Gas and liquid chromatography rely on different interactions.

The separation process in either case can still be described using the same general theory.

Chromatographic Theory

The separation process

Sample components are carried by a mobile phase through a bed of stationary phase.

Individual species are retarded by the stationary phase based on various interactions such as:

Surface adsorption, Relative solubility

Size, Charge, Vapor pressure

Phase of the moon

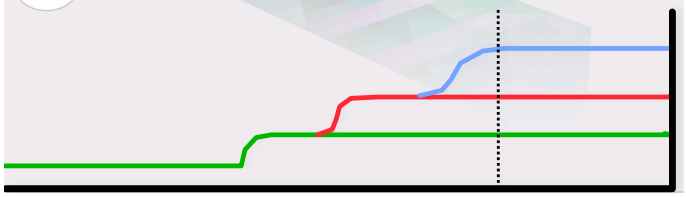
Power of positive thinking

Types of separation

Frontal analysis

- Continuously add your sample to the start of the column.
- Monitor components as they evolve.
- Gives a general measure of how things are retained.
- Example - charcoal filtration

Frontal analysis



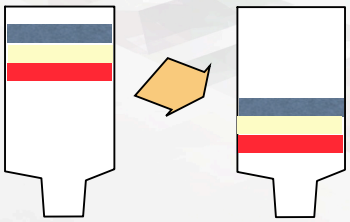
sample volume

Approach an be use to evaluate relative retention. Not useful as a method of separation.

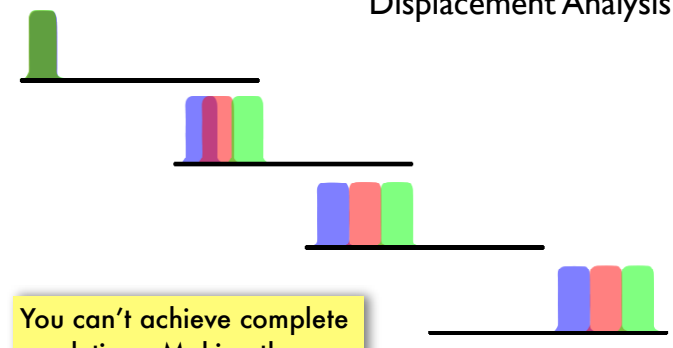
Displacement Analysis

Materials move down a column by being displaced by a more highly retained solute.

Example - ion exchange / water softeners

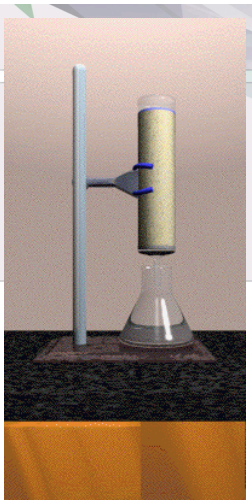


Displacement Analysis



You can't achieve complete resolution. Making the column longer has no effect

Elution

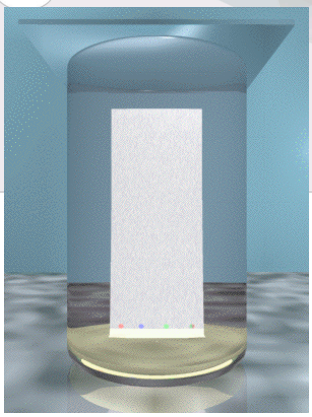


A solute partitions between two phases (equilibrium).

Separation is based on relative retention.

Making the column longer will increase the degree of separation.

Elution



Coated plates or paper can also be used - 2D methods.

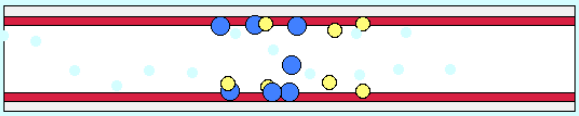
The basic competition for the solutes in the stationary and mobile phases remain the same.

Elution

Many types of competitive attractions can be used.

Example

In GLC it's vapor pressure vs. solute solubility in the stationary phase.



Theories

Two approaches can be taken to explain the separation process.

Plate theory - proposed in 1941 by Martin and Synge. Based on an analogy with distillation and countercurrent extraction.

Rate theory - accounts for the dynamics of a separation - 1956, J.J. van Deemter.

Each has its own advantages and limitations.

Plate theory

In distillation

- Actual plates exist where vapor passes through a liquid phase.
- During this mixing, equilibrium between the phases is assumed.
- The height of a plate can often be directly measured.
- In a chromatographic column, the plates can't be observed - called theoretical plates.

Plate theory

If the plate can be observed, we can measure the plate height.

If it can't be directly observed, we can calculate the height equivalent for a theoretical plate (HETP or h)

This concept was extended to explain the chromatographic process.

Plate theory

Some definitions

Partition coefficient

$$K = \frac{[\text{Solute}]_{\text{Mobile phase}}}{[\text{Solute}]_{\text{Stationary phase}}}$$

K is assumed to be independent of concentration. It can be altered by such factors as temperature.

$$K_P = \frac{[Z]_1}{[Z]_2}$$

From solvent extraction

Plate theory

Capacity ratio, k

$$k = K(V_m/V_s) = K\beta$$

where β, the phase ratio, is equal to the ratio between the volume of each phase.

Solute fractions

mobile phase = 1 / (1 + k)

stationary phase = k / (1 + k)

Plate theory

Retention factor, R_f

Measure of solute velocity through a column, compared to mobile phase.

It's a function of the capacity ratio (k) derived as:

$$U_{avg} = f_1v_1 + f_2v_2 + \ldots + f_nv_n = \Sigma f_iv_i$$

where f = the fraction of molecules at velocity v.

Plate theory

With chromatographic bands, there are only two fractions and velocities.

- one for the mobile phase
- another for the stationary phase

The fraction in the mobile phase moves at the same velocity as the mobile phase.

The fraction in the stationary phase is considered as having a v = 0.

Plate theory

$$u_{avg} = v_m\frac{1}{1+k} + v_s\frac{1}{1+k}$$

Since Vs ≅ 0 *(velocity in stationary phase)*

$$u_{avg} = \frac{v_m}{1+k}$$

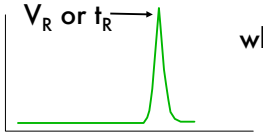
$$R_f = \frac{u_{avg}}{v_m} = \frac{v_c}{v_m} = \frac{1}{1+k}$$

where v_c is the velocity of a component with a capacity ratio of k

Retention time and volume

Retention volume, V_R - volume of mobile phase required to elute a solute to a maximum from a column.

Retention time, t_R, time required to reach the same maximum at constant flow.



where $V_R = t_R$ x flowrate

Retention time and volume

For a constant column length:

$$\frac{t_R}{t_m} = \frac{V_R}{V_M} = 1 + k$$

where t_m = retention time for mobile phase.

$$t_R = t_m(1 + k)$$

Retention times

- In GC, t_R is also a function of the inlet and outlet pressures.
- As a result, solute and mobile phase velocities will vary along the column.

- This expansion will contribute to some broadening of peaks.

Mechanism of component separation

If solutes 1 and 2 have capacity ratios of k_1 and k_2, then their retention times, t_{R1} and t_{R2} are given by:

$$t_{R1} = t_M (1 + k_1) \quad \text{and} \quad t_{R2} = t_M (1 + k_2)$$

Peak separation is equal to:

$$t_{R2} - t_{R1} = t_M (k_2 - k_1)$$

assuming $t_{R2} > t_{R1}$

Mechanism of component separation

Mobile phase volume is proportional to column length so retention is also increased for longer columns.

However, as peaks travel through the column, they broaden. Width increases with the square root of column length.

You can't just make a column longer to obtain a 'better' separation.

Plate theory of chromatography

According to plate theory, a column is mathematically equivalent to a distillation plate column.

Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
v_m 2	v_m 2	v_m 3	v_m 4	v_m 5
v_s 1	v_s 2	v_s 3	v_s 4	v_s 5

Total length is divided into N segments each representing an equilibrium stage or theoretical plate.

An equilibrium is established at each stage as the mobile phase passes from one stage to the next.

Plate theory of chromatography

Assuming that the phases are not compressible, we have:

$$V_m = v_m N$$

$$V_s = v_s N$$

where V = total volume and

v = volume of each stage

N = number of plates

Plate theory of chromatography

A differential material balance of a solute around plate n gives:

$$C_{n-1}dv - C_n dv = d[(v_m + kv_s)C_n]$$

where v_m = plate volume of mobile phase
C_n = [solute] in plate n
k = partition coefficient

so $\frac{dC_n}{dv} + aC_n = aC_{n-1}$ and $a = \frac{1}{v_m + kv_s}$

a is a constant for a given column and solute.

General elution equation

This approach assumes that initially there is solute in each plate at a concentration of C_n° and that mobile phase enters plate 1 free of solute.

$$C_0 = 0$$

$$\frac{dC_1}{dv} + aC_1 = 0$$

Integrating gives $C_1 = C_1^0 e^{-av}$ for plate 1

General elution equation

Going to plate 2 yields

$$\frac{dC_2}{dv} + aC_2 = C_1^0 e^{-av}$$

and with integration gives

$$C_2 = C_2^0 e^{-av} + C_1^0 (av) e^{-av}$$

For N plates, we get:

$$C_N = \sum_{r=1}^{N-1} C_r^0 \frac{(av)^{N-r}}{(N-r)!} e^{-av}$$

General elution equation

Under actual usage conditions, we only add solute at the start of the column - in the mobile phase. As a result:

[solute]=C_o in plate 1 but = 0 in all others.

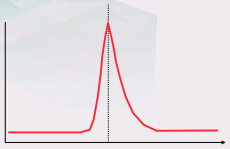
At plate 1 $\frac{dC_1}{dv} + aC_1 = aC_0$

At plate N $C_N = C_0\left(1 - \sum_{r=1}^{N-1} \frac{(av)^N}{(r)!}\right)e^{-av}$

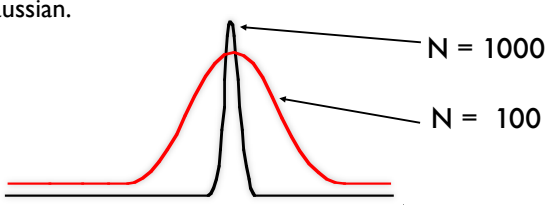
$= C_0 P_N^{av}$

General elution equation

P_N^{av} is a Poisson summation indicating that the peaks are not really Gaussian.



However, as the number of plates increase (n>>100), peak shape approaches Gaussian.



Determination of N

The peak produced by an eluent can be used to determine the number of theoretical plates in a column.

From the properties of a Poisson distribution we find that

$a\bar{v} = N$

Points where the peak intercepts the baseline can be used to determine peak width (w)

Determination of N

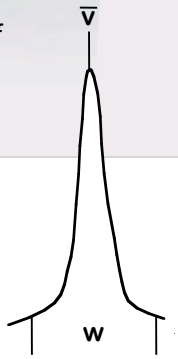
Once the width is known, the number of plates can be determined as:

$$a\ w = 4\ N^{1/2}$$

Combining the whole mess gives:

$$N = 16\ (\bar{v} / w)^2$$

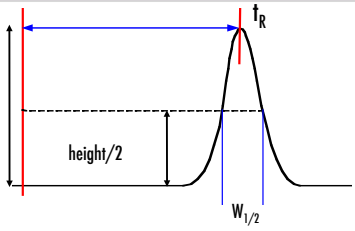
which can be used to calculate N from experimental data.



Determination of N

Since it is difficult to accurately measure the beginning and end of a peak, it is common to use the width at half height and assume the peak is Gaussian.

$$N = 5.54\left(\frac{t_R}{W_{\frac{1}{2}}}\right)^2$$



Resolution

Knowing how well a column can retain a component is nice but we need to deal with multiple eluents or why bother.

Resolution, R_s

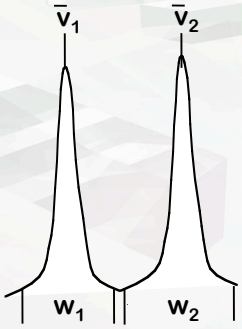
A measure of how completely two neighboring peaks are separated from each other.

Resolution

$$R_S = \frac{\bar{v}_2 - \bar{v}_1}{\frac{w_2 + w_1}{2}}$$

since $w = \frac{4\bar{v}}{\sqrt{N}}$

$$R_S = \frac{\sqrt{N}}{2}\frac{\bar{v}_2 - \bar{v}_1}{\bar{v}_2 + \bar{v}_1}$$



Resolution

Since $v = V_m(1 - k)$

resolution in terms of k is:

$$R_S = \frac{\sqrt{N}}{2}\frac{k_2 - k_1}{2 + k_2 + k_1}$$

From our coverage of distillation, the relative volatility, α, is:

$$\alpha = \frac{k_2}{k_1}$$

Resolution

$$R_S = \frac{\sqrt{N}}{2}\frac{\alpha - 1}{\alpha + 1 + \frac{2}{k_1}}$$

Finally, if we define k as $(k_1+k_2)/2$ - average k

$$R_S = \sqrt{\frac{N}{4}}\ \frac{\alpha - 1}{\alpha + 1}\ \frac{k}{1 + k}$$

This version of the resolution equation will be most useful with LC - or an approximation.

Approximate resolution equations

The exact resolution equation is:

$$R_s = \sqrt{\frac{N}{4}}\, ln\left(1 + \frac{k_2 - k_1}{1 + k_1}\right)$$

$k_2 \simeq k_1$, when $\frac{k_2 - k_1}{1 + k_1}$ is small compared to 1.

and $ln(1 + x) \simeq x$

For a small x, $R_s = \sqrt{\frac{N}{4}}\left(\frac{k_2 - k_1}{1 + k_1}\right)$

Approximate resolution equations

Since $\alpha = k_2/k_1$, we get the Knox equation.

$$R_s = \sqrt{\frac{N}{4}}(\alpha - 1)\frac{k_1}{1 + k_1}$$

Similarly, if we start with:

$$R_s = \sqrt{\frac{N}{4}}\, ln\left(1 - \frac{k_2 - k_1}{1 + k_1}\right)$$

This will lead to the Purnell equation.

$$R_s = \sqrt{\frac{N}{4}}\frac{\alpha - 1}{\alpha}\frac{k_2}{1 + k_2}$$

Approximate resolution equations

Each version of the equation will yield R_s values that deviate from the exact solution.

- Knox - R_s values will be higher
- Purnell - R_s values will be lower

  (a more conservative estimate)

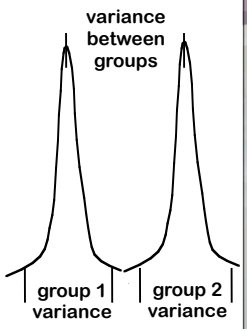
ANOVA basis for resolution

We can also define resolution in statistical terms.

mean square = σ^2

= variance

The F test can tell if the two groups are different



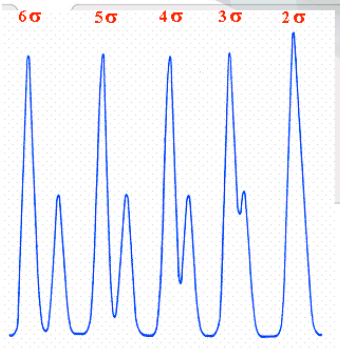
ANOVA basis for resolution

F can be determined by

$$F = \frac{\sigma^2_{between}}{\sigma^2_{within}}$$ **average for the two groups**

An F value of 1 indicates that there is no overlap between the groups. A value of 0.95 would indicate a 5% common area.

ANOVA basis for resolution



For baseline resolution, you must have a sigma between difference of 6.

However for most assays, a value of 4 is considered adequate.

This is assuming that the peaks have similar peak widths.

Working resolution equations

The following provides an easy way to calculate the resolution from experimental data.

$$R_S = \frac{\bar{v}_2 - \bar{v}_1}{\frac{w_2 + w_1}{2}} = \frac{t_{R_2} - t_{R_1}}{\frac{w_2 + w_1}{2}}$$

You can see that we are looking at the 'difference between groups(peaks)' divided by the 'average variance within the groups.

Working resolution equations

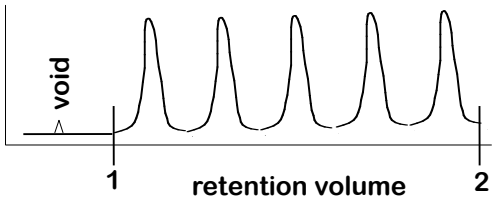
- It can be difficult to accurately measure the width of a peak due to low sensitivity near baseline or peak overlap.
- If we assume that the peaks are Gaussian, we can use the following:

$$R_S = 1.18\frac{t_{R_2} - t_{R_1}}{w_{1/2_2} + w_{1/2_1}}$$

$W_{1/2}$ = width at half height.

Peak capacity

A measure of how many peaks could be totally separated between any two points on a chromatogram.



Peak capacity

- A measure of the number of components that can be separated
- Based on isothermal/isocratic conditions.
- Assumes that peak width increases linearly with t_R
- Resolution may be considered as a special case of peak capacity.
- Points 1 and 2 correspond to the maximums for 2 adjacent peaks.

Peak capacity

The total peak capacity (n_t) is the peak capacity between the void peak maximum and the maximum of the last peak.

Totally separated peaks assume that the resolution is 1 or greater based on the Purnell equation.

Peak capacity

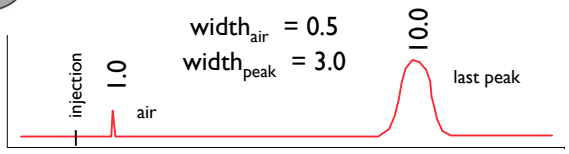
If we assume a constant peak width, the number of separated peaks possible between points 1 and 2 is:

$$n = (v_2 - v_1) / w$$

While this will provide a 'quick and dirty' estimate, it neglects that facts that peak width varies according to:

$$w = 4\bar{v} / N^{1/2}$$

Peak capacity example



w = (3.0+0.5)/2 = 1.75

n = ($\bar{v}_2$ - $\bar{v}_1$) / w = (10.0 - 1.0) / 1.75

= 5.1

The maximum peaks you could separate would be 5 as a best case example. This is only for packed columns - does not hold for capillary columns.

Example

Determine the k, N and HETP for toluene in the following analysis

Solute	t_R, min	$W_{1/2}$, min
air	1.5	
benzene	7.45	1.05
toluene	10.6	1.45

Column length = 10 meters

Flow rate = 30 ml/min

isothermal conditions

Example

$$k = \frac{t_R}{t_M} = 1 + k$$

$k = t_R / t_M - 1$

$= 10.6 / 1.50 - 1$

$= 6.07$

Example

$$N = 5.54 \left(t_R / W_{1/2} \right)^2$$

$= 5.54 (10.6 / 1.45)^2$

$= 296$

HETP

$h = 1 / N = 1000 \text{ cm} / 296$

$= 3.38$ cm

Example

Now calculate the peak capacity between the air and toluene peak.

Assume an average peak width of 2.0 min.

Peak capacity = ($t_{toluene}$ - t_{air}) / 2.0 min

= 4.55

Example

Finally, calculate the resolution between the benzene and toluene peaks.

$$R_S = 1.18 \frac{t_{R_2} - t_{R_1}}{W_{1/2_2} + W_{1/2_1}}$$

= 1.18 (10.6-7.45)/(1.05+1.45)

= 1.48 (quantitative separation)

Modified plate model

Simple peak capacity and resolution equations for packed columns don't work well for capillary columns.

Reason - we assumed that peak width was proportional to retention volume, measured from point of injection.

Modified plate model

When plotting width vs. retention time or volume, we get a straight line. For any 2 points on a chromatogram:

$$\frac{w_1}{\bar{V}_1} = \frac{w_2}{\bar{V}_2} = \frac{dw}{d\bar{V}}$$

and the number of theoretical plates is:

$$N = 16\left(\frac{\bar{V}_1}{w_1}\right)^2 = 16\left(\frac{\bar{V}_2}{w_2}\right)^2 = 16\left(\frac{d\bar{V}}{dw}\right)^2$$

Modified plate model

When point 1 is taken as the air peak, then

$\bar{V}_2 - \bar{V}_1 = \bar{V}'$ (adjusted retention volume)

$t_{R_2} - t_{R_1} = t'_R$ (adjusted retention time)

The number of plates can then be determined by

$$N = 16\frac{\bar{V}'^2}{(w - w_o)^2} = 16\frac{t_R'^2}{(w - w_o)^2}$$

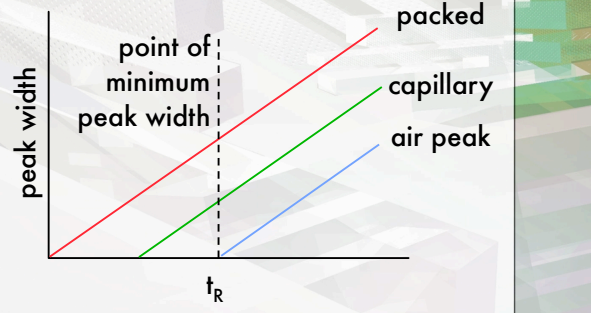
Where w_o is the width of the air peak.

Modified plate model

For capillary columns, a plot of width vs. retention volume still results in a straight line but it does not go through the point of injection.

The better the column, the closer the intercept is to the air peak.

Modified plate model



This shows that peaks produced on a capillary column are narrower than predicted by theory.

Modified plate model

To account for this behavior, a modified definition for number of plates is used.

$$N_{real} = 16\left(\frac{\bar{V}_2 - \bar{V}_1}{w_2 - w_1}\right)^2 = 16\left(\frac{d\bar{V}}{dw}\right)^2$$

If peak 1 is the air peak, then

$$N_{real} = 16\left(\frac{\bar{V}'}{w - w_o}\right)^2$$

$N_{real} \neq N$

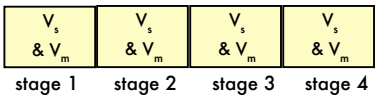
Modified plate model

A modified plate model is necessary.

From distillation theory

Our original model was based on the separation occurring in a series of stages.

V_s is in equilibrium with V_m

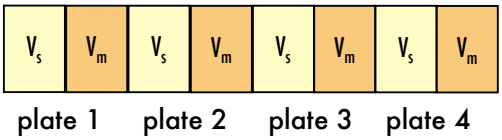


Modified plate model

Plate model

- from solvent extraction theory

In this model, V_S is considered as being in equilibrium with Δv - the V_M on either side.

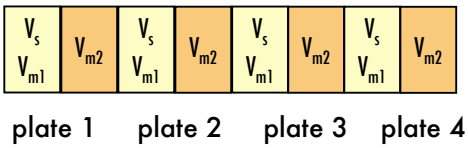


Modified plate model

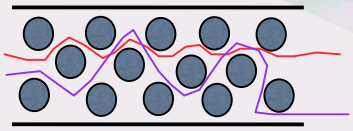
Modified plate model.

In this model, V_s is in equilibrium with V_{M1}

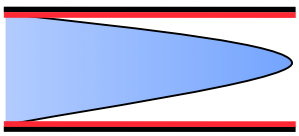
This assumes incomplete mixing of the phases due to streamline flow in the column.



Packed vs. capillary flow



Packed column



Capillary column

Modified plate model

The stage and plate models actually represents extremes.

Packed columns come close to a pure plate model.

Capillary columns are better represented by the modified model.

Rate theory of chromatography

Plate theory assumes that a column is mathematically equivalent to a plate column.

An equilibrium is established for the solute between the mobile and stationary phases one each plate.

It is a useful theory and can predict many aspects of chromatographic performance.

Rate theory of chromatography

Plate theory neglects the concepts of solute diffusion and flow paths.

Rate theory accounts for these and can be used to predict the effect on column performance factors such as:

- phase properties
- solute diffusivitivites
- partition coefficients
- phase velocity
- phase thickness
- support size
- support porosity
- flow rates

Rate theory of chromatography

A partial differential equation set up by van Deemter for a linear isotherm resulted in an effluent concentration function.

It is based on a Gaussian distribution similar to that of plate theory.

He was attempting to account for the dynamics of the separation process.

Van deemter equation

$$H = 2\lambda d_P + \frac{2\gamma D_g}{u} + \frac{8}{\pi}\frac{k d_f^2}{(1+k)^2 D_l}u$$

- λ - factor characteristic of packing
- d_p - particle diameter
- γ - factor for irregularity of interparticle spaces
- D_g - diffusion coefficient of compound in gas
- D_l - diffusion coefficient of compound in liquid
- u - linear gas velocity
- k - capacity ratio
- d_f - liquid phase effective film thickness
- H - height of a theoretical plate

Van deemter equation

The equation consists of three basic terms.

$2\lambda d_P$ Packing related term

$\frac{2\gamma D_g}{u}$ Gas (mobile phase) term

$\frac{8}{\pi}\frac{k d_f^2}{(1+k)^2 D_l}u$ Liquid (stationary phase) term

Van deemter equation

Commonly group the various constants into single terms and reduce the equation to:

$$H = A + B/u + Cu$$

- A - multipath or eddy diffusion $2\lambda d_P$
- B - molecular diffusion $\frac{2\gamma D_g}{u}$
- C - resistance to mass transfer $\frac{8}{\pi}\frac{k d_f^2}{(1+k)^2 D_l}u$

Note that A, B and C are constants but the effect of B and C is dependent of the velocity of the mobile phase.

Modifications to the Van deemter equation

Giddings modified the equation to account for the observed coupling of the A and C terms

$$H = \left(\frac{1}{\frac{1}{A} + \frac{1}{c_l u}}\right)\frac{B}{u} + c_l u + c_g u + H_e$$

c_l and c_g are functions of D_g

c_l is a function of D_l

H_e is a constant for the equipment

Modifications to the Van deemter equation

Golay also modified the relationship - to account for capillary columns. This relationship can be reduced to:

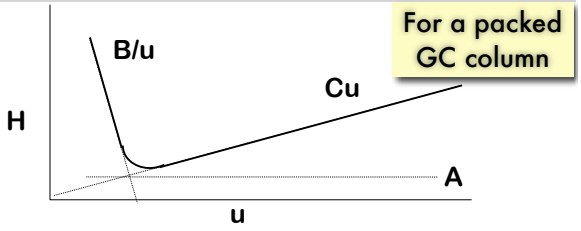
$$H = \frac{B}{u} + C_{1u} + C_{0g}$$

Note - the A term is missing because there is no packing in a capillary column.

Van deemter equation

Regardless of the relationship used, the goal is to find H_{min} for optimum column performance.

$$H = A + B/u + Cu$$



Van deemter terms

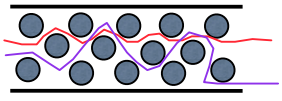
You don't need to calculate H for each column/eluent combination to be able to use this relationship.

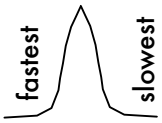
An understanding of the effects of each term will help you design/select appropriate columns and optimum flows.

A term

Multipath or eddy diffusion

This term accounts for the effects of packing size and geometry.





The range of possible solute paths results in a minimum peak width.

A term

Once the column is packed, nothing can be done to reduce the A term.

Its effect can be reduced by using

- regular sized packing
- small diameter packing
- firmly packed material
- no dead space in the column

B term

Molecular diffusion

Represents broadening due to diffusion in the mobile phase.

Reverse diffusion is more significant than forward due to mobile phase movement.

B term

The effect of the B term is flow dependent. As you increase the flow, the time for diffusion is reduced.

You should keep the flow as high as possible within the limits imposed by the instrument and the C term.

C term

Resistance to mass transfer.

- It take time for a solute to reach an equilibrium between the mobile and stationary phases.
- Thick or viscous stationary phases have larger C terms

C term

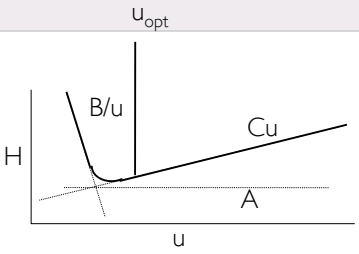
You can minimize the effect of the C term by:

- Using "thin" coatings of the stationary phase on a solid support.
- Use less viscous phases.
- Keep the flow as low as possible - limited by the effect of the B term.

Optimum velocity

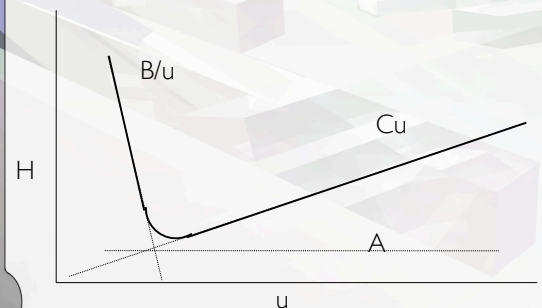
The best velocity is a function of the Van deemter equation and practical conditions. You need to have a useable analysis time.

Also, since the effects of B are greater than C, it is best to set the flow a little on the high side in case it changes slightly during the analysis.



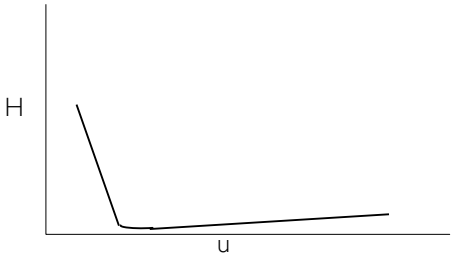
Van Deemter plots

This plot is for a typical packed column GC analysis. Gases have high diffusions and column packings are relatively large.



Capillary columns

Not much effect from A or C. There is no packing and the phase is very thin



Liquid chromatography

At first, LC relied on irregular packing. Now the packings are pretty good so the A term is very low.

The B and C terms are low because liquids diffuse much more slowly than gases.