VIVEKANANDA COLLEGE
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NAAC ACCREDITED 'A' GRADE

Topic:HPLC and Performance parameters

Course Title:Enzymes

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Performance parameters:

Retention time – When chromatography is carried out, the various sample components separate on the column because they are retained to different extents. We can describe this retention in terms of time or volume called retention time (t_R) and retention volume (V_R).

$$V_R = f\, t_R$$

f = flow rate ml/min.

The retention time t_R for each analyte has 2 components. The first is the time it takes for the analyte molecules to pass through the free spaces between the particles of the matrix coated with the stationary phase, known as the dead time denoted by (t_M).

The volume of the free space is referred to as the column

void volume V_0.

The second component is the time the stationary phase retains the analyte referred to as t'_R ~~ref~~ referred to as adjusted ~~retention~~ / retention time.

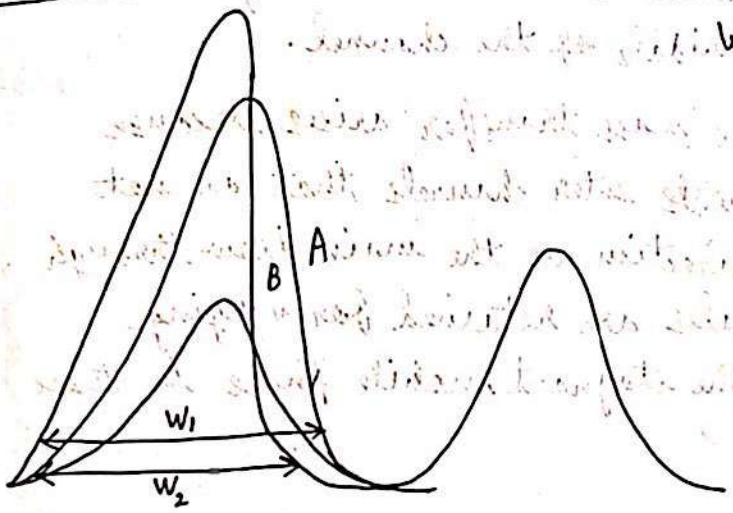
$$t'_R = t_R - t_M$$

~~Ref~~ Retention factor (Previously, capacity factor) ~~denter~~ denoted by K'

$$K' = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M}$$

24/3/19

Resolution:



W_{av} = W(average)

W = width between base of peaks

Resolution is defined ~~as~~ as the ratio of the difference in retention time (Δt_R) between 2 peaks (t_{RA} and t_{RB}) to the mean (W_{av}) of their base widths (W_A and W_B).

(t = retention time)

$$R_S = \frac{\Delta t_R}{W_{av}} = \frac{2(t_{RA} - t_{RB})}{W_A + W_B} = \frac{V_2 - V_1}{\frac{W_A + W_B}{2}}$$

(V = retention volume)

Physical bases of peak ~~band~~ broadening

If a sample of a single component is applied to a chromatography system in a small volume, it might be expected to elute in a very sharp peak of similar volume. This is not usually observed due to a phenomenon called band broadening, that is, the sample

elutes in a much larger volume than that in which it was applied. The applied sample may interact with the stationary phase resulting in diffusion of the sample or it may become involved in mass transfer phenomena within the mobile or stationary phase. Circular flow (eddy diffusion) in narrow channels is generally slower than that in wide channels. Since sample will diffuse through a variety of channel sizes, it will be gradually distributed between fast flowing wide channels and slow flowing narrow channels.

Mass transfer phenomena may be

i) Mobile phase mass transfer occurring because mobile phase adjacent to the particles move more slowly than mobile phase in the middle of the channel.

ii) Stagnant mobile phase mass transfer arises because different sample components enter channels that are not oriented in the same direction as the main flow through the column. These molecules are retained for varying amounts of time in the stagnant mobile phase in these channels.

Peak symmetry.

In practice, peak symmetry may be significantly distorted from Gaussian shape. This arises from a variety of factors such as the use of non-linear flow rates, incomplete separation of peaks and the use of gradient elution techniques, all of which are frequently encountered in chromatography.

Components of chromatography system

1) Pump
2) Mixer
3) Guard column — To prevent clogging and prolong the

life of the column.

4) Fraction collector

5) Auto sampler (for multiple samples)