)

- (b) There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. Describe;
non-selective, selective, specific, concentration dependant and mass flow dependant detectors.

(10 marks)

6. (a) There are three important parts of the gas chromatograph in which the temperature must be controlled briefly, comment on the requirement of those parts in a gas chromatograph. (6 marks)
- (b) The separation of paraffin mixture are shown in the chromatograms below. Explain the relationship of the separation efficiency of the mixture in relation to the temperature and the individual component of the paraffin.



Chromatograms of C5–C10 n-paraffin mixture.

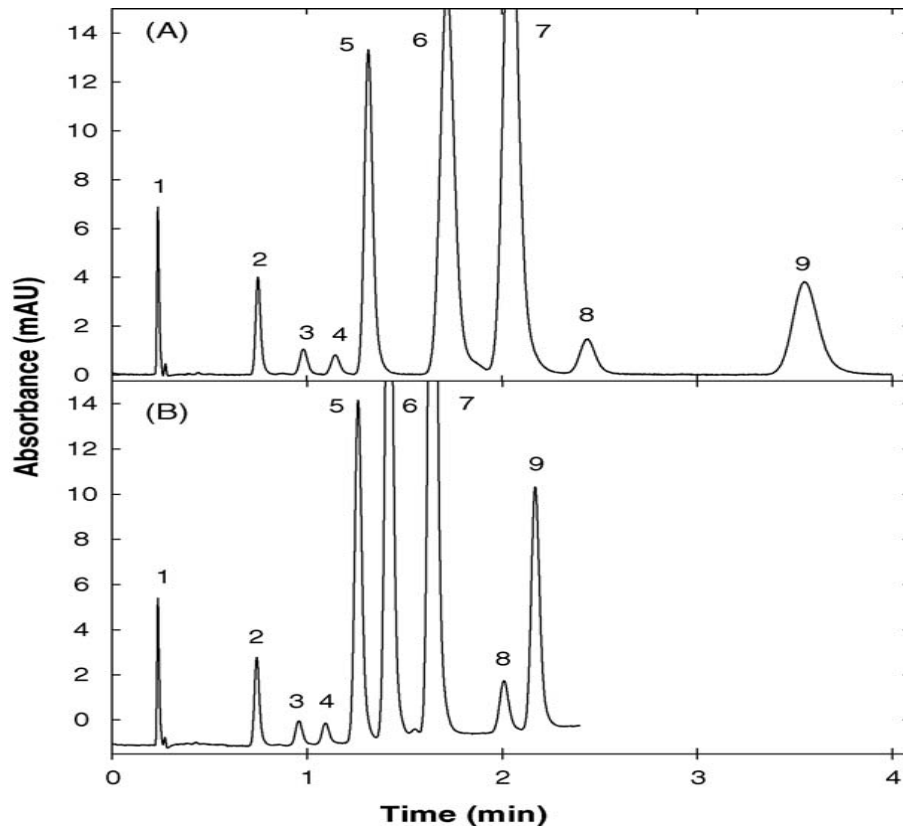
- A. Heating rate 30 °C/min, flow rate 100 mL/min, starting temperature 40 °C.
- B. Same conditions except heating rate 5 °C/min.
- C. Isothermal chromatogram, temperature: 75 °C; flow rate: 100 mL/min.

(14 marks)

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7. (a) The chromatograms below show the isocratic and gradient elution separations for a sample easily separated by isocratic elution.

Choose and justify the best system (A or B) to separate the same mixture.



Optimized isocratic (A) and gradient (B) elution separations for a sample. Conditions: Eluent A: 1/31/67.9/0.1 (v/v/v/v) *n*-butanol/acetonitrile/water/trifluoroacetic acid; Eluent B: 1/60/38.9/0.1 (v/v/v/v) *n*-butanol/acetonitrile/water/trifluoroacetic acid; detection at 254 nm; 2 mL/min flow rate; 2 μ L injection of stock solution diluted 10–500 mL; 25 °C. (A) 100/0 A/B and run ended at 4.0 min; critical pair: 3, 4. (B) 100/0 to 60/40 to 100/0 A/B in 2 and 0.01 min and the run was ended at 2.4 min; critical pair: 8, 9. Solutes: uracil (1), phenol (2), 1-(diphenylmethyl)-4-methylpiperazine (3), alprenolol (4), perphenazine (5), acetophenone (6), promethazine (7), methylbenzoate (8) and amitriptyline (9).

(6 marks)

- (b) In the analyses of chlorinated pesticides, in environmental, food, or other samples usually by liquid-liquid extraction, which takes 4 to 18 hours, or with solid phase extraction cartridges or disks, which takes 1 to 2 hours total time and 20 to 45 minutes of handling time per sample. In contrast, solid phase microextraction (SPME) is faster (15 minutes) and much less labor intensive (about 3 minutes of handling time per sample) than liquid-liquid extraction or solid phase extraction, and requires only small amounts of sample and no organic solvents. SPME reduces interfering background in pesticide or other analyses, making analyte identification and quantification more reliable. Elaborate the working principle of each technique and highlight advantages and disadvantages of each technique.

(14 marks)

TERJEMAHAN

Sila pastikan bahawa kertas peperiksaan ini mengandungi EMPAT BELAS muka surat bahan bercetak.

Arahan:

Jawab **LIMA** (5) soalan . Jika calon menjawab lebih daripada lima soalan hanya lima soalan pertama mengikut susunan dalam skrip jawapan akan diberi markah.

Jawab setiap soalan pada muka surat yang baru.

Anda dibenarkan menjawab soalan ini sama ada dalam Bahasa Malaysia atau Bahasa Inggeris.

Sekiranya terdapat sebarang percanggahan pada soalan peperiksaan, versi Bahasa Inggeris hendaklah digunakan.

Jawab **LIMA** (5) soalan

1. (a) Satu gram asid benzoik asalnya terlarut di dalam 100 mL air akan diseimbangkan dengan 100 mL eter. Pekali taburan, K_D , adalah 100 dan pemalar peruraian, K_a , adalah 6.5×10^{-5} . Hitung nisbah taburan, D , jika lapisan akueus adalah pada pH 3, pH 5 and pH 7.
- (10 markah)
- (b) Andaikan 4 g asid butirik hendak diekstrakkan daripada 500 mL air dengan 500 mL eter. Pekali taburan bagi sistem ini adalah pada 25 °C. Hitung banyaknya asid butirik terekstrak.
- i. Jika eter digunakan secara tunggal.
 - ii. Jika eter digunakan dua kali berturutan dengan 250-mL.
 - iii. Jika eter dibahagikan kepada lima bahagian 100-mL.
- (10 markah)
2. (a) Hitunglah taburan suatu bahan di dalam setiap bekas sesudah 3 pemindahan pada radas Craig yang mana $V_s = 2$ mL dan $V_m = 4$ mL. Pekali taburan adalah 3.0. (Peratus bahan di dalam empat bekas pertama)
- (6 markah)
- (b) Suatu sampel mengandungi kira-kira 1 g sebatian organik A dilarutkan didalam 100 mL air. Larutan akueus kemudiannya digoncang dengan 100 mL eter. Analisis lapisan eter menunjukkan 0.7 g daripada A telah terekstrak.
- i. Berapakah nilai $K_D = C_{et}/C_{aq}$ bagi sistem ini?
 - ii. Berapakah tambahan pengekstrakan dengan 100- mL bahagian eter yang akan diperlukan untuk mengekstrak 95% sebatian tersebut?
 - iii. Berapakah pecahan A yang akan terekstrak dengan empat bahagian 25-mL eter?
- (6 markah)
- (c) Formaldehida mempunyai pekali taburan, $K_D = 0.111$ at 25°C.
- i. Berapa liter air yang akan diperlukan untuk menyingkirkan dengan sekali pengekstrakan, 95% formaldehida daripada 1 L eter mengandungi 0.5 g, formadehida?
 - ii. Berapakah banyaknya formaldehida yang akan berbaki didalam 50 mL eter yang pada asalnya mengandungi 5 g formaldehida selepas lima pengekstrakan berturutan dengan 25-mL bahagian air?
- (8 markah)

3. (a) Apakah yang dapat diterangkan oleh faktor muatan, k' , dan faktor kepilihan, α ?
(4 markah)

- (b) Turunkan persamaan van Deemter.

- i. Terangkan bagi setiap sebutan di dalam persamaaan ini dan kenapa sebahagiannya dipengaruhi oleh aliran dan sebahagiannya tidak.
- ii. Tunjukkan secara grafik kesan saiz zarah terhadap lengkung van Deemter dan terangkan kenapa?

(6 markah)

- (c) Suatu ekstrak air minuman menunjukkan dua puncak GC dengan Pengesan Tangkapan Elektron (ECD) terelusi-rapat yang dipercayai datang daripada metil trikloroasetat dan metil dikloroasetat. Turus yang digunakan adalah 2 m x 4 mm turus kaca dipadatkan dengan 3% SP-1000 diatas 100/200 mesh Supelcoport. Berat fasa pegun adalah 2.54 g, dan ketumpatannya adalah 0.699 g/mL. Tekanan inlet turus adalah 22 psig, kadar aliran adalah 60 mL/min, dan suhu turus isothermal pada 65°C.

Masa Penahanan (min)	Lebar Puncak (min)	Pengenalan
0.40	-	Pelarut Taktertahan
8.85	0.63	metil trikloroasetat?
9.55	0.71	metil dikloroasetat?

- i. Hitunglah k' bagi setiap ester yang disyaki.
- ii. Hitunglah purata bilangan plat teoritis.
- iii. Terangkan bagaimana anda dapat menentukan pengenalan sebenar kedua-dua ester yang disyaki itu. Anda hanya dapat gunakan GC dengan pengesan pengionan nyalaan (FID) dan ECD.

(10 markah)

4. Anda mempunyai suatu sampel air permukaan yang disyaki tercemar dengan asid pentabromobenzoik (C_6Br_5COOH). Sebatian aromatik ini dikatakan amat beracun, jadi anda berminat ditahap jumlah surihan. Salah satu kebimbangan anda adalah gangguan daripada bahan organik hadir secara semulajadi dengan kepekatan 3 atau lebih tertib magnitud lebih besar daripada analit. Cadangkan bagaimana anda dapat menganalisis sampel sedemikian bagi asid pentabromobenzoik. Jelaskan alasan bagi kaedah dan tatacara pilihan anda. Ambil perhatian kepada fakta berikut; pengekstrakan dan/atau pemekatan, pencucian sampel, penerbitan, pemisahan dan pengkuantifikasikan, dan pengenalpastian.

(20 markah)

5. (a) Data berikut diperolehi dari turus kromatografi cecair. Panjang turus adalah 35.7 cm, isipadu fasa bergerak dan fasa pegun adalah masing-masing 1.6 mL dan 0.134 mL. Turus bekerja pada kadar aliran 0.5 mL/min. Suatu campuran 4 komponen (A,B,C dan D) memberikan data berikut:

	Masa penahanan (min)	Lebar dasar puncak (min)
-	tak-tertahan, 2.1	-
A.	5.4	0.51
B.	10.3	1.03
C.	12.1	1.25
D.	20.6	1.62

- i. Gunakan puncak C, hitung bilangan plat (N) dan tinggi plat (H) bagi turus tersebut.
- ii. Bagi puncak D, hitung faktor muatan (k') dan pekali pembahagian (K).
- iii. Bagi puncak B dan C, hitung resolusi (R) dan penahanan relatif.

(10 markah)

- (b) Terdapat banyak pengesan yang dapat digunakan didalam kromatografi gas. Pengesan berbeza memberikan perbezaan jenis kepilahan. Jelaskan;
Pengesan tak-selektif, selektif, spesifik, bergantung kepekatan dan bergantung aliran jisim.

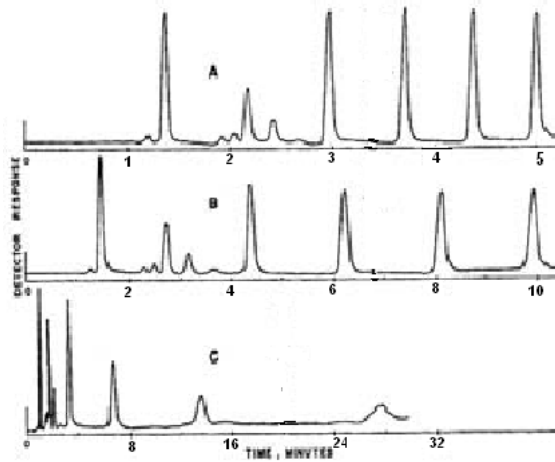
(10 markah)

6. (a) Terdapat tiga bahagian penting pada kromatograf gas yang mana suhu mesti dikawal, secara ringkasnya, komen ke atas keperluan bahagian-bahagian tersebut daripada suatu kromatograf.

(6 markah)

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- (b) Pemisahan campuran paraffin adalah seperti ditunjukkan didalam kromatogram dibawah. Terangkan hubungan kecekapan pemisahan berkaitan dengan suhu dan setiap komponen daripada paraffin.



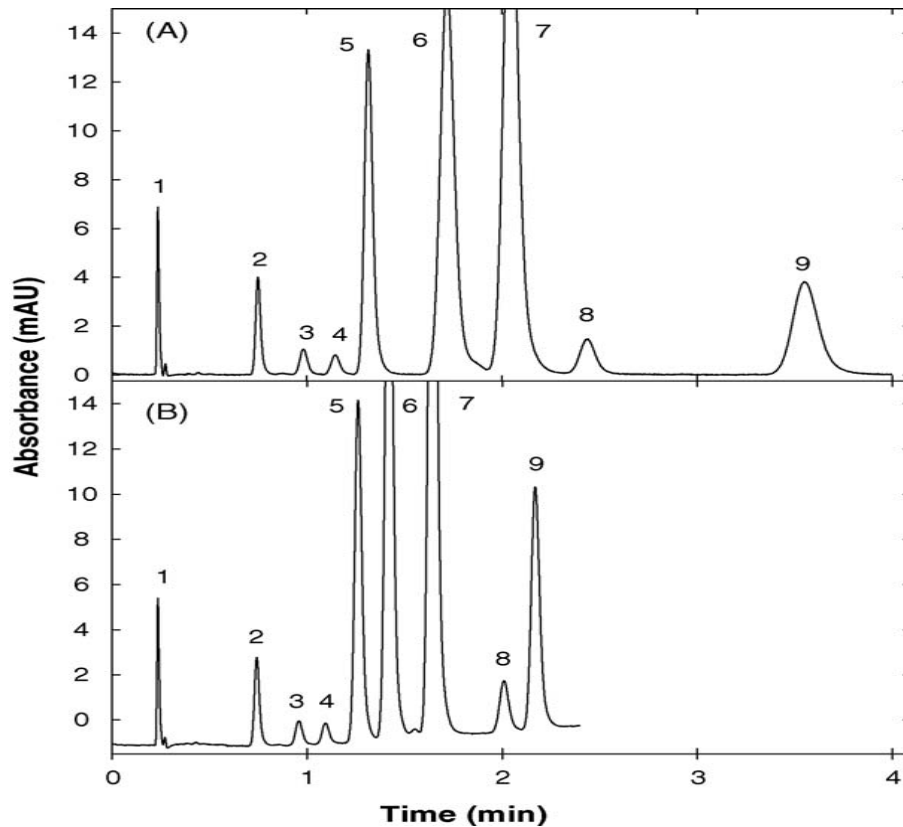
Chromotogram C5–C10 campuran n-paraffin.

- A. *Kadar pemanasan 30 °C/min, kadar aliran 100 mL/min, suhu awal 40 °C.*
 B. *Keadaan sama kecuali kadar pemanasan 5 °C/min.*
 C. *Kromatogram isoterma, suhu: 75 °C; kadar aliran: 100 mL/min.*

(14 markah)

8. (a) Kromatogram dibawah menunjukkan pengelusan pemisahan isokratik dan gradien bagi sampel yang mudah terpisah dengan secara elusi isokratik.

Pilih dan beri justifikasi system terbaik (A atau B) bagi memisahkan campuran yang sama.



Pengelusan isokratik teroptimum (A) dan gradien (B) pemisahan suatu sampel. Keadaan: Eluen A: 1/31/67.9/0.1 (v/v/v/v) *n*-butanol/asetonitril/air/trifluoroasetik asid; Eluent B: 1/60/38.9/0.1 (v/v/v/v) *n*-butanol/asetonitril/air/trifluoroasetik asid; pengesanan pada 254 nm; kadar aliran 2 mL/min; suntikan 2 μ L larutan stok dicairkan 10–500 mL; 25 °C. (A) 100/0 A/B dan eksperimen dihentikan setelah 4.0 min; pasangan kritikal: 3, 4. (B) 100/0 hingga 60/40 ke 100/0 A/B pada 2 dan 0.01 min dan eksperimen di tamatkan pada 2.4 min; pasangan kritikal: 8, 9. Solut: uracil (1), fenol (2), 1-(difenilmetil)-4-metilpiperazina (3), alprenolol (4), perphenazina (5), acetofenon (6), prometazina (7), metilbenzoate (8) dan amitriptilina (9).

(6 markah)

- (b) Didalam suatu analisis pestisid terklorin, didalam alam sekitar, makanan, atau sampel yang lain, kebiasaannya jika secara pengekstrakan cecair-cecair, akan mengambil masa 4 hingga 18 jam, atau dengan pengekstrakan kartrij atau piring fasa pepejal, akan mengambil masa 1 hingga 2 jam masa keseluruhan dan 20 hingga 45 minit masa kendali setiap sampel. Sebaliknya, mikropengekstrakan fasa pepejal (SPME) lebih cepat (15 minit) dan jauh kurang penggunaan tenaga manusia (kira-kira 3 minit masa kendali setiap sampel) daripada pengekstrakan cecair-cecair atau pengekstrakan fasa pepejal, dan memerlukan cuma sejumlah kecil sampel dan tanpa pelarut organik. SPME mengurangkan gangguan latarbelakang bagi analisis pestisid atau lain-lain, membuatkan pengenalpastian dan pengkuantitatifan lebih terpakai. Jelaskan prinsip kerja setiap teknik dan ketengahkan kebaikan dan keburukan setiap teknik.

(14 markah)