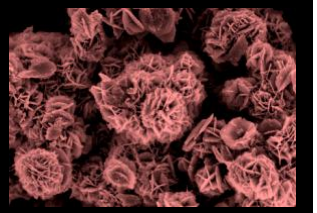
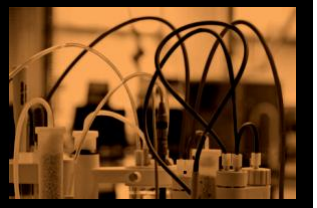




Chemical Engineering

Inspiring Potential · Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

CHROMATOGRAPHIC METHOD

CEB 4032/CFB3032: ANALYTICAL CHEMISTRY/ANALYTICAL INSTRUMENTATION

TS CHM DR. MOHD DZUL HAKIM WIRZAL

Chemical Engineering
Inspiring Potential • Generating Futures

Outline

UNIVERSITI TEKNOLOGI PETRONAS

- Principle of Chromatography
- Classification of Chromatographic Techniques
- Qualitative Analysis and Quantitative Analysis
- Gas Chromatography (GC) -Principles and Instrumentation
- Liquid Chromatography (LC) – Principles and Instrumentation
- Thin Layer Chromatography (TLC)

Chemical Engineering
Inspiring Potential • Generating Futures

Learning Outcomes

UNIVERSITI TEKNOLOGI PETRONAS

At the end of this chapter:

1) The principles of chromatography and the classification of chromatographic method.

2) Qualitative and quantitative analyses using chromatographic method.

3) Principles and instrumentation of GC, LC and TLC.

Chemical Engineering
Inspiring Potential • Generating Futures

Outline

UNIVERSITI TEKNOLOGI PETRONAS

- Principle of Chromatography
- Classification of Chromatographic Techniques
- Qualitative Analysis and Quantitative Analysis
- Gas Chromatography (GC) -Principles and Instrumentation
- Liquid Chromatography (LC) – Principles and Instrumentation
- Thin Layer Chromatography (TLC)

Chemical Engineering
Inspiring Potential • Generating Futures

Principle of Chromatography

UNIVERSITI TEKNOLOGI PETRONAS

- Chromatography is a powerful separation method that finds applications in all branches of science.
- Chromatography encompasses a diverse and important group of methods that allow the separation, identification, and determination of closely related components of complex mixtures.
- In all chromatographic separations, the sample is dissolved in a **mobile phase**, which may be a gas, a liquid or a supercritical fluid.

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- The mobile phase is then forced through an immiscible **stationary phase**, which is fixed in a place in a column or on a solid phase.

- The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phases to varying degrees.

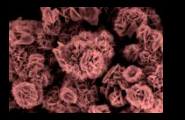
- Components strongly retained by the stationary phase move only slowly with the flow of mobile phase.

Chemical Engineering
Inspiring Potential · Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- Components are weekly held by the stationary phase travel rapidly.

- As a consequence of these differences in migration rates, sample components can be separated and analyzed qualitatively and quantitatively.





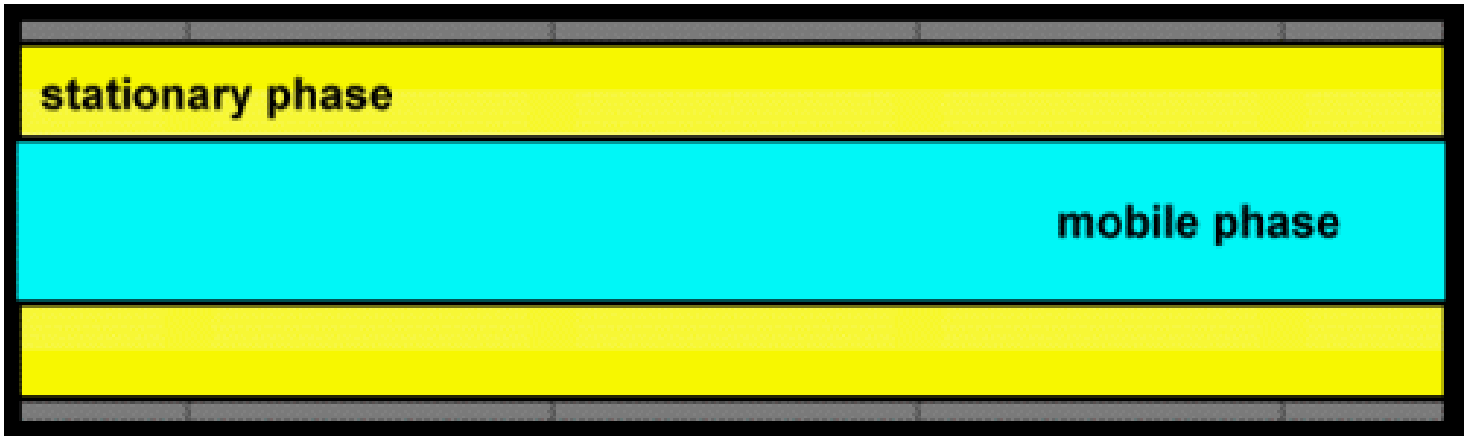
Chemical Engineering
Inspiring Potential · Generating Futures

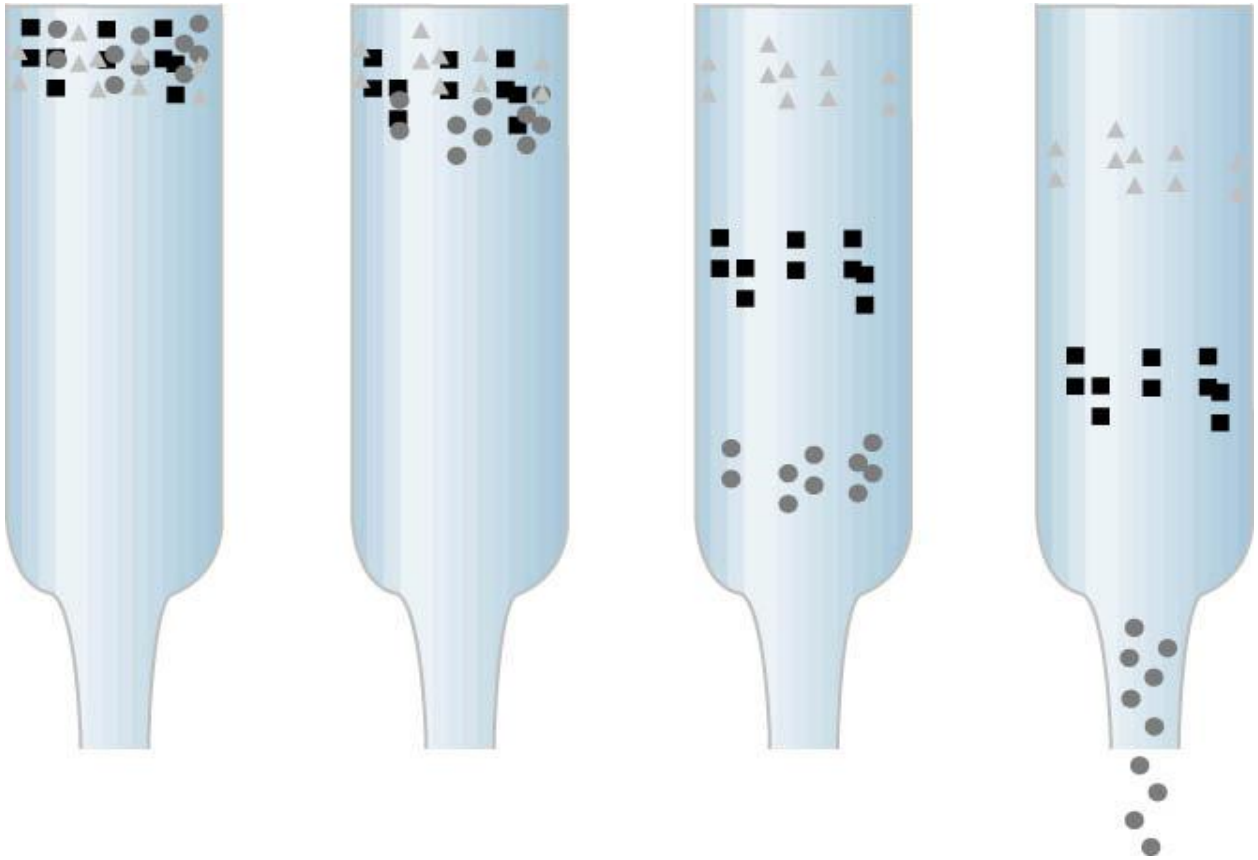
Sample
Mobile phase
A + B
B
A
Packed column
Stationary phase
B
A
B
A
B
Detector
t_0 t_1 t_2 t_3 t_4
(a)

Detector signal
A
B
t_0 t_1 t_2 t_3 t_4
Time
(b)

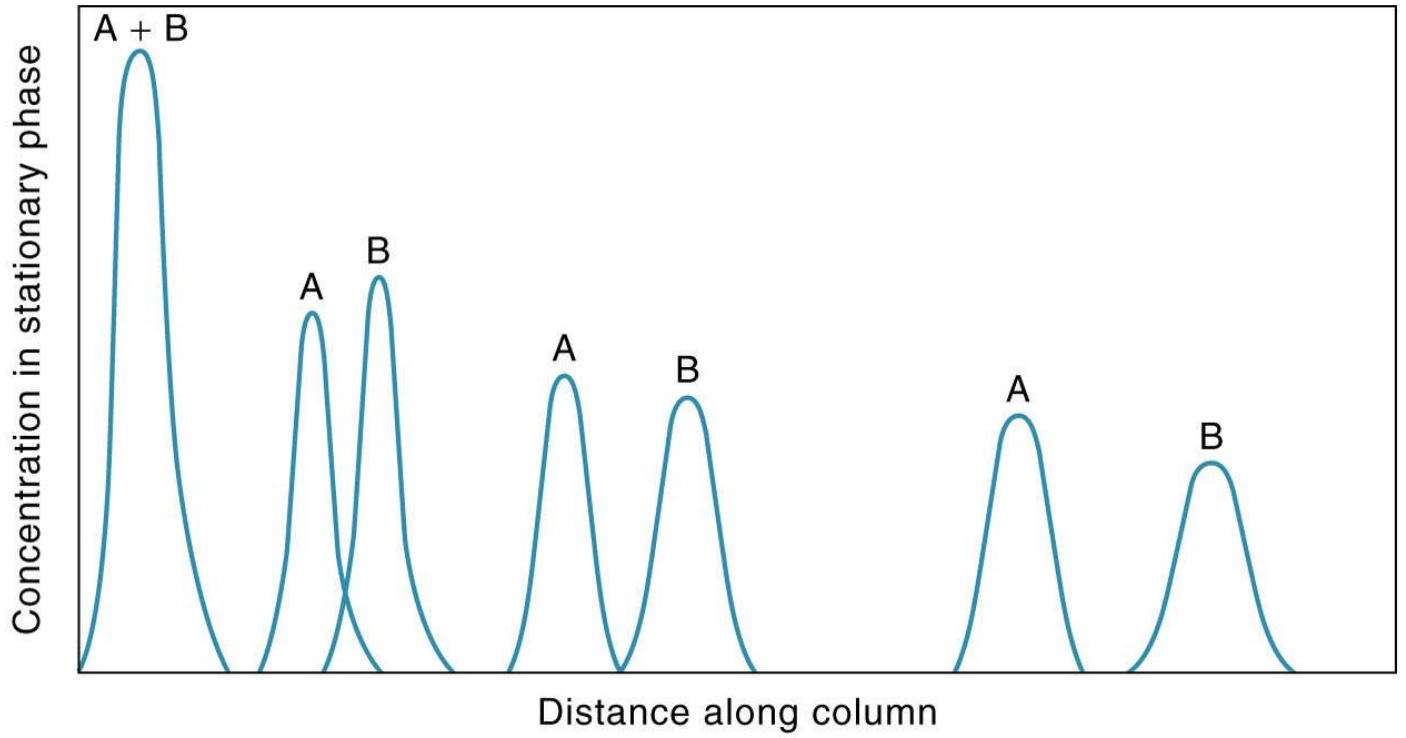
Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS





Principle of Chromatographic Separations



Distribution of two substances, A and B, along a chromatographic column in a typical chromatographic separation.

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- The two principal types of chromatography are:
  i. Gas chromatography (GC)
  ii. Liquid chromatography (LC)

i. Gas Chromatography (GC)

- separates gaseous substances based on adsorption on or partitioning in a stationary phase from a gas phase.

ii. Liquid Chromatography (LC)

- separates liquid substances using techniques such as size exclusion (separation based on molecular size), ion exchange (separation based on charge), adsorption and partitioning from a liquid phase.

Chemical Engineering
Inspiring Potential • Generating Futures

Outline

UNIVERSITI TEKNOLOGI PETRONAS

- Principle of Chromatography
- Classification of Chromatographic Techniques
- Qualitative Analysis and Quantitative Analysis
- Gas Chromatography (GC) -Principles and Instrumentation
- Liquid Chromatography (LC) – Principles and Instrumentation
- Thin Layer Chromatography (TLC)

Chemical Engineering
Inspiring Potential • Generating Futures

Classification of Chromatographic Techniques

UNIVERSITI TEKNOLOGI PETRONAS

- Chromatography processes can be classified according to the type of equilibrium process involved.

- Governed by the type of stationary phase.

- Various bases of equilibration are:
  i. Adsorption
  ii. Partition
  iii. Ion exchange
  iv. Molecular exclusion
  v. Affinity

Chemical Engineering
Inspiring Potential · Generating Futures

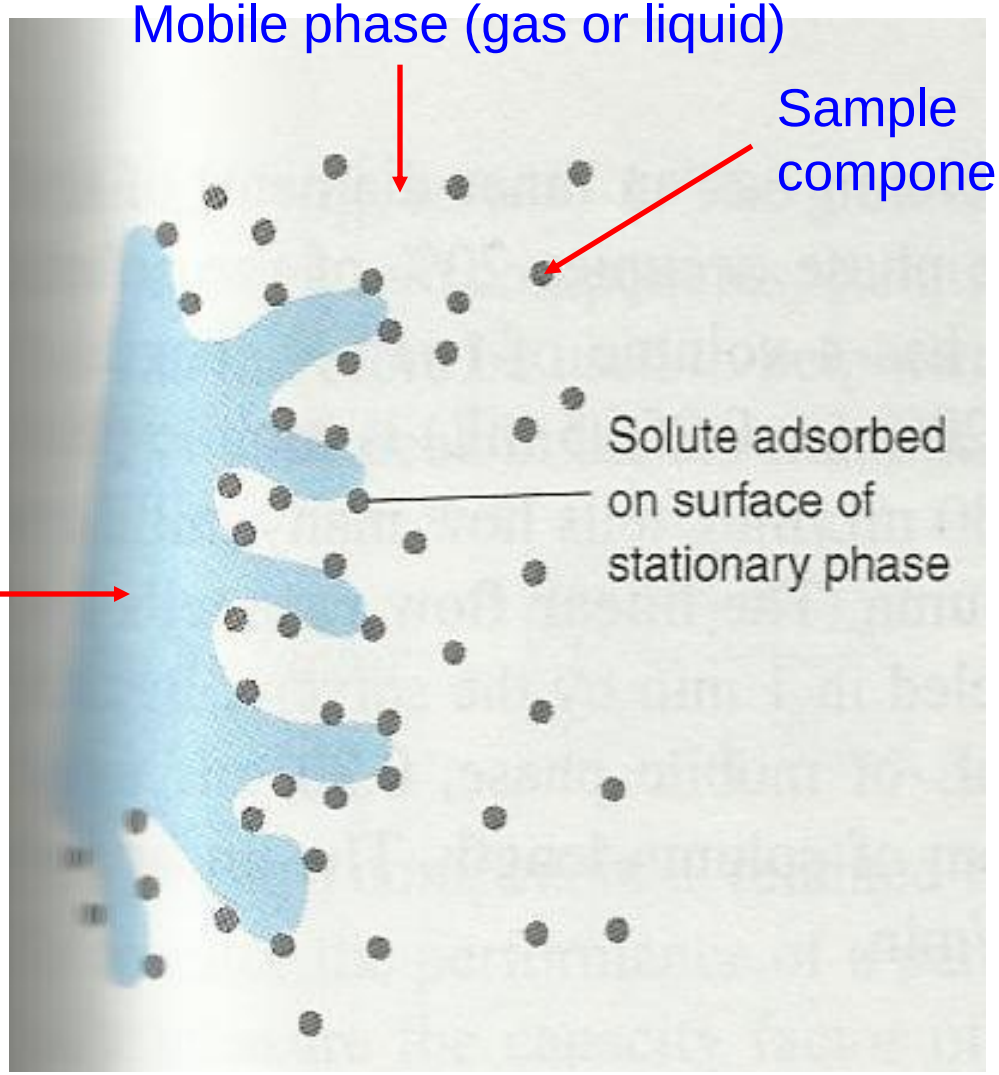
i) Adsorption Chromatography

UNIVERSITI TEKNOLOGI PETRONAS

- The stationary phase is a solid on which the samples components are adsorbed.
- The mobile phase may be a liquid (liquid-solid chromatography) or a gas (gas-solid chromatography).
- The components distribute between the two phases through a combination of sorption and desorption processes.

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS



Adsorption Chromatography

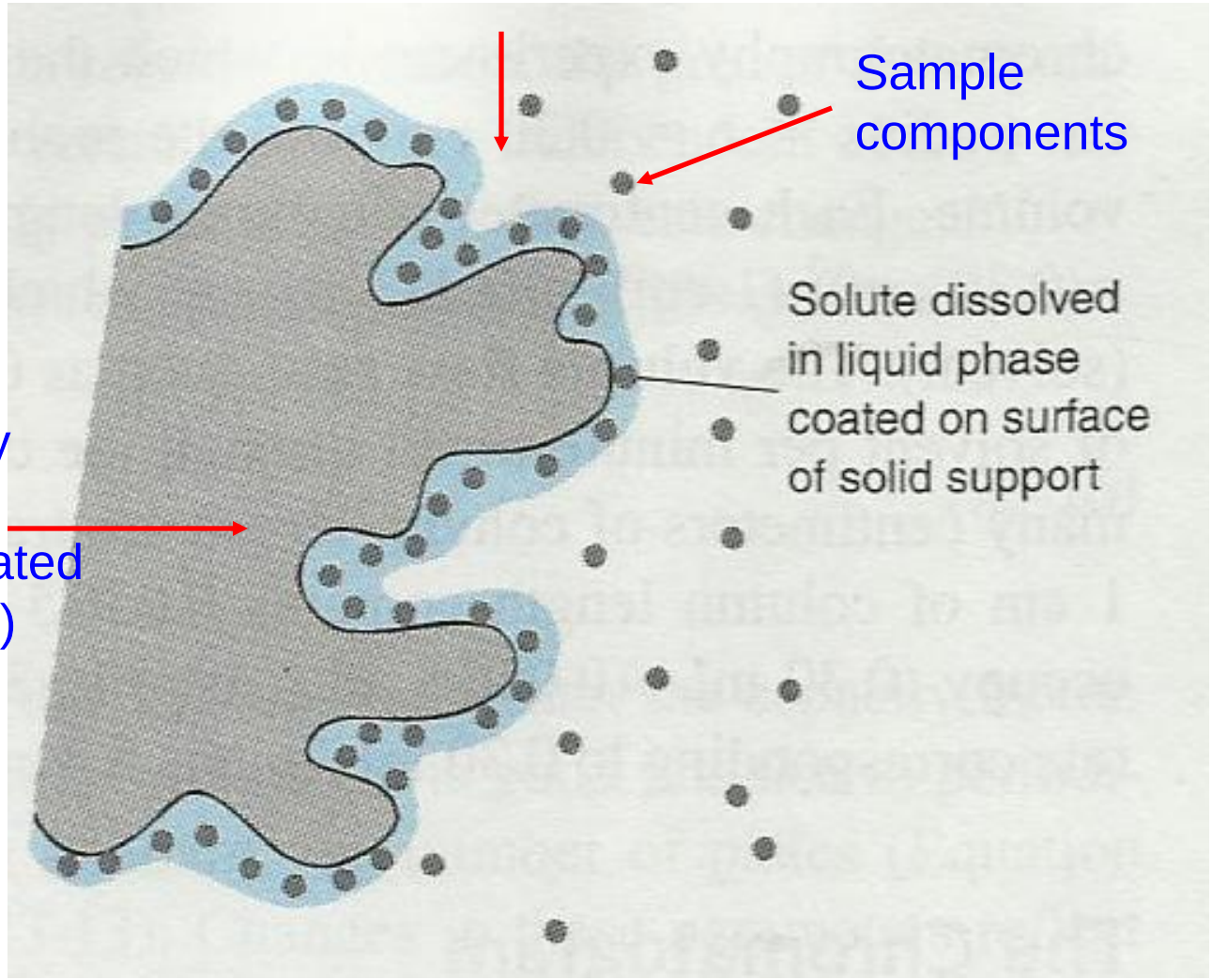
ii) Partition Chromatography

- Stationary phase → liquid supported on an inert solid.
- Mobile phase → liquid (liquid-liquid chromatography) or a gas (gas-liquid chromatography).
- In liquid-liquid partition:
- Normal phase chromatography → consists of polar stationary phase used with a nonpolar mobile phase → favors retention of polar compounds and elution of nonpolar compounds.
- Reversed- phase chromatography → consists of nonpolar stationary phase used with a polar mobile phase→ nonpolar solutes are retained more and polar solutes more readily eluted.

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

Mobile phase (gas or liquid)



Partition Chromatography

Chemical Engineering
Inspiring Potential • Generating Futures

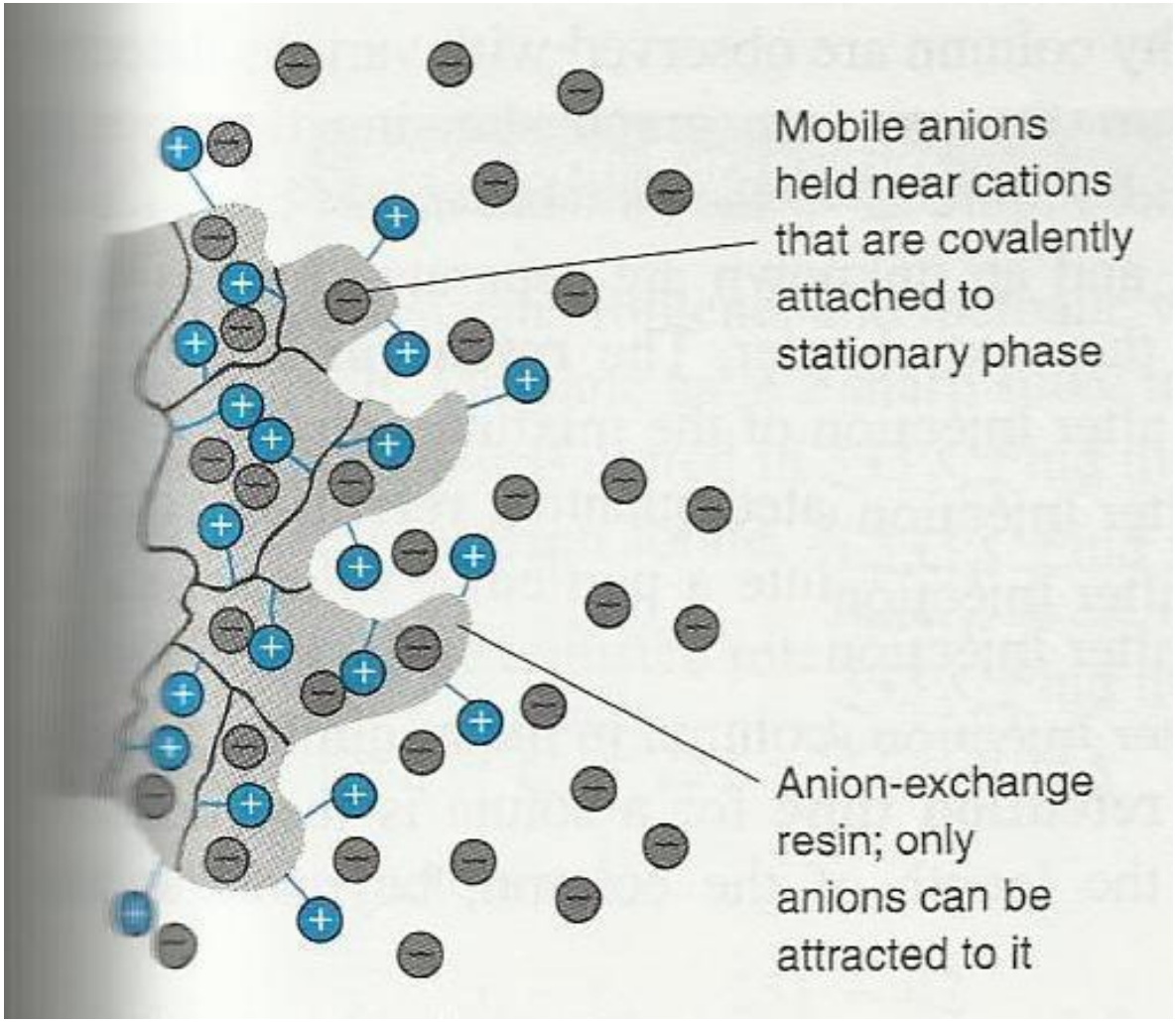
iii) Ion-Exchange Chromatography

UNIVERSITI TEKNOLOGI PETRONAS

- Uses an ion exchange resin as the stationary phase.
- The mobile phase is a liquid.
- Mechanism of separation→ based on ion exchange equilibria.
- Solute ions of the opposite charge are attracted to the stationary phase by electrostatic force.

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS



Ion-exchange Chromatography

Chemical Engineering
Inspiring Potential · Generating Futures

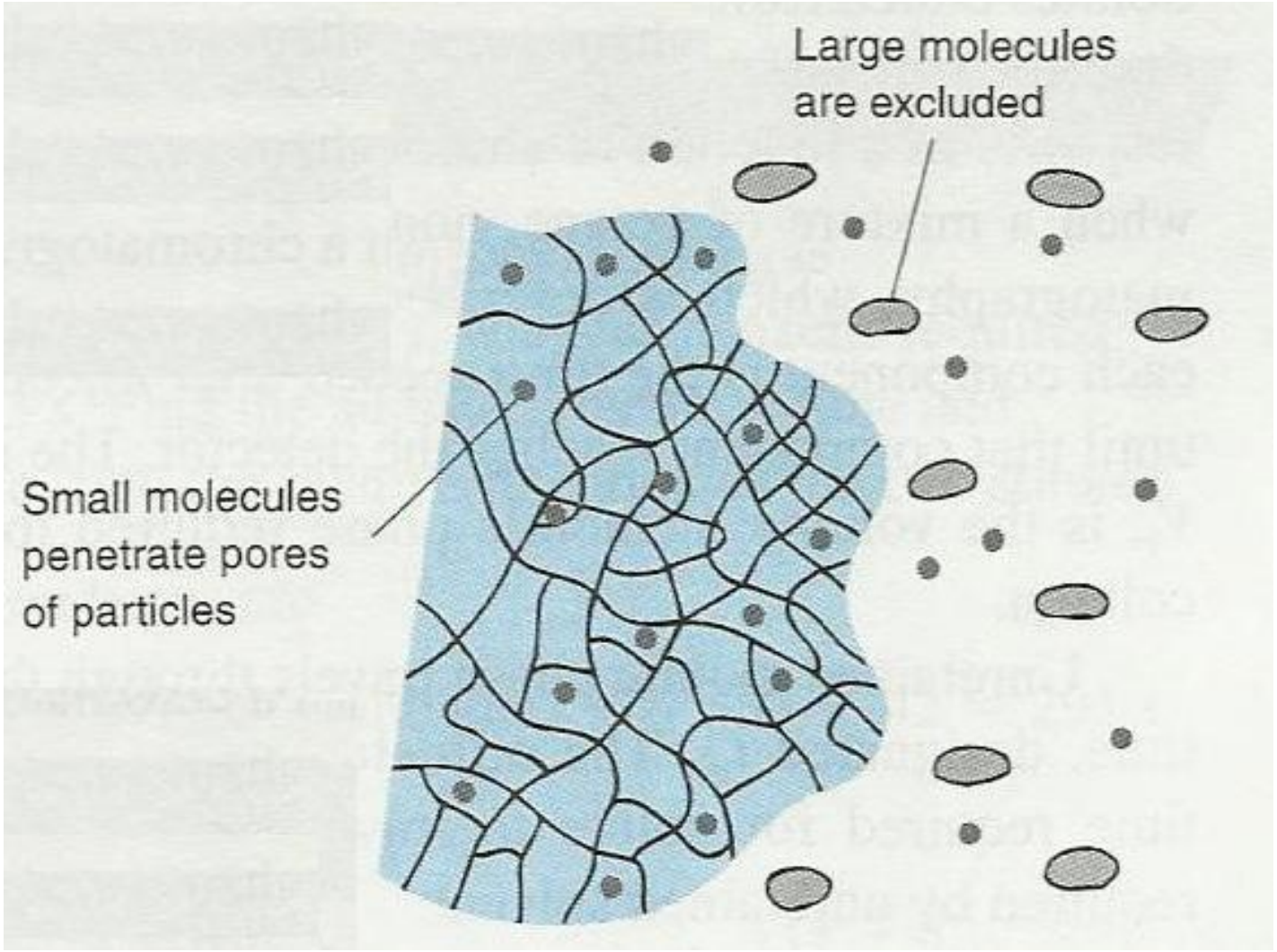
iv) Molecular Exclusion Chromatography

UNIVERSITI TEKNOLOGI PETRONAS

- This technique separates molecules by size, with the larger solutes passing though most quickly.

- The pores are small enough to exclude large solute molecules → the large molecules past without entering the pores.

- The small molecules take longer time to pass through the column → they enter the gel and therefore must flow through a larger volume before leaving the column.



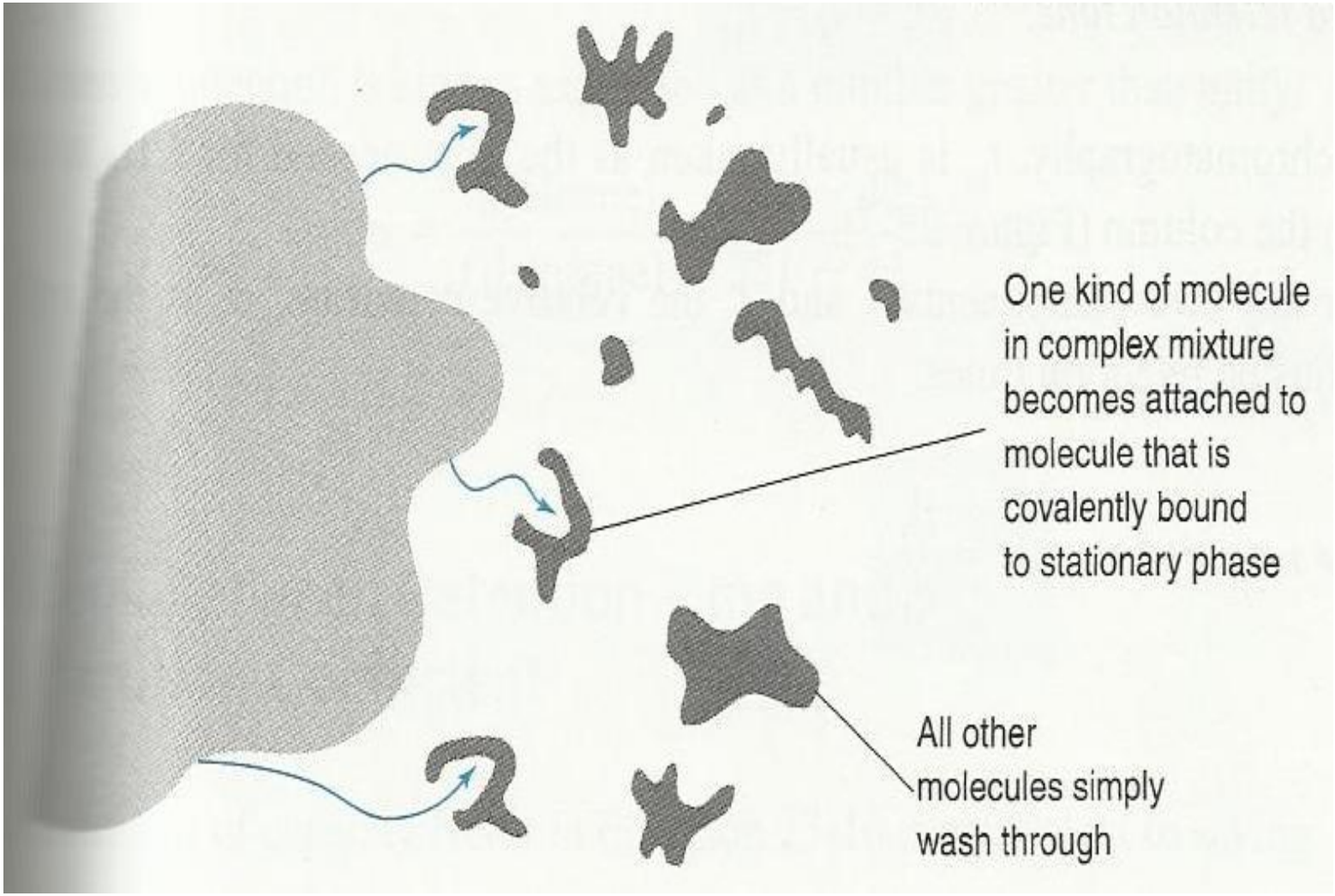
Molecular Exclusion Chromatography

Chemical Engineering
Inspiring Potential • Generating Futures

v) Affinity Chromatography

UNIVERSITI TEKNOLOGI PETRONAS

- Most selective kind of chromatography.
- Employs specific interactions between one kind of solute molecule and a second molecule that is covalently attached (immobilized) to the stationary phase.
- The immobilized molecule might be an antibody to a particular protein.
- When a mixture containing a thousand protein is passed through the column → only one protein that reacts with the antibody bonds to the column.



Affinity Chromatography

Classification of Column Chromatographic Methods

TABLE 30-4

Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Gas chromatography (GC)	Gas-liquid (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	Gas-solid	Solid	Adsorption
Liquid chromatography (LC)	Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	Affinity	Group-specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
Supercritical fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Chemical Engineering
Inspiring Potential · Generating Futures

The Chromatogram

UNIVERSITI TEKNOLOGI PETRONAS

- Solutes eluted from a chromatography column are observed with various detectors.
- A chromatogram is a graph showing the detector response as a function of elution time.
- **Retention time**, t_r for each component is the time needed after injection of the mixture into the column until that component reaches the detector.

Chemical Engineering
Inspiring Potential · Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- Unretained mobile phase travels through the column in the minimum possible time → t_m

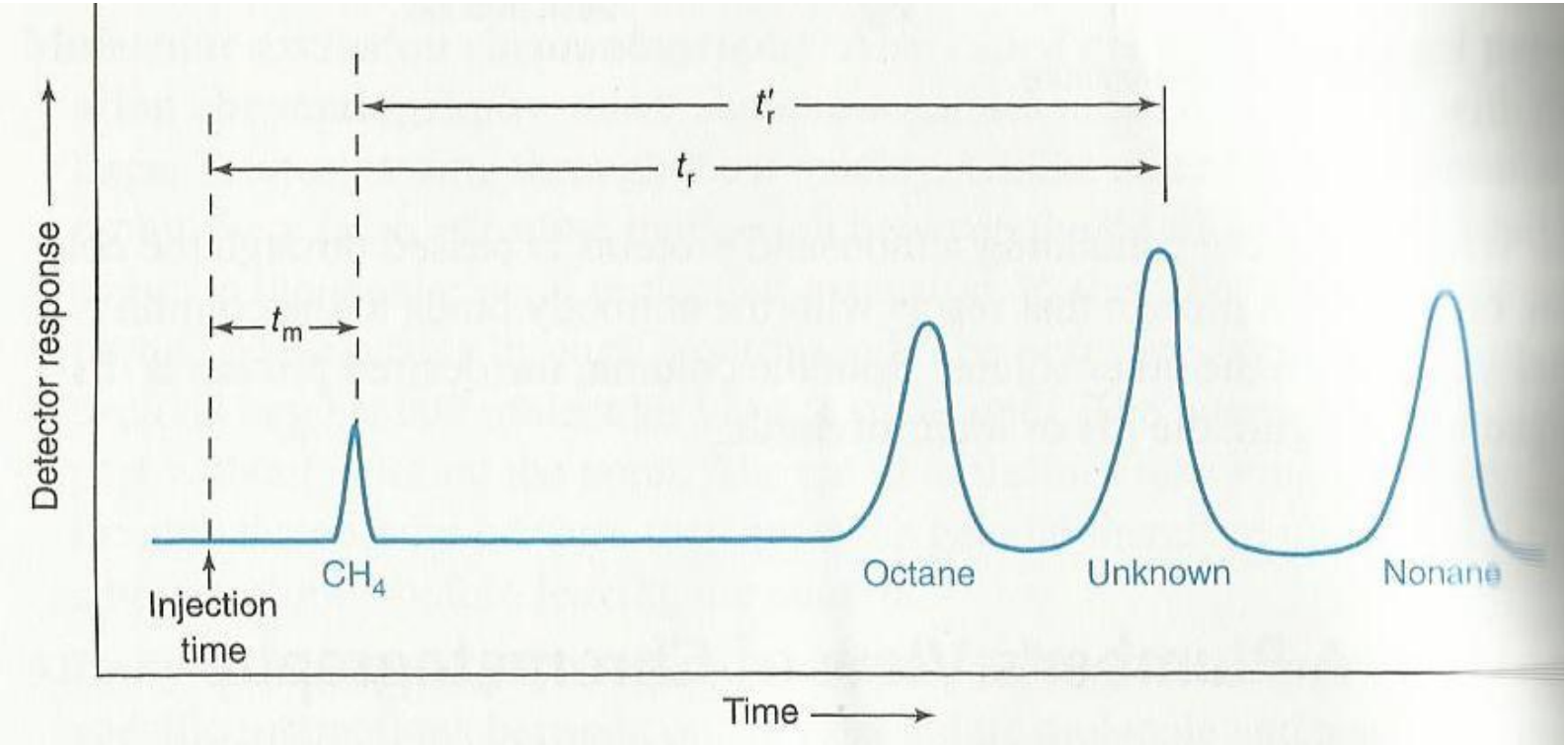
- The **adjusted retention time, t'_r** for a solute is the time required for solute to travel the length of the column, beyond the time required by unretained solvent.

$$t'_r = t_r - t_m$$

- The **relative retention, $\alpha = t'_{r2}/t'_{r1}$**

- Where $t'_{r2} > t'_{r1}$; so $\alpha > 1$. The greater the relative retention, the greater the separation between two components.

Schematic gas chromatogram showing measurement of retention times



Chemical Engineering
Inspiring Potential · Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- **Capacity factor: $k' = (t_r - t_m)/t_m$**

$$k' = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}}$$

- Relative retention, α, can also be expressed as

$$\alpha = t'_{r2}/t'_{r1} = k'_2/k'_1$$

- The longer a component is retained by the column, the greater the capacity factor.

Chemical Engineering
Inspiring Potential • Generating Futures

Example 1

UNIVERSITI TEKNOLOGI PETRONAS

A mixture of benzene, toluene and methane was injected into a gas chromatograph. Methane gave a sharp spike in 42.0 s, whereas benzene required 251 s and toluene was eluted in 333 s.

Find the adjusted retention time and capacity factor for each solute.

Also, find the relative retention of the two solutes.

Chemical Engineering
Inspiring Potential • Generating Futures

Solution

UNIVERSITI TEKNOLOGI PETRONAS

The adjusted retention times, t'_r, are

Benzene: $t'_r = t_r - t_m = 251 - 42 = 209$ s

Toluene: $t'_r = 333 - 42 = 291$ s

The capacity factors, k', are

Benzene: $k' = (t_r - t_m)/t_m = (251-42)/42 = 5.00$

Toluene: $k' = (333-42)/42 = 6.93$

- The relative retention is always expresses as a number greater than unity.

$$\alpha = \frac{t'_r(\text{toluene})}{t'_r(\text{benzene})} = \frac{333\text{-}42}{251\text{-}42} = 1.39$$

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

Chemical Engineering
Inspiring Potential • Generating Futures

Efficiency of Separation

UNIVERSITI TEKNOLOGI PETRONAS

- Two factors contribute to how well compounds are separated by chromatography.

i. One is the difference in elution times between peaks: the further apart, the better their separation.

ii. How broad the peaks are: the wider the peaks, the poorer their separation.

→ Two methods: i) Resolution, ii) Theoretical plates

Chemical Engineering
Inspiring Potential · Generating Futures

i) Resolution (Peak Broadening)

UNIVERSITI TEKNOLOGI PETRONAS

- Solute moving through a chromatography column tends to spread into Gaussian shape with standard deviation σ.
- The longer a solute spends passing through a column, the broader the band become.
- Common measures of breadth are:
  i. the width $w_{1/2}$ measured at height equal to half of the peak height.
  ii. The width w at the baseline between tangents drawn to the steepest parts of the peak.
- For Gaussian peak, it is possible to show that $w_{1/2} = 2.35\sigma$ and $w = 4\sigma$

Chemical Engineering
Inspiring Potential • Generating Futures

Area of Gaussian peak
= 1.064 x *h* x $W_{1/2}$
= 1.064 x *h* x 2.35 σ

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

Chemical Engineering
Inspiring Potential • Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- In chromatography, the resolution **of** two peaks from each other is defined as:

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}}$$

The ability to resolve two consecutive peaks

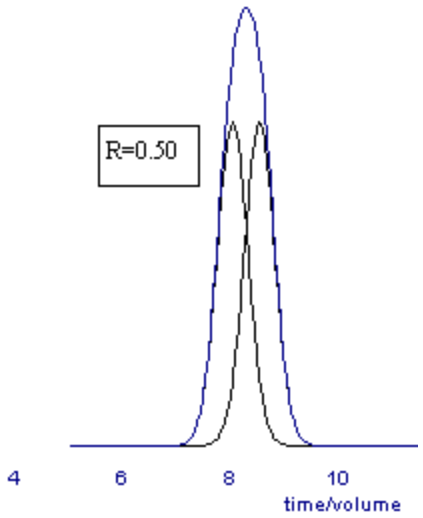
Δt_r = separation between peaks

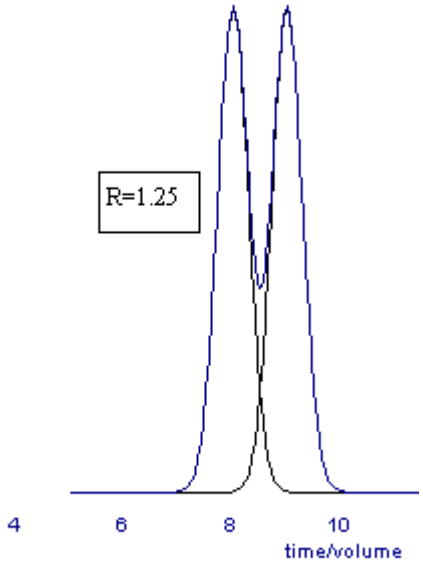
W_{av} = average width of the two peaks in corresponding units $[(w_1 + w_2)/2]$.

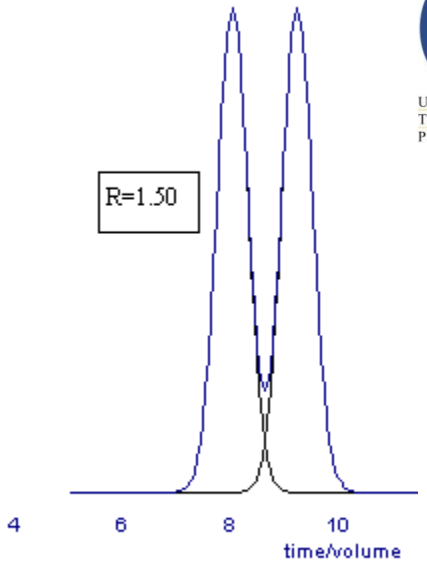
- A value of 1.0 results in 2.3 % overlap of two peaks of equal width → minimum for a separation to allow good quantitation.

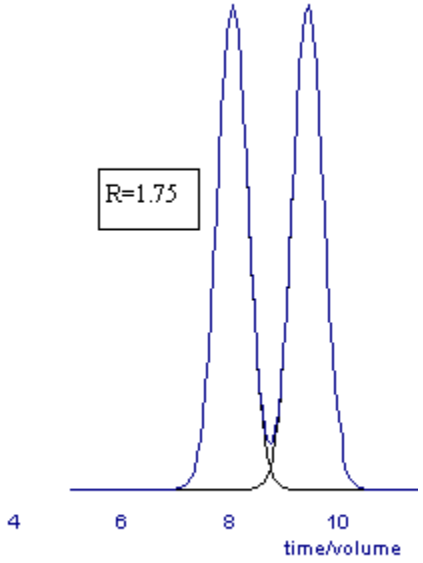
Chemical Engineering
Inspiring Potential • Generating Futures

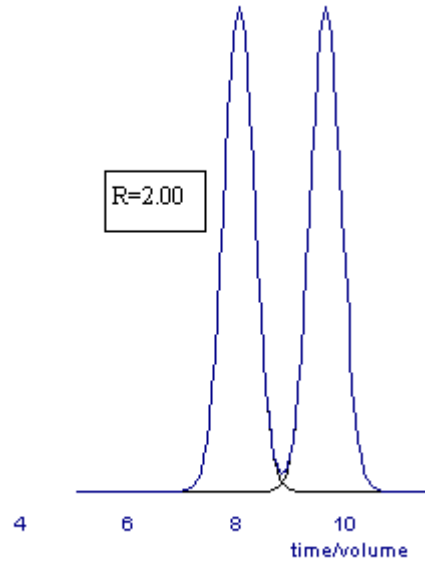
UNIVERSITI TEKNOLOGI PETRONAS

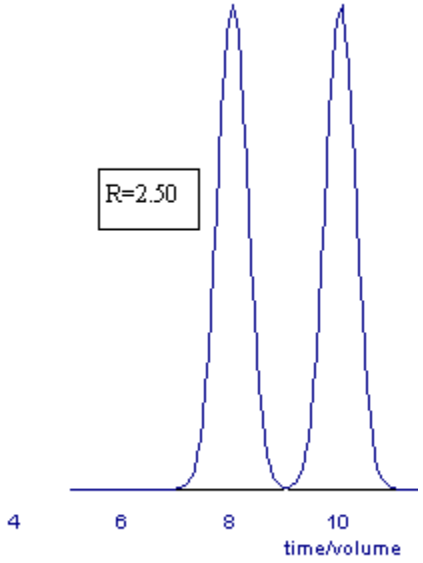












Chemical Engineering
Inspiring Potential · Generating Futures

Example 2

UNIVERSITI TEKNOLOGI PETRONAS

A peak with a retention time of 407 s has a width at the base of 13 s. A neighboring peak is eluted at 424 s with a width of 16 s.

Find the resolution for these two components.

Chemical Engineering
Inspiring Potential · Generating Futures

Solution

UNIVERSITI TEKNOLOGI PETRONAS

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = \frac{424 - 407}{\frac{1}{2}(13 + 16)}$$

$$= 1.2$$

Chemical Engineering
Inspiring Potential • Generating Futures

ii) Theoretical Plates

UNIVERSITI TEKNOLOGI PETRONAS

- The band broadening that occurs in column chromatography is the results of several factors → influence the efficiency of separations.

- Theoretical plates
  i. separation efficiency → number of theoretical plate in the column.
  ii. concept defined from distillation column → each theoretical plate in chromatography can be thought of as representing a single equilibrium step.
  iii. for high efficiency, large number of plate is necessary.
  iv. plate height, H = length of a column divided by the number of theoretical plates

Chemical Engineering
Inspiring Potential · Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- For a solute emerging from a column of length , L, the number of theoretical plate or efficiency, N:

$$N = \frac{L}{H} = \frac{L^2}{\sigma^2}$$

where H = plate height

- σ =w/4, therefore,

$$N = \frac{16\ L^2}{w^2}$$

- If we express L and *w* in units of time instead of length, the most useful equation for N:

$$N = 16\left(\frac{t_r}{w}\right)^2$$

Where t_r = retention time of the peak and *w* – the width at the base (unit of time)

Chemical Engineering
Inspiring Potential · Generating Futures

UNIVERSITI TEKNOLOGI PETRONAS

- If we use the width at half-height instead of the width at the base, we get

- number of theoretical plate or efficiency, N:

$$N = \frac{5.55\ {t_r}^2}{{w^2}_{1/2}}$$

Chemical Engineering
Inspiring Potential • Generating Futures

Example 3

UNIVERSITI TEKNOLOGI PETRONAS

A solute with a retention time of 407 s has a width at the base of 13.0 s on a column of 12.2 m long.

Find the number of plates and plate height.

Chemical Engineering
Inspiring Potential • Generating Futures

Solution

UNIVERSITI TEKNOLOGI PETRONAS

Number of plates, N

$$N = 16\left(\frac{t_r}{w}\right)^2$$

$= 16 \times (407^2/13^2)$

$= 1.57 \times 10^4$

Plate height

$H = L/N$

$= 12.2\ m / 1.57 \times 10^4$

$= 0.780\ mm$

Chemical Engineering
Inspiring Potential • Generating Futures

Resolution and Number of Plates

UNIVERSITI TEKNOLOGI PETRONAS

- The greater the resolution, the better the separation between two peaks.

- The relationship between the number of plates on a column and the resolution is

$$\text{Resolution} = \frac{\sqrt{N}}{4}\left(\frac{\alpha\text{-}1}{\alpha}\right)\left(\frac{k'_2}{1+k'_{av}}\right)$$

N = number of theoretical plate

α = relative retention of two peaks

k'_2 = capacity factor for the more retained component

k'_{av} = average capacity factor for both component

Chemical Engineering
Inspiring Potential · Generating Futures

Example 4

UNIVERSITI TEKNOLOGI PETRONAS

Two solute have a relative retention of $\alpha = 1.08$ and capacity factor $k'_1 = 5.00$ and $k'_2 = 5.40$. The number of theoretical plates is nearly the same for both compounds.

How many plates are required to give a resolution of 1.50? If the plate height is 0.200 mm, how long must the column could be ?.

Chemical Engineering
Inspiring Potential • Generating Futures

Solution

UNIVERSITI TEKNOLOGI PETRONAS

$$\text{Resolution} = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_2}{1+k'_{av}}\right)$$

$$1.5 = \frac{\sqrt{N}}{4}\left(\frac{1.08-1}{1.08}\right)\left(\frac{5.4}{1+[(5.4+5.0)/2]}\right)$$

$N = 8.65 \times 10^3$ plates

For a resolution of 1.5, the length of column required is

0.20 mm/plate x 8.65×10^3 plates = 1.73 m

$N = L/H$

$L = N \times H$

Chemical Engineering
Inspiring Potential · Generating Futures

Example 5

UNIVERSITI TEKNOLOGI PETRONAS

Ethanol and methanol are separated in a capillary GC column with retention times of 370 and 385 s, respectively, and base width w of 16 and 17 s. An unretained air peak occurs at 10 s.

Calculate the relative retention and the resolution.

Chemical Engineering
Inspiring Potential · Generating Futures

Solution

UNIVERSITI TEKNOLOGI PETRONAS

t_{r1} = 370 s, t_{r2} = 385 s, t_m = 10 s , w_1 = 16 s, w_2 = 17 s

$k' = (t_r - t_m)/t_m$

$k'_1 = (370-10)/10 = 36$

$k'_2 = (385-10)/10 = 37.5$

Relative retention, $\alpha = t'_{r2}/t'_{r1} = k'_2/k'_1 = 37.5/36 = 1$

Resolution $= \Delta t_r/w_{av}$

$= (385-370)/ [(16+17)/2]$

$= 0.9$

Chemical Engineering
Inspiring Potential · Generating Futures

Summary

UNIVERSITI TEKNOLOGI PETRONAS

i. Adjusted retention time, $t'_r = t_r - t_m$

ii. Relative retention, $\alpha = t'_{r2}/t'_{r1} = k'_2/k'_1$

iii. Capacity factor, $k'=(t_r-t_m)/t_m = t'_r/t_m$

iv. Number of plates, $N = 16\ t^2_r/w^2 = 5.55\ t^2_r/w^2_{1/2}$

v. Plate height, $H = L/N$

vi. Resolution $= \Delta t_r/w_{av}$

$$= \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_2}{1+k'_{av}}\right)$$

Chemical Engineering
Inspiring Potential · Generating Futures

Exercise

UNIVERSITI TEKNOLOGI PETRONAS

Q1. A chromatogram of a mixture of Species A and B provided the following data:

	Retention time, min	Width of Peak Base (W), min
Nonretained	3.10	-
A	13.3	1.07
B	14.1	1.16

Calculate:

i. The resolution of these two species.
ii. The relative retention, α.
iii. The number of plate from each species.

Chemical Engineering
Inspiring Potential · Generating Futures

Exercise

UNIVERSITI TEKNOLOGI PETRONAS

Q2. Consider a chromatography experiment in which two components with capacity factors k'_1 =4.00 and k'_2 = 5.00 are injected into a column with N = 1.00 x 10^3 theoretical plates. The retention time for the less-retained component is t_{r1} = 10.0 min.

a) Calculate t_m and t_{r2}.

b) Find $w_{1/2}$ (width at half height) and w (width at the base) for each peak.

Chemical Engineering
Inspiring Potential • Generating Futures

Outline

UNIVERSITI TEKNOLOGI PETRONAS

- Principle of Chromatography
- Classification of Chromatographic Techniques
- Qualitative Analysis and Quantitative Analysis
- Gas Chromatography (GC) -Principles and Instrumentation
- Liquid Chromatography (LC) – Principles and Instrumentation
- Thin Layer Chromatography (TLC)

Chemical Engineering
Inspiring Potential • Generating Futures

Outline

UNIVERSITI TEKNOLOGI PETRONAS

- Principle of Chromatography
- Classification of Chromatographic Techniques
- Qualitative Analysis and Quantitative Analysis
- Gas Chromatography (GC) -Principles and Instrumentation
- Liquid Chromatography (LC) – Principles and Instrumentation
- Thin Layer Chromatography (TLC)

Qualitative Analysis

- A chromatogram provides only a single piece of qualitative information about each species in a sample.
- It is widely used tool for recognizing the presence and absence of components of a mixtures.
- If the sample does not produce a peak at the same retention time as standard run under identical conditions → the compound is absent or is present at a concentration level below the detection limit.

Quantitative Analysis

- Quantitative column chromatography is based on a comparison of either the height or the area of the analyte peak with that of one or more standards.

i. Analyses based on Peak Height
ii. Analyses based on Peak Areas
iii. Calibration and Standards
iv. The Internal-Standard Method
v. The Area-Normalization Method

Chemical Engineering
Inspiring Potential · Generating Futures

i) Analyses based on Peak Height

UNIVERSITI TEKNOLOGI PETRONAS

- The height of a chromatographic peak is obtained by connecting the baselines on either side of the peak by a straight line and measuring the perpendicular distance from this line to the peak.

- Relative error → 5-10%

Chemical Engineering
Inspiring Potential · Generating Futures

ii) Analyses based on Peak Areas

UNIVERSITI TEKNOLOGI PETRONAS

- Areas are a more satisfactory analytical parameter than peak heights → are independent to the broadening effects → usually preferred method for quantification.

- % Error = 2 - 5%

iii) Calibration and Standards

- Involves preparation of a series of external-standard solutions that approximate the composition of unknown.
- Chromatograms for the standards are then obtained and peak heights or areas are plotted as a function of concentration.
- A plot of data should yield a straight line passing through the origin, determination are based on this calibration curve.

Chemical Engineering
Inspiring Potential · Generating Futures

iv) The Internal-Standard Method

UNIVERSITI TEKNOLOGI PETRONAS

- In this procedure, a careful measured quantity of an internal-standard substance is introduced into each standard and sample, and the ratio of analyte to internal standard peak areas (or heights) serves as the analytical variable.

- Precision better than 1%

v) The Area-Normalization Method

- Complete elution of all components of the sample is required.
- The areas of all eluted peaks are computed; the concentration of the analyte is found from the ratio of its area to the total area of all peaks.
- Has limited applications.