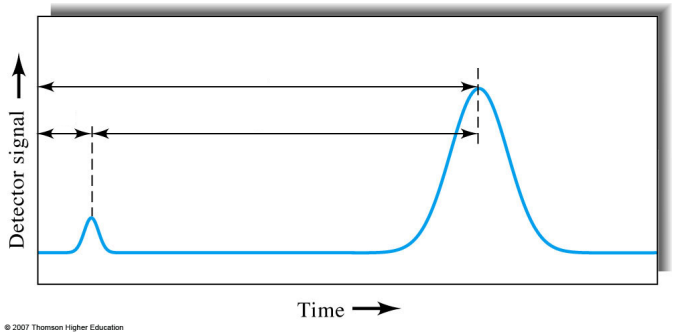
Chromatography- Separation of mixtures

Define the following terms in words and with an equation, if applicable.

Term	Symbol	Equation	Description
Mobile phase	--		
Stationary phase	--		
Retention time	t_r		
Linear mobile phase velocity	u		
Average linear solute migration velocity			
Distribution coefficient	K_c		
Retention factor	k_a		
Selectivity factor	α		
Plate height	H		
Theoretical plates	N		
FWHM	$W_{½}$		
Resolution	R_s		

Describe the relationship between t_r, t_m, and t_s with respect to the interaction of the mobile phase, stationary phase, and solute. Label t_r, t_m, and t_s in the diagram below.



Describe the basis of separation for each of the types of chromatography described below.

Absorption Chromatography

Partitioning

Ion chromatography

Size exclusion chromatography

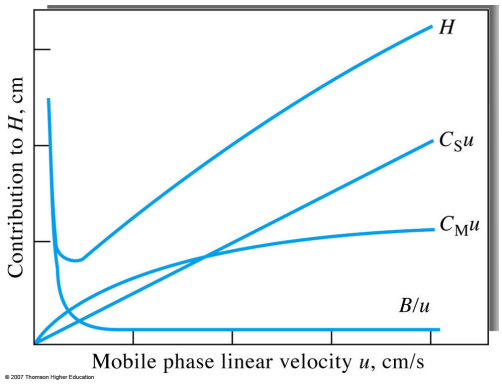
Affinity chromatography

Chiral chromatography

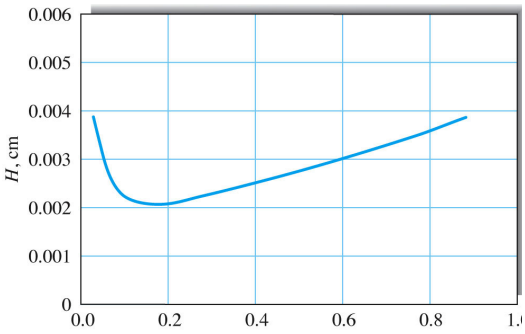
Factors affecting peak broadening

1. Peaks are never narrower than injection width

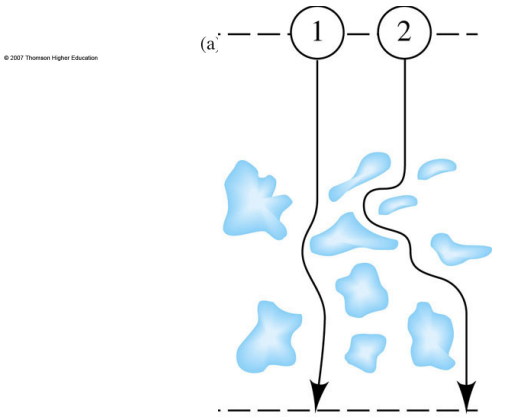
Column efficiency- Plate height and number theoretical plates



2. Longitudinal broadening- Band width $\alpha \sqrt{t_R}$



3. Mobile phase flow rate- Van Deemter plot



4. Multiple flow paths

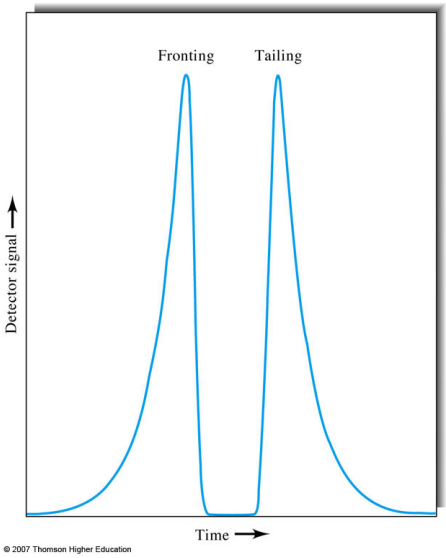
5. Particle or column diameter

6. Finite equilibration time

Factors affecting peak shapes

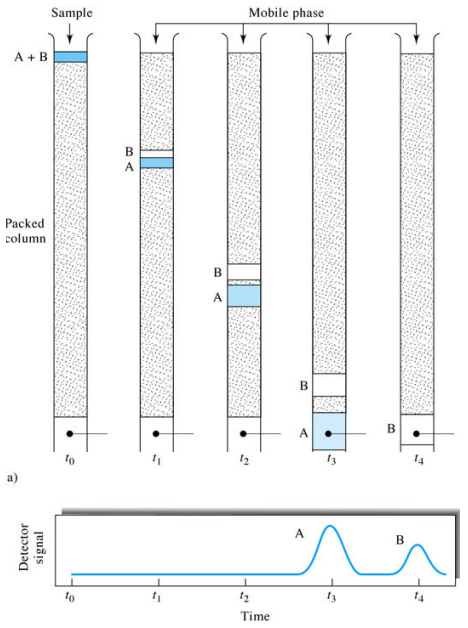
7. Fronting

8. Tailing



Chromatography- Separation of mixtures

1. Sample dissolved in mobile phase
2. Mobile phase passes over stationary phase
3. Analyte molecules partition between mobile and stationary phase
4. Different solutes will elute at different times

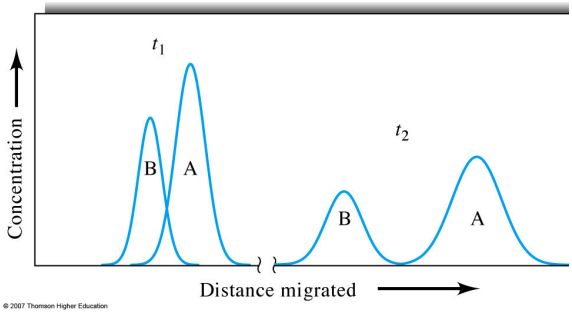


Describing solute- column interactions

Distribution Coefficient

$A_{mobile} \Leftrightarrow A_{stationary}$

Retention factor

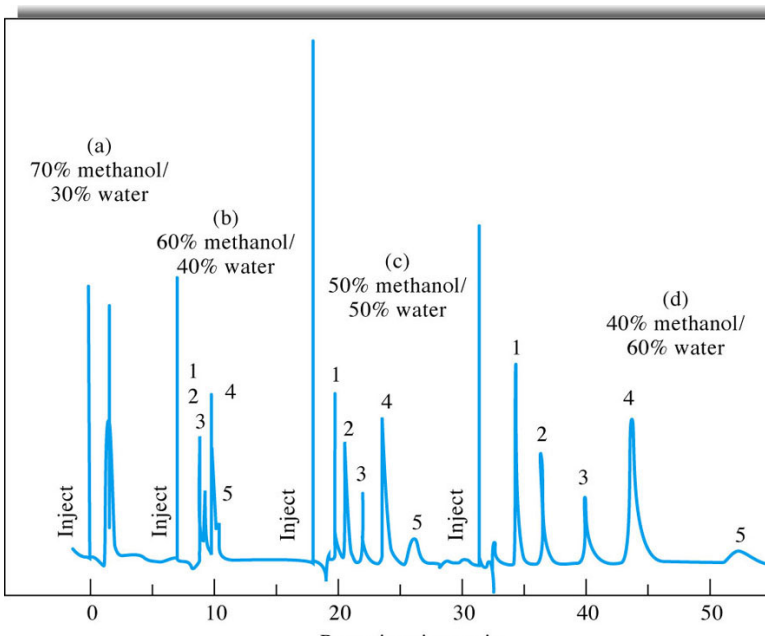


Separation of mixtures

Better separations require either:

1. increase in rate of separations
2. decrease in rate of band spreading

Resolution of separations



General elution problem

The following data are for a liquid chromatographic column:
length = 24.7 cm flow rate= 0.313 mL min^{-1} V_m= 1.37 mL V_s= 0.164 mL

A chromatogram of a mixture of species A, B, C, D provided the following data:

Species	retention time (min)	width of peak (FWHM; min)
t_m	3.1	
A	5.4	0.41
B	13.3	1.07
C	20.1	3.16
D	21.6	3.72

Calculate the number of plates from each peak, the mean and standard deviation of N and H for this mixture of solutes. Then calculate the distribution constant and retention factor for each species.

species	N	H	K_c	k_c
A				
B				
C				
D				
Mean			--	--
St. Dev			--	--

For species C and D, calculate the resolution and selectivity factor. Then calculate the column length required for a resolution of 1.5.

Comparing chromatographic techniques

	TLC	GC	HPLC
Stationary phase	Silica gel on glass plate	Capillary with liquid or solid phase	Silica beads- often derivitized with C-18, etc
Mobile phase	solvent	Gas, usually He	Organic or polar solvent or mixture of solvents
Column length	~10cm	15-100m	~5-25 cm
Pressure	ambient	low	Up to 1,-000 bar
Temperature	ambient	variable	Variable

High Performance Liquid Chromatography (HPLC)
HPLC pressure- up to 1,000 bar (~100-400 bar normal)
Column length ~10-25 cm with ~5um C-18 derivativized silica particles
Solvent:
Isocratic elution

Gradient elution

Detectors:
UV-Vis

ELSD

Mass Spectrometry