We start with reviewing the key for Test 3

Section 4 – Analytical Separations

Chp 23
Pages 549-551, 553-570
Problems 1-3, 15-19, 27, 29-32, 36, 37, 43, 44

Chp 24
Pages 579-591
Problems 1-3, 6, 8a, 10, 13, 18, 20

Chp 25
Pages 608-612, 614-617, 621-623, 627-629, 631-633
Problems 1a, 3, 5, 6, 9a, 15, 16, 19, 21-23, 28

Chp 26
Pages 641-644, read section on CE methods but mainly use my notes
Problems 2-7, 22-26, 29, 31, 33, 39

Choose 3 of
Multiple Choice questions are 4 pts each

1. Which of the following will not cause deviation from the normal linear relationship of Beer’s Law?
   (A) Self absorption
   (B) Variation in light intensity due to a double beam instrument
   (C) Light source that is monochromatic
   (D) Changes in refractive index of the sample solution at high sample concentrations
   (E) (B) and (D)

2. The amount of a sample is measured at 30% when the sample is diluted by a factor of 3 and the optical path length is increased by a factor of 3. The absorbance of the solution is ______.
   (A) 0.20
   (B) 0.30
   (C) 0.30 absorbance
   (D) 0.40 absorbance
   (E) 0.60 absorbance

3. The absorbance of a solution of CuSO4 at 300 nm is measured with a spectrophotometer. The mean value of the 48 replicate readings is 0.36. The absorbance now becomes
   (A) 0.46
   (B) 0.37
   (C) 0.38
   (D) 1.0
   (E) 2.5
   3.5 \times 10^{-2} \times 2 = 0.36

4. Which of the following is not a correct statement regarding optical spectroscopy?
   (A) Beer’s Law is a good descriptor for all absorptions
   (B) A double beam instrument is not required to obtain an absorbance spectrum
   (C) The PUR spectrometer produces a one-to-one conversion of optical photon to electron stream
   (D) none of the above is correct (all are correct)
   (E) A, B, and C are not correct

5. Molecular spectroscopy is more sensitive than absorptometry as an analytical technique because ______.
   (A) Fluorescence appears efficiently one approach only while no such term exists in absorptometry
   (B) sensitivity depends on E_{0} in spectrophotometry, but absorptometry is less affected
   (C) Monochromatic in absorptometry means low energy photon
   (D) Absorption spectrometries are based on interactions with a relatively large optical background
   (E) (A) and (B)

6. A) An example of an interaction between in AAS spectrometry to reduce ionization of the analyte is ______.
   (B) EDTA
   (C) CaCl2
   (D) NaOH
   (E) HCl

7. Energy level changes that occur in the visible region of the EM spectrum correspond to ______ transitions.
   (A) electronic
   (B) electronic
   (C) electronic
   (D) vibrational
   (E) electronic

Provide a short (5-3 sentences) answer for 9 & 10 (5 pts each).

9. Most AAS analyses occur in the spectral region that can be achieved with a conventional tungsten continuum source. However, a tungsten lamp – monochromator, as it is often used in molecular absorption work, is not suitable for use in AAS work. Why?

The absorption lines are very narrow. Thus, the light source must be very monochromatic for Beer’s Law to be valid. The bandwidth (range of wavelengths scanned) of a monochromator is too wide to satisfy this monochromatic requirement (see Fig 23-15).

10. Why is an iron standard necessary for ICP for a “true” determination of Fe concentration?

The standard (solution of Fe) is necessary to determine the instrument response for Fe in the sample. Without this standard, the instrument response for Fe in the sample cannot be accurately determined, resulting in inaccurate measurements of Fe concentration. The standard allows the instrument response to be calibrated and ensures that the instrument is properly configured for the analysis.
Separations

1. Take a mixture of chemical species and separate it into individual chemical compounds.

2. Analyze the separated components (off-line or on-line).

3. Extractions, precipitations, chromatography, electrophoresis, etc.
Extractions (Partition)

- A solute partitions between two immiscible liquids (2 phases)
  
  \[ m = \text{moles } S \]
  
  \[ q = \text{fraction of } S \text{ in phase 1} \]
  
  \[ V_1 = \text{volume of phase 1} \]
  
  \[ V_2 = \text{volume of phase 2} \]
  
  \[
  K = \frac{[S]_2}{[S]_1} = \frac{(1-q)m/V_2}{qm/V_1}
  \]

Rearrange to obtain….

\[
q = \frac{V_1}{V_1 + KV_2}
\]

- \( n \) extraction steps

\[
q^n = \left( \frac{V_1}{V_1 + KV_2} \right)^n
\]
Extractions Meet Acid-Base Chemistry

1. Neutral compounds primarily dissolve in the organic phase.
2. Charged compounds only dissolve in the aqueous phase.

Consider:

$$B + H_2O \rightleftharpoons BH^+ + OH^-$$

Extractions Meet Acid-Base Chemistry

$$D = \frac{[B]_2}{[B]_i + [BH^+]_i}$$

$$K = \frac{[B]_2}{[B]_i} \quad \text{and} \quad K_a = \frac{[H^+]_i[B]}{[BH^+]_i}$$

$$D = \frac{K \cdot K_a}{K_a + [H^+]}$$
Chromatography Basics

Definition – Separation technique based on the differential distribution of introduced solutes between a moving mobile phase and a stationary phase. Important elements of phase equilibria, mass transport, and kinetics are involved.

Often performed in columns packed with the stationary phase...

Chromatography Basics

Definition – Separation technique based on the differential distribution of introduced solutes between a moving mobile phase and a stationary phase. Important elements of phase equilibria, mass transport, and kinetics are involved.

Forms→

Adsorption chromatography Partition chromatography
**Chromatography Basics**

**Definition** – Separation technique based on the differential distribution of introduced solutes between a moving mobile phase and a stationary phase. Important elements of phase equilibria, mass transport, and kinetics are involved.

**The Chromatogram**

- Plot of the response of a Detector at end of column
- Versus The length of time it take to elute from column
- Notice Gaussian profile (many random processes shape the band)
**Adjusted Retention Time**

\[ t'_r = t_r - t_m \quad \alpha = \frac{t'_r}{t'_r} \]

**Capacity Factor**

\[ k' = \frac{t_r - t_m}{t_m} \]

\[ K = \frac{[\text{stat}]}{[\text{mob}]} \quad \& \quad k' = \frac{\text{amt}_{\text{stat}}}{\text{amt}_{\text{mob}}} \]

\[ k' = K \frac{V_{\text{stat}}}{V_{\text{mob}}} \]
**Separation Efficiency**

\[ N = \frac{16t_r^2}{w^2} = \frac{5.55t_r^2}{w_{1/2}^2} \]

\[ H = \frac{L}{N} \]

**Resolution (the main objective)**

\[ R = \frac{\Delta t_r}{w_{av}} = \frac{\sqrt{N}}{4} \left( \frac{\alpha}{\alpha - 1} \right) \left( \frac{k_2'}{1 + k_2'} \right) \]

- \( R = 1.0 \) good
- \( R = 1.5 \) excellent
- \( R > 2.0 \) only good

efficiency \[ \uparrow \]
selectivity \[ \uparrow \]
capacity \[ \uparrow \]
Resolution (the main objective)

- Band width at baseline = $4 \sigma$
- Recall area under Gaussian

How to Resolve B and C

Increase peak separation through better selectivity; a matter of thermodynamics

\[ R = \frac{\Delta t_r}{w_{av}} = \frac{\sqrt{N}}{4} \left( \frac{\alpha}{\alpha - 1} \right) \left( \frac{k'_2}{1 + k'_{av}} \right) \]
How to Resolve B and C

Increase peak sharpness through efficiency;
A matter of kinetics (mass transport)

\[ R = \frac{\Delta t_r}{w_{av}} = \frac{\sqrt{N}}{4} \left( \frac{\alpha}{\alpha - 1} \right) \left( \frac{k_2'}{1 + k_{av}'} \right) \]

What Causes Peak Broadening?

\[ H = A + \frac{B}{u} + Cu \]

- **A is the Multiple Path Term**
  - Packed (particles) columns result in broadening since different analyte molecules can take different paths down the column.

- **B is the Longitudinal Diffusion Term**
  - If the analytes move down the column and equilibrium is not established, peaks broaden. The faster the analytes move the less time they have to equilibrate (u dependence).

- **C is the Mass Transfer Term**
  - A concentrated band of analytes diffuses down the column. The slower the band moves the more it diffuses (u dependence).
What Causes Peak Broadening?

A Term; eddy diffusion
- With smaller particles more decisions \( \rightarrow \) averages out
- Open tubular columns have no eddy diffusion problem

B Term; axial diffusion
- Standard deviation of Gaussian band \( \sigma = (D_m t)^{1/2} \)
- Use high flow rates
- See Table 23-1

C Terms; \( C_m \) & \( C_s \);
Use slow Flow rate

Van Deemter Plot
Table 23-2  Summary of chromatography equations

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Equation</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partition coefficient</td>
<td>( K = C_s/C_m )</td>
<td>( C_s ) = concentration of solute in stationary phase ( C_m ) = concentration of solute in mobile phase</td>
</tr>
<tr>
<td>Adjusted retention time</td>
<td>( t_r' = t_r - t_0 )</td>
<td>( t_r ) = retention time of solute of interest ( t_0 ) = retention time of unretained solute</td>
</tr>
<tr>
<td>Retention volume</td>
<td>( V_r = V_i )</td>
<td>( V_i ) = volume flow rate ( V_r ) = volume/retention time</td>
</tr>
<tr>
<td>Capacity factor</td>
<td>( k' = \frac{t_r'}{t_m} = \frac{V_r}{V_m} )</td>
<td>( V_m ) = volume of mobile phase ( V_r ) = volume of stationary phase</td>
</tr>
<tr>
<td>Relative retention</td>
<td>( \alpha = \frac{K_2}{K_1} = \frac{V_2}{V_1} )</td>
<td>Subscripts 1 and 2 refer to two solutes</td>
</tr>
<tr>
<td>Number of plates</td>
<td>( N = \frac{16d^2}{w_{1/2}^2} = \frac{5.56d^2}{w_{1/2}^2} )</td>
<td>( w ) = width at base ( w_{1/2} ) = width at half-height</td>
</tr>
<tr>
<td>Plate height</td>
<td>( H = \frac{x^2}{N} )</td>
<td>( x ) = distance traveled by center of band ( N ) = number of plates on column</td>
</tr>
<tr>
<td>Resolution</td>
<td>Resolution = ( \frac{\Delta t}{w_{1/2}} )</td>
<td>( \Delta t ) = difference in retention times ( \Delta V_r ) = difference in retention volumes</td>
</tr>
<tr>
<td></td>
<td>Resolution = ( \sqrt{\frac{N}{4}} \left( \alpha - 1 \right) \left( \frac{k_2'}{1 + k_2'} \right) )</td>
<td>( N ) = number of plates ( \alpha ) = relative retention ( k_2' ) = capacity factor for second peak ( k_i' ) = average capacity factor</td>
</tr>
</tbody>
</table>

Problems From Chp 23

23.2 If you wish to extract aqueous acetic acid into toluene, is it more effective to adjust the aqueous phase to pH 3 or pH 8?

23.3 Why is it difficult to extract the EDTA complex of aluminum into an organic solvent, but easy to extract the 8-hydroxyquinoline complex?
Problems From Chp 23

23-17. Solute $S$ has a partition coefficient of 4.0 between water (Phase 1) and chloroform (phase 2) in Equation 23-1.
(a) Calculate the concentration of $S$ in chloroform if $[S_{aq}]$ is 0.020 M.
(b) If the volume of water is 80.0 mL and the volume of chloroform is 10.0 mL, find the quotient (mol S in chloroform)/(mol S in water).

23-20. The solute in the previous problem is initially dissolved in 80.0 mL of water. It is then extracted six times with 10.0-mL portions of chloroform. Find the fraction of solute remaining in the aqueous phase.

Problems From Chp 23

23-17. (a) Write the meaning of the capacity factor, $k'$, as a term of time spent by solute in each phase.
(b) Write an expression in terms of $k'$ for the fraction of time spent by a solute molecule in the mobile phase.
(c) The retention ratio in chromatography is defined as:

$$R = \frac{\text{time for solvent to pass through column}}{\text{time for solute to pass through column}} = \frac{t}{t_s}$$

Show that $R$ is related to the capacity factor by the equation:

$$R = \frac{1}{1 + \frac{k'}{N}}$$
Problems From Chp 23

23.21. Chromatograms of compounds A and B were obtained at the same flow rate with two columns of equal length.

(a) Which column has more theoretical plates?
(b) Which column has a larger plate height?
(c) Which column gives higher resolution?
(d) Which column gives a greater retention time?
(e) Which compound has a higher capacity factor?
(f) Which compound has a greater partition coefficient?

23.22. What is longitudinal diffusion a more serious problem in gas chromatography than in liquid chromatography?

Gas Chromatography – Separations based on both analyte volatility and specific interactions with the stationary phase

INSTUMENTATION

<table>
<thead>
<tr>
<th>Common stationary phases in capillary gas chromatography</th>
<th>Polymer</th>
<th>Temperature range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fused silica capillary (FSC)</td>
<td>Glass</td>
<td>40°C - 120°C</td>
</tr>
<tr>
<td>Packed column</td>
<td>Glass</td>
<td>20°C - 200°C</td>
</tr>
<tr>
<td>Porous graphitized carbon (PGC)</td>
<td>Carbon</td>
<td>20°C - 200°C</td>
</tr>
<tr>
<td>Cross-linked silica gel</td>
<td>silica</td>
<td>20°C - 200°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>0.1 – 0.53 mm inner diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>Flow</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>0.1 – 5 μm thick</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td></td>
</tr>
</tbody>
</table>
Example of a more exotic stationary phase (chiral discrimination)

Stationary phase containing l-valine covalently attached to column wall

<table>
<thead>
<tr>
<th>Gas</th>
<th>30K</th>
<th>m</th>
<th>ν·cm⁻¹-s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂</td>
<td>0.079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N₂</td>
<td>0.140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂</td>
<td>0.0015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂</td>
<td>0.00007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₄</td>
<td>0.0004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₂H₆</td>
<td>0.0006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₃H₆</td>
<td>0.0011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₄H₆</td>
<td>0.0014</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thermal conductivity at 32°C and 1 atmosphere
Gas Chromatography (GC)

\[ R = \frac{\Delta t_r}{w_{av}} = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k'_2}{1 + k'_{av}} \right) \]

**Temperature** – Analytes must be near or above their boiling points to spend a significant amount of time in the mobile phase (gas). Analytes must be thermally stable for GC analysis!

**Adorption/Partition** – Analytes move more slowly when they spend more time interacting with the stationary phase. Remember, “Like dissolves like”.

Above illustrates both boiling pt & stationary phase interactions influence the separations
Effect of Column Temperature and T-Programming

**Gas Chromatography (GC)**

\[ R = \frac{\Delta t_r}{w_{av}} = \sqrt{\frac{N}{4}} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k'}{1 + k'_{av}} \right) \]

**Interaction with Stationary Phase** – The primary way to change \( \alpha \) is to change stationary phases. This requires changing the column! Remember that the mobile phase is inert, so changing the gas doesn’t help.
Gas Chromatography (GC)

\[ H = A + \frac{B}{u} + Cu \]

Originally most GC columns had a packed stationary phase (solid particles or particles coated with a polymer).

Today most GC is performed in thin capillaries with the stationary phase coated on the walls of the capillary.

Diffusion in a gas is much faster than diffusion in a liquid; hence, the B-term is very significant in determining efficiency in GC.

---

**Table 23-3** Comparison of packed and wall-coated open tubular column performance<sup>a</sup>

<table>
<thead>
<tr>
<th>Property</th>
<th>Packed</th>
<th>Open tubular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column length, ( L )</td>
<td>2.4 m</td>
<td>100 m</td>
</tr>
<tr>
<td>Linear gas velocity</td>
<td>8 cm/s</td>
<td>16 cm/s</td>
</tr>
<tr>
<td>Plate height for methyl oleate</td>
<td>0.73 mm</td>
<td>0.34 mm</td>
</tr>
<tr>
<td>Capacity factor, ( k' ), for methyl oleate</td>
<td>58.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Theoretical plates, ( N )</td>
<td>3 290</td>
<td>294 000</td>
</tr>
<tr>
<td>Resolution of methyl stearate and methyl oleate</td>
<td>1.5</td>
<td>10.6</td>
</tr>
<tr>
<td>Retention time of methyl oleate</td>
<td>29.8 min</td>
<td>38.5 min</td>
</tr>
</tbody>
</table>

<sup>a</sup> Methyl stearate \((\text{CH}_3\text{CH}_2)_{16}\text{CO}_2\text{CH}_3\) and methyl oleate \((\text{cis-CH}_3\text{CH}_2\text{CH}≡\text{CH}[(\text{CH}_2)_x\text{CO}_2\text{CH}_3])\) were separated on columns with poly(diethylene glycol succinate) stationary phase at 180°C.

**Source:** L. S. Ettre, *Introduction to Open Tubular Columns* (Norwalk, CT: Perkin-Elmer Corp., 1979).
Chapter 24 Problems

24-1. (a) What is the advantage of temperature programming in gas chromatography?

(b) What is the advantage of a bonded stationary phase in gas chromatography?

24-3. (a) Why do open tubular columns provide greater resolution than packed columns in gas chromatography?

(b) Why does a thermal conductivity detector respond to all analytes except the carrier gas? Why isn’t the flame ionization detector universal?

Table 24-3: Retention indices for several compounds on common stationary phases

<table>
<thead>
<tr>
<th>Phase</th>
<th>Retention index</th>
<th>Hydrocarbon</th>
<th>OH</th>
<th>2-Pentanone</th>
<th>1-Nitropropane</th>
<th>Pyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dimethylsiloxane)</td>
<td>657</td>
<td>646</td>
<td>630</td>
<td>708</td>
<td>732</td>
<td></td>
</tr>
<tr>
<td>(Diethyl)dimethylsiloxane</td>
<td>579</td>
<td>565</td>
<td>549</td>
<td>628</td>
<td>652</td>
<td></td>
</tr>
<tr>
<td>(Diethyl)dimethylsiloxane</td>
<td>792</td>
<td>777</td>
<td>751</td>
<td>830</td>
<td>854</td>
<td></td>
</tr>
<tr>
<td>Poly(dimethylsiloxane)</td>
<td>797</td>
<td>799</td>
<td>784</td>
<td>863</td>
<td>887</td>
<td></td>
</tr>
<tr>
<td>Polychloroether (chlor)</td>
<td>956</td>
<td>943</td>
<td>928</td>
<td>941</td>
<td>965</td>
<td></td>
</tr>
<tr>
<td>Dichloroacetylene (dichloro)</td>
<td>1161</td>
<td>1142</td>
<td>1127</td>
<td>1206</td>
<td>1230</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Note</th>
<th>Retention index</th>
<th>Hydrocarbon</th>
<th>OH</th>
<th>2-Pentanone</th>
<th>1-Nitropropane</th>
<th>Pyridine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dimethylsiloxane)</td>
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<td>549</td>
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<td>(Diethyl)dimethylsiloxane</td>
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<td>965</td>
<td></td>
</tr>
<tr>
<td>Dichloroacetylene (dichloro)</td>
<td>1161</td>
<td>1142</td>
<td>1127</td>
<td>1206</td>
<td>1230</td>
<td></td>
</tr>
</tbody>
</table>

24-9. Using Table 24-3, predict the order of elution of the following compounds from columns containing di(n-hexyl)poly(dimethyl) siloxane, (b) (diethyl)dimethyl(polydimethylsiloxane), and (c) poly(ethylene glycol): 
1. n-pentanol (n-C₅H₁₀OH, bp. 138°C) 
2. 2-hexanone (CH₃C=O)n-C₆H₁₃, bp. 128°C) 
3. heptane (n-C₇H₁₅, bp. 98°C) 
4. octane (n-C₈H₁₈, bp. 126°C) 
5. nonane (n-C₉H₂₀, bp. 151°C) 
6. decane (n-C₁₀H₂₂, bp. 174°C) 

Chapter 24 Problems


(a) Which term is 0 for an open tubular column? Why?

(b) Express the value of B in terms of measurable physical properties.

(c) Express the value of C in terms of measurable physical quantities.

(d) The linear flow rate that produces minimum plate height (optimum resolution) is found by setting the derivative d²H/dx² to 0. Find an expression of the minimum plate height in terms of measurable physical quantities used to measure the column.

β ≈ C

Efficiency

\[ H = \frac{2d_p}{u} + \frac{2D_m}{u} + \frac{2yD_p^2k_u}{D_e(1+k')^2} + \frac{(1+6k^2+11k^2)u^2}{96(1+k^2)D_m} \]
Why use polymeric phases as GC stationary phases?

Why is OTGC preferred to packed column GC?

To improve H, would you like to increase or decrease the values of the following?
- Stationary Phase thickness
- Column diameter
- Diffusion Coefficient
- Flow rate

Of the two GC detectors discussed which is more sensitive?

Why are internal standards often used in GC analysis?

Doubling the length of a GC column will do what to plate number (N)?

The two most important factors in determining the retention time of a compounds in GC are what?

What is an ideal value for Rs?

The A-term in efficiency pertains to what efficiency factor?

What is a typical length of a OTGC column?

Etc.
**HPLC Instrumentation**

**Fixed Loop**

**High Pressure**

**Injector – Typical V = 10 µL**

**Reciprocating Piston Pump**

**Typical Flow 1 mL/min**

**Column**

**Typical L = 25 cm**

---

**HPLC Instrumentation**

**Table 25.3** Comparison of commercial HPLC detectors

<table>
<thead>
<tr>
<th>Detector</th>
<th>Approximate Limit of detection (ng)</th>
<th>Useful with gradient?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultraviolet</td>
<td>0.1-1</td>
<td>Yes</td>
</tr>
<tr>
<td>Refractive index</td>
<td>0.001-1000</td>
<td>No</td>
</tr>
<tr>
<td>Evaporative light-scattering</td>
<td>0.1-1</td>
<td>Yes</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>0.001-1</td>
<td>No</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>0.001-0.01</td>
<td>Yes</td>
</tr>
<tr>
<td>Nitrogen (N&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>0.3</td>
<td>Yes</td>
</tr>
<tr>
<td>Conductivity</td>
<td>0.5-1</td>
<td>No</td>
</tr>
<tr>
<td>Mass spectrometry</td>
<td>0.1-1</td>
<td>Yes</td>
</tr>
<tr>
<td>Fourier transform infrared</td>
<td>1000</td>
<td>Yes</td>
</tr>
</tbody>
</table>

HPLC

The Packing Particles

Silica Particle
Typically 10 µm dia; whopping surf area

Silica particle with bonded “reversed phase” (C-18)

HPLC

\[
R = \frac{\Delta t_r}{w_{av}} = \sqrt{\frac{N}{4}} \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{k'_2}{1 + k'_av}\right)
\]

Solvent Composition – Solvent polarity is adjusted. Typically a mixture of water and a miscible organic solvent is changed to control solvent polarity

Adsorption/Partition – Analytes move more slowly when they spend more time interacting with the stationary phase. Remember, “Like dissolves like”.

Interaction with Stationary Phase – The primary way to change \( \alpha \) is to change phases. This requires changing the column or the solvent; the latter is obviously easier.
**HPLC**

- A wide range of detectors are used for HPLC. Absorbance detection is very important.

- A wider range of analytes can be analyzed by HPLC compared to GC. Reproducible injection is easier than for GC.

- Plumbing is a significant issue (H) in HPLC.

- Solvent programming means to change the solvent composition during an experiment. This is used to adjust $k'$ continuously during the separation.

---

**HPLC**

*The effect of solvent strength & S-Programming*

Reversed phase (C18 stationary phase) separations of aromatic cmpds. The base solvent is generally water and modified with a more organic solvent like acetonitrile to increase the eluting strength of the mobile phase.

In a manner analogous to T-programming in GC, Solvent Programming in LC addresses the general elution problem.
We will focus on “Reversed Phase HPLC” but know when exclusion and ion exchange are used and the difference between reversed and normal phase.

**HPLC**

Stationary phases – PS-DVB modified with charged groups
Mobile phases - aqueous and contain acids or bases

Exchange rxn - \( Ca^{2+} + 2\text{Resin}\text{-H}^+ \leftrightarrow 2\text{Resin}^- + Ca^{2+} + 2H^+ \)
**HPLC**

**Table 25-4** Starting conditions for reversed-phase chromatography

Stationary phase: C\textsubscript{18} or C\textsubscript{8} on 5-µm-diameter spherical silica particles. Less acidic Type B silica (Figure 25-7) is preferred. For operation above 50°C, sterically protected silica (Figure 25-8) is preferred.

Column: 0.46 × 15 cm column for 5-µm particles\(^a\)
0.46 × 7.5 cm column for 3.5-µm particles (shorter run, same resolution)

Flow rate: 2.0 mL/min

Mobile phase: CH\textsubscript{3}CN/H\textsubscript{2}O for neutral analytes
CH\textsubscript{3}CN/aqueous buffer\(^b\) for ionic analytes
5 vol % CH\textsubscript{3}CN in H\textsubscript{2}O to 100% CH\textsubscript{3}CN for gradient elution

Temperature: 35°C–40°C if temperature control is available

Sample size: 25–50 µL containing ~25–50 µg of each analyte

---

\(a\) A 0.30 × 15 cm column reduces solvent consumption to (0.30/0.46)\(^3\) = 43% of the volume required for 0.46-cm diameter, reducing the flow to (0.43/2.0 mL/min) = 0.86 mL/min.

\(b\) Buffer is 25–50 mM phosphate/pH 2–3 made by treating H\textsubscript{3}PO\textsubscript{4} with KOH. K\textsuperscript{+} is more soluble than Na\textsuperscript{+} in organic solvents and leads to less tailing. Add 0.2 g sodium azide per liter as a preservative if the buffer will not be used quickly.

---

**HPLC**  
**Solvent Optimization**
Chapter 25 Problems

25-1. (a) Why does solvent strength increase as solvents become less polar in reversed-phase chromatography, whereas solvent strength decreases as solvents become more polar in normal-phase chromatography?

25-2. If a 15-cm-long column has a plate height of 5.0 mm, what will be the half-width (in seconds) of a peak eluting at 10.0 min if the plate height is 25.0 mm, what will be the half-width?

1. (a) The mobile phase competes with the stationary phase for interactions with the analytes. Competition is more effective when they become more similar with regard to polarity.

2. N = 15 cm / 5.0x10^-4 cm = 30,000
   30,000 = 5.56 (600/W)^2
   W = 8.2 sec

(b) H = A + B/u + C u
   C proportional to dp^2
   And dominates the plot at large u

Chapter 25 Problems

25-14. Peak areas and relative detector responses are to be used to determine the concentrations of the five analytes in a sample. The chromatogram for the mix is described in Problems 25-13 to 25-15. Also shown are the relative detector responses of the detector. Calculate the percentage of each component in the mixture.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative Peak Area</th>
<th>Relative Detector Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32.5</td>
<td>0.50</td>
</tr>
<tr>
<td>B</td>
<td>20.7</td>
<td>0.72</td>
</tr>
<tr>
<td>C</td>
<td>40.1</td>
<td>0.75</td>
</tr>
<tr>
<td>D</td>
<td>30.2</td>
<td>0.78</td>
</tr>
<tr>
<td>E</td>
<td>15.3</td>
<td>0.76</td>
</tr>
</tbody>
</table>

15. Liquid chromatography is used to separate styrene (A), toluene (B), ethylbenzene (C), and propylbenzene in the order of increasing Rf. The elution times for the separation were as follows.

| Rf       | Column 25 cm    | Flow 0.1 mL/min | Chromatogram
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>X-rf</td>
<td>15 sec</td>
<td>20 sec</td>
<td>10 mm</td>
</tr>
<tr>
<td>X-s</td>
<td>25 sec</td>
<td>20 sec</td>
<td>3.5 mm</td>
</tr>
<tr>
<td>X-b</td>
<td>25 sec</td>
<td>20 sec</td>
<td>3.0 mm</td>
</tr>
</tbody>
</table>

Calculate the following:
(a) X-rf height (mm) = 
(b) X-s rotation factor (Rf) = 
(c) Resolution between X-rf and X-s = 
(d) The length of column needed to provide a resolution of 1.5 = 
(e) If the distribution coefficient of C is 0.001, then the volume of the stationary phase must be.
What is the most common detector in HPLC and explain why its effective volume must be small?

Why is HPLC sometimes called “high performance” & sometimes “high pressure”?

What are typical flow rates?, injection volumes?, peak volumes?, plate counts?

Define: Bonded Phase, Normal Phase, and Reversed Phase?

In what form of LC is the stationary phase charged?

In HPLC what terms in the VanDeemter (A, B, Cm, Cs) equation are most significant?

What is the equivalent of T-programming in LC?

What is a common solvent mixture used for a mobile phase in reversed phase LC & how does one increase its eluting strength?

What is the principle of size exclusion chromatography?

Draw the stationary phase for a “C-18” packing

Etc.

---

Capillary Electrophoresis
(Last 310 Lecture)

- Capillary Zone Electrophoresis, CZE
- Capillary Electrokinetic Chromatography
  Micellar Electrokinetic Capillary Chromatography, MECC
  Cyclodextrin Distribution Capillary Electrochromatography, CDCE
- Size-Selective Capillary Electrophoresis, SSCE
Capillary Electrophoresis

- Separation technique based on the mobility differences in an electric field for ions injected into narrow-bore capillaries
- Mobilities scale roughly with charge to mass ratios of the ions
- Running buffer is electrokinetically pumped through the capillary
- Neutrals can be separated based on differential association with charged running buffer additives (e.g., micelles in MECC)
- CDs can be used to selectively modify the effective migration rates of ions or neutrals in CE
- Entangled polymers used for size selective separations
- Attributes of CE include very high efficiency, wide-ranging versatility, low sample and reagent consumption and waste generation, speed

HPLC vs. CE

<table>
<thead>
<tr>
<th></th>
<th>HPLC</th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Sample Size</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Large Sample Size</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Efficiency</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Limited</td>
<td>X</td>
</tr>
<tr>
<td>Speed</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Flexibility</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sample Variety</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
**CE Instrumentation**

- The bulk flow of the liquid in the capillary and is a consequence of the surface charge on the capillary wall
- Electromotive pumping action which originates at the wall of the capillary and results in a “plug-like” flow profile.

**Electroosmotic Flow**

- The bulk flow of the liquid in the capillary and is a consequence of the surface charge on the capillary wall
- Electromotive pumping action which originates at the wall of the capillary and results in a “plug-like” flow profile.
EOF Formation

\[ \mu_{eo} = \frac{\ell}{V} \frac{L}{t_o} \]

- \( l \) = length to the detection window
- \( L \) = total length of capillary (meters)
- \( V \) = applied voltage (volts)
- \( t_o \) = retention time of a neutral species

Note: \( V_{eo} = \mu_{eo} E \)

Separation Mechanism

Velocity = \( (\mu_{eof} + \mu_e) E \)  :  \( E = V / L \)

Note: \( \mu_e \) is roughly proportional to size/charge
**Separation Mechanism**

- Cartoon of CE separation

**CE Characteristics:** efficient, fast, versatile, diminutive

**Efficiency**

\[ N = \frac{\mu_{\text{net}} V}{2D} \]

Equation assumes only axial diffusion

(Einstein Eq. \( \sigma^2 = 2Dt \) and \( H = N/L = \sigma^2/L \) are used)

Note that there is no length dependence
Surfactants are polar/charged hydrophobic molecules that can aggregate into micelles.

Critical Micelle Concentration (CMC)

Example sodium dodecyl sulfate (SDS) C12 – SO₃⁻ (CMS ~ 8 mM, ~60 SDSs/micelle)

Micelles are dynamic entities and can be destroyed by organic solvents.

Figure 26-5: Electropherogram showing the separation of 30 anions. Capillary internal diameter: 50 μm (tissue silicon). Detection: indirect UV, 254 nm. Peaks: 1 = thiosulfate (4 ppm), 2 = bromide (4 ppm), 3 = chloride (2 ppm), 4 = sulfate (4 ppm), 5 = nitrite (4 ppm), 6 = nitrate (4 ppm), 7 = methanol (10 ppm), 8 = ethanol (4 ppm), 9 = triisopropylbenzene (10 ppm), 10 = sodium hydrogen phosphate (4 ppm), 11 = nitrate (4 ppm), 12 = citrate (2 ppm), 13 = fluoride (1 ppm), 14 = formate (5 ppm), 15 = phosphate (4 ppm), 16 = phosphoric acid (4 ppm), 17 = chlorite (4 ppm), 18 = galactose (5 ppm), 19 = carbonate (4 ppm), 20 = acetate (4 ppm), 21 = ceric sulfate (4 ppm), 22 = propionate (5 ppm), 23 = propylsulfate (4 ppm), 24 = benzoate (5 ppm), 25 = sodium dodecyl sulfate (4 ppm), 26 = valerate (2 ppm), 27 = benzoate (4 ppm), 28 = 3-glucuronic (5 ppm), 29 = pentane- sulfate (4 ppm), 30 = 4-glucuronic (5 ppm). (From W. M. Jones and P. Landis. J. Chromatogr. 1991, 546, 445. With permission.)
MECC for Separations of Neutrals

Ordered Mobilities

\[
\begin{align*}
\mu_{\text{obs}} &= \mu_{\text{micelle}} \\
\mu_{\text{obs}(N)} &= \mu_{\text{obs}(C)} \\
\mu_{\text{obs}(A)} &=
\end{align*}
\]

MECC for Separations of Neutrals

Separation

Use of SDS Surfactant for Separation of Neutral Fluorescently Derivatized Alkyl Amines

Elution Window (or Range) – \( t_0 \)-to- \( t_m \)
is very important
Cyclodextrin Characteristics

Macroyclic (small nanometer dimensions) sugar molecules with 6 (alpha), 7 (beta), or 8 (gamma) sugars in the structure. Apolar cavity forms inclusion complexes with analytes based on size, shape, and chemi-physical properties.

We have employed molecular mechanics, molecular dynamics, simulated annealing, and various visualization techniques to study and predict separation behavior.

The hydroxyl groups can be derivatized to influence inclusion or impart a charge for migration.

CDCE for Separations of Neutrals

Capillary Electrophoretic Separations of Neutrals by “CDCE”

Observed Mobilities

$\mu_{\text{Ref}} = \mu_{\text{CMCD}} \rightarrow$ depends on inclusion

$\mu_{\text{Ref}} = \mu_{\text{CD}}$

Wide windows are very desirable
CDCE for Separations of Neutrals

A goal was to create designer CD cocktails as Running buffer systems Using molecular modeling techniques

Figure 2. Separation of a mixture of n-alkylanthracene compounds by the CDCE technique. The running buffer contained a mixture of charged and neutral cycloextrins at low mM concentrations. Excellent resolution of positional isomers of these compounds is shown. Using cycloextrins as running buffer additives also permits chiral separations.

MolCAD and Molecular Mechanics & Dynamics Experiments Guide Creation of a New Reagents Cyclodextrin Reagents Investigated

MolCAD structures with electrostatic potential surfaces

Interaction energies from systematic grid search docking experiment

1,8-DNN:-19.9 kcal/mol  1,8-DNN:-31.3 kcal/mol

MolCAD structures & MM interaction energy values support efforts to synthesize new CD reagent
**Improved Separation Performance With HDMCM-β-CD (dihydroxynaphthalenes)**

- Small window → 1-CM-β-CD
- Poor inclusion → HDMS-β-CD
- Wide window →
  - Good inclusion → mixed CDs systems: important for rationally designed systems
  - HDMCM-β-CD with β-CD

**SSCE for Separations of DNA**

*CE of DNA in Soluble Polymer Matrix*

Polymers like high MW methyl cellulose added to RB at concentrations above the entanglement threshold.
SSCE for Separations of DNA

Complete Digestion of \( \phi X-174 \) DNA

• Super efficiency (why?)

• Example of uses:
  - Studies of genetic complexity
  - Studies of Protein–DNA interactions

• Notice increasing peak heights (why)

Methods of Detection in CE

• Absorbance
• Laser Induced Fluorescence (LIF)
• Surface Enhanced Raman Scattering (SERS)
• Others (electrochemical, conductivity, etc)
• Usually accomplished on-column by removal of a section of the polyamide coating on the capillary
LIF Detection of DNA

We have detected as little as $10^{-19}$ moles injected!

Figure 3.3: DNA-ethidium bromide (EB) interaction event (A) and related spectroscopy (B).

SFC Design
the problem: competing optical processes have cross-sections:
\[ \sigma_{\text{fluorescence}} \approx 10^{-19} \text{ cm}^2 \]
\[ \sigma_{\text{Ravleigh}} \approx 10^{-26} \text{ cm}^2 \]

\[ \sigma^R \text{ only} \approx 10^{-29} \text{ cm}^2 \]
Large EM Field Enhancements

Depends on polarizability and field

Chemical and EM models (SERS intensities are proportional to the 4th power of the field at the scattering molecule.)

Closely spaced metal nanospheres (or other shapes) can generate large enhancements of incident fields due to concerted plasmon field effects.

Potential “Hot Spot”

CE-SERS Detection Approaches

- Electro-filament onto moving substrate for off-capillary approach

- Ag colloid in RB for on-capillary approach

- Integrated µ-fluidic-SERS platforms molded from composites

- On Ag-colloid sprayed on frosted glass

- On Ag - pliable polymer composites
**SERS Spectra Obtained On-the Fly (On-Capillary)**

*CE separation of rhodamine6G and riboflavin*

**LIF detection**  
**SERS detection**

**Conditions:**
- Dilor spectrometer;
- 75 µm capillary;
- ~15 mW at 514 nm;
- 0.5 w/v% Ag;
- 1 sec acquisition

The high S/N spectrum of Rh6G obtained from less than 10⁻¹⁶ moles injected!

---

**CE-SERS of With EF Transfer and Ag Colloid on Frosted Glass Slide (Off-Capillary)**

*CE separation of riboflavin and resorufin*

**LIF detection**  
**SERS detection**

finding bands  
actual spectra

resorufin  
riboflavin

1 2 3  
Time, min.