Chapter 26 Separation Methods

Problems: 1-10, 12-15, 20

This chapter a general overview, then next week GC and following week HPLC

Chromatography - powerful separation method
term coined by Biologist Mikhail Tswett early 1900's
using various techniques to separate plant pigments
would see various colored bands, hence the term
Chroma - color
Graphein - to write

Field has grown immensely in past 50 years
Lots of new materials
Lots of new needs
At least 12 Nobel prizes between 1937 and 1972 had vital links to chromatography

26A General Description
Diverse methods use to separate closely related compounds in a mixture
Usually a mobile phase may be a gas, liquid or supercritical fluid
mobile phase is then forced through an immiscible stationary phase
- fixed in a column or on a surface
Two phases chosen so components in sample distribute between the phases to varying degrees
Compounds strongly attracted to mobile phase move quickly in the mobile phase, while other compounds that are more attracted to the stationary phase move more slowly
Components usually end up in discrete Bands or zones that can then be isolated and characterized

26A-1 Classification of Chromatographic Methods
Table 26-1
Two different ways to catagorize
1. Based on physical way mobile and stationary phases are brought together
   A. Column Chromatography - stationary phase in narrow tube and mobile phase is forced through under pressure
   B. Planar Chromatography - stationary phase is a flat surface, mobile phase moves through it by capillary action or gravity

Equilibria for both methods are closely related so if you understand one, you understand the other
2. Based on types of mobile and/or liquid phase
   A. Gas Chromatography - Gas mobile phase
   B. Liquid Chromatography - Liquid mobile phase
   C. Supercritical-fluid Chromatography - supercritical fluid mobile phase

1 & 3 are column methods
2 can be column or planar

26A-2 Elution on Columns
Figure 26-1
Apply 2 substances A and B to column at time $t_0$
Wash continuously with fresh mobile phase
- When applied, sample distribute between solid and liquid bases on attraction to each (partitioning)
- Application of fresh mobile phase (eluant) forces solvent containing sample to move down column
- Now sample redistributes itself
- Application of more solvent continues movement

- net effect a series of partitioning events between stationary and mobile phase
- Average rate that a solute zone moves depends on fraction of time it stays in mobile phase
- Differences in partition coefficient between phases results in different average speeds, results in peaks coming off column at different times and being separated from each other

Analyte Dilution
Example shows one important characteristic
Analytes are almost always diluted

Usually apply sample in a small, concentrated volume
When get to end components are separated so that makes it more dilute, + usually mixed with additional solvent so this makes it even more dilute

Net result, detectors have to be more sensitive

Chromatograms
Typically place a detector at end of column
Then plot detector response vs time (or eluant volume)
This is called a chromatogram
Typical chromatogram figure 26-2
Position of peak can be used to identify peak  
Area under peak can be used to quantitate amount of material

**Effects of Migration Rates and Zone Broadening on resolution**  
Figure 26-2 compares 2 materials  
In 1 case eluted quickly because column is short  
In 2\textsuperscript{nd} case eluted more slowly because column is longer  
B is retained by stationary phase so is always slower

Note that when separation takes more time, generally get more distance between the peaks, but peaks become broader and more dilute. This is called *zone broadening*

Resolution refers to cleanly separating one component from another  
Resolution will depend BOTH on distance between two peaks and zone broadening of the peak  
**Figure 26-3**  
Can achieve resolution by either increasing distance between peaks or reducing broadening around a peak  

Conversely can lose resolution by losing distance between peaks or by increase zone broadening

Hence need to understand how these factors arise

**26B Migration Rates of Solutes**  
Lets start with how fast a component moves down the column  
As you have seen this depends on how a component partitions (or distributes) between stationary and mobile phase

**26B-1 Distribution Constant**  
Often can describe chromatography with straightforward equilibrium equations

\[ A_{\text{mobile}} = A_{\text{stationary}} \]

Distribution constant or partition ratio, \( K = \frac{[S]}{[M]} \)

Ideally \( K \) is independent of solute concentration, so the distribution is the same no matter what the concentration is, but this is not always true

When \( K = \frac{[S]}{[M]} \) works, this is termed *linear Chromatography*  
And peaks are nice Gaussian shapes
And retention times independent of [ ]
While this isn’t always the case in practice, we will develop our theory under these conditions

26B-2 Retention Time
Figure 26-4 typical chromatogram for a 1 component sample
Time from injection to component reaching detector called *retention time* $t_R$

Peak at left is from a species that is not retained by column
Samples often contain these components
$t_m$, time for unretained component to reach detector
Called the *dead time* or *dead volume*
Simply related to motion of mobile phase itself

Average linear rate of solute migration $\bar{v}$

$$\bar{v} = \frac{L}{t_R}$$

Where $L$ is length of column packing

Average linear rate of solvent is $u$

$$u = \frac{L}{t_m}$$

26B-3 Relationship between Retention time and Distribution Constant
Let’s start by expressing the migration rate as a fraction of velocity of the mobile phase

$$\bar{v} = u \times \text{fraction of time in mobile phase}$$

But fraction of time in mobile phase is = moles of solute in mobile phase/total number of solute molecules

$$\bar{v} = u \times \frac{\text{moles of solute in mobile phase}}{\text{total moles of solute}}$$

$$\bar{v} = u \times \frac{c_m V_m}{c_m V_m + c_s V_s}$$

Total # of moles = molarity x volume so:

$$\bar{v} = u \times \frac{1}{1 + c_s V_s / c_m V_m}$$
And $C_u/C_M = K$ so

$$= u \times \frac{1}{1 + K(V_s/V_M)}$$

and you can estimate the $V$'s from how the column was made.

**26B-4 Rate of Solute Migration: the Retention Factor**

$K' = \text{retention factor or capacity factor}$

widespreadly used parameter to describe migration of solutes on a column

For component $A$

$$k'_A = \frac{K_A V_s}{V_M}$$

You can see how this fits into the velocity equation we are developing

$$\bar{v} = u \times \frac{1}{1 + k'_A}$$

Now let's substitute in the definitions of $\bar{v}$ and $u$

$$\frac{L}{t_R} = \frac{L}{t_s} \times \frac{1}{1 + k'}$$

Now let's rearrange to get $k'$ alone

$$k'_A = \frac{t_R - t_M}{t_M}$$

And now you can see that you can obtain $k'$ by simple measurements off the chromatogram

If $k'$ is $<1$ then comes off so fast that hard to measure properly
If $K > 20$ or 30, elution so long that other problems tend to mess up separation
Bottom line want $k'$ to be between 2 and 10

**26B-5 Relative migration rates: Selectivity factor**
Selectivity factor (α) for two species on a column
\[ \alpha = \frac{K_B}{K_A} \]
where B is most strongly retained
A is least strongly retained
So as defined \( \alpha > 1 \)

Doing some substitutions you can find that
\[ \alpha = \frac{k'_B}{k'_A} \]
also

Then substituting this back in to the last equation in the last section
\[ \alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} \]

And we will use this equation later when we talk about resolving peaks

26C Zone Broadening and Column Efficiency
How broad a peak gets can be modeled using the rate or the kinetic theory of Chromatography. This theory, based on random motion is too complex for this level, so will just present the results and what they mean

26C-1 Shapes of peaks
Get Gaussian peak shape
This implies that position of a particular molecule in the distribution is random
Essentially this is because each molecule has a different velocity through the column, and the distribution of these velocities is random

Zones get larger as you go down the column because the longer time the material sits on the column, the more the molecule randomly diffuses away

26C-2 Methods for describing Column efficiency
2 measures widely used,
Plate Height (H)
Plate count N
\[ N = \frac{L}{H} \]
Where L = length of column (usually in cm)

A wide variety in these numbers as you compare different methods and medium
Terms plate height and number of theoretical plates originated with Martin and Synge in 1941. Their work and nomenclature was based on a model of equilibria between mobile and stationary phases. New theory is better at describing what is going on, but still use the older nomenclature. This leads to definitions that don’t seem to have any resemblance to the terms.

The Definition of Plate Height
Zone look like a Gaussian distribution
Characterize width of a Gaussian with the variance $\sigma^2$

Plate height is related to how broad something is relative to the elution time:

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

See figure 26-5

Experimental values of H and N

Figure 26-6
Measure $X$ in time
Designate $\tau^2$ to distinguish from $\sigma^2$ (measured in cm$^2$)

These two parameters can be related

$$T = \sigma / (L/t_r)$$

$L/t_r$ is linear velocity of solute in cm/sec)

Use this figure to approximate these values from chromatogram
Use tangents at inflection points to make a triangle
Area of triangle is 96% of area under gaussian
So close match with 2 $\sigma$ units
Therefore intercepts occur at about +/-2 $\tau$

$W = \text{magnitude of base of triangle}$
$W = 4\tau$

Substituting into previous equation and rearranging

$$\sigma = KW / 4t_R$$

Further

$$H = LW^2 / 16t^2_R$$
And \( N = 16(t/W)^2 \)

So get \( N \) from 2 time measurements, \( t_1 \) and \( W \)
Only need \( L \), length of column packing to get it all

Alternate equation for \( N \) based on \( W_{1/2} \) width at \( 1/2 \) height
Thought by some to be more reliable
\[ N = 5.54(t_{1/2}/W_{1/2})^2 \]

\( N \) and \( H \) are widely used to evaluate instruments, columns, etc. For them to be a meaningful comparison need to be determined for the same compound

26C-3 Kinetic Variable Affecting Zone Broadening
Zone broadening due to rate at which mass moves in transfer process
Table 26-2 list the most important parameters that can affect zone broadening

Mobile phase flow rate
How does \( H \) change with speed of stationary phase?

- Figure 26-7
- Minimum of \( H \) (a maximum of efficiency) at low flow rates
  - Note flow rate in HPLC is << less than GC
- Plot is called a van Deemter Plot after originator of theory

Note since optimum flow rate is much slower for liquid than for gas, GC is usually much faster than HPLC

Plate height (\( H \)) about 10X for GC
This means that for the same separation an HPLC column can be 10x shorter!

- Somewhat deceiving. Liquids columns can only be 25-50 cm before pressure is so large can push anything through. GC columns can be 50m or more in length, so can make very long to match HPLC efficiency

Plate Height and Column Variable
Lots of different theories and models
One that has worked the best was done by van Deemter in 1950's

VanDeemter equation
\[ H = A + B/\mu + C\mu \]
\[= A + B/\mu + (C_s + C_m)\mu\]

A coefficient for multiple flow path
B coefficient for longitudinal diffusion
C coefficient for mass transfer between phases
   Can be broken down into 1 # for each phase

Fancier equation have been created since, but this equation works quite well

Note that it has terms that are independent of flow, directly proportional to flow and inversely proportional to flow

Terms summarized in table 26-3, use figure 26-9 to follow

**Multipath term (A)**
As shown in figure 26-8 molecules have a variety of paths they can take

These different paths are one of the reasons that there is a spread in the time that molecules get to the end of the column

This is directly proportional to diameter of particles

So if have small particles can make this term small

Also if slow flow, then molecular diffusion dominates so this term is also small at low flow rates

**Longitudinal Diffusion term (B/\mu)**
Solutes naturally diffuse from high conc to low conc
So the faster we get the matter through the column, the less broadening due to diffusion, and as it slow down the broadening can get enormous

Diffusion slower in liquids so not as much of a problem in HPLC

**Mass-Transfer Coefficient (C_s and C_m)**
While theory is based on equilibrium conditions, since the mobile phase is moving don’t really have equilibrium conditions!

It takes a finite time for a molecule to bind into the stationary
phase and to unbinds back into the mobile phase

The slower the flow, the better the equilibrium and broadening slows, the faster the flow rate, the worse the equilibrium and the worse the broadening

Let’s skip the rest and cut to the chase

**Summary of Methods for reducing Zone Broadening**

From above theory find 2 major factors that affect column efficiency, diameter of particles making the column and diameter of column itself

See figure 26-10

- Need narrower columns
- Make particle size smaller
- If liquids adsorbed to particle, make its thickness as small as possible
- Lower T of GC to lower diffusion

**26D Optimization of Column Performance**

Vary experimental conditions until get all components cleanly separated in minimum time. Will try to minimize broadening to make peaks narrower (section C) and alter retention rates of components (Section B) for optimum resolution

**26D-1 Column Resolution**

Figure 26-11

\[ R_s = \frac{\Delta Z}{W_a / 2 + W_B / 2} = \frac{2 \Delta Z}{W_A + W_B} \]

\[ = \frac{2(t_r)_b - (t_r)_a}{W_A + W_B} \]

Resolution \(>= 1.5\) give complete resolution

To increase resolution need more plates
easiest way is to make column longer
However this increases run time so not always the best choice

**26D-2 Effect of Retention and Selectivity on Resolution**

Getting an equation that relates resolution, retention time, selectivity, and plates on a column (Will use in section D-4 just showing where these came from)

Have 2 compounds in about equal amounts so
ie \( W_A \approx W_B = W \)

Resolution equation becomes

\[
R = \frac{\left( t_{R_B} - t_{R_A} \right)}{W}
\]

Now go back to previous section and get equation for \( W \) in terms of \( t_r \) and \( N \)

\[
R = \frac{\left( t_{R_B} - t_{R_A} \right)}{\left( t_{R_B} \right)} \times \frac{q}{4}
\]

Now substitute retention factors for \( t_r \)'s

\[
R = \left( k_A' - k_B' \right) / (1+k_B') \times \sqrt{N/4}
\]

Eliminate \( k_A' \) with \( \alpha = k_B'/k_A' \) and rearrange

\[
R = \sqrt{N/4}[[\alpha-1]/\alpha][k_B'/(1+k_B')]
\]

And if we want to isolate \( N \), so we know the number of plates we need for a resolution then:

\[
N = 16R^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k_B'}{k_B'} \right)^2
\]

When A and B have similar retention times so come off close together simplify equations to:

\[
R = \sqrt{N/4}[\alpha-1][k'/(1+k')]
\]

\[
N = 16R^2(1/(\alpha-1))^2((1+k')/k')^2
\]

**26D3 Effect of Resolution on retention time**

Need one more equation, relating retention time to these parameters

\[
\bar{\tau}_B = L/(t_r_B)
\]

\[
\bar{\tau} = u \times 1/(1+k') \quad N = L/H
\]

\[
(t_r_B) = [NH(1+k')]/u
\]

where \( t_r \) is time required to bring component b to the end of the column at flow rate \( u \)

Combine this with our equation for \( N \) based on \( R \)
26D-4 Variable that Affect Column Performance

Now let’s see what this last equation and the \( R \) equation tell us.

All equations have 3 major terms, each is independent for the others:

1st depends on \( \sqrt{n} \) or \( H/u \)
- Change \( N \) by changing length of column
- Change \( u \) by changing column flow

\[
(t_R)_B = 16R_s^2 \frac{H}{u} \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + k'_B}{k'_B} \right)^2
\]
flow rate, particle size, thickness of packing film
2nd depends on \( \alpha \)
- Fixed by relative interactions of 2 solutes or 2 distribution coefficient
- Changed by changing composition of mobile phase or \( t \)

3rd depends on \( k' \)
- Fixed by relative interaction between mobile and stationary phases
- Also changed by changing composition of mobile phase or \( t \)

Variation in \( N \)
- Easiest to change, get a longer column
- Costs more time
- May cost in purchase of a new column
- Get same effect by reduction in \( H \)

Variation in \( H \)
- If you can reduce plate height it is the same as increasing \( N \)
- Already saw that ways to reduce \( H \) are: ???
  - Reduce particle size
  - Reduce thickness of coating on particles
  - Reduce column diameter
  - Reduce solvent viscosity
  - Etc

Variation in Retention Factor
- Increase \( k'_B \) so slow peak is retained longer
- Generally increase resolution, but again make run longer

Optimum range of values for \( k' \) found my re-writing equation

\[ R = Qk'(1 + k') \]
\[ t_w = q'(1 + k')^3/(k')^3 \]

\( Q \) and \( q' \) are constants containing all the other terms we don’t want to vary.
Plot (figure 26-12)
If \( k' > 10 \) elution time goes up, but little increase in resolution is seen
\( k'' = 2 \) is about as small as you can go
typically want in 1-5 range

Change \( k' \) in GC by increasing \( T \)
Change \( k' \) in HPLC by changing solvent composition
Illustration of changing \( k' \) with solvent shown in figure 26-13
best condition is 2, get resolution with minimum time

**Variation in Selectivity Factor**
as \( \alpha \) approaches 1 thing get sticky
Need to increase \( \alpha \) but keep \( k' \) in 1-10 range

Several things to try (in order)
- Change solvent composition
- Change column temp
- Change stationary phase
- Use special chemical effects

If separation involves weak acids or bases changing solvent composition
includes changing pH

**26D-5 The general Elution Problem**
figure 26-14
with 1 \( k' \) can never get all 3 pairs of components reasonably resolved
this is called the General Elution Problem

Fix
- Change \( k' \) in the middle of the run
- In HPLC change solvent mixture
- In GC change column temp
- Either in step or in gradient

**26E Summary of Important Relationships**
Table 26-4 and 26-5 Summarize terms and equations needed in next 3 chapters

**26F Applications**

**26F-1 Qualitative Analysis**
Compared to IR or NMR not much information, just a single retention time under
a single set of conditions

In a well researched system can confirm the absence or presence of a
component, (but really doesn’t prove its there, it could be something else with an
identical retention time

26F-2 Quantitative analysis
highly quantitative relying on peak height or area

Analyses based on peak height
make a straight line from start to end of peak base
Measure to top of peak

Peak heights inversely proportional to width
so width must be constant or at least same for sample and standard

Other problems
overloading can cause changes in peak shape that will affect the height
Manual sample injection introduce 5-10%

Analyses based on peak areas
makes analysis independent of peak broadening artifacts
harder to do manually, but built in to computer in most modern instruments
if you have to do it manually this introduces a 2-5% error

Calibration and Standards
usually have a set of standard with about the same concentration as sample
plot volume (or height) vs concentration for a calibration curve

Biggest problem is reproducibility of injection
Even worse in GC, injection of 1 ul, + evaporation of sample in heated injection port

Internal Standard Method
because of injection errors, best method is internal standard
comes off close to sample
but where nothing else can interfere
look at relative areas of sample and reference independent of injection volume
can reduce precision to <1% error

Area Normalization Method
look at total volume of all the peaks, and then use the % of total for your analysis. Pretty hard to do properly so usually only applied to special systems