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UNIVERSITI SAINS MALAYSIA

Second Semester Examination  
2008/2009 Academic Session

**KAA 505 – Separation Techniques**  
**[Kaedah Pemisahan]**

Duration : 3 hours  
*[Masa : 3 jam]*

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

**Instructions:**

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

Begin your answer on a new page.

You may answer either in Bahasa Malaysia or in English.

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

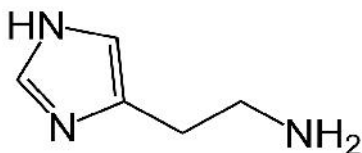
In the event on any discrepancies, the English version shall be used.

...2/-

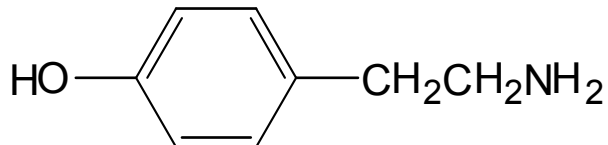
- 2 -

1. (a) A urine sample containing ppm levels of histamine and tyramine was obtained from a cancer patient.
- Describe (with the help of a schematic diagram) a liquid phase microextraction technique that can be used for the extraction of the analytes.
  - What are the main parameters that need to be optimized for the proposed method in (i)?
  - What will be the most suitable analytical technique that can be used for the analysis of the extracts of the proposed method in (i)? Provide justifications for your answer.
  - What are the advantages of the proposed method in (i) over the solid-phase extraction technique?

(13 marks)



Histamine



Tyramine

- (b) A mixture of benzoic acid ( $\text{Ar-COOH}$ ) ( $\text{pK}_a = 4.19$ ) and aniline ( $\text{Ar-NH}_2$ ) ( $\text{pK}_b = 9.4$ ) was prepared in a buffer ( $\text{pH } 2.0$ ) and was extracted using 15 mL chloroform (Ar represents benzene ring).
- Predict the extraction efficiency under these conditions (provide justifications for your answer).
  - Describe a green technology that can be used for the isolation of the analytes.

(7 marks)

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- 3 -

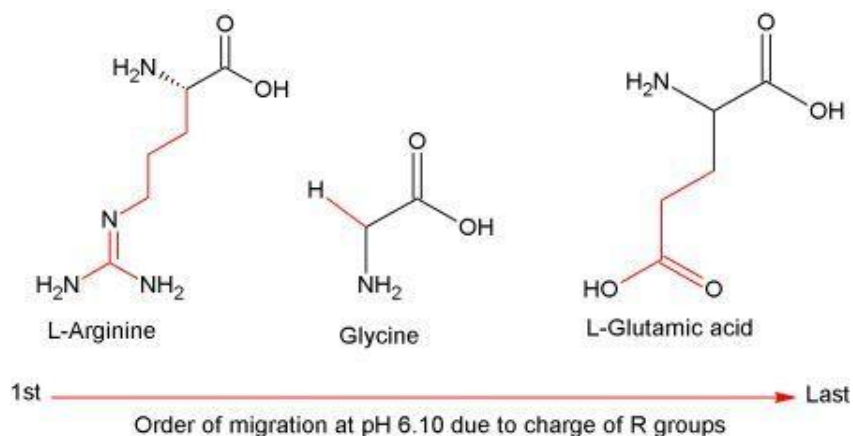
2. The identification and quantification of amino acids in different matrices, such as food, plant tissues and physiological samples, are of great importance. Amino acids are also an important class of neuroactive compounds and are implicated in many physiological effects and several diseases.

(a) Describe a capillary electrophoresis (CE) method that can be used for the determination of ppm levels of the amino acids namely glutamic acid, glycine and arginine in a biological fluid sample. Provide details on the CE conditions and give justifications for your choice.

(10 marks)

(b) Describe how you can separate the enantiomers of glutamic acid (i.e., the L- and the D-forms) using a CE technique.

(5 marks)



(c) What are the major causes of peak tailings in CE? How can peak tailings be minimized?

(5 marks)

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- 4 -

3. (a) Describe the operating principles of chemiluminescence detectors. Discuss the prospects of this type of detector in portable CE units.

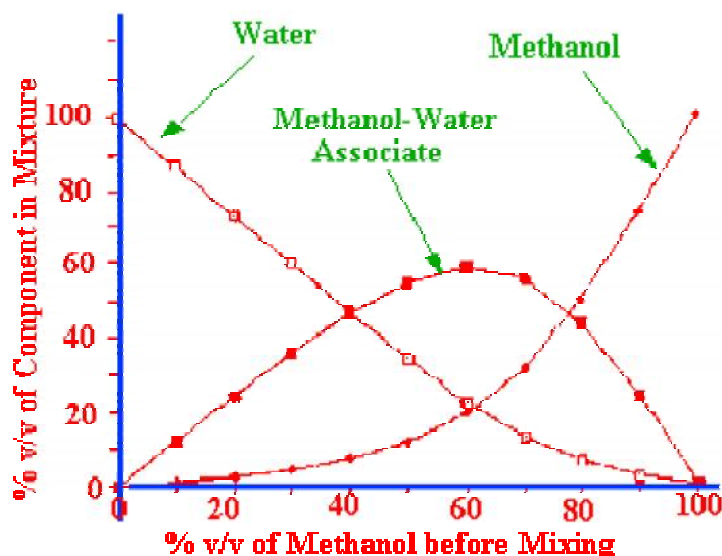
(13 marks)

- (b) A student was trying to determine the levels of the anions fluoride, chloride and sulphate in an ultrapure water sample. He found that the migration times of the analytes are abnormally long (more than 1 hour). Give suggestions (and justifications) how the problem can be overcome.

(7 marks)

4. The figure below shows the observation on the association of methanol-water by their variation in the composition as liquid chromatographic mobile phase as reported by Scott & Kucera (R.P.W.Scott and P.Kucera, *J. Chromatogr* **149** (1978) 93.).

- (i) Explain the mobile phase interaction phenomena of methanol-water and relate with the interaction of the analytes (at least two) of your choice leading towards their separation in isocratic and gradient elution modes.
- (ii) Explain why the order of elution can be reversed by changing the mobile phase composition.



(20 marks)

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5. (i) Derive the van Deemter Equation.
- (ii) Explain why the same molecules in the column travel at slightly different rates, even though the molecules themselves are identical. How can these effects be minimized or eliminated?
- (20 marks)

6. Nitrous oxide (N<sub>2</sub>O), carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) are considered to be very important greenhouse gases. These gases absorb the sun's rays and thus, influence the global warming. Continuous measurement provides insights into the sources of the different gases. Propose a chromatographic system that can be used to analyze simultaneously all the three gases in a matrix of atmospheric air that contain water vapor. Hint: begin with the sampling followed by the column (or columns) and finally the detector (or detectors)

OR

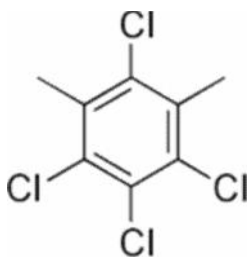
As a chemist you are assigned to analyse the common pollutants as listed below. Propose a cost-effective chromatographic system to be purchased to accomplish the task.

- |                         |                        |
|-------------------------|------------------------|
| 1. Tetrachloro-m-xylene | 12. 4,4' DDE           |
| 2. Alpha BHC.           | 13. Dieldrin           |
| 3. Gamma BHC            | 14. Endrin             |
| 4. Beta BHC             | 15. 4,4' DDD           |
| 5. Delta BHC            | 16. Endosulfan II      |
| 6. Heptachlor           | 17. 4,4' DDT           |
| 7. Aldrin               | 18. Endrin aldehyde    |
| 8. Heptachlor epoxide   | 19. Endosulfan sulfate |
| 9. Gamma chlordane      | 20. Methoxychlor       |
| 10. Alpha chlordane     | 21. Endrin ketone      |
| 11. Endosulfan          | 22. Decachlorobiphenyl |

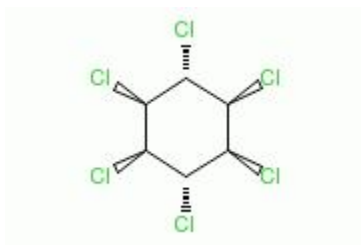
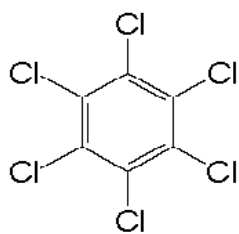
(20 marks)

**SOME PERSISTENT ORGANIC POLLUTANTS**

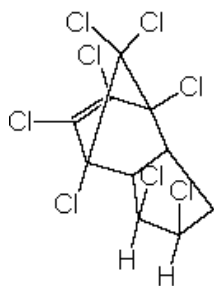
Tetrachloro-m-xylene



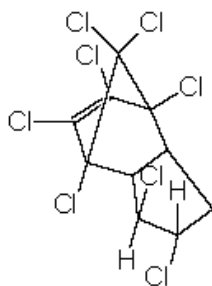
Hexachlorobenzene: Alpha BHC , Gamma BHC, Beta BHC , Delta BHC,



Chlordane



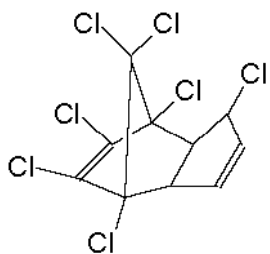
Cis



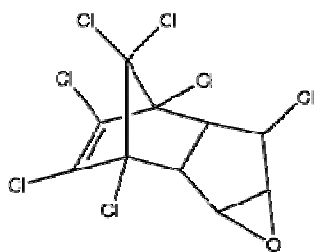
Trans

- 7 -

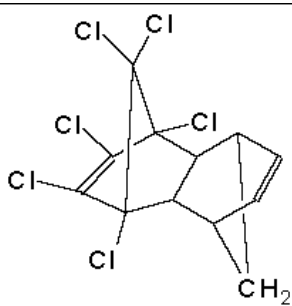
Heptachlor



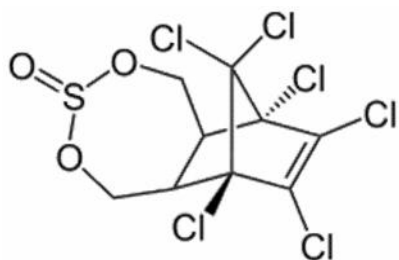
Heptachlor epoxide



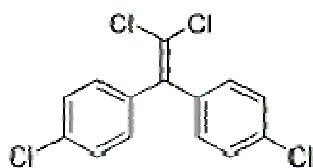
Aldrin



Endosulfan

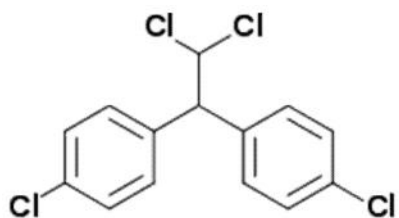


4,4' DDE

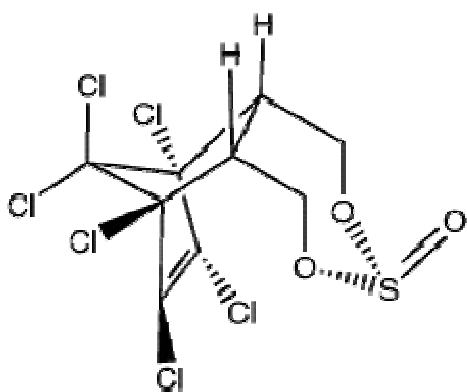


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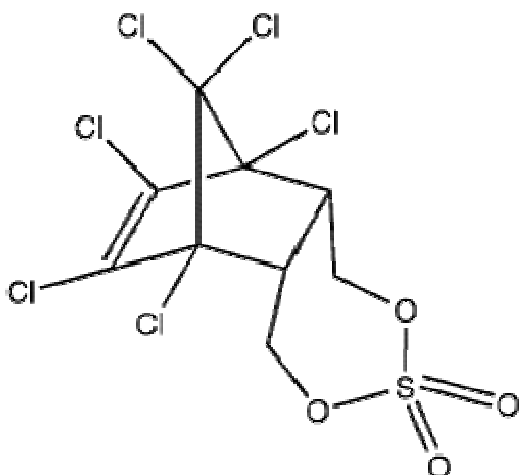
Dichlorodiphenyldichloroethane, 4,4' DDD



Endosulfan II

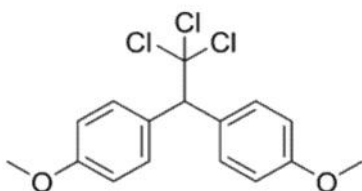


Endosulfan sulfate

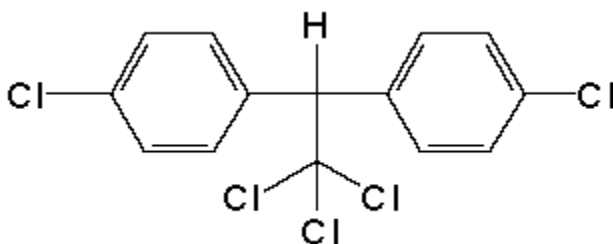




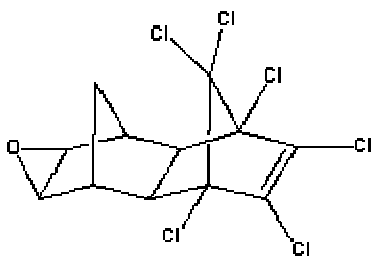
Methoxychlor



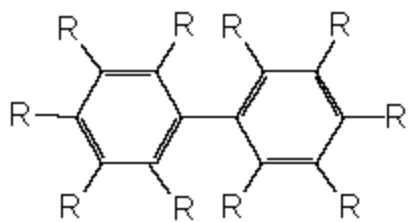
DDT



Endrin



POLYCHLORINATED BIPHENYLS



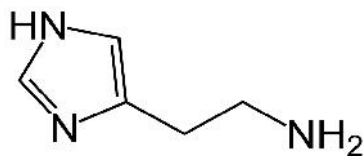
R = Cl

Monochlorobiphenyl  
Dichlorobiphenyl  
Trichlorobiphenyl  
Tetrachlorobiphenyl  
Pentachlorobiphenyl  
Hexachlorobiphenyl  
Heptachlorobiphenyl  
Octachlorobiphenyl  
Nonachlorobiphenyl  
Decachlorobiphenyl

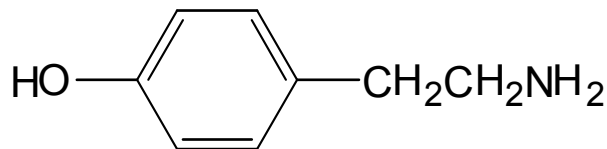
- 12 -

1. (a) Suatu sampel air kencing mengandungi paras ppm histamina dan tiramina telah diperolehi daripada seorang pengidap kanser.
- Terangkan (dengan bantuan gambarajah skema) satu kaedah pengekstrakan mikro fasa cecair yang boleh digunakan untuk mengekstrak analit.
  - Apakah parameter utama yang perlu dioptimumkan bagi kaedah yang dicadangkan di (i)?
  - Apakah kaedah analisis yang paling sesuai untuk menganalisis ekstrak dalam (i)? Beri alasan untuk jawapan anda.
  - Berikan kebaikan kaedah yang anda cadangkan di (i) daripada kaedah pengekstrakan fasa pepejal.

(13 markah)



Histamina



Tiramina

- (b) Satu campuran asid benzoik ( $\text{Ar-COOH}$ ) ( $\text{pK}_a = 4.19$ ) dan anilina ( $\text{Ar-NH}_2$ ) ( $\text{pK}_b = 9.4$ ) telah disediakan di dalam suatu larutan tampan ( $\text{pH} 2.0$ ) dan telah diekstrak menggunakan 15 mL kloroform.
- Ramalkan kecekapan pengekstrakan di bawah keadaan ini (berikan alasan untuk jawapan anda).
  - Terangkan satu teknologi hijau yang boleh digunakan untuk pemencilan analit.

(7 markah)

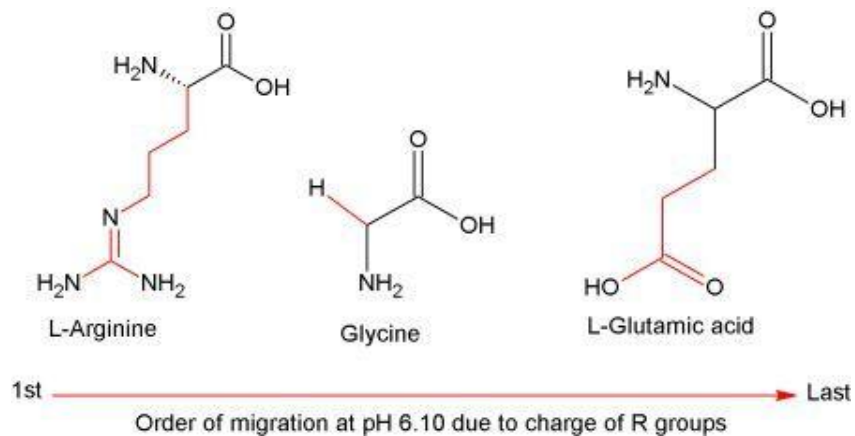
2. Pengenalan dan pengkuantitian asid amino di dalam pelbagai matriks seperti makanan, tisu tumbuhan dan sampel fisiologi adalah amat mustahak. Asid amino juga adalah kumpulan sebatian neuroaktif yang penting dan telah dikaitkan dengan banyak kesan fisiologi dan beberapa penyakit.

- (a) Terangkan satu kaedah elektroforesis rerambut (CE) bagi penentuan paras ppm asid amino iaitu glutamat, glisina and arginina di dalam sampel cecair biologi. Berikan maklumat lanjut bagi CE dan beri alasan untuk pemilihan anda.

(10 markah)

- (b) Terangkan bagaimana anda boleh menentukan kepekatan enantiomer glutamat (iaitu, bentuk L- dan D-) menggunakan satu kaedah CE.

(5 markah)



- (c) Apakah punca utama ekor puncak di dalam CE? Bagaimanakah ekor puncak boleh dikurangkan?

(5 markah)

3. (a) Terangkan prinsip operasi pengesanan "chemiluminescence". Bincangkan prospek pengesanan ini dalam unit CE mudahalih.

(13 markah)

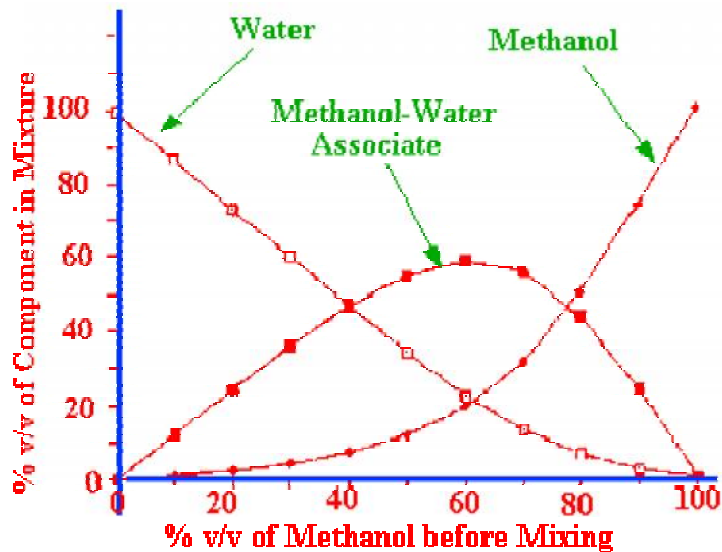
- 14 -

- (b) Seorang pelajar telah mencuba untuk menentukan paras anion fluorida, klorida and sulfat di dalam sampel air ultratulen. Beliau mendapati masa penghijrahan analit adalah terlalu panjang (lebih dari 1 jam). Berikan cadangan (dan alasan) bagaimana masalah ini boleh diatasi.

(7 markah)

4. Gambarajah di bawah menunjukkan pemerhatian terhadap persekutuan metanol-air hasil dari perubahan komposisi sebagai fasa bergerak kromatografi cecair seperti di laporkan oleh Scott & Kucera (R.P.W.Scott and P.Kucera, *J. Chromatogr* **149** (1978) 93.).

- (i) Terangkan fenomena interaksi fasa bergerak metanol-air dan kaitkan dengan interaksi analit (sekurang-kurangnya dua) pilihan anda ke arah pemisahan kandungannya bagi elusi mod isokratik atau cerun.
- (ii) Terangkan kenapa tertib elusi dapat diterbalikkan dengan mengubah komposisi fasa gerak.



(20 markah)

- 15 -

5. (i) Terbitkan persamaan van Deemter.
- (iii) Terangkan kenapa molekul yang sama di dalam turus bergerak pada kadar yang sedikit berbeza walaupun molekul-molekul tersebut adalah seiras. Bagaimanakah kesan ini dapat dikurangkan atau dihapuskan?

(20 markah)

6. Nitrus oksida ( $N_2O$ ), karbon dioksida ( $CO_2$ ) dan metana ( $CH_4$ ) dianggap gas rumah hijau yang amat penting. Gas tersebut menyerap cahaya dan dengan itu mempengaruhi pemanasan global. Pengukuran berterusan memberi maklumat kepada sumber gas yang berbeza-beza. Cadangkan sistem kromatografi yang dapat digunakan untuk menganalisis secara serentak kesemua gas tersebut di dalam matrik atmosfera udara yang mengandungi wap air. Bayangan: mulakan dengan pensampelan diikuti dengan turus (atau turus-turus) dan akhirnya pengesanan (atau pengesanan-pengesanan)

ATAU

Sebagai ahli kimia anda di tugaskan untuk menganalisis pencemar yang lazim seperti senarai di bawah. Cadangkan suatu sistem kromatografi yang berkesan – kos untuk dibeli untuk menjayakan tugas tersebut.

- |                         |                        |
|-------------------------|------------------------|
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| 11. Endosulfan          | 22. Decachlorobiphenyl |

(20 markah)

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