Chromatographic Analysis

Chapter 23

Distribution of Analytes between Phases

An analyte is in equilibrium between the two phases

\[
[S_1] \quad [S_2] \\
\text{(in phase 1)} \quad \text{(in phase 2)}
\]

\[
K = \frac{\frac{A_2}{A_1}}{\frac{[S_2]}{[S_1]}}
\]

Activity coefficients in phase 1 and 2 Concentration of the solute S

Partition coefficient is an equilibrium constant

Two solutes of differing Partition Coefficients will therefore distribute differently in same pairs of phases

Intermolecular Interactions
Distribution of Analytes between Phases

**Chromatography**

- A solid phase - stationary
- A liquid phase - mobile
- Partition coefficient $K$, will be defined as the molar concentration of analyte in the stationary phase divided by the molar concentration of the analyte in the mobile phase.

Distribution of Analytes between Phases

- Mobile phase moves on - elution
- Separation of the solutes occurs

Column Chromatography

**Chromatogram**

A graph showing detector's response as a function of elution time.
The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the retention time (t_R). Each analyte in a sample will have a different retention time. The time taken for the mobile phase to pass through the column is called t_M.

Adjusted retention time = t'_R = (t_R - t_M).

What occurs in a column? 
- Components migrate differentially
- Component bands spread

Capacity Factor (k')
A term called the capacity factor, k', is often used to describe the migration rate of an analyte on a column. You may also find it called the retention factor. The capacity factor for analyte S is defined as:

$$k'_S = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}} = \frac{t_R - t_M}{t_M}$$
Column Chromatography

Relative Retention ($\alpha$)

The relative retention of two solutes $S_1$ and $S_2$ is defined as:

$$\alpha = \frac{t'_{R(S_1)}}{t'_{R(S_2)}}$$

Ratio of adjusted retention times

It can be shown that

$$\alpha = \frac{k'_{S_1}}{k'_{S_2}} = \frac{K_{S_1}}{K_{S_2}}$$

Ratio of capacity factors

Ratio of partition coefficients

Efficiency of Separation

Idealized Gaussian Chromatogram

Longer the solute travels through the column broader is the peak

Resolution, $R_s = \frac{\Delta t}{w_{1/2}} = 0.589 \frac{M_k}{w_{1/2}}$

$\sigma$ represents the standard deviation

$w_{1/2}$ is the peak width at half-height

Resolution: Separation Efficiency

Resolution, $R_s = \frac{\Delta t}{w_{1/2}} = 0.589 \frac{M_k}{w_{1/2}}$
1. A peak with a retention time of 407 s shows a poorly resolved second peak which appeared 5 s after it. The calculated resolution was 0.67. The first peak had a width of 8 s. The width of the second peak is

- a. 11 s
- b. 22 s
- c. 7 s

2. Octanoic acid (CH₃CH₂CH₂CH₂CH₂CH₂CH₂COOH) and 1-aminooctane (CH₃CH₂CH₂CH₂CH₂CH₂CH₂CH₂NH₂) were passed through an octadecyl (C₁₈) bonded phase column, using an eluent of 20 % methanol/80% buffer (pH 3.0). Choose the correct statement

- a) Being polar, octanoic acid will be retained more in the column and will come out after 1-aminooctane.
- b) Being nonpolar, 1-aminooctane will be retained more in the column and will come out after octanoic acid.
- c) At pH 3.0, octanoic acid will not dissociate and act as a nonpolar molecule, while 1-aminooctane being protonated will act as a polar molecule and therefore will come out first.
- d) The column will be unable to distinguish the two chemicals and both will come out roughly at the same time.

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### The Theoretical Plate Model of Chromatography

The chromatographic column hypothetically contains a large number of separate layers, called theoretical plates.

Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.

Theoretical plate

### Plate height

\[ H = \frac{L}{N} = \frac{\sigma^2}{N \bar{x}} = \frac{16 \sigma^2}{w^2} = \frac{5.55 \sigma^2}{w_{1/2}^2} \]

- \( L \) = the total length of the column
- \( N \) = number of theoretical plate
- \( \sigma \), the standard deviation, can be obtained from the HPLC peak and
- \( \bar{x} \) = the distance traveled by the solute in the column
- \( w \) = the width of the peak at its base
- \( w_{1/2} \) = width at half height
Factors Affecting Resolution

Resolution, \( R_s = \frac{\sqrt{N}}{4} (\gamma - 1) \)

\( N = \text{no. of theoretical plates} \)
\( \gamma = \text{separation factor} = \frac{t'_{R(s)}}{t'_{R(S)}} > 1 \)

Increasing \( N \), increasing \( \gamma \)

Factors Affecting Resolution

Resolution, \( R_s = \frac{\sqrt{N}}{4} (\gamma - 1) \)

Increasing \( N \), increasing \( \gamma \)

\( N = \text{no. of theoretical plates} \)
Can be increased by increasing the length of the column
\( \gamma = \text{separation factor} \)

Factors Affecting Resolution

Resolution, \( R_s = \frac{\sqrt{N}}{4} (\alpha - 1) \left( \frac{1 + k'}{k'} \right) \)

Initial Chromatogram

Varying \( N \)

Varying \( \gamma \)
Two compounds with capacity factors of 2.00 and 2.20 are separated on a column with a plate height 0.5 mm. The length of column is 0.8 m. What is the resolution? What length required to give a resolution of 1.00?

Chromatographic Analysis

The Process
1. Make $R_S > 1.0$
2. Increase $N$
3. Measure standards, peak height or area. Make calibration curve
4. Measure sample peak(s)
5. Calculate concentration

High Performance Liquid Chromatography (HPLC) (or High Pressure Liquid Chromatography)

The Instrument
High Pressure Liquid Chromatography (HPLC)

The Instrument

A guard column to absorb impurities.

The Column

- **Mobile phase**: Carrying the solutes
- **Elution time**: Depends on the mutual attraction of the solutes to the stationary phase. Solutes which have small attraction to the stationary phase go down the column at the speed of the mobile phase.
- **These solutes elute when the old volume of mobile phase in the column is completely replaced by a new volume of the mobile phase.**
- **Void volume**: The volume of the mobile phase in the column (≠ Volume of the column – volume occupied by the packing material)
High Pressure Liquid Chromatography (HPLC)

Normal Phase and Reverse Phase Columns

- **Normal Phase**: Polar stationary phase and less polar solvent
- **Reverse Phase**: Nonpolar stationary phase and more polar solvent

Column type 1: Pure Silica is the stationary phase – Normal Phase

- **Adsorption Chromatography**: Difference in analyte adsorption affinities results in their separation.
- **Normal Phase**: Analytes with polar functionalities are retained longer and nonpolar analytes are retained less.

Column type 2: Reversed Phase Separation Principle

- **Reversed Phase**: Octadecyl group (i.e. \((\text{CH}_2)_17\text{CH}_3\))
High Pressure Liquid Chromatography (HPLC)

Column type 2: Reversed Phase Separation Principle

Separation Principle

- Adsorption Chromatography:
  Difference in analyte adsorption affinities results in their separation

- Reverse Phase:
  Analytes with larger hydrophobic part are retained longer and more polar analytes are retained less

Theoretical Plate Model of Chromatography

The chromatographic column hypothetically contains a large number of separate layers, called theoretical plates.

Why Do Bands Spread?

Separate equilibrations of the sample between the stationary and mobile phase occur in these “plates”. The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.
van Deemter equation: relates the theoretical plate (height equivalent theoretical plate, $H$) of a separation column to the linear mobile phase velocity

$$H = \frac{B}{u} + C u$$

where $u$ is the flow-rate and $A$, $B$, and $C$ are constants for a given column of a stationary phase.

High Pressure Liquid Chromatography (HPLC)

Band Spreading due to **Multiple Path**

The existence of multiple path increases the band width in a manner independent of the flow rate.

Band Spreading due to **Longitudinal Diffusion**

The band spread due to longitudinal diffusion is inversely proportional to the flow rate.
High Pressure Liquid Chromatography (HPLC)

Band Spreading due to Slow Equilibration

Chapter 25

The band spread due to equilibration is directly proportional to the flow rate.

High Pressure Liquid Chromatography (HPLC)

Two Characteristics of Reversed Phase Column Help to Minimize Band Spreading

- Stationary phase does not strongly adsorb solute i.e. the longitudinal diffusion is decreased
- Less sensitive to polar impurities such as water i.e. the equilibration is diminished

Increasing Retention time (tR)

1. Pressure (flow rate)
2. The nature of the stationary phase (not only what material it is made of, but also particle size)
3. Composition of the solvent
4. Temperature of the column

Two Primary Experimental Factors that Affect Retention Time

- Flow rate
- Polarity of the Solvent Composition of the Mobile Phase
The Variable Wavelength UV Detector uses a monochromator (slits and a grating) to select one wavelength of light to pass through the sample cell.

The Photodiode Array Detector passes all wavelengths of light through the sample cell, then focuses each wavelength on a single sensor element.