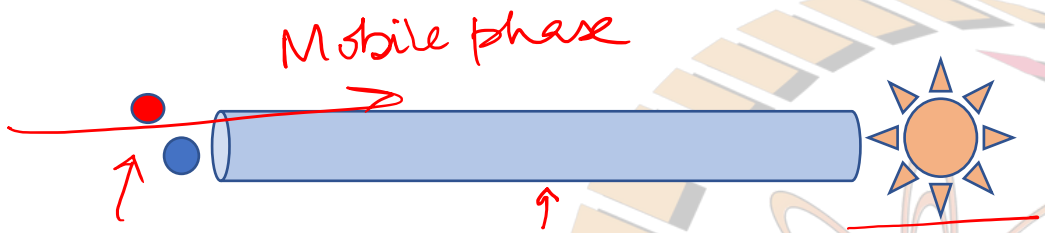
Quantitative Methods in Chemistry

Week 10, Lecture 1

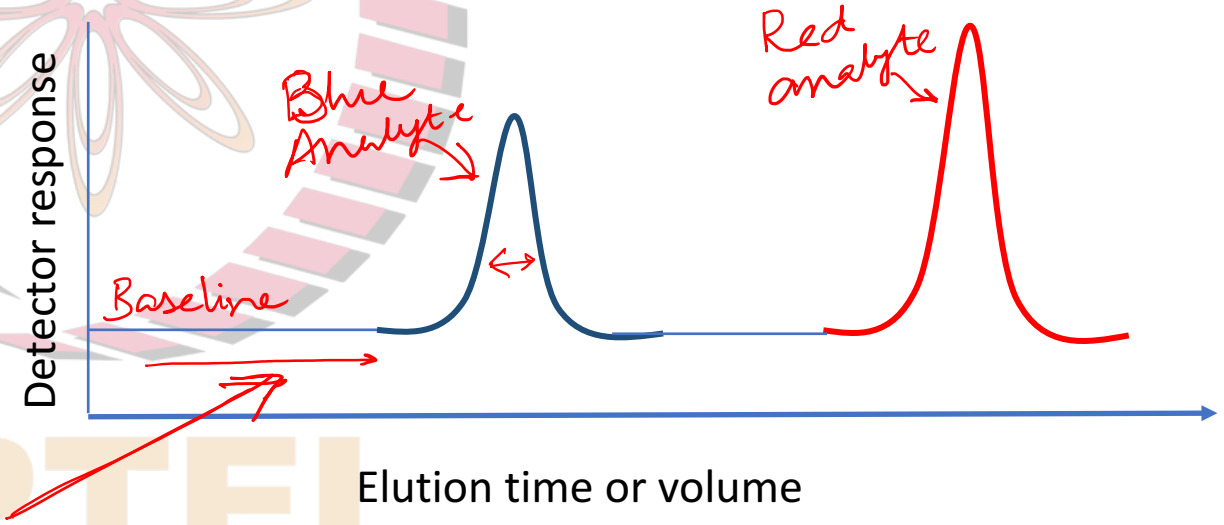
This week: Theoretical basis of chromatography (concept of plates, theoretical plate height, plate count, resolution, retention time, retention factor, selectivity factor)

This lecture: Generation and analysis of chromatograms – retention time, retention factor, selectivity factor, resolution

Chromatogram



Eluting analyte produces a response in the detector



Chromatogram is the detector response plotted as a function of time/ volume

Migration of solutes within the column

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

Chromatographic separations are dependent on the extent to which the analyte distributes between the mobile and the stationary phase.

The analyte can come out of the chromatography column by dissolving in the mobile phase.

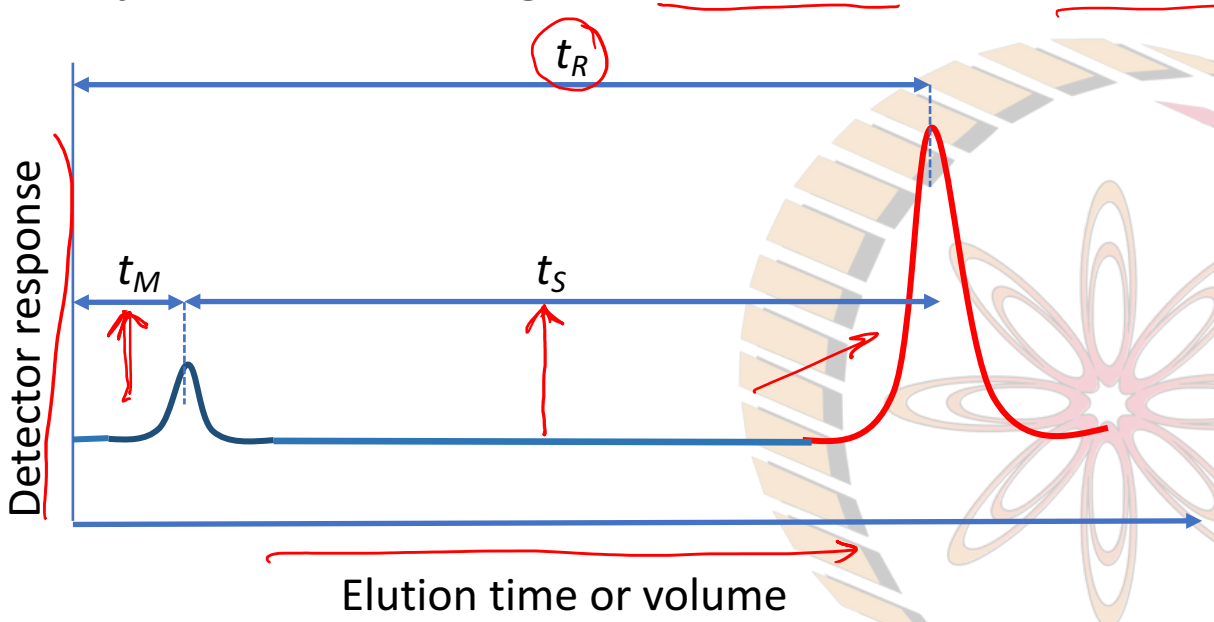
Distribution constant of the analyte:

$$K_c = \frac{[A]_{stationary}}{[A]_{mobile}}$$

Conc. of Analyte on stationary phase / Conc. of analyte in the mobile phase

K_c is expected to be independent of solute concentration.

Analysis of the chromatogram: Retention time and dead time



$t_R = t_S + t_M$

t_R = **Retention time** of the analyte

t_M = Void/ **dead time** time taken by the mobile phase or an unretained analyte to come out of the column of length L

t_S = **time spent on stationary phase** by the analyte

For a column of length L,

Analyte linear velocity $v = L/t_R$

L/t_R

Similarly, linear velocity of solvent $u = L/t_M$

$u > v$

Relationships between ν, u and K_C

Avg. linear velocity for solute

$\nu = u$ x fraction of time analyte spends in mobile phase

$$\nu = u \times \frac{moles\ of\ solute\ in\ mobile\ phase}{total\ moles\ of\ solute\ taken}$$

fraction of analyte which is present in the mobile phase at a particular instance of time.

$$\nu = u \times \frac{C_M V_M}{C_M V_M + C_S V_S}$$

C_M = Conc. of analyte in the mobile phase

V_M = Volume of the mobile phase in the column.

C_S = conc. of the analyte present on the stationary ph

V_S = volume of the stationary phase

$$\nu = u \times \frac{1}{1 + K_c V_S / V_M}$$

$$K_c = \frac{C_S}{C_M}$$

Retention Factor (k_A) of an analyte

$$k_A = K_c V_S / V_M$$

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

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1+k_A}$$

$$k_A = \frac{t_R - tM}{t_M} = \frac{t_s}{t_M}$$

t_R = **Retention time** of the analyte

t_M = Void/ **dead time**

t_S = **time spent on stationary phase** by the analyte

L = Length of the column

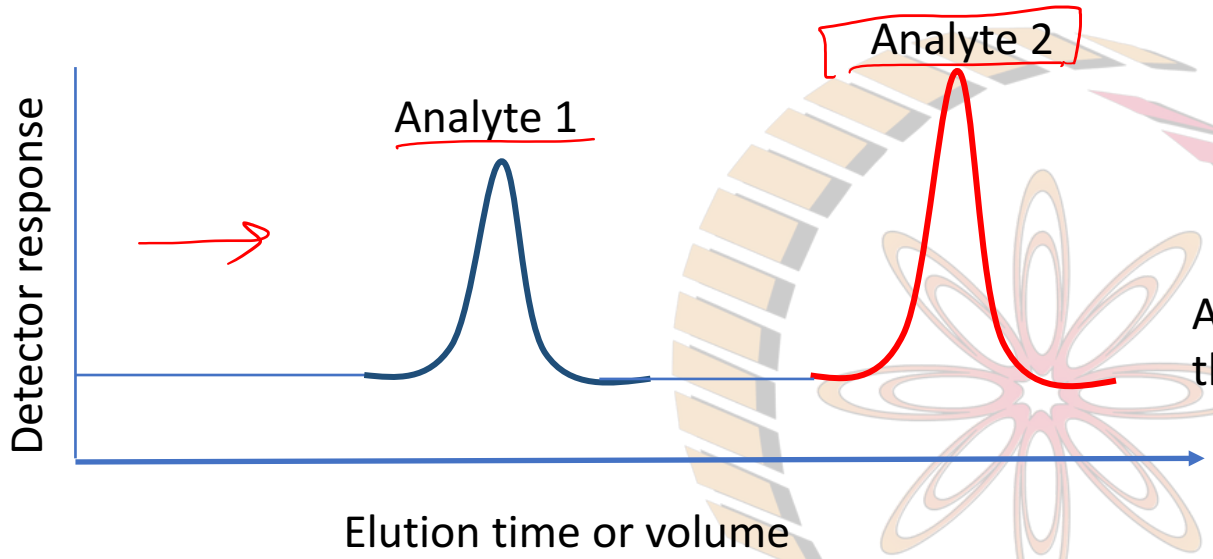
If k_A << 1, then the analyte is not retained in the column

If k_A ~ 15-20, then the analyte is retained for too long in the column

k_A ~ 1-5 are best for chromatographic separation

Selectivity Factor (α)

Two or more analytes



Analyte 2 is retained longer in the column than analyte 1.

Selectivity Factor $\alpha = K_{c2} / K_{c1}$ Selectivity Factor in terms of Distribution Constant.

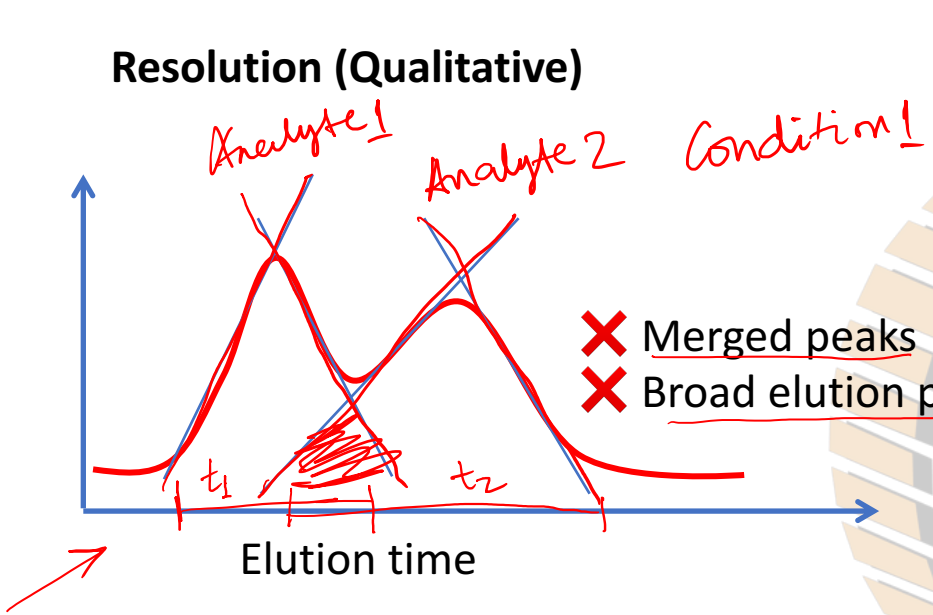
$\alpha = k_2 / k_1$ Selectivity Factor in terms of retention factor.

$$\alpha = \frac{t_{R2} - t_M}{t_{R1} - tM}$$

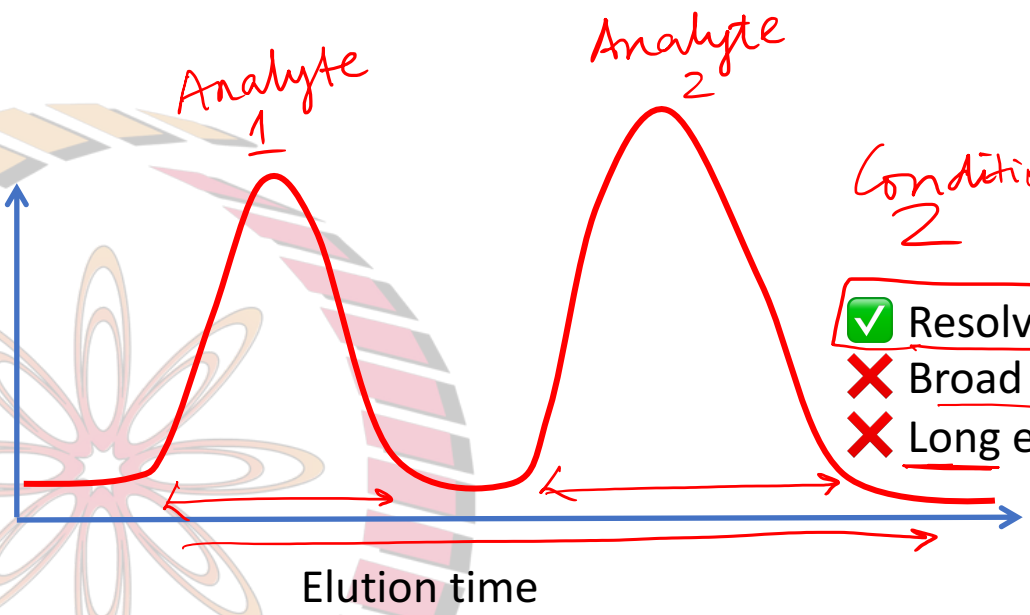
α is always >1

Numerator → Analyte retained longer in the column.

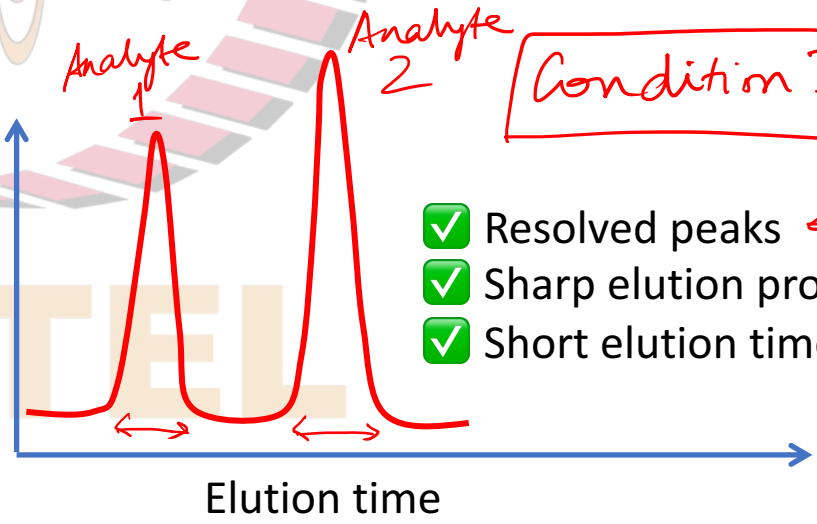
Resolution (Qualitative)



❌ Merged peaks
❌ Broad elution profile



✅ Resolved peaks
❌ Broad elution profile
❌ Long elution time



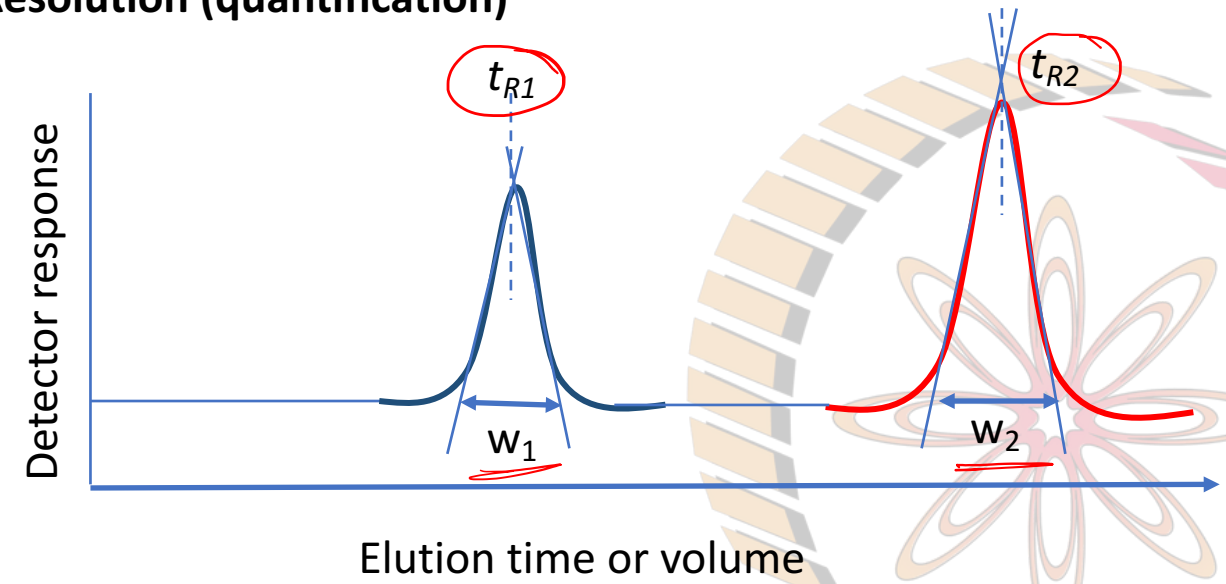
✅ Resolved peaks
✅ Sharp elution profile
✅ Short elution time

Improving R_s by interaction between the analyte and the stationary phase

Sometimes we can exploit specific interactions between the stationary phase and the analyte to improve the resolution of the chromatogram.

For example, silica particles are sometimes impregnated with Ag^+ ions to improve their interaction with alkenes and achieve more efficient separation of isomeric alkenes.

Resolution (quantification)



Resolution (R_s) tells us how far apart the analyte bands are in relation to their width at the baseline

t_{R1}, t_{R2}, w_1, w_2

$$R_s = \frac{t_{R2} - tR_1}{(w_1 + w_2)/2} = \frac{t_{R2} - tR_1}{(w_1 + w_2)} \times 2$$

At R_s = **1.0**, the bands are just resolved but contain **4% contaminant**

At R_s = **1.5**, the bands are well resolved and contain about **0.3% contaminant**

R_s can also be expressed in term of selectivity factor α and the retention factor k_A