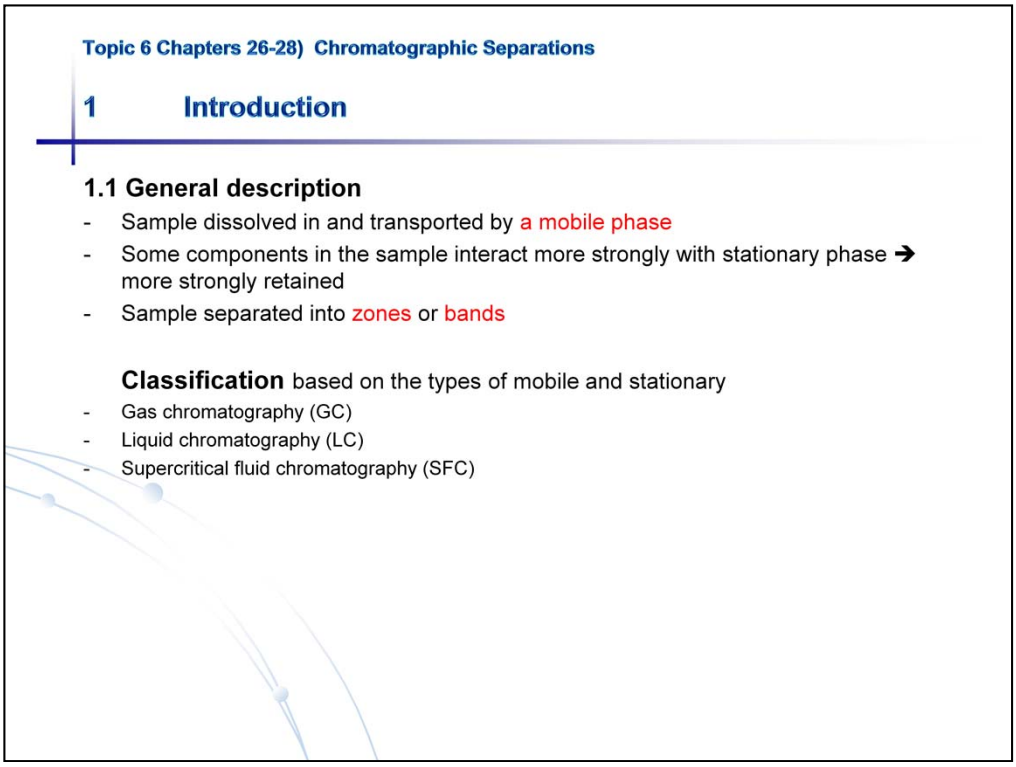


Many determinations start with separation, followed by analysis using spectrophotometers or mass spectrometers.

In short, in chromatography:

1. Sample dissolved in and transported by a mobile phase. Mobile phase can be gas or liquid, and it is forced through an immiscible stationary phase.

2. Some components in sample interact more strongly with stationary phase and are therefore more strongly retained. The interaction between components and stationary phase can be electrostatic or van der Waal forces.

3. As a consequence, the sample separated into zones or bands. This method was originally developed for separating pigments, so separated species appear as different colored bands.

TABLE 26-1 Classification of Column Chromatographic Methods

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

There are several ways to characterize chromatographic methods. A more fundamental classification is based on the types of mobile and stationary phases.

1. Gas chromatography (GC)

2. Liquid chromatography (LC)

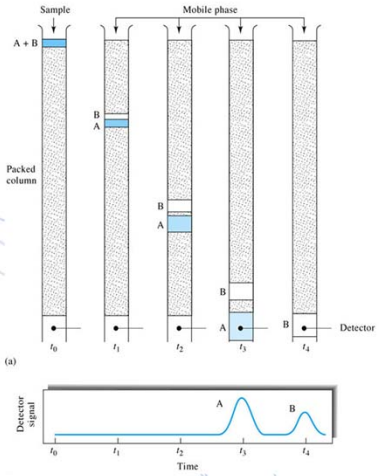
3. Supercritical fluid chromatography (SFC)

As the names imply, their mobile phases are gas, liquid, and supercritical fluid, respectively. The stationary phase could be liquid absorbed or bonded to a solid surface, or a solid.

All these are indicated in Table 1.

1.2 Elution chromatography:

Flush sample through column by continual mobile phase addition



- only eluent (portion of sample in mobile phase) moves down

- migration rate $\propto$ fraction of time in mobile phase

Fig. 26-1 (p764) (a) The separation of a mixture of components A and B by column elution chromatography, (b) the output of the signal detector

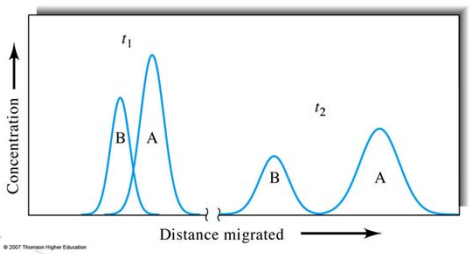
Let's look at how chromatography works.

Assuming the sample contains a mixture of two components A and B. The sample is flushed through a column by continuously adding mobile phase.

1. When first introduction of fresh mobile phase, the eluent moves down (the portion of sample contained in mobile phase). In the meantime, the eluent partitions between fresh mobile phase and stationary phase.

2. The point is only the part of solute contained in the mobile phase can move down, but not the part retained in the stationary phase. As a result, the migration rate of solute zone depends on the fraction of time it spends in mobile phase.

3. Consequently, if a solute is strongly retained in the stationary phase, it will take more time to get to the bottom of the column. In other words, different components get to the bottom of the column at different time. If a detector is located at the bottom of the column and detect the signal as a function of time, it will observe two signals.



- Chromatogram (concentration vs. elution time)

- More strongly retained species elutes last (elution order)

- Analyte is diluted during elution (dispersion)

- Zone broadening proportional to elution time

Fig. 26-2 (p765) Concentration profiles of solute bands A and B at two different times in their migration down the column

There are several important concepts about the chromatogram

1. concentration versus elution time
2. In this figure, more strongly retained specie B elutes last.
3. Analyte is diluted during elution.
4. Related to this, as the analyte migrates over the distance, the zone becomes broaden, this of course lowers the separation efficiency.

- Adjust migration rates for A and B (increase band separation)
- Adjust zone broadening (decrease band spread)

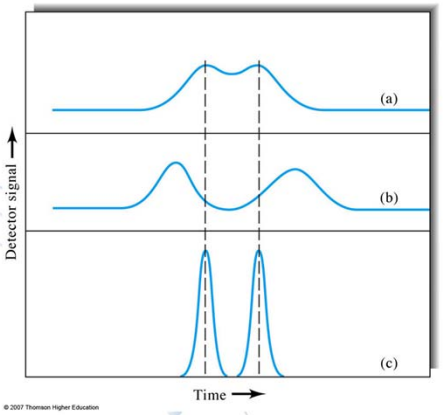
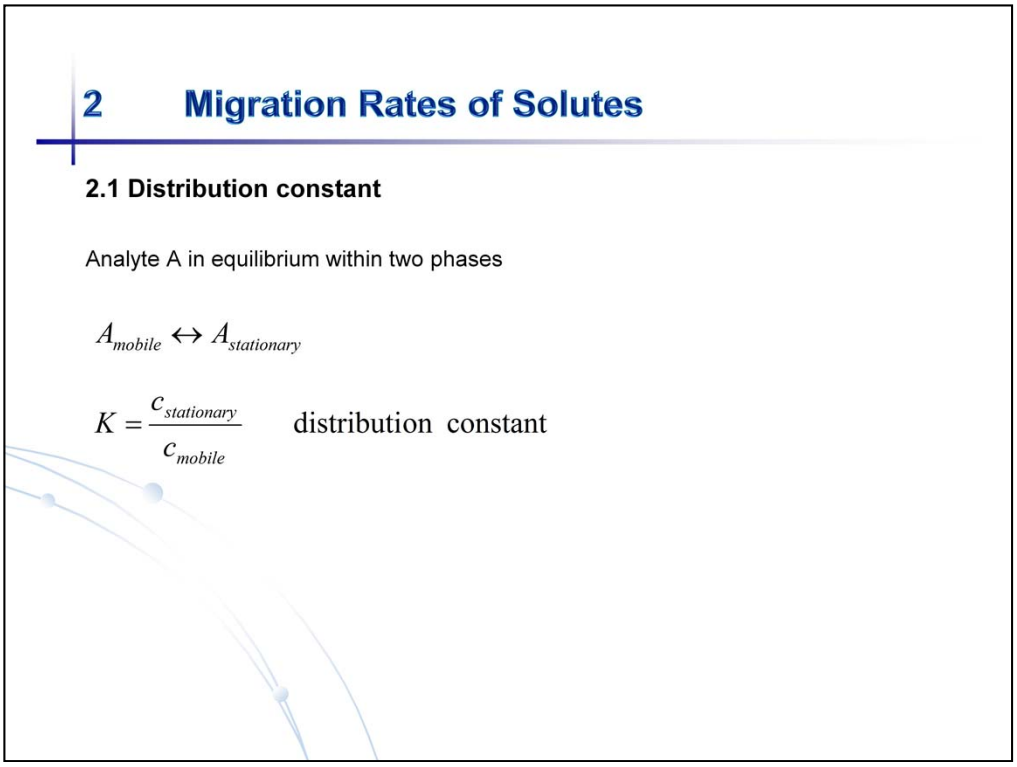


Fig. 26-3 (p765) Two-component chromatogram illustrating two methods for improving overlapping peaks

In order to increase separation efficiency, experimental conditions can be changed, so **non-separating bands be separated**

There are two method.

1. {see slide}
2. {see slide}



Let's talk about the first method, adjusting the migration rates of solutes.

Analyte A is in equilibrium with two phases.

The equilibrium *K* is equal to $c_{stationary}/c_{mobile}$, where $c_{stationary}$ is the concentration in the stationary phase and c_{mobile} is the concentration in the mobile phase. *K* is also called distribution constant or partition coefficient.

K is the fundamental quantity affecting distribution of components between two phases, but how can we calculate its value?

2.2 Retention time

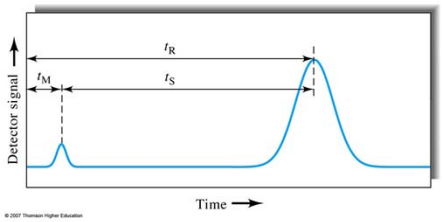


Fig. 26-4 (p767) A typical chromatography for a two-component mixture

t_M time for unretained species (dead time), same rate as mobile phase molecules average migration rate

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

t_R retention time for retained species average migration rate

$$\bar{v} = \frac{L}{t_R} \quad \text{L: column length}$$

Ideally: t_R independent of volume injected, produces a Gaussian peak

This is a typical chromatogram consisting of two peaks.

1. The small peak represents a solute that is not retained on the column and so reaches the detector almost immediately after elution begins. Thus, its retention time t_M is approximately equal to the time required for a molecule of the mobile phase to pass through the column.
2. The large peak on the right is that of an analyte species. The time required for this zone to reach the detector after sample injection is called retention time t_R.
3. {see slide}

From this two time, we can calculate the

1) Average linear rate of solute migration through the column
2) Average linear rate of mobile phase molecules through the column

2.3 Relationship between t_R and K

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

$$= u \times \frac{\text{moles of A in mobile phase}}{total \text{ mols of A}}$$

$$= \mathrm{u} \times \frac{\mathrm{c_M} V_M}{c_M V_M + c_S V_S}$$

$$= \mathrm{u} \times \frac{1}{1 + \mathrm{c_S} V_S / c_M V_M}$$

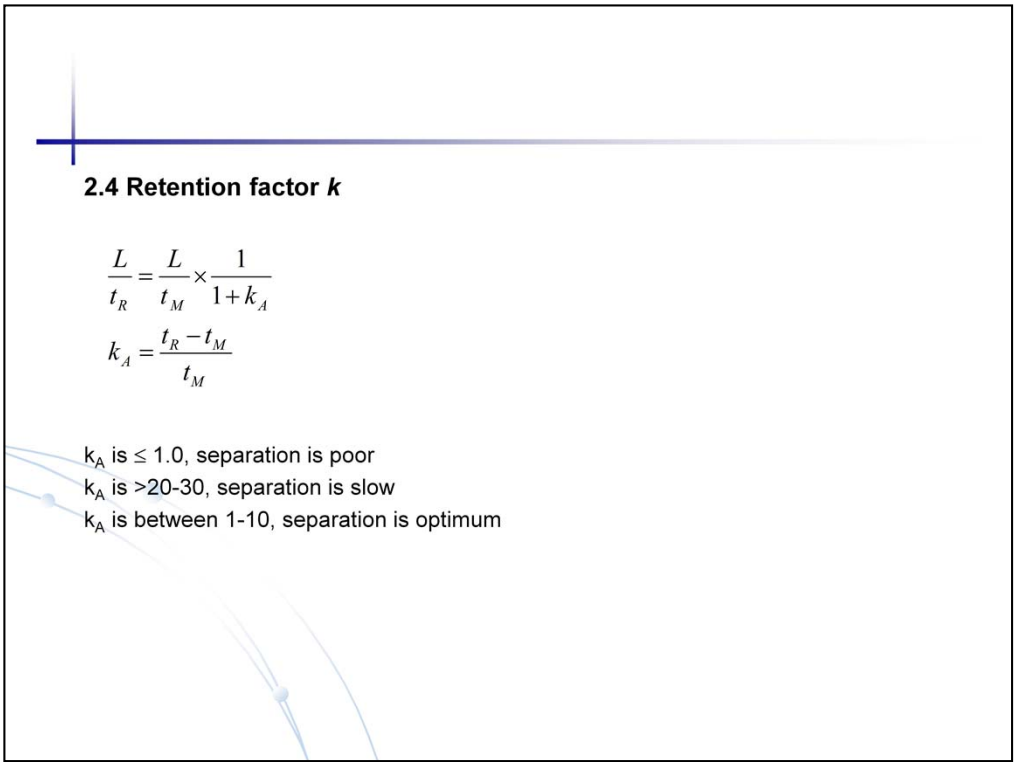
$$= \mathrm{u} \times \frac{1}{1 + \mathrm{K_A} (V_S / V_M)}$$

V_S / V_M : *estimeated* from column packing

$K_A V_S / V_M$: retention factor $\mathrm{k_A}$

$$= u \times \frac{1}{1 + k_A}$$

We cannot directly calculate this from the concentration, but we can use an alternative method through the retention time. Let's figure out the relationship between t_R and K.



A bit more about the parameter retention factor *k*:

How is k_A related to t_R and t_M?

Rewrite the previous equation and get equations shown above.

Dependence of separation efficiency on values of *k*

2.5 Relative migration rate: Selectivity factor (α)

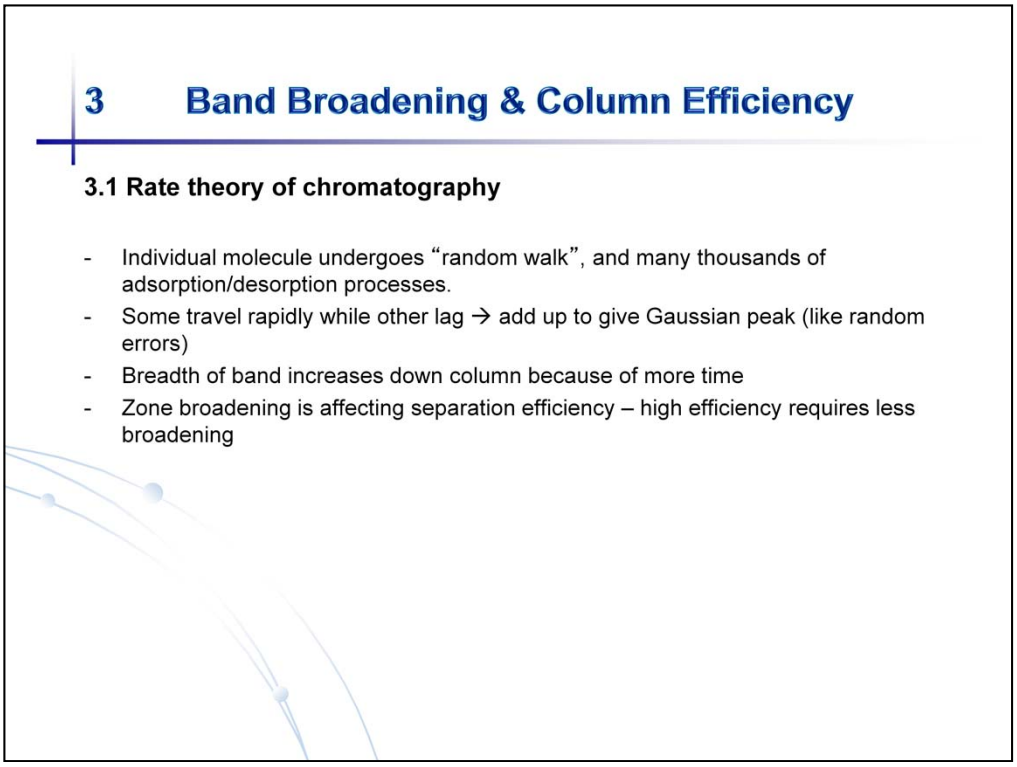
Selectivity factor $a = \frac{K_B}{K_A}$ distribution constants

$= \frac{k_B}{k_A}$ retention factors

$= \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$ retention times

Larger α = better separation

How to compare elution of two components A and B?



The rate theory describes the shape of elution bands.

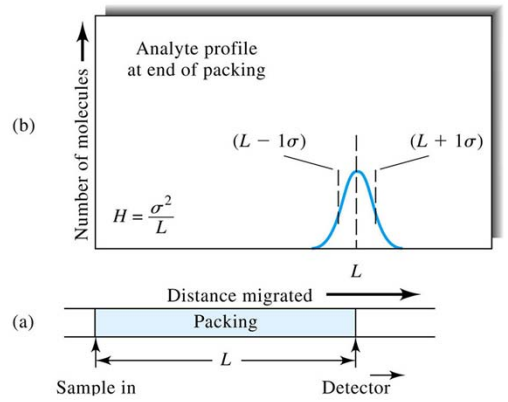
A good separation desires a narrow band.

3.2 Column efficiency

$$N = \frac{L}{H}$$

N: number of plates
L: length of column
H: height of 1 theoretical plate

Plates are only theoretical –
column efficiency increase with N



Efficient column has small plate height – less zone broadening

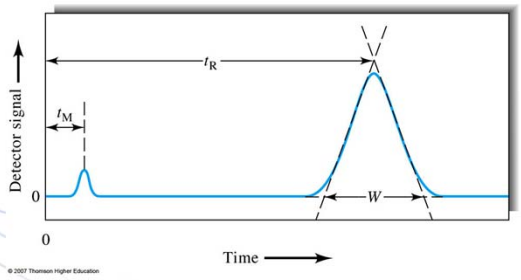
Fig. 26-6 (p770) Definition of H

The previous slide is a qualitative description of column efficiency. The column efficiency can also be quantitatively described using the plate height and number of plates as in distillation column. However, these are theoretical only.

Look at this figure, the elution band has a Gaussian shape with deviation of σ.

In (a) the column length is shown as the distance from the sample entrance point to the detector. In (b) the Gaussian distribution of sample molecules is shown.

Experimentally, H and N can be approximated from the width of the base of chromatographic peak.



$$N = 16\left(\frac{t_R}{W}\right)^2$$

Fig. 26-7 (p770) Determination of N

Tangents at the inflection points at the two sides of the peak, then extend to form a triangle with the baseline.

3.3 Kinetic variables affecting column efficiency (H)

Mobile phase velocity

- Higher mobile phase velocity, less time on column, less zone broadening
- However, plate height H also changes with flow rate

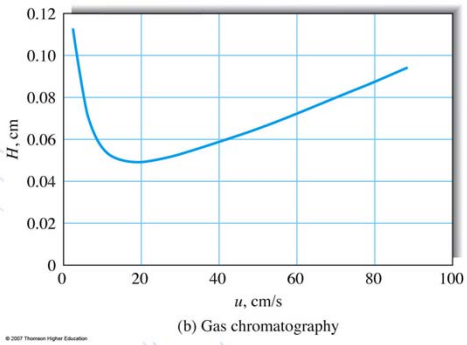


Fig. 26-7 (p770) Effect of mobile-phase flow on plate height for GC

van Deemter Equation

$$H = A + \frac{B}{u} + C_s u + C_M u$$

A: multipath term

- Molecules move through different paths
- Larger difference in path length for larger particles
- At low flow rates, diffusion allows particles to switch between paths quickly and reduces variation in transit time

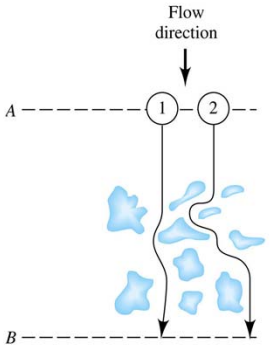


Fig. 26-9 (p773) Typical pathways of two molecules during elution

In the figure, the distance traveled by molecule 2 is greater than that traveled by molecule 1. Thus, molecule 2 will arrive at place B later than molecule 1.

At a low flow rate, because of diffusion, each molecule will approach the average.

At a high flow rate, the time is not sufficient for diffusion averaging to occur, so we will see broadening.

B/μ: Longitudinal diffusion term

- Diffusion from central zone to front and tail
- Proportional to analyte diffusion coefficient
- Inversely proportional to flow rate
- high flow, less time for diffusion

Cμ: Mass transfer coefficients (C_S and C_M)

- C_S is rate for adsorption onto stationary phase
- C_M is rate for analyte to desorb from stationary phase
- Effect proportional to flow rate – at high flow rates less time to approach equilibrium

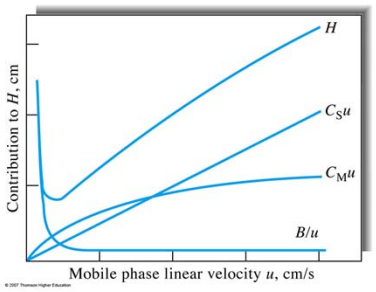


Fig. 26-10 (p774) van Deemter plot

Contribution of various mass-transfer terms to plate height.

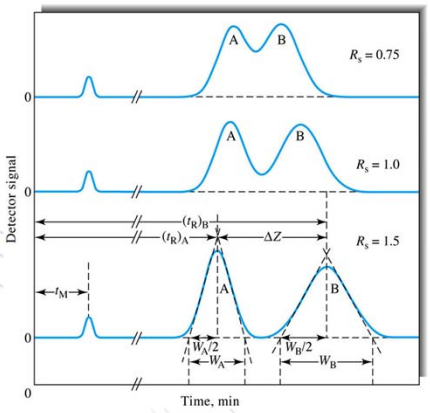
B/μ is associated with longitudinal diffusion.

In theory, an equilibrium needs to be established between mobile and stationary phases. In practice, the elution time is short and the column is often operated under non-equilibrium conditions. Some solute molecules are swept ahead before they have time to equilibrate with the stationary phase, while others are left behind in the stationary phase by the fast-moving mobile phase.

4 Optimization of Column Performance

(cf. Tables 26-4 & 26-5 for summary)

Column resolution $R_s = \dfrac{2\Delta Z}{W_A + W_B} = \dfrac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$



- u (linear flow rate): low flow rate favors increased resolution (van Deemter plot)

-H (plate height) (or N number of plates): use smaller particles, lengthen column, reduce viscosity of mobile phase (diffusion)

- α (selectivity factor): vary temperature, composition of column/mobile phase

- k_A (retention factor): vary temperature, composition of column/mobile phase

Fig. 26-12 (p776) Separation at three resolution values

The column resolution for two bands is defined by the separation between two bands relative to their widths.

The figure shows that the best separation is achieved when R = 1.5. At this value we believe the separation is essentially complete.

There are a lot of factors affecting the column resolution,

1. The solvent average linear flow rate u.{see slide}
2. Reducing plate height H or increasing the number of plates N. This can be achieved by using small particles of the packing, reduce the viscosity of the mobile phase (longitudinal diffusion term).
3. Selectivity factor α, the value of selectivity factor can be increased by varying temperature, changing the composition of column and mobile phase.
4. Finally, improve the retention factor *k* by varying temperature and column/mobile phase compositions. But KEEP in MIND the optimum value of *k* is between 1-10.

Effect of *k* and α on R

When A and B have similar retention

$$W_A = W_B \approx W$$

$$R_s = \frac{(t_R)_B - (t_R)_A}{W}$$

$$[N = 16(\frac{(t_R)_B}{W})^2]$$

$$R_s = \frac{(t_R)_B - (t_R)_A}{(t_R)_B} \times \frac{\sqrt{N}}{4}$$

$$[k = \frac{t_R - t_M}{t_M}]$$

$$R_s = \frac{k_B - k_A}{1 + k_B} \times \frac{\sqrt{N}}{4}$$

$$[a = k_B / k_A]$$

$$R_s = \frac{\sqrt{N}}{4}(\frac{a-1}{a})(\frac{k_B}{1+k_B}) \cong \frac{\sqrt{N}}{4}(\frac{a-1}{a})(\frac{k}{1+k}) \quad \mathrm{k} = \frac{\mathrm{k_A} + k_B}{2}$$

$$N = 16R_s^2(\frac{a}{a-1})^2(\frac{1+k_B}{k_B})^2 \cong 16R_s^2(\frac{a}{a-1})^2(\frac{1+k}{k})^2$$

Effect of *R* on t

$$(t_R)_B = \frac{L}{\overline{\overline{v_B}}}$$

$$[k_B = \frac{(t_R)_B - t_M}{t_M} = \frac{\frac{L}{\overline{\overline{v_B}}} - \frac{L}{\overline{u}}}{\frac{L}{\overline{u}}}, \overline{\overline{v_B}} = u \times \frac{1}{1+k_B}]$$

$$(t_R)_B = \frac{L(1+k_B)}{u} = \frac{NH(1+k_B)}{u}$$

$$= \frac{16R_s^2 H}{u}(\frac{\alpha}{\alpha - 1})^2 \frac{(1+k_B)^3}{k_B^2}$$

General elution problem: for multiple components, conditions rarely optimum for all components.

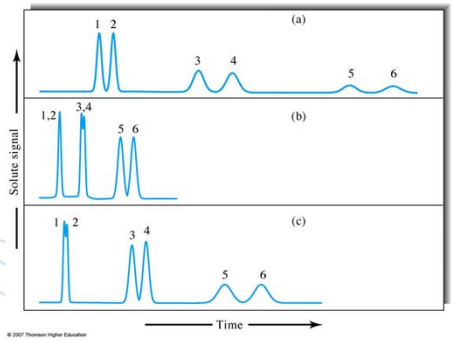


Fig. 26-15 (p780) The general elution problem in chromatography

1. Change liquid mobile phase composition – gradient elution or **solvent programming**
2. Change temperature for gas chromatography – **temperature programming**

One general elution problem we will frequently encounter is for multiple components.

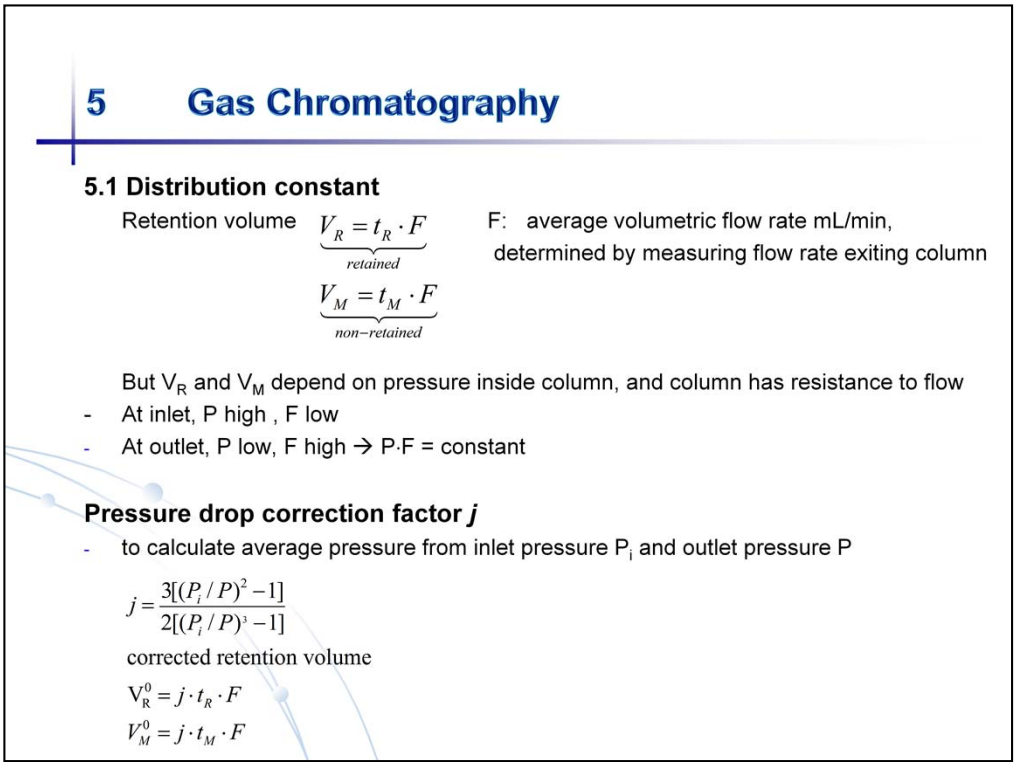
As shown in these hypothetical chromatograms, resolving of first two eluting peaks result in long retention time for the following peaks. Acceptable t_R's for last eluting peaks results in poor resolution of first eluting peaks.

Solutions?

Change column conditions during elution.

1. Gradient elusion for HPLC

2. Temperature programming for GC



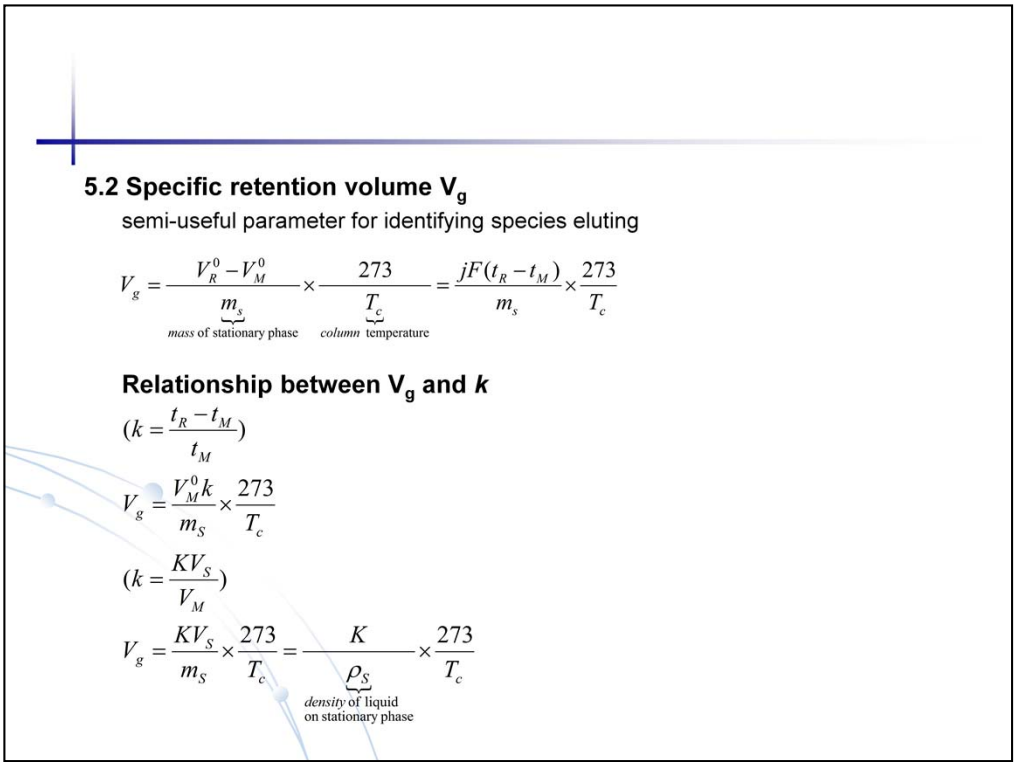
There are two types of GC.

1. Gas-solid chromatography, where the stationary phase is solid. The application of gas-solid chromatography is limited because the semipermanent retention of polar molecules in solid stationary phase.
2. A more popular GC is gas-liquid chromatography, where the stationary phase is liquid phase immobilized on the surface of inert solid packing or walls of a capillary tube.

All the general principles and equations we have discussed can be applied to GC, with some minor modifications because of gaseous mobile phase.

In GC we use retention volume rather than retention time, {see eqs on slide} where F is the average volumetric flow arte ml/min.

F can be determined by measuring flow rate exiting the column using soap bubble meter (caution: some gases dissolving in soap solution).



In GS we use specific retention volume V_g as a semi-useful parameter for identifying species eluting.

Rationalization: …

5.3 GC Instrumentation

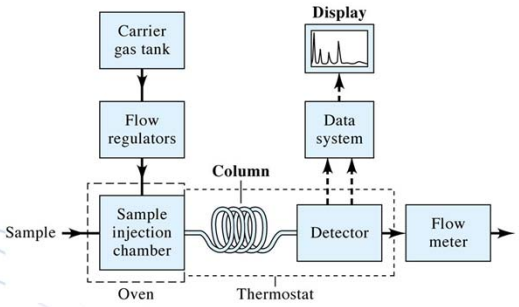


Fig. 27-1 (p790) Block diagram of a typical GS

Carrier gas (mobile-phase gas)

He (common), N_2, H_2, P_i 10-50 psi above atom, F = 25-150 mL/min for packed column, 1-25 mL/min for open tubular capillary

This a block diagram for a GS.

The mobile-phase gas is called carrier gas. He is the most common one, N_2 and H_2 can be used, too. The inlet pressure is about 10 - 60 psi. The flow rate depends on the type of column.

Sample injection

- Direct injection into heated port to promote fast vaporization
- Sample volume
  1-20 μL for packed column

  10^{-3} μL for capillary column, a sample splitter is needed (1/50-1/500 to column, rest to waste)
  or use a purge valve

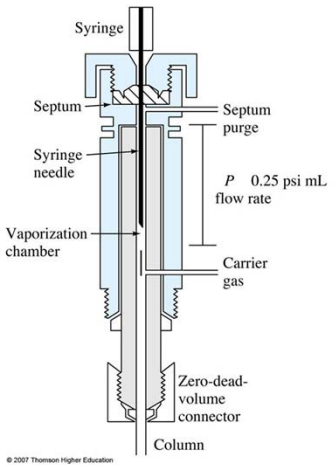


Fig. 27-4 (p791) Cross-section view of a microflash vaporizer direct injector

Sample is injected into the column using a microsyringe, through a septum.

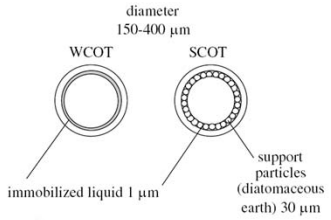
The sample port is heated 50 °C above the boiling point of the least volatile component of the sample.

Purge vale: it stays closed for 30 - 60 seconds after injection during which all sample vapor goes to the column. Purge valve will then open, and let any remaining vapor vent to atmosphere.

Column

Open tubular columns (Capillary)

- Wall coated (WCOT) <1 μm thick liquid coating on inside of silica tubing
- support-coated (SCOT) 30 μm thick coating of liquid-coated support on inside of silica tubing
- Best for speed and efficiency but only small samples



Packed columns

- Liquid-coated **silica particles** (<100-300 μm diameter) in glass tubing
- Best for large scale but slow and inefficient

Column is housed in an oven. The column temperature is an important variable, so it is controlled to be accurate within one degree or better.

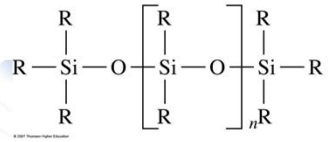
The optimal column temperature depends on the sample boiling point.

Two kinds of open tubular columns

1. Wall-coated, which gas 1 micrometer thickness of liquid coating on the inner surface
2. Support-coated, the inner surface of the capillary is lined with a thin layer of support material, and liquid is coated at the surface of the support particles. The advantage of SCOT is that the sample capacity increases, but the efficiency drops.

Immobilized liquid stationary phase

- Low volatility
- High decomposition temperature
- Chemically inert (reversible interaction with solvent)
- Chemically attached to support (prevent "bleeding")
- Appropriate k and α for good resolution
- Many based on polysiloxanes or polyethylene glycol (PEG)



[Check Table 27-2 for common stationary phases for GC]

Stationary phases usually bonded and/or cross-linked for longer-lasting

- Bonding – covalent linking of stationary phase to support
- Cross-linking – polymerization reactions after bonding to join individual stationary phase molecules

Film thickness (0.5-1 μM) affects retention and resolution

- thicker films for volatile analytes

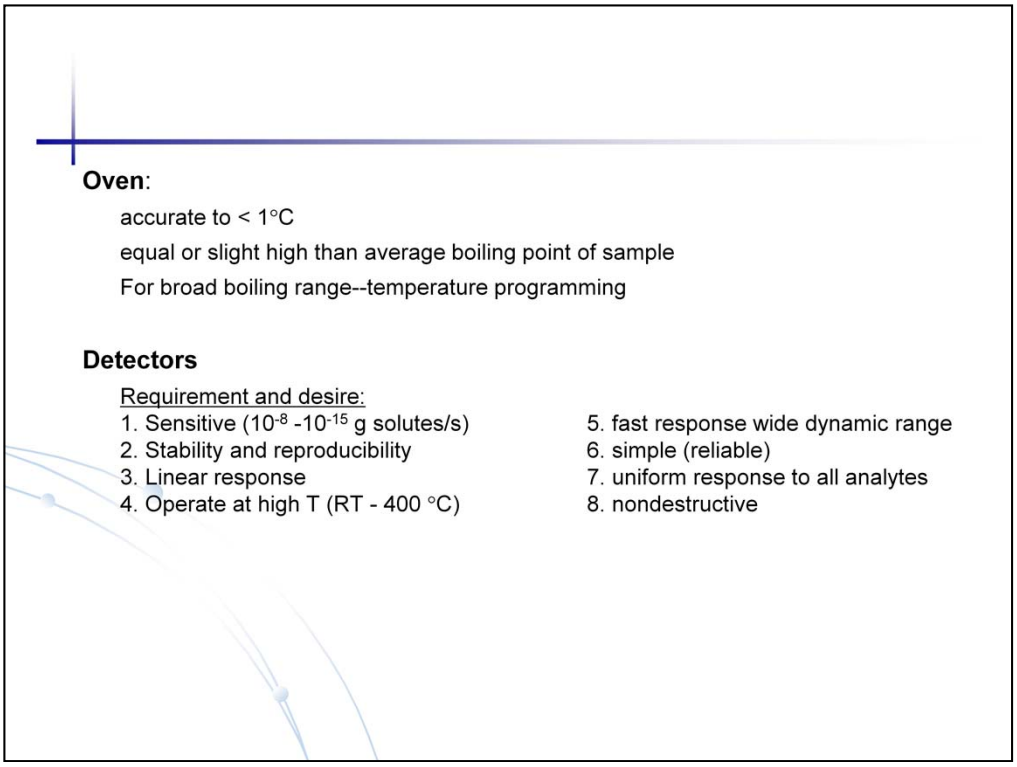
Non-polar stationary phases best for non-polar analytes

- non-polar analytes retained preferentially

Polar stationary phases best for polar analytes

- polar analytes retained preferentially

Chiral phases being developed for enantiomer separation (pharmaceutical)



Column is housed in an oven. The column temperature is an important variable, so it is controlled to be accurate within 1 degree or better.

The optimal column temperature depends on the sample boiling point.

Flame Ionization Detector (FID)

- Sample pyrolyzed and produce current in electrical field.
- Signal depends on #C atoms in organic analyte –mass sensitive not concentration sensitive
- Weakly sensitive to carbonyl, halogen, alcohol, amine groups
- Insensitive to non-combustibles – H_2O, CO_2, SO_2, NO_X

Advantages

- Rugged, general applicability, ease of use
- Sensitive (10^{-13} g/s)
- Wide dynamic range (10^7), low noise

Disadvantage

- Destructive

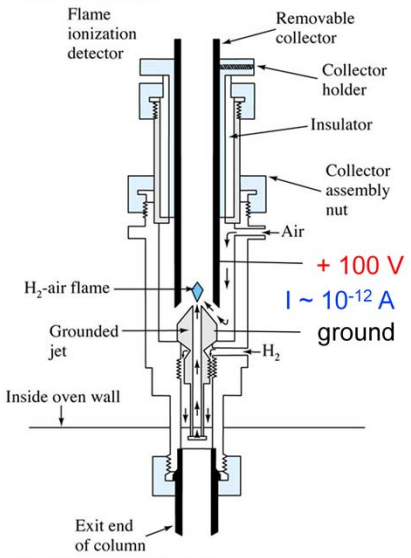


Fig. 27-8 (p794) FID

This is the most widely used detector.

- Elute from the column is directed into a small air-hydrogen flame.

- Most organic compounds produce ions and electrons when pyrolyzed at the temperature of hydrogen-air flame.

- Between the burner tip and collector electrode an electrical field of several hundred volts is applied. The electrical field causes ions (or electrons) to move toward the collector, producing current.

GC-MS

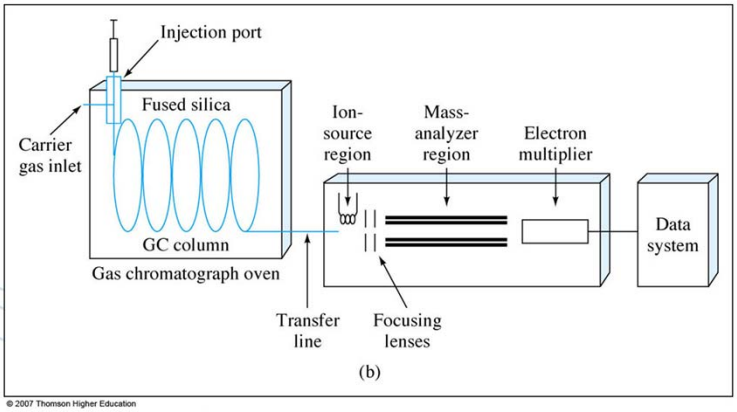


Fig. 27-14 (p799) GC/MS

One of the most powerful detectors, employed in GC-MS, is mass spectrometer. The eluent from the GC is passed into the inlet of a mass spectrometer, where the molecules in the eluent is vaporized by heat, ionized by a ionization source, mass analyzed by a quadrupole mass filter and detected by an electron-multiplier.

GC-MS offers mass spectra besides chromatograms. It measures mass spectrum for each individual component.

6 Liquid Chromatography

Small particles (3-10 μm) give high performance

$$N \approx \frac{3500L(cm)}{d_P(\mu m)}$$

but require high pressure

6.1 Scope of LC

Four types of liquid chromatography

- Partition
- Adsorption (liquid-solid)
- Ion-exchange
- Size exclusion or gel

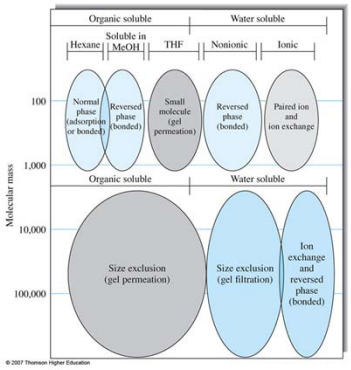


Fig. 28-1 (p817) Selection of LC modes

In liquid chromatography instruments, column is usually made up of stainless steel or glass tube with diameter of 10 - 50 mm, and packed with small diameter solid particles. It is found that decreasing the size of stationary phase particles reduces the plate height and improves the performance.

As can be imagined, a column with a length of 15 cm, packed with micrometer particles, the gravity flow rate would be very slow. So it is necessary to speed the flow rate using a high pressure. For this reason, the instrument is named high performance liquid chromatography, to distinguish with traditional gravity-flow method. In fact, nowadays, almost all liquid chromatography instruments use high pressures, and belong to HPLC. As a result, LC and HPLC mean the same thing, and they are interchangeable.

Scope — Four major types of liquid chromatography:
1) partition chromatography,
2) adsorption chromatography,
3) ion-exchange chromatography, and
4) size exclusion chromatography.

We will discuss each of these in detail later.
Among these four types, the liquid-solid absorption chromatography uses solid as stationary phase. As we discussed in gas chromatography, the disadvantage of solid stationary phase is that there exits semipermanent retention. For this reason, the application of adsorption chromatography is limited.

6.2 HPLC instrumentation

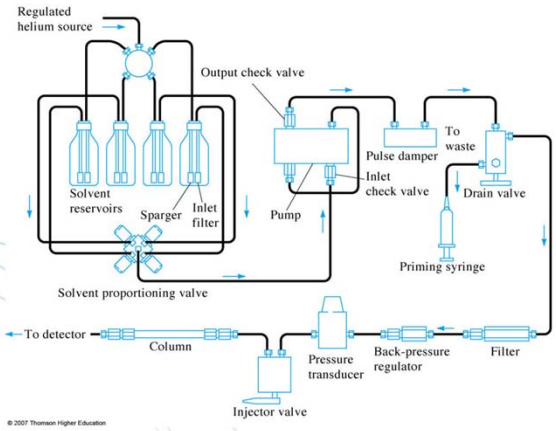
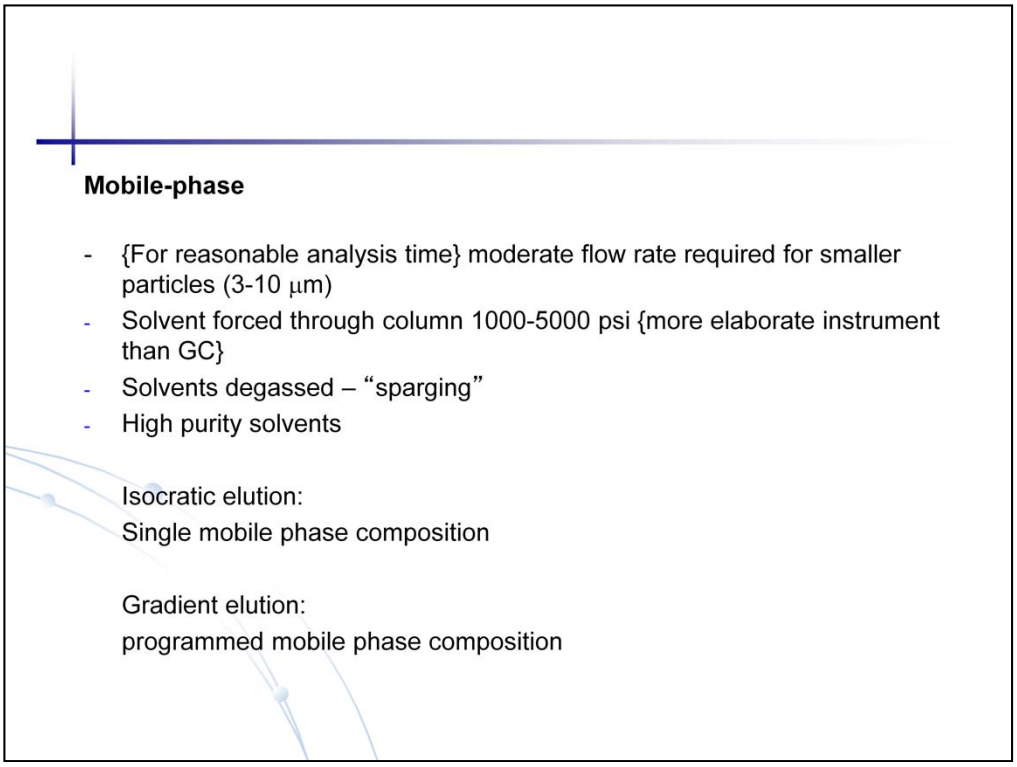


Fig. 28-3 (p819) Block diagram of HPLC

Now let's look at the HPLC instrument.



The first thing to deal with is mobile phase.

1. {see slide}

2.

3. Sparging means the dissolved gas are swept out of solution by fine bubbles of an inert gas. The reason for this is to avoid interference with the flow rate and band broadening by dissolved gas.

4. Need high purity solvents.

A modern HPLC can introduce liquid from more than one solvent bottles use proportioning valve.

If only one solvent or one single composition of mixed solvents is used during the elution, this is isocratic elution.

If the composition of the solvent changes continuously, it is gradient elution. As we discussed in chapter 26, a general elution problem for multicomponent sample is that it is difficult to find one optimal column condition for all components. One solution is to change the column condition during elution. For GC, we can change the oven temperature, using temperature programming; for LC, we use gradient elution (can be preprogrammed).

Pumping system for solvent

1) Reciprocating pumps

- Up to 10,000 psi, small internal volumes (35-400 μL)
- Produces pulsation

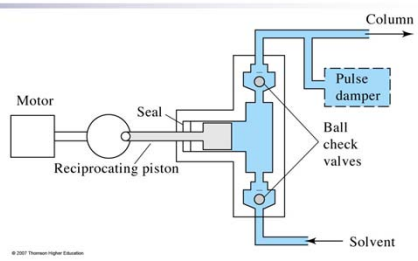


Fig. 28-5 (p820) A reciprocating pump

2) Displacement pumps

- A syringe equipped with a plunger activated by a screw-driven mechanism (the screw powered by a stepping motor)
- Pulse-free output
- Limited solvent capacity (250 mL), and inconvenience for solvent change

There are two major types of pumping system.

One is reciprocating pump. This pump has a small cylinder indicated in blue shaded color, the solvent in the cylinder is pumped by back and forth motion of a motor-driven piston. There are two ball check valves, open and close alternatively. Pull the piston, …; and push the piston back, ….

The advantage for this pump is delivery of high pressure. But the downside is that the forth-back motion of piston produces pulsation.

The other type is displacement pump. Here is a picture. Essentially, this is a syringe equipped with a plunger activated by a screw-driven mechanism. It is not directly connected to the reservoir and the syringe has limited capacity.

Sample injection

- Similar to GC
- Introduce small sample (0.1-100 μL) w/o depressurization

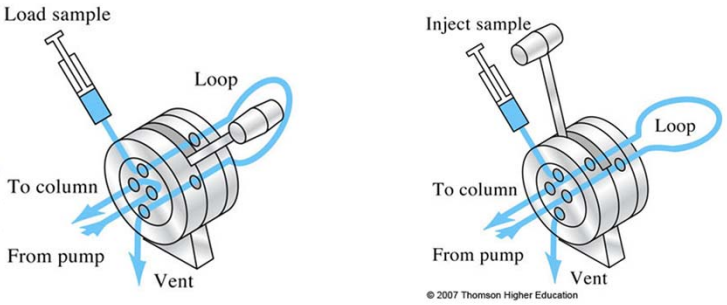


Fig. 28-6 (p821) A sampling loop for LC

New look at how the sample is actually injected into the column.

This injection is quite similar to GC. We only need small sample, around 0.1 to 100 microliter.

However, this column is pressurized by the pump. How to prevent the column depressurize during sample injection?

Here is a smart design for the injection valve. This is an injection valve with interchangeable sample loops. When place the valve in this position, the loop is filled with sample. Then switch the valve to the left position, now the loop is inserted between the pump and the column, and the solvent from the pump will sweep the sample into the column.

Columns and packings

- Stainless steel
- 5-25 cm long
- 3-5 mm internal diameter
- 3-5 μm particle (pellicular or porous, coated with liquid stationary phase)
- 40,000-70,000 plates/m

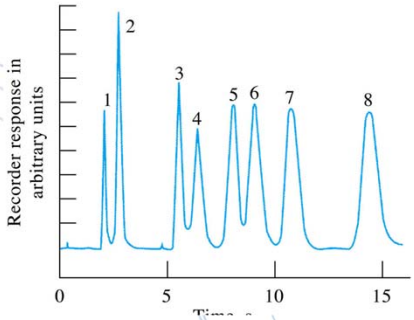


Fig. 28-7 (p822)
High speed isocratic separation. N: 100,000 plates/m. **Column**: 4cm x 0.4 cm inside diameter.
Packing: 3 μm
Mobile phase: 4.1% ethyl acetate in n-hexane.
Compounds: …

1. LC columns are constructed from stainless steel tubing or heave wall glass tubing, or polymer PEEK (ployetheretherketone). Columns are quite expensive (several hundred $$ each).

2. The length of column is 5 - 25 cm, and the internal diameter is 3 to 5 centimeter.

3. The packing particles for stationary phase is 3 - 5 um in diameter. They can be pellicular or porous particles, i.e., glass or polymer beads coated with silica, alumina, or synthetic resin.

Use the formula N = 3500L/d, the plate number is estimated to be around 40 to 70 thousand per meter.

This a chromatogram using isocratic elution, with specified column parameters and resulted performance. Now you can realize how efficient the LC is.

Detectors

Requirements and desires (similar as GC)

1. Sensitive (10^{-8} -10^{-15} g solutes/s)
2. Stability and reproducibility
3. Linear response
4. ~~Operate at high T (RT-400 °C)~~
5. fast response wide dynamic range
6. simple (reliable)
7. uniform response to all analytes
8. nondestructive
9. **Small internal volume to reduce zone broadening**

TABLE 28-1 Performance of HPLC Detectors

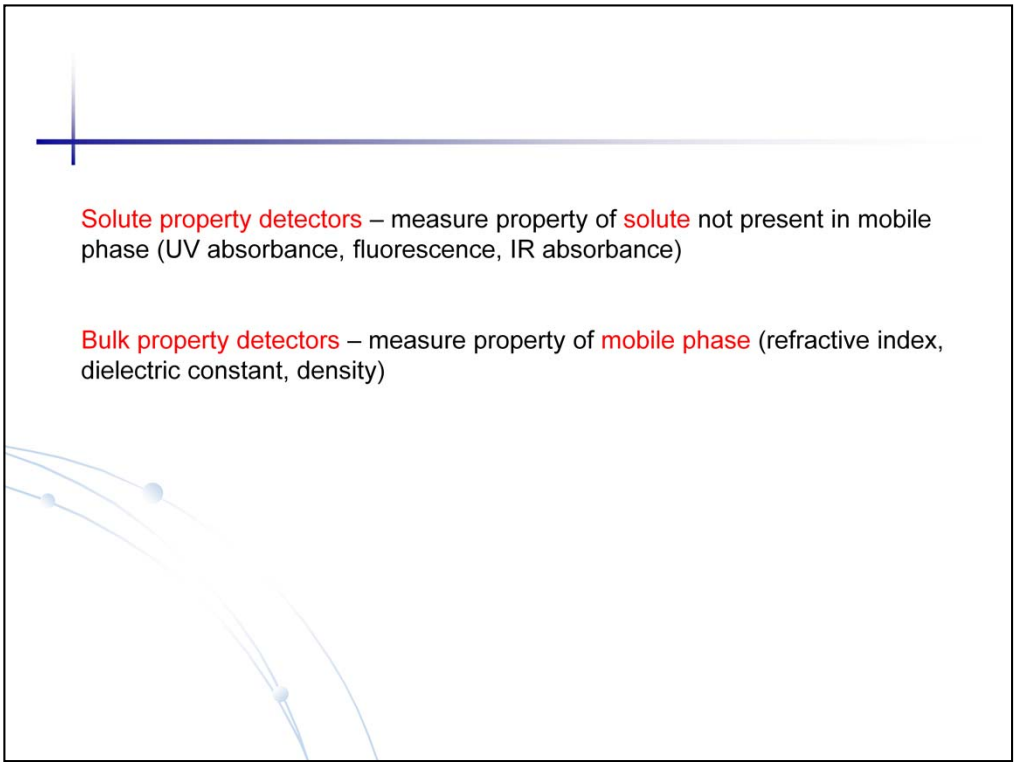
HPLC Detector	Commercially Available	Mass LOD* (typical)	Linear Range† (decades)
Absorbance	Yes	10 pg	3–4
Fluorescence	Yes	10 fg	5
Electrochemical	Yes	100 pg	4–5
Refractive index	Yes	1 ng	3
Conductivity	Yes	100 pg–1 ng	5
Mass spectrometry	Yes	<1 pg	5
FTIR	Yes	1 μg	3
Light scattering	Yes	1 μg	5
Optical activity	No	1 ng	4
Element selective	No	1 ng	4–5
Photoionization	No	<1 pg	4

The last part of the HPLC is the detector.

We have talked about GC detectors, such as flame ionization detector, thermal conductivity detector, and electron capture detector. All these only work for gas sample. In LC, we need to find something works for liquid flow.

The requirements for such detector are similar as LC's, except that high temperature is not required. In addition, the detector should have minimum internal volume to reduce zone broadening. The reason is that the flow rate changes when they flow through the detector, and the flow rate of the center part is different from those near the wall, causing extra broadening.

This table lists commercially available detectors.



They can be grouped into two categories. Give one example for each type.

UV-Vis detection

Sources:

- Single line (arc or hollow cathode lamp, laser)
- Continuum (Xe, D_2 lamp)

Detector:

- Photodiode/photomultiplier tube
- Photodiode array

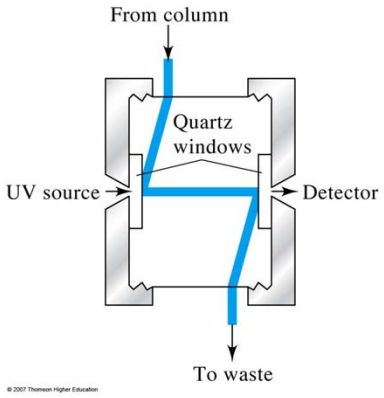


Fig. 28-8 (p824) A UV-Vis cell for HPLC

An example of solute property detector is UV-Vis detection. Briefly speaking, the source for this detector is either single line source from hollow cathode lamp or laser, or a continuum source from Xe or D_2 lamp.

The detector can be photodiode, photomultiplier tube, or photodiode array.

Some HPLC offers grating optics, so the different wavelengths can be chosen.

Double beam (elute cell/reference beam)

Chopped beam with single photon transducer.

Combination of separation and analysis (HPLC-UV-Vis)

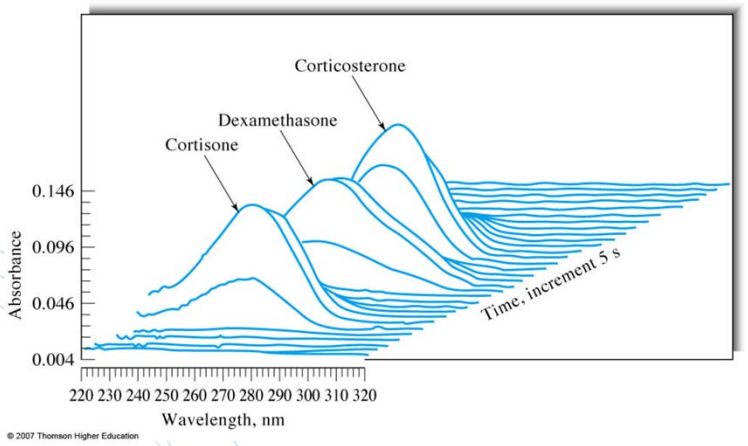


Fig. 28-9 (p825)

This a 3D plot showing the UV-Vis spectra as a function of elution time, recorded every 5 seconds. This measurement combines separation and analysis. The appearance and disappearance of three steroids in the eluent can be clearly revealed.

GC-MS and HPLC-MS may also present powerful 3D spectra.

Refractive-index detection

Advantages:

- Responds to all solutes
- Reliable and unaffected by flow rate

Disadvantages

- Needs constant temperature (a few thousandths of degree)
- Not sensitive

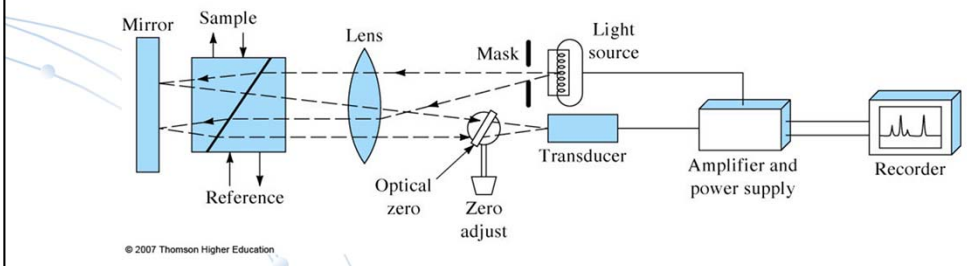


Fig. 28-10 (p826) A differential refractive-index detector

Refractive-index detector is an example of bulk property detector. This detector has two separated half compartments, one holds the blank solvent as a reference, and the eluate flows through the other half. Two sections are separated by a glass plate at an angle. Because the refractive index of eluate is different from that of the blank solvent, the incident beam bends and causes displacement, and consequently changes the output signal intensity of the photocell.

Advantages: …

Disadvantages: …

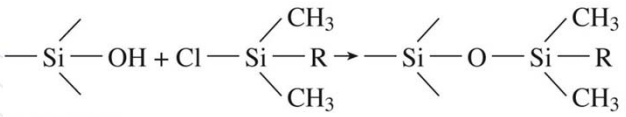
The sensitivity is 1000 times lower than UV detector.

6.3 Partition Chromatography

- Most popular method
- Low molecular weight (mw < 3000) analytes
- Polar or non-polar
- Liquid-bonded-phase column (liquid stationary phase chemically bonded to support particles)

Columns & packings

3,5 or 10 μm hydrolyzed silica particles coated with siloxanes



Normal phase HPLC: nonpolar solvent/polar column

Reversed phase HPLC: polar solvent/nonpolar column (advantages? Water can be used as mobile phase, mass transfer is rapid in nonpolar stationary phase, faster elution, and high resolution)

There are four types of liquid chromatography, based on their separation mechanisms and stationary phases. The most widely used one is partition chromatography. Most of the columns in partition chromatography is liquid-bonded-phase(liquid stationary phase chemically bonded to support particles rather than physical absorption).

The packing material for liquid-bonded-phase column are prepared from hydrolyzed silica particle coated with siloxane, with 3, 5 or 10 um diameter.

Through the reaction of hydrolyzed surface with organocholorsilane. R is alkyl group.

In literatures we often see terms of normal phase HPLC and Reversed phase HPLC. In earlier days, LC was based on polar stationary phase/nonpolar solvent, this is called normal phase. Reverse phase is just opposite. The difference is that in reversed phase, the most polar component elutes first.

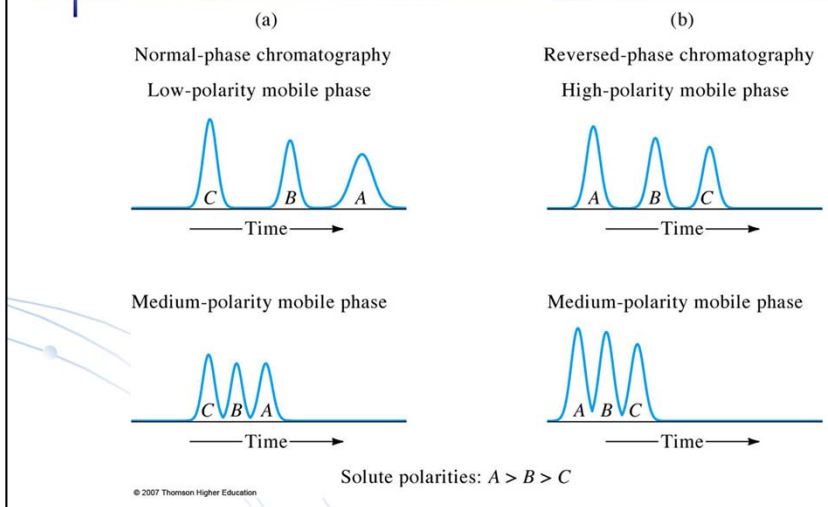


Fig. 28-14 (p829) Relationship between polarity and the elution times for normal phase and reversed-phase.

Column optimization

- optimize k and α (more difficult than GC)
- In GC mobile phase (He) just transports solute
- In HPLC mobile phase interacts with solute

Analyte polarity:

Hydrocarbons < ethers < esters <ketones < aldehydes < amines < alcohols

Stationary phase choice:

with similar polarity to analyte for maximum interaction

Reversed-phase column (nonpolar) : R hydrocarbon

Normal-phase column (polar) : R cyano (C_2H_4CN) most polar

Regarding column optimization for partition liquid chromatography, essentially we have to optimize the values of *k* and α. However, this is much more difficult than GC.

The reason is that in GC, carrier gas only carries solute. In HPLC, the mobile phase interacts with solute . The interaction is related to the polarities of solute, mobile phase, and stationary phase. Have to balance the intermolecular forces among different components.

For analyte: the order of polarity is as above. The polarity of stationary phase has to roughly match that of analyte.

Mobile phase choice:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{a-1}{a}\right)\left(\frac{k}{1+k}\right)$$

Optimization of *k*

Polarity index P'

$P'_{AB} = \phi_A P'_A + \phi_B P'_B$ where ϕ is the volume fraction

$$\frac{k_2}{k_1} = 10^{(P_1' - P_2')/2} \quad \text{for normal phase}$$

$$\frac{k_2}{k_1} = 10^{(P_2' - P_1')/2} \quad \text{for reversed phase}$$

Recall that we have derived this equation in previous lecture.

R depends on N, α and *k*. *k* is strongly depending on the composition of mobile phase. We can change the mobile phase to manipulate *k*. [reminder of *k* value: 1 to 10].

The reason is that polar ("strong") solvent interacts most with polar analyte (solute) – elutes faster but less resolution.

The polarity of mobile phase can estimated using polarity index P'.

For mixed solvent, P'_{AB}= {see slide}

Assume we change mobile phases polarity index P_1 prime to P_2 prime. A change of two in polarity index results a order of magnitude change in the retention factor.

TABLE 28-2 Properties of Common Chromatographic Mobile Phases

Solvent	Refractive Index[a]	Viscosity, cP[b]	Boiling Point, °C	Polarity Index, P'	Eluent Strength,[c] ε^0
Fluoroalkanes[d]	1.27–1.29	0.4–2.6	50–174	<–2	−0.25
Cyclohexane	1.423	0.90	81	0.04	−0.2
n-Hexane	1.372	0.30	69	0.1	0.01
l-Chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
i-Propyl ether	1.365	0.38	68	2.4	0.28
Toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
Tetrahydrofuran	1.405	0.46	66	4.0	0.57
Chloroform	1.443	0.53	61	4.1	0.40
Ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
Dioxane	1.420	1.2	101	4.8	0.56
Methanol	1.326	0.54	65	5.1	0.95
Acetonitrile	1.341	0.34	82	5.8	0.65
Nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
Water	1.333	0.89	100	10.2	Large

This table shows some common mobile phases for LC. The most polar solvent is water.

Optimization of selectivity α with two or three organic solvents (with predetermined value of *k*)

Step 1. optimize the separation with acetonitrile/water to generate chromatogram A (water is used to maintain solvent strength and keep *k* value)

Step 2. optimize the separation with methanol/water to generate chromatogram B

Step 3. optimize the separation with THF/water to generate chromatogram C

Step 4. Mix the solvents used in A, B and C, one pair at a time, in 1:1 proportion, to generate chromatograms D, E and F

Step 5. Construct a 1:1:1 of solvents A, B,C to generate G

Step 6: If some of the results A thru. G are almost good enough, select the two best points and mix the solvents to obtain points between two

In many cases, adjusting *k* is enough to achieve a good separation. But in some cases, two bands overlap although the value of *k* is reasonable. In this situation, more than one solvent can be used to change the selectivity while still keeping *k* in the appropriate range.

The following slide shows an optimizing procedure for reversed phase partition chromatography, and water is added to solvent.

For normal-phase separation, a similar solvent triangle can be used, for example "ethyl ether, methylene chloride, and chloroform", and n-hexane added to adjust solvent strength.

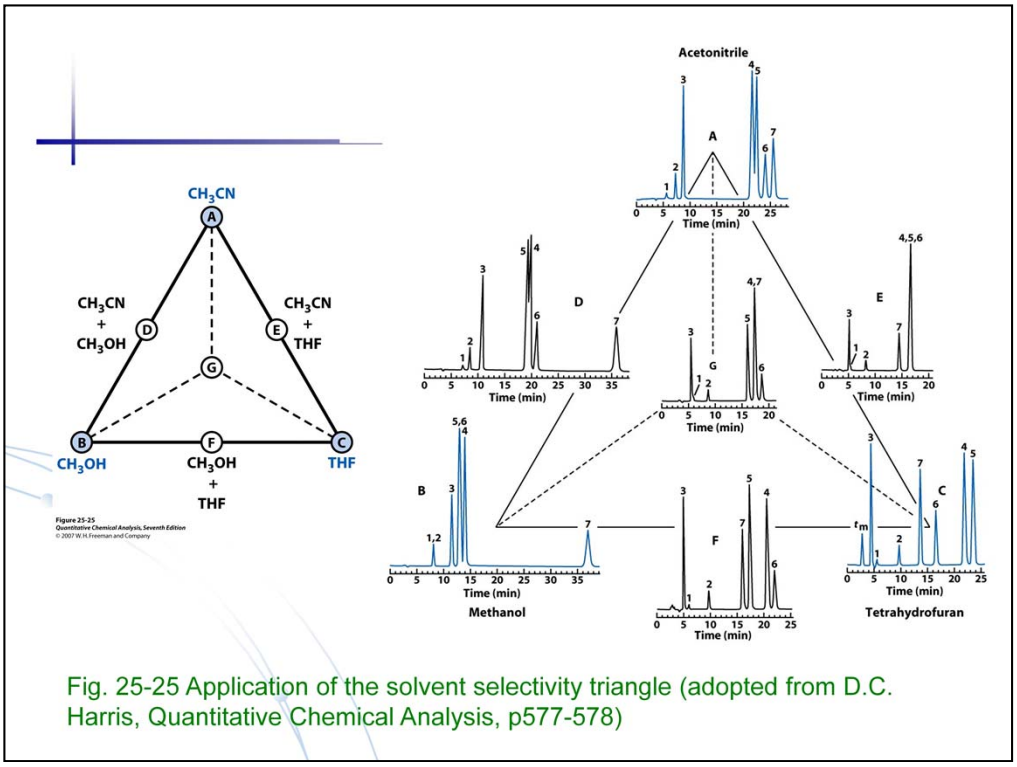


Fig. 25-25 Application of the solvent selectivity triangle (adopted from D.C. Harris, Quantitative Chemical Analysis, p577-578)

This figure shows use of a solvent selectivity triangle to optimize the selectivity.

Three solvents are methanol, acetonitrile, and tetrahydrofuran THF. Water is used to adjust the solvent strength and maintain a reasonable value of k.

{The reason for using multiple solvents is that different solvent have different proton donor, proton acceptor, and dipolar interactions.}

In this triangle, three solvents (and water) define three vertices of the triangle.

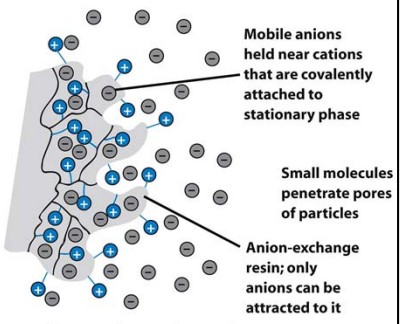
7 Ion-Exchange Chromatography

7.1 Ion-Exchange

- retention is based on the attraction between solute ions and charge sites bound to the stationary phase

Anion exchanger

- positive charged groups on the stationary phase attract solute anions



Ion-exchange chromatography

Figure 23-6 part 3
Quantitative Chemical Analysis, Seventh Edition
© 2007 W. H. Freeman and Company

$$xRN(CH_3)_3^+OH^-(s) + A^{x-}(aq) \Leftrightarrow [RN(CH_3)_3^+]_x A^{x-}(s) + xOH^-(aq)$$

Cation exchanger

- contain covalently bound, negatively charged sites that attract solute cations

$$xRSO_3^-H^+(s) + B^{x+}(aq) \Leftrightarrow (RSO_3^-)_x B^{x+}(s) + xH^+(aq)$$

Another category of liquid chromatography is ion-exchange chromatography.

Here, retention is based on the attraction between solute ions and charge sites bound to the stationary phase, as shown in this cartoon.

If these are anion exchangers, as shown in this cartoon, then the stationary phase contains positive charge groups, and these positive groups attract solute anions.

If these are cation exchangers, the stationary phase contains covalently bound, negatively charged sites that attract solute cations.

7.2 Ion-exchange equilibrium
(example of retention of B^+ by RSO_3^- resin]

$$RSO_3^-H^+(s) + B^+(aq) \Leftrightarrow RSO_3^-B^+(s) + H^+(aq)$$

$$K_{ex} = \frac{[RSO_3B^+]_s[H^+]_{aq}}{[RSO_3H^+]_s[B^+]_{aq}} \qquad (1)$$

$$\frac{[RSO_3^-B^+]_s}{[B^+]_{aq}} = K_{ex}\frac{[RSO_3^-H^+]_s}{[H^+]_{aq}} \qquad (2)$$

$$[RSO_3^-H^+]_s >> [RSO_3^-B^+]_{aq}$$

$$[H^+]_{aq} >> [B^+]_{aq}$$

$$\frac{[RSO_3^-B^+]_s}{[B^+]_{aq}} = K = \frac{c_S}{c_M} \qquad (3)$$

All equations in Table 26-5 can be applied to ion-exchange chromatography

Let's consider the exchange reaction between analyte B^+ ions and the sulfonic acid groups attached to the stationary phase. This is an cation exchange column, we use this column to separate B^+, and use dilute chloride acid as eluent.

The equilibrium constant K for this exchange reaction is described in Eq.1, where the subscript s means stationary phase, and aq mean the solution phase.

Rearrange this equation to Eq. 2.

Because the exchangers has enormous number of exchange sites RSO_3^-, $[RSO_3H]_s >> [RSO_3B]$, so its overall concentration can be treated as a constant. We use chloride acid as eluent, so $[H^+]$ can also be treated as a constant, therefore we have Eq. 3.

It turns out that *K* is a constant, and equal to distribution constant we defined before.

The implication for this is that all equations listed in Table 26-5 can be applied to ion-exchange chromatography.

7.3 Ion-exchange packing

- Resin: amorphous particles of organic material, such as coploymer of styrene and divinylbenzene
- Divinylbenzene content 1-16%, varied to increase the extent of cross-linking
- Benzene ring can be modified to produce a cation-exchange resin containing sulfonate group (SO_3^-), or an anion-exchange resin containing ammonium groups($-NR_3^+$)

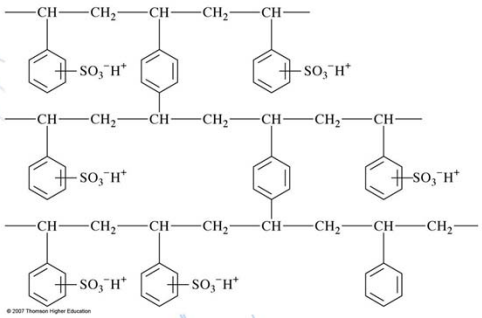
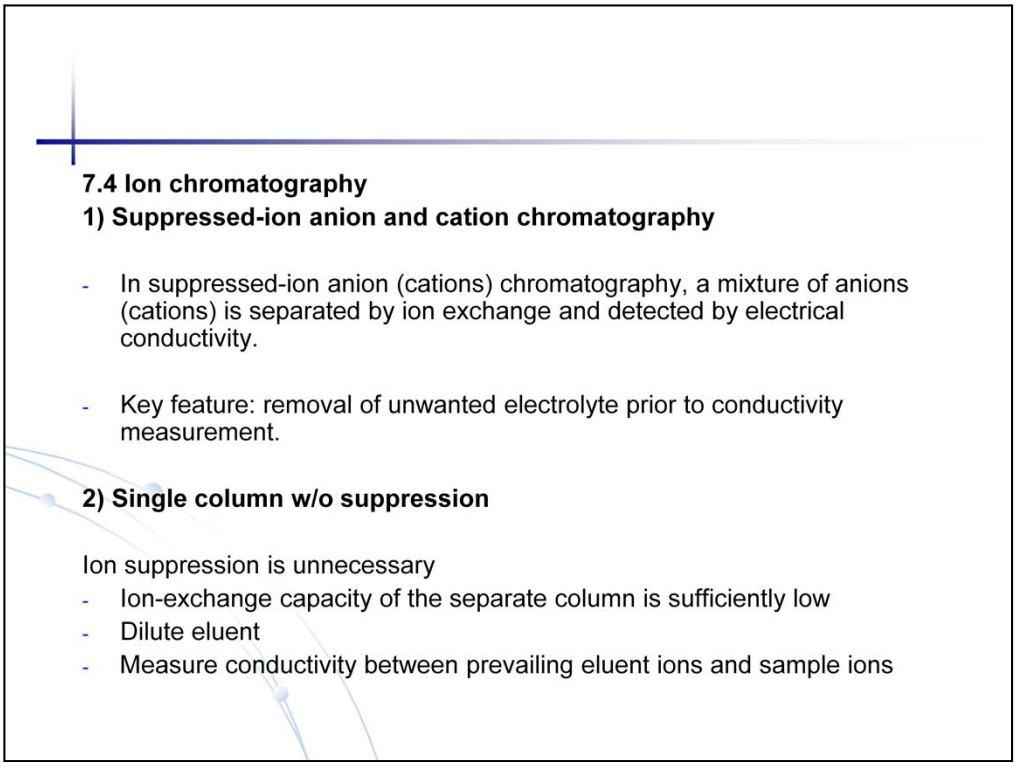


Fig. 28-22 (p841) Structure of a cross-linked polystyrene ion-exchanger resin.

The ion-exchange chromatography is made of resin, which is {see slide }

The content of divinylbenzene varies from 1 % to 16 %, to change the extent of cross-linking.

The benzene ring can be modified to produce a cation-exchange resin containing sulfonate groups, or anion-exchange resin containing ammonium groups.



Suppressed ion chromatography is one type of ion chromatography.

In suppressed-ion anion chromatography, a mixture of anions is separated by ion exchange and detected by electrical conductivity.

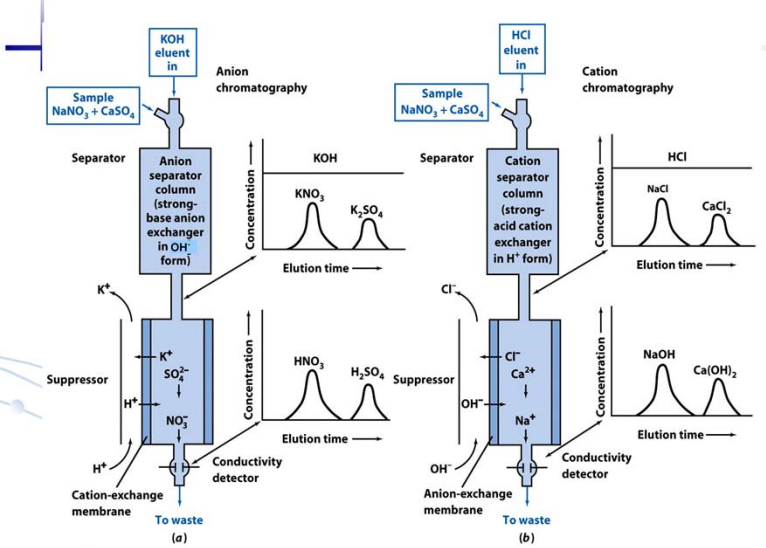


Fig. 26-4. Suppressed-anion and -cation chromatography (adopted from D.C. Harris, Quantitative Chemical Analysis, p595)

For an example, this is an anion exchange chromatography. We want to separate NO_3 and SO_4 in the sample.

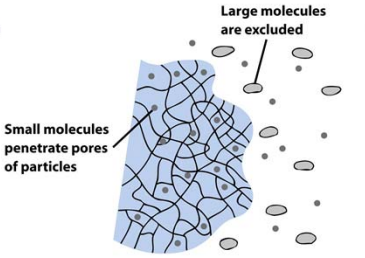
A sample containing $NaNO_3$ and $CaSO_4$ is injected into an anion-exchange containing OH^- sites – followed by elution with KOH. NO_3^- and SO_4^{2-} equilibrate with resin and are slowly displaced by OH^- eluent. Na^+ and Ca^{2+} are not retained and simply wash through. Eventually, KNO_3 and K_2SO_4 are eluted from the column, as shown in this figure. These species cannot be easily detected, however, because the solvent contains a high concentration of KOH, whose high conductivity obscures analyte species.

To remedy this problem, the solution passes thru. A suppressor, in which cations are replaced by H^+. H^+ replaces K^+ and form water with OH^-. The net result is eluent KOH converts to water and has low conductivity. HNO_3 and H_2SO_4 can be detected based on conductivity.

Suppressed-cation chromatography is conducted in a similar manner, but the suppressor replaces Cl^- from eluent with H+, so the eluent HCL converts to water. Only NaOH and $Ca(OH)_2$ are detected.

8 Size Exclusion Chromatography (Gel Permeation)

- Used for large mw compounds – proteins, polymer
- Separation mechanism is sieving not partitioning
- Stationary phase is porous silica or polymer particles (5-10 μm), with well defined pore size (10-1000 Å)



Molecular exclusion chromatography

Figure 23-6 part 4
Quantitative Chemical Analysis, Seventh Edition
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TABLE 28-4 Properties of Typical Packings for Size-Exclusion Chromatography

Type	Particle Size, μm	Average Pore Size, Å	Molecular Mass Exclusion Limit*
Polystyrene-divinylbenzene	10	100	700
		1000	$(0.1 \text{ to } 20) \times 10^4$
		10^4	$(1 \text{ to } 20) \times 10^4$
		10^5	$(1 \text{ to } 20) \times 10^5$
		10^6	$(5 \text{ to } >10) \times 10^6$
Silica	10	125	$(0.2 \text{ to } 5) \times 10^4$
		300	$(0.03 \text{ to } 1) \times 10^5$
		500	$(0.05 \text{ to } 5) \times 10^5$
		1000	$(5 \text{ to } 20) \times 10^5$

*Molecular mass above which no retention occurs.

This LC method is based on sieving rather than partitioning, and is mostly used for separation of large biomolecules.

large molecule excluded from pores
– not retained, first eluted (exclusion limit – terms of mw)

intermediate molecules
– retained, intermediate elution times

small molecules permeate into pores
– strongly retained, last eluted (permeation limit – terms of mw)

Retention related to size (and shape) of molecule

$$\underbrace{V_t}_{total} = \underbrace{V_g}_{gel\ or\ solid} + \underbrace{V_i}_{inside\ pores} + \underbrace{V_o}_{outside\ pores\ free\ space}$$

V_0: retention volume for non-retained (large) molecules
$V_0 + V_i$: retention volume for retained (small) molecules
$V_e = V_0 + KV_i$: retention volume for intermediate molecules
($K = c_s/c_M$) → $K = (V_e - V_0)/V_i$

Regarding the theory of size-exclusion chromatography, the total volume of a column is {see equation on the slide},

V_g: volume occupied by the solid matrix of the polymer.

V_i: volume of solvent held in pores

V_0: free volume outside the particles.

K: distribution constant.

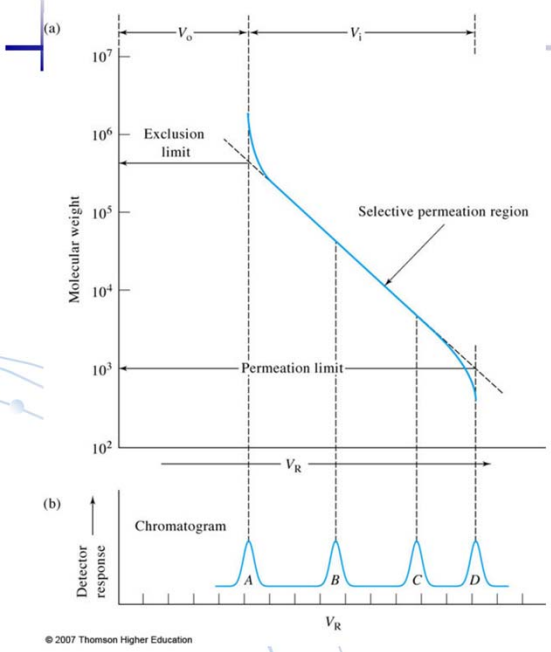

Fig. 28-27 (p846) a) calibration curve for a size exclusion column, and b) different mass-weight components

Such relationships are shown in the calibrated curve. In the figure, A is the peak corresponding to all molecules with masses higher than the exclusion limit, and D is the peak corresponding to all molecules with masses below the permeation limit.

Applications

1. Determination of mw distribution of large polymers or natural products – mass calibration.
2. Separation of high mw, natural-product molecules from low mw species and from salts.
3. Advantages and disadvantages (p847)

- well defined separate time
- narrow bands
- free from sample loss
- absence of column deactivation (because of interaction with solute)

But

- only a limited number of bands because time scale is short
- require 10% mass difference for separation