VIVEKANANDA COLLEGE

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NAAC ACCREDITED 'A' GRADE

Topic:Chromatography continuation

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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 mm/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 space 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 time~~ / retention time.

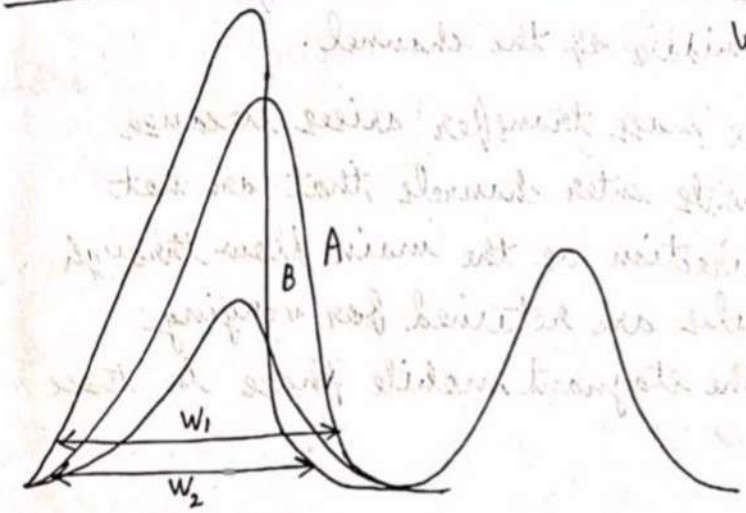
$$t'_R = t_R - t_M$$

~~Ref~~ Retention factor (Previously, capacity factor) ~~denotes~~ 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 base of peak ~~broad~~ 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~~ 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)

Modes of chromatography

Characteristics	Procedure
1) Solubility	1) Salting in 2) Salting out
2) Ionic charge	Ion exchange chromatography, electrophoresis, iso-electric focusing (IEF)
3) Polarity	Adsorption chromatography, paper chromatography Reverse phase chromatography Hydrophobic interaction chromatography
4) Molecular size	Dialysis and ultrafiltration, gel electrophoresis, gel filtration chromatography Ultracentrifugation
5) Binding specificity	Affinity chromatography

Ion exchange chromatography

In the process of ion ~~exct~~ exchange that are electrostatically bound to an ~~it~~ insoluble and chemically inert matrix are reversibly replaced by by ions in solution. It is a form of adsorption chromatography in which charged proteins or other biomolecules are ~~sub~~ exchanged for small ions of like charge originating in salt.

Three.
~~Three~~ ions are attached to chemical structure on the surface of the stationary phase called ion exchange groups or ion exchangers. Two types of ion exchangers are ~~com~~ common.

1) ~~An ion~~ Anion exchanger (which exchange negatively charged ions or anions)
2) Cation exchanger (which ~~exch~~ exchange positively charged ions or cations).

Ion exchangers are attached to a wide variety of solid supports such as cellulose, agarose and vinyl benzene.

Example of cation exchangers : CM cellulose (Carboxy methyl cellulose), pH range - 6 - 11.

Example of anion exchangers : ~~DE~~ DEAE (diethyl amino ethyl) cellulose.

Refer Voet for structure.

Reverse phase liquid chromatography

In this form of liquid chromatography which has similarities with hydrophobic interaction chromatography, the stationary phase is non polar and the mobile phase relatively polar hence the name reversed phase.

The most ~~comm~~ commonly used type is bonded phase form in which alkylsilane groups are chemically attached to silica. Butyl, octyl, ~~octadecy~~ octadecyl (C_{18}) groups are ~~chemically~~ commonly used.

Hydrophobic interaction chromatography

Soluble proteins require a salvation shell of water on their surface to maintain solubility in aqueous solution. This water masks hydrophobic groups that also exist on the protein surface. In the presence of reagents, that are capable

of binding water from the salvation shell (ammonium sulphate $(NH_4)_2SO_4$) it is possible to disrupt salvation and expose these hydrophobic groups. Using this hydrophobic stationary phase such as those provided by bonded octyl (strongly hydrophobic) or phenyl (more weakly hydrophobic) groups, it is possible to promote hydrophobic interactions between proteins and such a bonded phase. This is the basis of the adsorption chromatography technique called hydrophobic interaction chromatography.

Gel filtration chromatography

Gel filtration chromatography or molecular sieve, or size exclusion chromatography takes advantage of the fact that the proteins and other biomolecules' mass and shape. This difference of retaining biomolecules of a given size range and fractionating them in a manner related to their mass in a form of partition chromatography.

The stationary phase in this technique consists of beads of hydrated sponge like material containing pores that span a relatively narrow size range of molecular dimensions. The molecular mass of the smallest molecule unable to penetrate the pores of a given gel is said to be a gel's exclusion limit. If V_R is the volume occupied by the gel beads and V_o is the void volume, is the volume of the solvent space surrounding the beads, then V_t is the total bed volume of the column.

$$V_t = V_R + V_o$$

The elution volume of a given solute, V_E is the volume of solvent required to elute the solute from the column. Molecules with molecular masses ranging below the exclusion limit of a gel will elute from the gel in the order of their molecular masses with the largest eluting first.

Fast protein liquid chromatography (FPLC)

It is a type of liquid chromatography where the pumped solvent velocity is microprocessor controlled through a software interface to ensure constant flow rates of solvents. It was introduced in 1982 by Pharmacia as fast performance liquid chromatography. This system generally consists of a pump, a UV detector, conductivity meter, a fraction collector that operates at a pressure of nearly 3500 pounds per square inch (psi). Some ~~fast~~ of them also have column switching valves that allows the user to switch ~~betwe~~ between columns, sample pumps to apply the sample to a column and buffer blending valves that can generate desired buffer gradients. ~~This~~ This system has wavelength detectors for monitoring sample elutions at several wavelengths. This feature can be particularly useful while working with chromogenic or fluorescently tagged proteins that absorb at wavelengths other than 280 nm as elution of the protein of interest can be tracked separately from elution of contaminating proteins based on differences in absorption.