Quantitative Methods in Chemistry

Week 10, Lecture 2

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

This lecture: Theoretical basis of chromatography – Plate theory

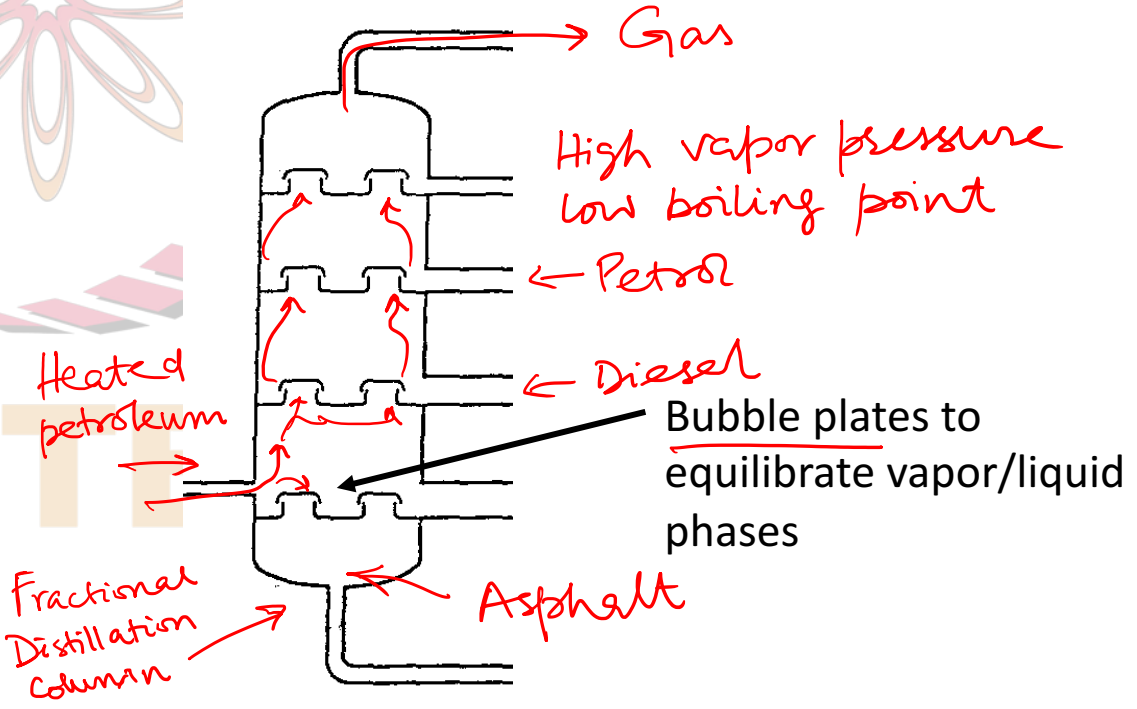
Plate theory of chromatography

Oldest theory to explain chromatograms. Very basic, and has been superseded by other theories.

Proposed by Martin and Synge (Nobel prize in 1952) as a theoretical framework for chromatography.

Takes inspiration from the fractional distillation columns where the vapor and liquid phases equilibrate on multiple bubble plates present in the column

Martin Synge

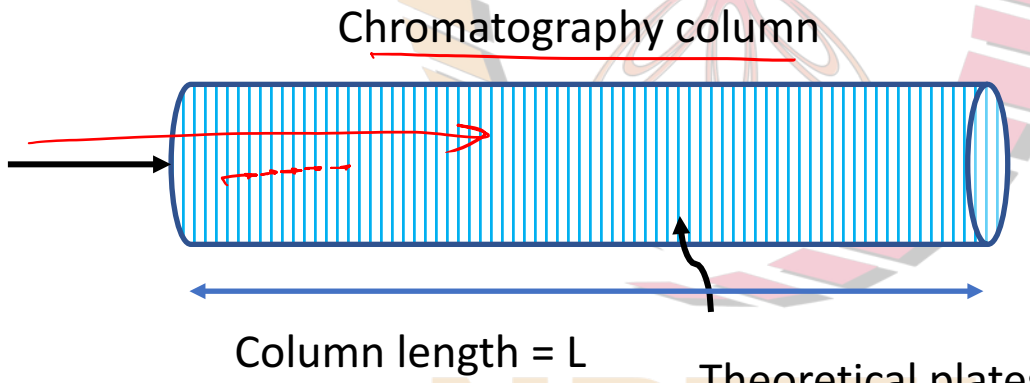


A column for Fractional Distillation

Plate theory of chromatography

Plate theory considers the existence of analogous “plates” inside chromatography column.

The solute moves down the column by transfer of the equilibrated mobile phase from one plate to the next.



The theoretical plates is an imaginary construct to explain the chromatographic profiles

Theoretical plates –
Over which mobile phase equilibrates while passing through the chromatography column

Concept of plate number and plate height

Total number of plates present in a column = N

By increasing/ decreasing the column length, the plate count can be increased/ decreased.

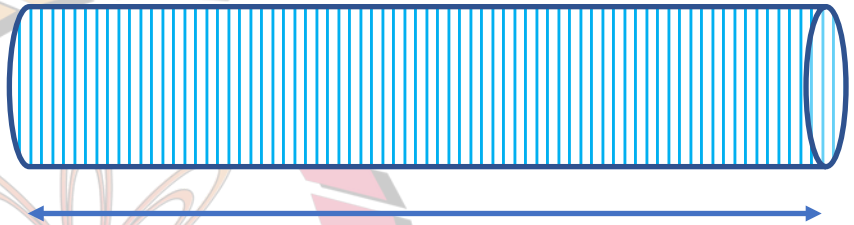
So, height of each plate is given by

$H = L/N$

Plate height is also abbreviated as HETP.

HETP = Height equivalent of theoretical plate

Chromatography column

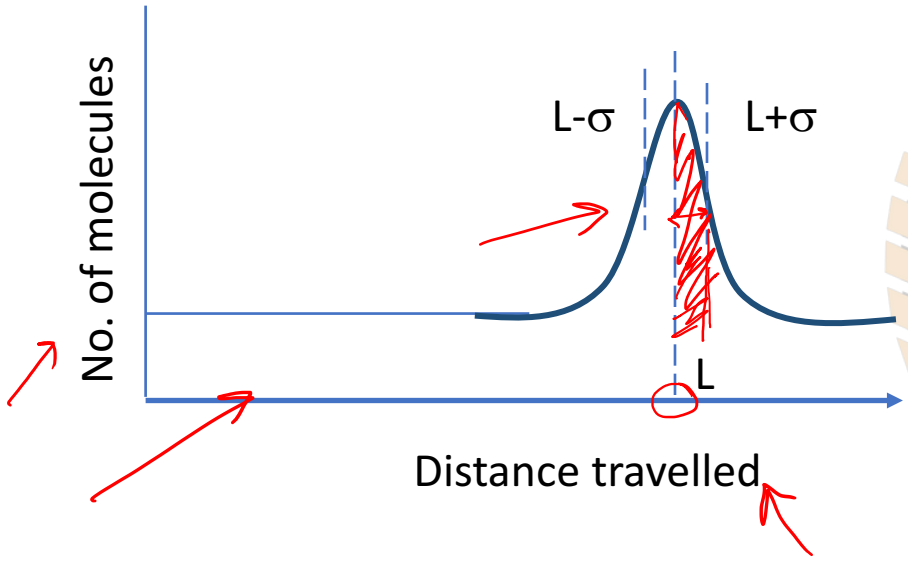


Column length = L

Note, Resolution $R_s \alpha \sqrt{N}$

For **increasing the efficiency** of separation through a column, the plate count N should be increased without changing the length of the column. This can be achieved by **reducing the plate height**.

Plate height in terms of peak variance



The eluting analyte band follows a **Gaussian profile** under optimized elution.

The **efficiency** of the column is reflected in the **breadth** of the eluting peak. More efficient column => Small band breadth.

The **spread** of the band can be described in terms of its variance σ^2.

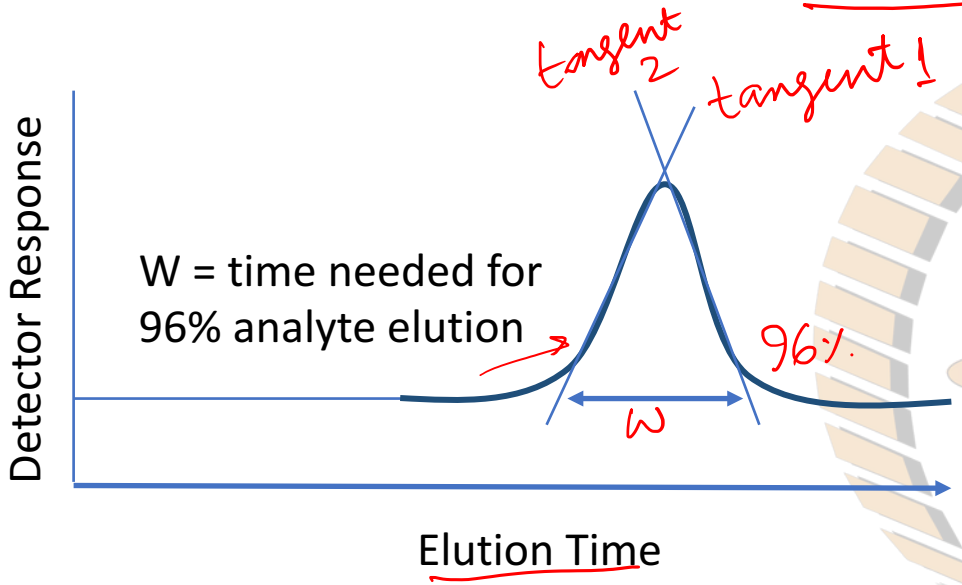
Std. dev.^{2} σ = Std. dev.

So, plate height H = σ^2/ L (variance of the peak/ length of column)

Since for Gaussian curves, $\mu \pm \sigma$ = 68% of the readings,
Plate height H is the length of the column that contains 68/2 = 34% analyte during elution.

H has units of length (cm)

Experimental determination of Plate Count N



Width of the peak at the baseline is the time/ volume of mobile phase required to elute out **96% of the analyte**.

96% data-points in a Gaussian profile imply $\mu \pm 2\sigma$

Since the variance is in terms of time, we designate it as τ^2 where,

σ = std. dev. of peak

$\tau = \sigma/\nu$ where t = time based variance in elution peak

σ = Length based variance in the elution peak

ν = Average linear velocity of the solute = L/t_R

$W = 4\tau$

So, $w = \frac{4\sigma}{L/tR}$

And $\sigma = LW/4t_R$ Variance based on length/distance

Now, $H = \sigma^2/L$ $cm^2/cm = H(cm)$

So, $H = LW^2/16t_R^2$

Finally $N = 16 \cdot t_R^2/W^2$

} H and N in terms of retention time and width at base

Solved Example

Analytes 1 and 2 exhibit retention times of 6.40 and 7.63 min on a 20 cm column while the unretained species can come out in 1.0 min. The peak widths at the baseline for 1 and 2 are 0.85 and 1.05 min respectively.

With this data, Calculate the following:

(a) Resolution of the column

(b) Average number of plates in the column

(c) Average height of the column

(d) Length of column required to obtain a resolution of 1.5.