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Print version

CEE 772:
Instrumental Methods in Environmental Analysis

Lecture #14

Chromatography: Theory

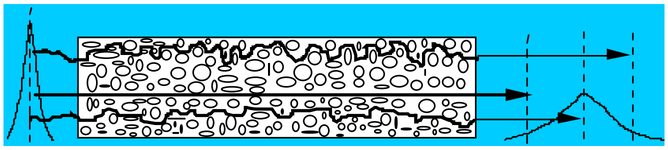
(Skoog, Chapt. 26, pp.674-693)

(Harris, Chapt. 23)
(641-664)

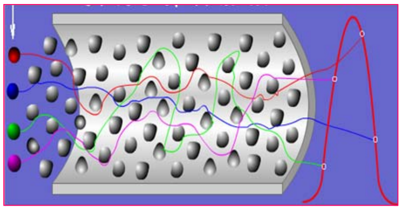
Rate Theory of Chromatography

- takes account of the time taken for the solute to equilibrate between the stationary and mobile phase
  - unlike the plate model, which assumes that equilibration is infinitely fast
  - The resulting band shape of a chromatographic peak is therefore affected by the rate of elution. It is also affected by the different paths available to solute molecules as they travel between particles of stationary phase. If we consider the various mechanisms which contribute to band broadening, we arrive at the Van Deemter equation for plate height;
  - where *u* is the average velocity of the mobile phase. *A, B,* and *C* are factors which contribute to band broadening

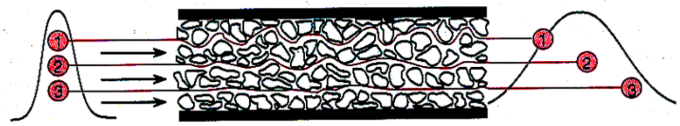
$$\mathrm{HETP} = A + B / u + C\,u$$

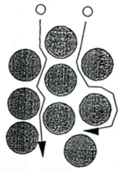


- ***A* - Eddy diffusion**
  The mobile phase moves through the column which is packed with stationary phase. Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths.



A) Flow and Diffusion in mobile phase (Eddy or multi-path diffusion) H_E





Eddy Diffusion

Profile of flow

$$H_E = \left(\frac{2\lambda\, d_p^{\,1+x}}{(D_m)^x}\right) u^x$$

λ:column packing factor (0.5~1.5)
d_p: average size of the filling particles
D_m: solute diffusion coefficient in mobile phase
u: linear velocity
x:constant of system (0 ~ 1/3)
In general, x=0 for GC. And x=1/3 for LC

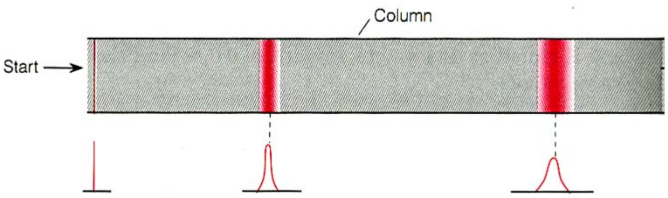
Smaller the d_p, smaller the H_E!

The effects from D_m and u is opposite to those for H_L!

Every thing has two sides!

- ***B* – Molecular (Longitudinal) diffusion**
  The concentration of analyte is less at the edges of the band than at the center. Analyte diffuses out from the center to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.
- ***C* - Resistance to mass transfer**
  The analyte takes a certain amount of time to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is high, and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes.

B) Diffusion: (molecular or longitudinal)



$$C_x = C_0\left(\frac{1}{2\sqrt{\pi D t}}\right) e^{-(x^2/4Dt)}$$

$$\sigma^2 = 2Dt = 2D\left(\frac{L}{u}\right)$$

$H_L = (\sigma^2)_L/L = 2D_m/u$

Packed bed

$H_L = (\sigma^2)_L/L = 2D_m/[u(1+\varepsilon_p/\varepsilon_e)]$

ε_p: intraparticle porosity
ε_e: interparticle porosity
D_m: solute diffusion coefficient in mobile phase.
u: linear velocity of flow

Longitudinal Diffusion is significant in GC but has much less effect in LC

C) Non-equilibrium (resistance to mass transfer) H_R (II)

(1) Resistance to mass transfer from stationary phase to mobile phase

$$H_s = q_s \left(\frac{k}{(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right) u$$

k:capacity factor
d_f: thickness of stationary phase
D_s:solute diffusion coefficient in stationary phase.
q_s:shape factor for the stationary phase coating coating (2/3 for a thin layer on the support).
u: linear velocity of flow

(2) Resistance to mass transfer from mobile phase to stationary phase

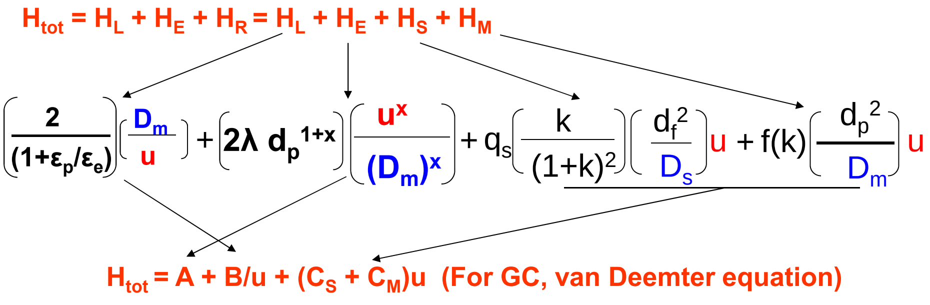
$$H_M = f(k)\left(\frac{d_p^2}{D_m}\right) u$$

f(k): a function of k, increasing with k
d_p:average size of the filling particles
D_m: solute diffusion coefficient in mobile phase
u: linear velocity

D_m → **Less effect on GC**

(3) $H_R = H_S + H_M$

Simplified Expressions



$H_{tot} = H_L + H_E + H_R = H_L + H_E + H_S + H_M$

$$\left(\frac{2}{(1+\varepsilon_p/\varepsilon_e)}\right)\left(\frac{D_m}{u}\right) + \left(2\lambda\, d_p^{1+x}\right)\left(\frac{u^x}{(D_m)^x}\right) + q_s\left(\frac{k}{(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u + f(k)\left(\frac{d_p^2}{D_m}\right)u$$

$H_{tot} = A + B/u + (C_S + C_M)u$ **(For GC, van Deemter equation)**

$H_{tot} = Au^{1/3} + B/u + (C_S + C_M)u$ **(For LC, Knox equation)**

Overall Solution

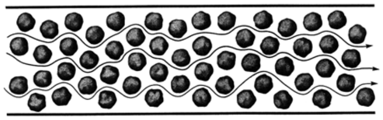
$$H_{tot} = H_L + H_E + H_R = H_L + H_E + H_S + H_M$$

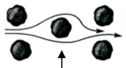
$$\left(\frac{2}{(1+\varepsilon_p/\varepsilon_e)}\right)\left(\frac{D_m}{u}\right) + \left(2\lambda\, d_p^{1+x}\right)\left(\frac{u^x}{(D_m)^x}\right) + q_s\left(\frac{k}{(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u + f(k)\left(\frac{d_p^2}{D_m}\right)u$$

	H_{tot} = H_L	+ H_E	+ H_S	+ H_M
u ↑	↓	↑	↑	↑
D ↑	↑	↓	↓	↓
d_p ↑	–	↑	–	↑
d_f ↑	–	–	↑	–
k ↑			↓	↑

Rate theory-- Van Deemter Equation

1. Packed-bed system

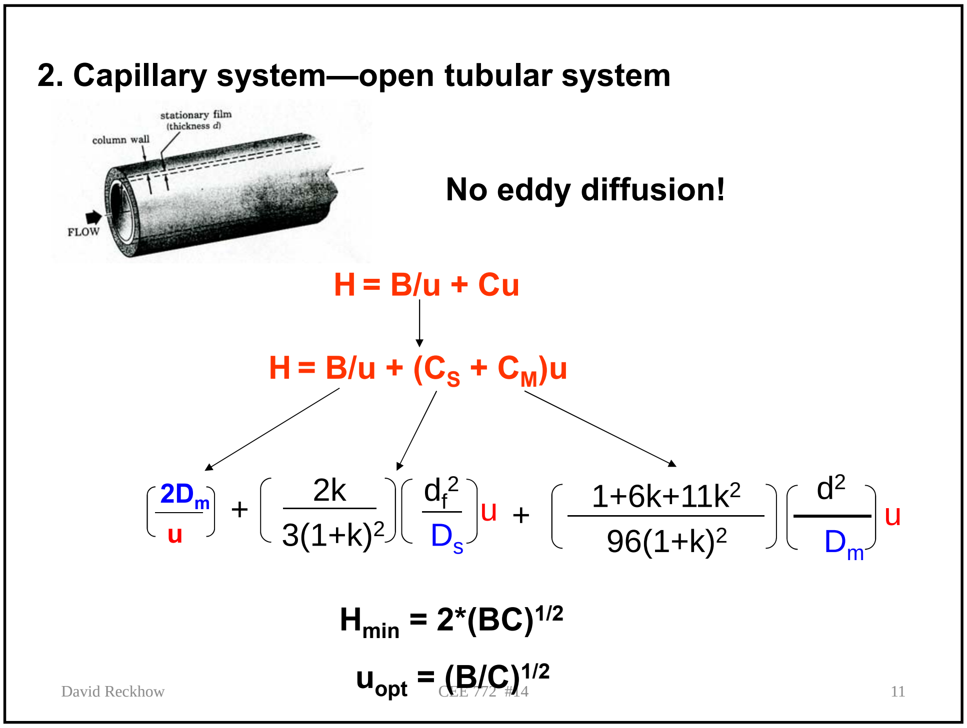




$$H = A + B/u + (C_S + C_M)u$$

$$2\lambda\, d_p + \left(\frac{2}{(1+\varepsilon_p/\varepsilon_e)}\right)\left(\frac{D_m}{u}\right) + q_s\left(\frac{k}{(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u + f(k)\left(\frac{d_p^2}{D_m}\right)u$$

λ:column packing factor (0.5~1.5)
d_p: average size of the filling particles
ε_p: intraparticle porosity
ε_e: interparticle porosity
D_m: solute diffusion coefficient in mobile phase.
k: capacity factor ⟶ $k = K\,(V_s/V_m)$
D_s: solute diffusion coefficient in stationary phase.
q_s:shape factor for the stationary phase coating coating (2/3 for a thin layer).
d_f: thickness of stationary phase



2. Capillary system—open tubular system

No eddy diffusion!

$$H = B/u + Cu$$

$$H = B/u + (C_S + C_M)u$$

$$\left(\frac{2D_m}{u}\right) + \left(\frac{2k}{3(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u + \left(\frac{1+6k+11k^2}{96(1+k)^2}\right)\left(\frac{d^2}{D_m}\right)u$$

$$H_{min} = 2^*(BC)^{1/2}$$

$$u_{opt} = (B/C)^{1/2}$$

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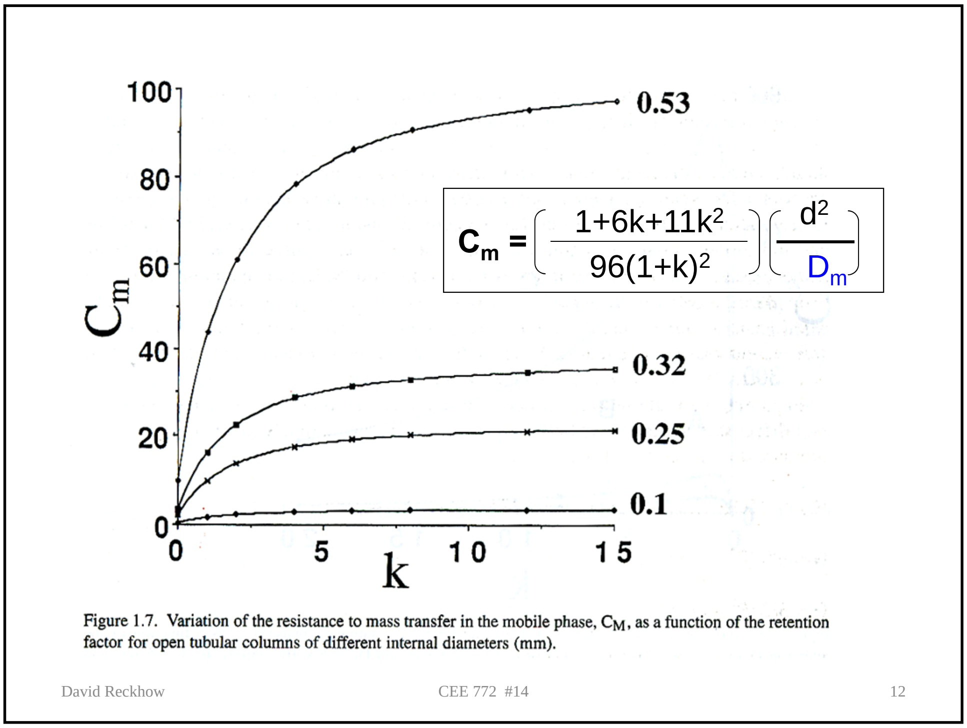

$$C_m = \left(\frac{1+6k+11k^2}{96(1+k)^2}\right)\left(\frac{d^2}{D_m}\right)$$

Figure 1.7. Variation of the resistance to mass transfer in the mobile phase, C_M, as a function of the retention factor for open tubular columns of different internal diameters (mm).

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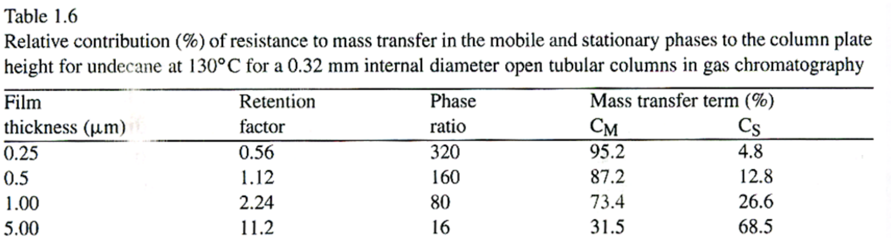


Table 1.6

Relative contribution (%) of resistance to mass transfer in the mobile and stationary phases to the column plate height for undecane at 130°C for a 0.32 mm internal diameter open tubular columns in gas chromatography

Film thickness (μm)	Retention factor	Phase ratio	Mass transfer term (%) C_M	C_S
0.25	0.56	320	95.2	4.8
0.5	1.12	160	87.2	12.8
1.00	2.24	80	73.4	26.6
5.00	11.2	16	31.5	68.5

$$C_S + C_M = \left(\frac{2k}{3(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right) + \left(\frac{1+6k+11k^2}{96(1+k)^2}\right)\left(\frac{d^2}{D_m}\right)$$

$$H = B/u + (C_S + C_M)u$$

The ratio of C_S and C_m contributions to the term of resistance to mass transfer is determined by the phase ration.

$$(V_m/V_s) = d/4d_f\text{ , when, } d >> d_f$$

The Effect of Carrier Gas

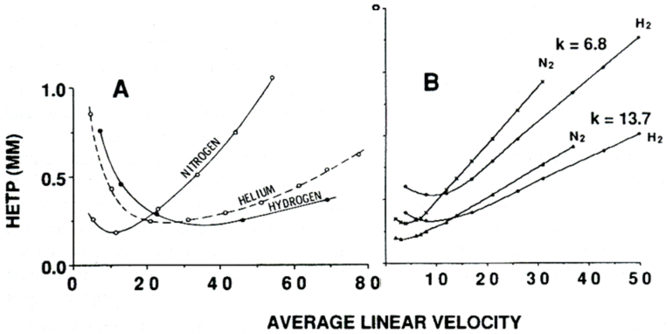


Figure 2.1. Van Deemter plots indicating the influence of the choice of carrier gas on column efficiency for thin-film (A) and thick-film (B) open tubular columns for solutes with different retention factors.

$$H = B/u + (C_S + C_M)u$$

$$H_{min} = 2*(BC)^{1/2}$$

$$u_{opt} = (B/C)^{1/2}$$

gas

$$\boxed{D_{AB} = \frac{1.00 \times 10^{-3}\, T^{1.75}}{P[(\text{sum } v_i)_A^{1/2} + (\text{sum } v_i)_B^{1/2}]}\left(\frac{1}{MW_A}\ \frac{1}{MW_B}\right)}$$

$$\boxed{D_{AB} = kT/(6\pi\eta_B r_A)}$$ liquid

Parameters affecting plate height

$$H = B/u + (C_S + C_M)u$$

$$\left(\frac{2D_m}{u}\right) + \left(\frac{2k}{3(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u + \left(\frac{1+6k+11k^2}{96(1+k)^2}\right)\left(\frac{d^2}{D_m}\right)u$$

	$\frac{2D_m}{u}$	$\left(\frac{2k}{3(1+k)^2}\right)\left(\frac{d_f^2}{D_s}\right)u$	$\left(\frac{1+6k+11k^2}{96(1+k)^2}\right)\left(\frac{d^2}{D_m}\right)u$
T	↑	↓	↓
u	↓	↑	↑
d_f	—	↑	—
d	—	—	↑
k	—	↓	↑

Preparation of Capillary Column

1. Materials

a. glass: soda-lime (soft) *alkaline*

SiO2 67.7%, Na2O 15.6%, CaO 5.7%, MgO 3.9%, Al2O3 2.8%, BaO 0.8%, and K2O 0.6%

borosilicate (hard), *acidic*

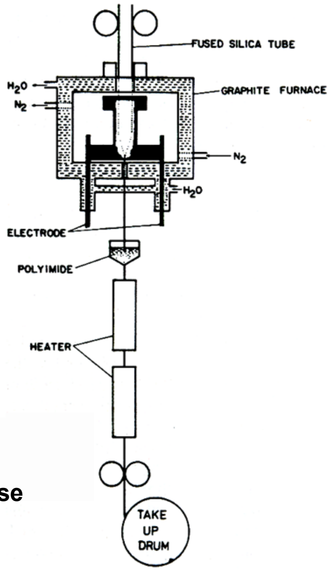
SiO2 67.7%, B2O3 13 %, Na2O 3.0%, Al2O3 2.0%, and K2O 1.%

b. fused silica

$SiCl_4 + O_2 \longrightarrow SiO_2$

Surface: Si—OH, O--SiH-O

Silanol Siloxane



2. Film Formation on Inner Surface of Tubes

(A) Uniform stationary film is essential for high-efficiency separation

Thin, smooth, and homogeneous film

(1) Surface tension (wettability): the surface tension of stationary phase should be smaller than that of glass or fused silica.

(1) The stability of the film depends on the viscosity of liquid and thickness of film (surface tension).

(B) Surface modification

(1) Improvement of wettability of glass surface: HCl (gas)
(2) Deactivation: silylation

(C) Coating Techniques

Dynamic coating, and Static coating

Evaluation of Column Quality

1. Activity test for uncoated columns

2. Grob test for coated columns

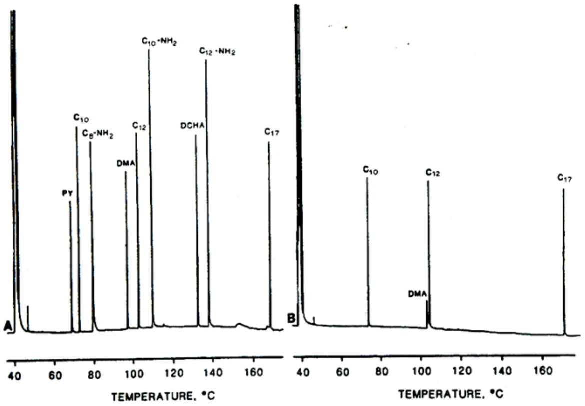


Figure 2.15. Activity test for an uncoated fused silica capillary column after (A) deactivation with poly(phenylmethylhydrosiloxane) and (B) before deactivation. Precolumn: 15 m x 0.20 mm I.D. coated with SE-54. Test columns 10 m x 0.20 mm I.D. The column tandem was programmed from 40 to 180°C at 4°C/min after a 1 min isothermal hold with a hydrogen carrier gas velocity of 50 cm/s. The test mixture contained C_{10} = n-decane, C_8NH_2 = 1-aminooctane, PY = 3,5-dimethylpyrimidine, C_{12} = n-dodecane, $C_{10}NH_2$ = 1-aminodecane, DMA = 2,6-dimethylaniline, DCHA = N,N-dicyclohexylamine, $C_{12}NH_2$ = 1-aminododecane, and C_{17} = n-heptadecane. (From ref. [355]. ©Wiley-VCH).

Grob Test

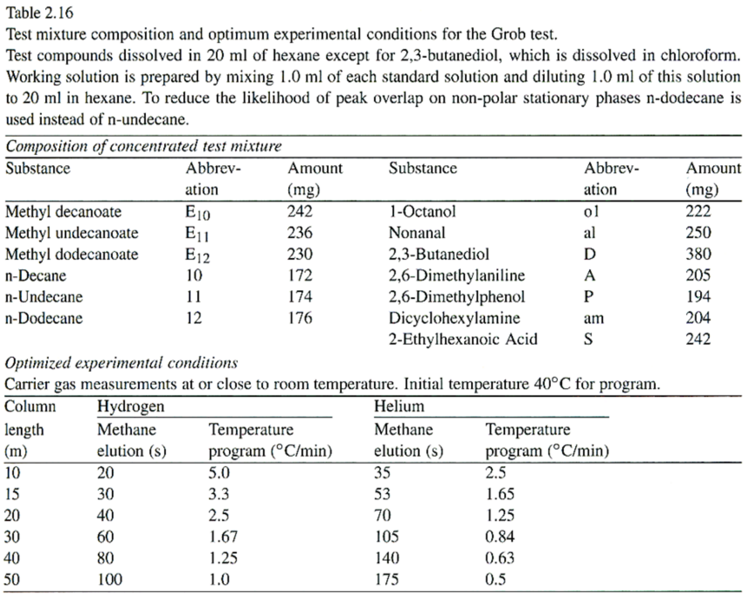


Table 2.16

Test mixture composition and optimum experimental conditions for the Grob test.

Test compounds dissolved in 20 ml of hexane except for 2,3-butanediol, which is dissolved in chloroform. Working solution is prepared by mixing 1.0 ml of each standard solution and diluting 1.0 ml of this solution to 20 ml in hexane. To reduce the likelihood of peak overlap on non-polar stationary phases n-dodecane is used instead of n-undecane.

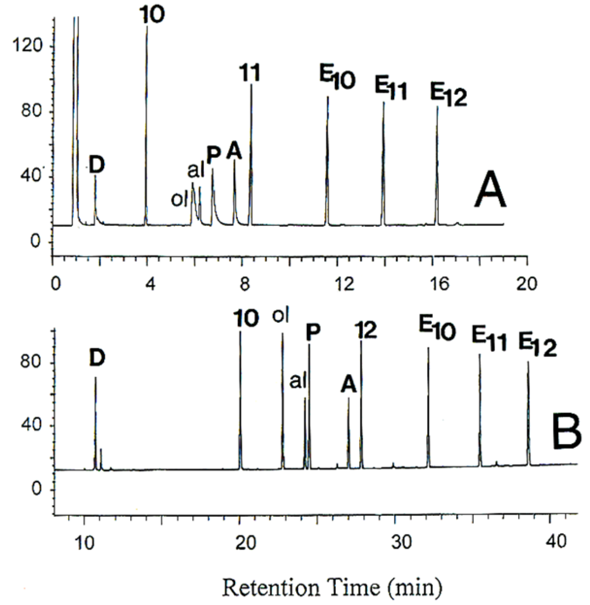
Composition of concentrated test mixture

Substance	Abbrev- ation	Amount (mg)	Substance	Abbrev- ation	Amount (mg)
Methyl decanoate	E_{10}	242	1-Octanol	ol	222
Methyl undecanoate	E_{11}	236	Nonanal	al	250
Methyl dodecanoate	E_{12}	230	2,3-Butanediol	D	380
n-Decane	10	172	2,6-Dimethylaniline	A	205
n-Undecane	11	174	2,6-Dimethylphenol	P	194
n-Dodecane	12	176	Dicyclohexylamine	am	204
			2-Ethylhexanoic Acid	S	242

Optimized experimental conditions

Carrier gas measurements at or close to room temperature. Initial temperature 40°C for program.

Column	Hydrogen		Helium	
length (m)	Methane elution (s)	Temperature program (°C/min)	Methane elution (s)	Temperature program (°C/min)
10	20	5.0	35	2.5
15	30	3.3	53	1.65
20	40	2.5	70	1.25
30	60	1.67	105	0.84
40	80	1.25	140	0.63
50	100	1.0	175	0.5



Old column

New column

(1) The height of the peaks
(2) The shape of the peaks

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3. Columns Thermal Stability

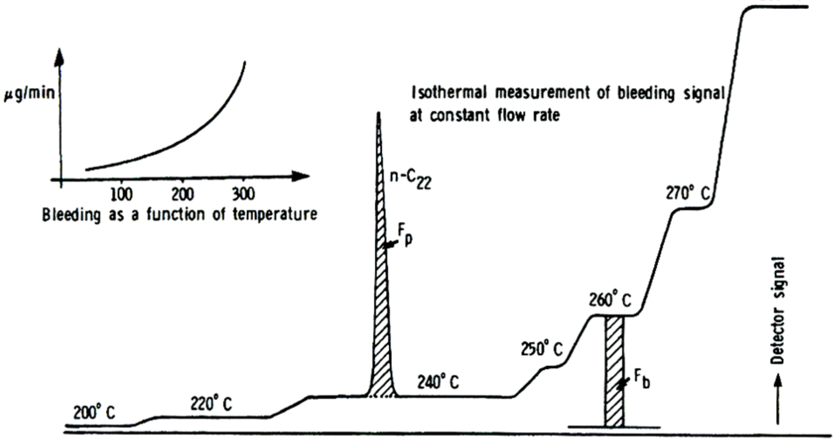


Figure 2.17. Standardized column bleed test. (From ref. [369]. ©Elsevier)

The bleed products from stationary phase consist primarily of low molecular weight impurities. Fused silica columns show very low levels of thermally induced catalytic phase decomposition

Capillary Gas-Liquid Chromatography

A. Separation efficiency and rate theory

B. Preparation of Capillary Column

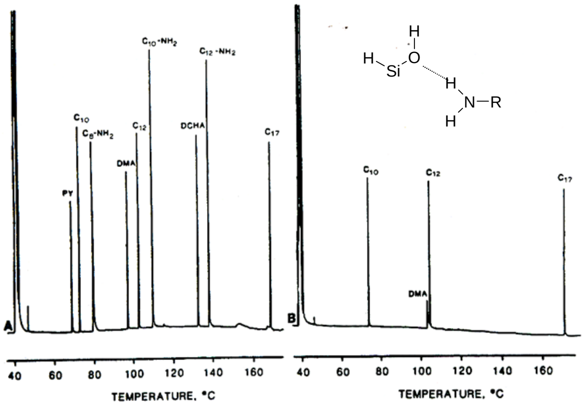
C. Evaluation of Capillary Column

Gas Chromatography

1. Introduction
2. Stationary phases
3. Retention in Gas-Liquid Chromatography
4. Capillary gas-liquid chromatography
5. Sample preparation and inlets
6. Detectors

(Chapter 2 and 3 in The essence of chromatography)

Evaluation of Column Quality



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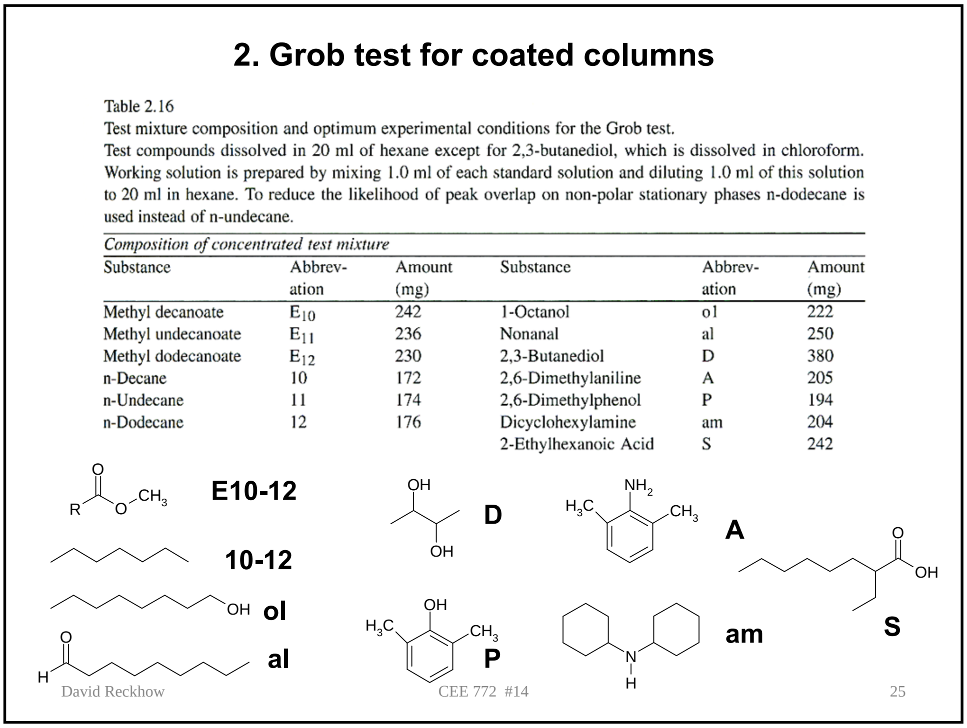
1. Activity test for uncoated columns

-SiO-H



2. Grob test for coated columns

Table 2.16

Test mixture composition and optimum experimental conditions for the Grob test.

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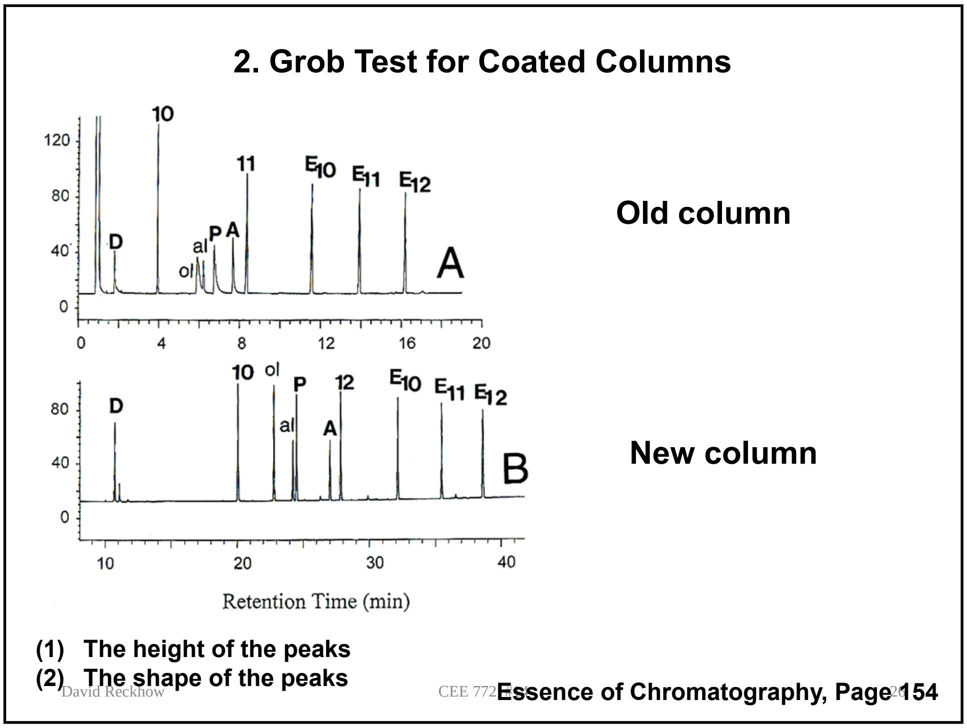
n-Undecane	11	174	2,6-Dimethylphenol	P	194
n-Dodecane	12	176	Dicyclohexylamine	am	204
			2-Ethylhexanoic Acid	S	242



2. Grob Test for Coated Columns

Old column

New column

(1) The height of the peaks
(2) The shape of the peaks

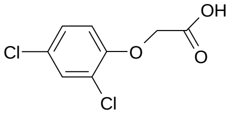
Essence of Chromatography, Page 154

Sample preparation and inlet

A. Sample Preparation:

1. The prerequisite in GC separation is that all solutes being separated must be: (a) fairly volatile, and (b) thermally stable.
   (c) Usually, the solute should be dissolved in a non-aqueous matrix (H_2O changes column behevir).

2. Lack of volatility prevents the direct use of GC for many solute. One way to overcome this difficulty is to *derivatize* the solutes into more volatile forms.



2,4-dichlorophenoxyacetic acid
(A cancer suspect agent).

Silylation

3. Derivatization of a solute can be used for any of the following reasons
   - (a) To increase the volatility of the solute.
   - (b) To increase the thermal stability of solute
   - (c) To improve the response for the solute on certain detectors (e.g., incorporating halogen atoms into a solute so that it can be detected using an electron capture detector).
   - (d) To improve the separation of the solute from other sample components (i.e., changing the structure of a solute will also affect its retention on the column)

4. Most derivatization reactions can be classified into one of three group:
   - (a) Silylation
   - (b) Alkylation
   - (c) Acylation

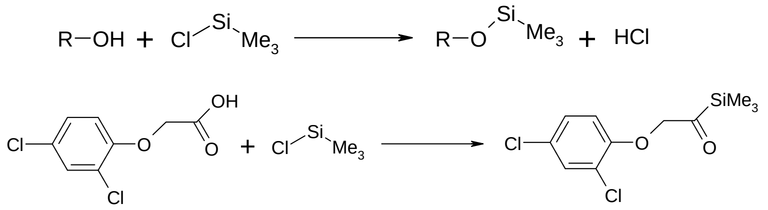
   Most of these reactions are performed using minimal amount of sample and reagents (i.e., 0.1~2.0 mL) are typical carried out at room temperature. Some, however, do require heating to moderate temperatures (60 ~ 100 ^{O}C).

5. Silylation

(a) This is the most common type of derivation techniques used in GC.

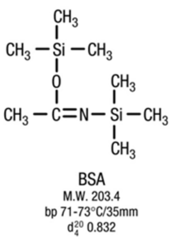
(b) It involves replacing an active hydrogen on the solute (i.e. R-OH, RCOOH, R-NH_2, etc.) with an alkylsilyl group (usually –$SiMe_3$). The result of this reaction is that the solute is converted into a less polar, more volatile and more thermally stable form.

(c) The most common reagent used in silylation is trimethylchlorosilane (TMS). Examples of its use are shown below:

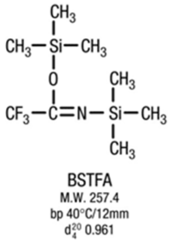


The resulting Product of this reaction is usually just referred to as a TMS-derivative.

(d) Besides trimethylchlorosilane, a number of other silylation reagents can also be used. These reagents have slightly different reactivity from trimethylchlorosilane.



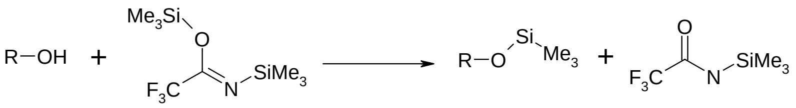
N, O-Bis(trimethylsilyl)acetamide



The byproduct of BSTFA is highly Volatile.

N,O-bis(Trimethylsilyl)trifluoroacetamide

BSA and BSTFA are highly stable TMS derivatives, with most organic functional groups, under mild reaction conditions.



(e) Alylation

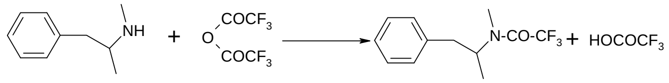
i. Alkylation involves the addition of alkayl group to some active function group on the solute. A common example is esterification of a carboxylic acid, forming a volatile methyl ester. This is commonly done using borontrifluoride in methanol as the reagent.

$$RCOOH + BF_3/MeOH \longrightarrow RCOOMe_3$$

(f) Acylation

i. Acylation involves the conversion of a solute into an acylate derivates. This is often used to improve the volatility of alcohols, phenols, thiols and amine (e.g., -OH, -SH and -NH) containing compounds. As is true for other GC derivations, acylation can also be used to increase the response of a solute to a given detector (e.g., allowing the use of electron capture in solute's detection by including fluorine atoms in the derivitizing agent.

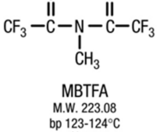
ii. Trifluoroacetic anhydride (TFAA) is one common reagent used for acylation.



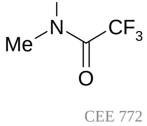
methamphetamine

Drug-of-abuse confirmation testing by GC/MS

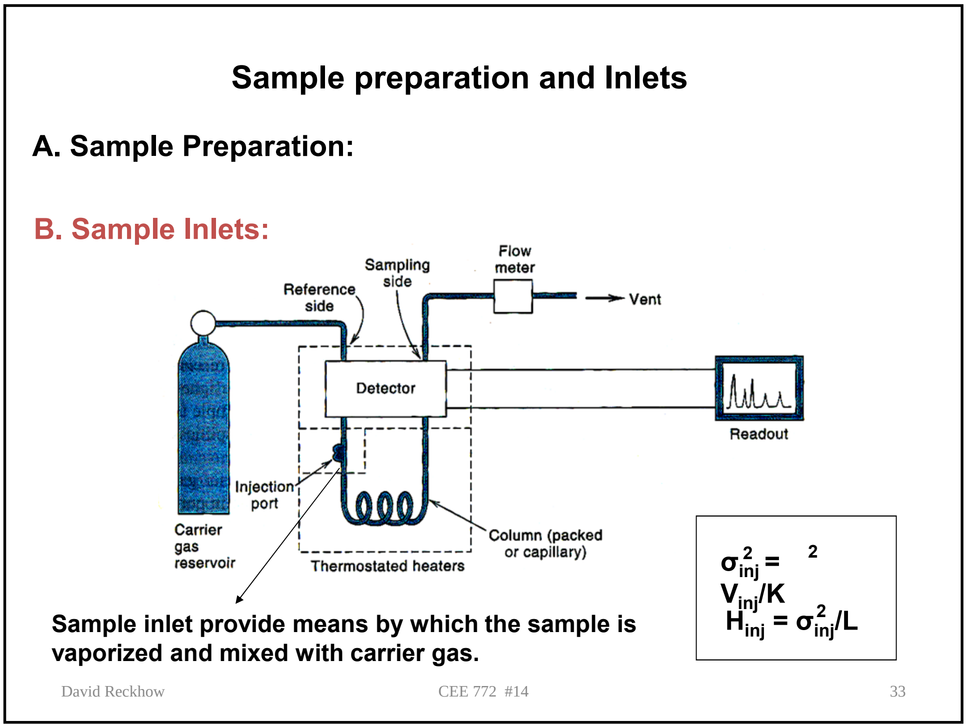
iii.Anther set of reagents used for solute with primary and secondary amines, as well as hydroxyl and thiol groups are N-Methyl-bis[trifluoroacetamide] (MBTFA). The reaction is under mild nonacidic conditions.



MBTFA
M.W. 223.08
bp 123-124°C
d_4^{20} 1.55



Byproduct is volatile



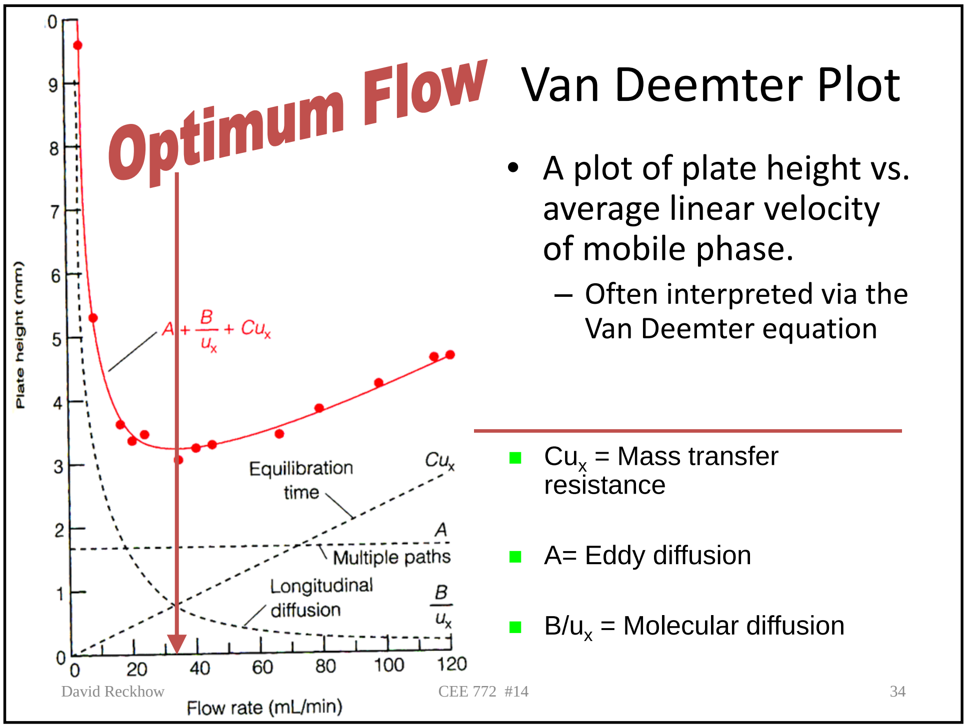
Sample preparation and Inlets

A. Sample Preparation:

B. Sample Inlets:

Sample inlet provide means by which the sample is vaporized and mixed with carrier gas.

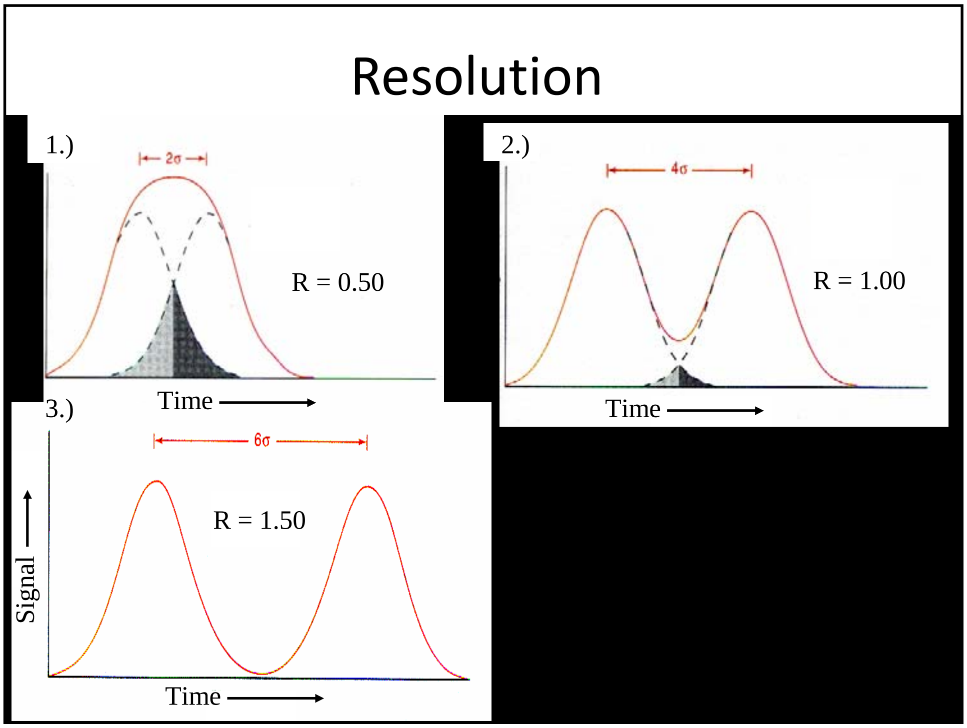
$\sigma_{inj}^2 = \quad ^2$
V_{inj}/K
$H_{inj} = \sigma_{inj}^2/L$



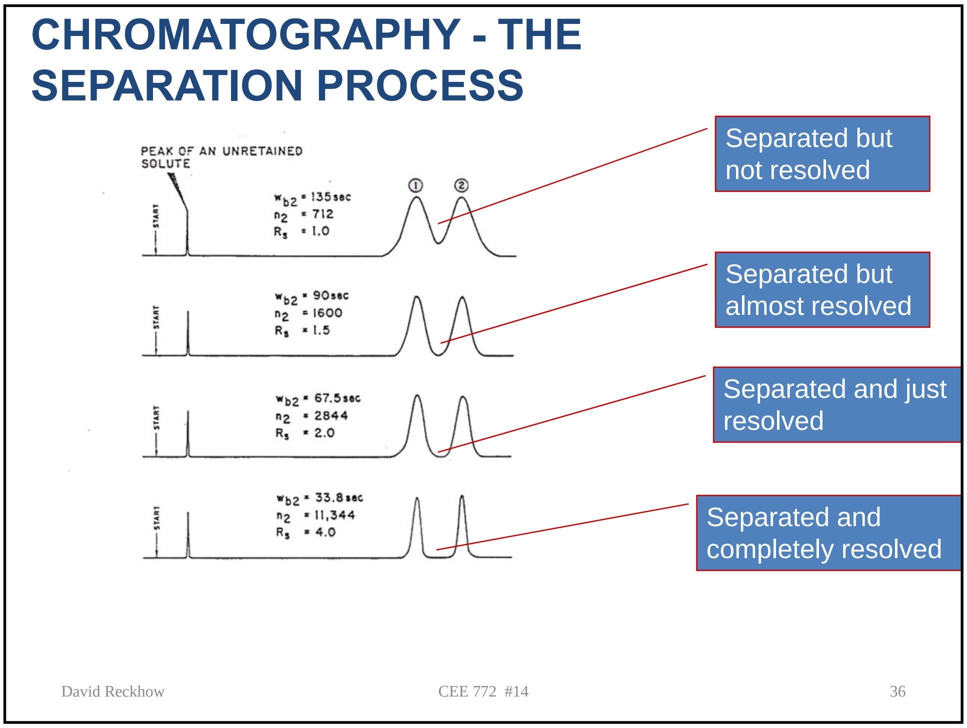
Van Deemter Plot

- A plot of plate height vs. average linear velocity of mobile phase.
  - Often interpreted via the Van Deemter equation

- Cu_x = Mass transfer resistance
- A= Eddy diffusion
- B/u_x = Molecular diffusion



Resolution



CHROMATOGRAPHY - THE SEPARATION PROCESS

Separated but not resolved

Separated but almost resolved

Separated and just resolved

Separated and completely resolved

Resolution

- Although the selectivity factor, α, describes the separation of peaks centers, it does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the *resolution*. The resolution of two species, A and B, is defined as

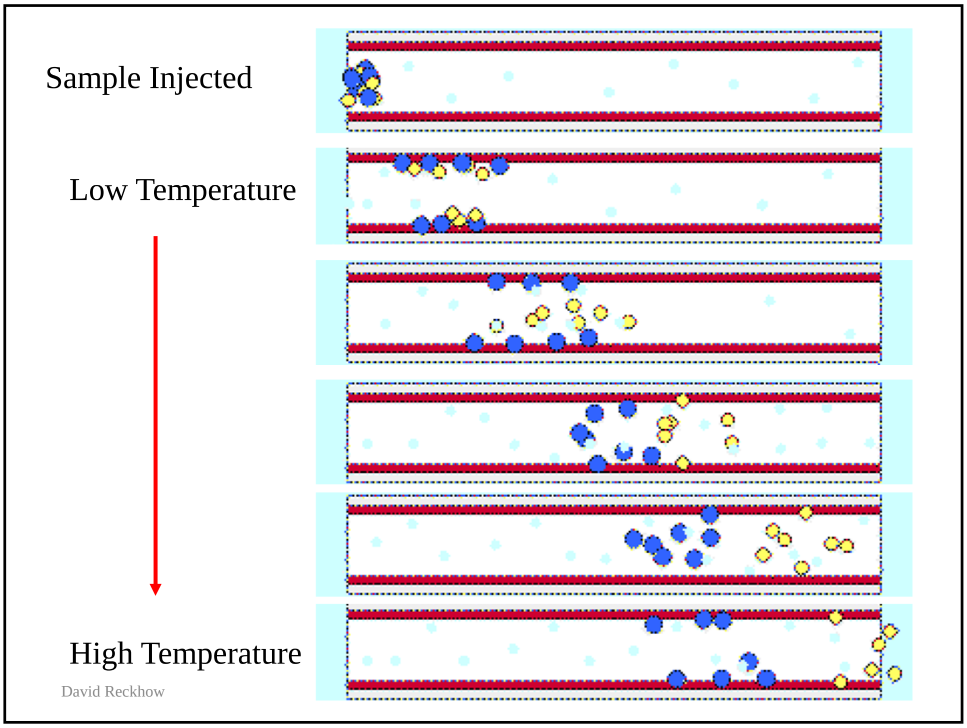
$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

  - Baseline resolution is achieved when *R* =
- It is useful to relate the resolution to the number of plates in the column, the selectivity factor and the retention factors of the two solutes;

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k'_B}{k'_B}\right)$$

Resolution (cont.)

- To obtain high resolution, the three terms must be maximized. An increase in *N*, the number of theoretical plates, by lengthening the column leads to an increase in retention time and increased band broadening - which may not be desirable. Instead, to increase the number of plates, the height equivalent to a theoretical plate can be reduced by reducing the size of the stationary phase particles.
- It is often found that by controlling the capacity factor, *k'*, separations can be greatly improved. This can be achieved by changing the temperature (in Gas Chromatography) or the composition of the mobile phase (in Liquid Chromatography).
- The selectivity factor, α, can also be manipulated to improve separations. When a is close to unity, optimizing *k'* and increasing *N* is not sufficient to give good separation in a reasonable time. In these cases, *k'* is optimized first, and then a is increased by one of the following procedures:
  - Changing mobile phase composition
  - Changing column temperature
  - Changing composition of stationary phase
  - Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase)



Sample Injected

Low Temperature

High Temperature

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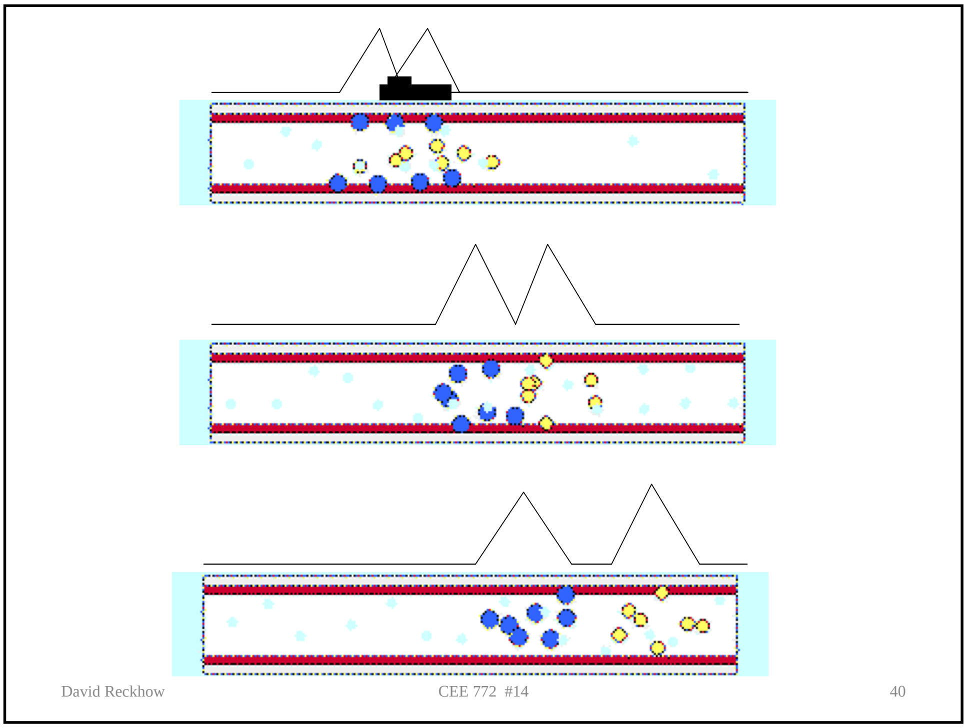

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CEE 772 #14

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GC: Major Components

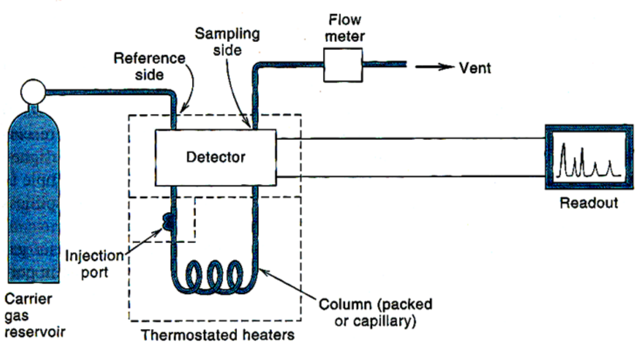
- Injectors
  - Need to rapidly convert liquid sample into vapor
  - Flash vaporization, splitless, split
- Columns
  - Packed, capillary
- Detectors
  - FID, ECD, TCD, NPD, PID

Mobile Phase

- **Carrier Gas:**
  - E.g. : - Hydrogen, Helium and Nitrogen
- Properties of carrier gas :
  - Inert
  - Able to minimize gas diffusion
  - Readily available and pure
  - Inexpensive
  - Suitable for the detector used
- Control
  - Flow controller and pressure regulator
  - Desire constant flow rate even with changes in temperature
    - Gas viscosity changes,

Sample Inlets: injectors

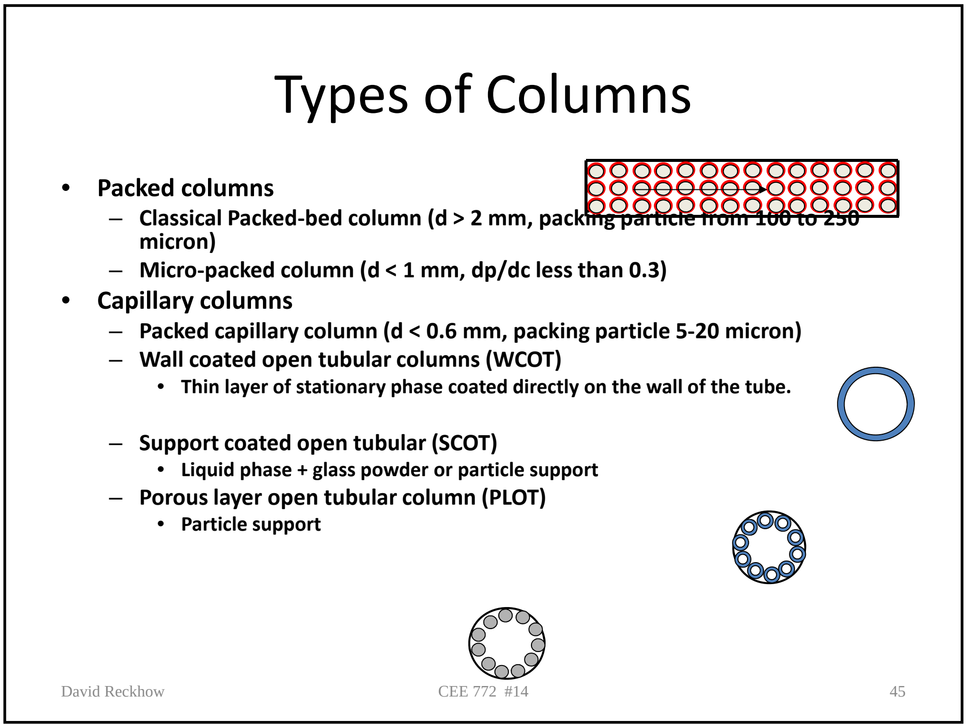
- **Sample inlet provide means by which the sample is vaporized and mixed with carrier gas.**



$\sigma_{inj}^2 =$ ^{2}
V_{inj}/K
$H_{inj} = \sigma_{inj}^2/L$

Sample Introduction

- Injectors
  - – Need to rapidly convert liquid sample into vapor
  - – Flash vaporization, splitless, split
    - Introduced instantaneously as a plug onto the column.
    - Gases are introduced by gas tight syringes.
    - Liquids are handled with syringes.
    - Solids are usually introduced as solution in a solvent



Types of Columns

- **Packed columns**
  - **Classical Packed-bed column (d > 2 mm, packing particle from 100 to 250 micron)**
  - **Micro-packed column (d < 1 mm, dp/dc less than 0.3)**
- **Capillary columns**
  - **Packed capillary column (d < 0.6 mm, packing particle 5-20 micron)**
  - **Wall coated open tubular columns (WCOT)**
    - **Thin layer of stationary phase coated directly on the wall of the tube.**
  - **Support coated open tubular (SCOT)**
    - **Liquid phase + glass powder or particle support**
  - **Porous layer open tubular column (PLOT)**
    - **Particle support**

- To next lecture