Simulated Moving Bed Chromatography

Simulated moving bed (SMB) is a chemical processing aid that uses the principles of chromatography to purify a mixture of chemicals.

SMB Terminology*

Adjusted retention time (t_r'). A measure of the retention time adjusted for the void volume. $t_r' = t_r - tm$ (or t0).

Adsorption. The process of interaction between the solute and the surface of an adsorbent. The forces involved can be strong (for example, hydrogen bonds) or weak (van der Waals forces). For silica gel, the silanol group is the driving force for adsorption, and any solute functional group that can interact with this group can be retained by liquid-solid chromatography on silica.

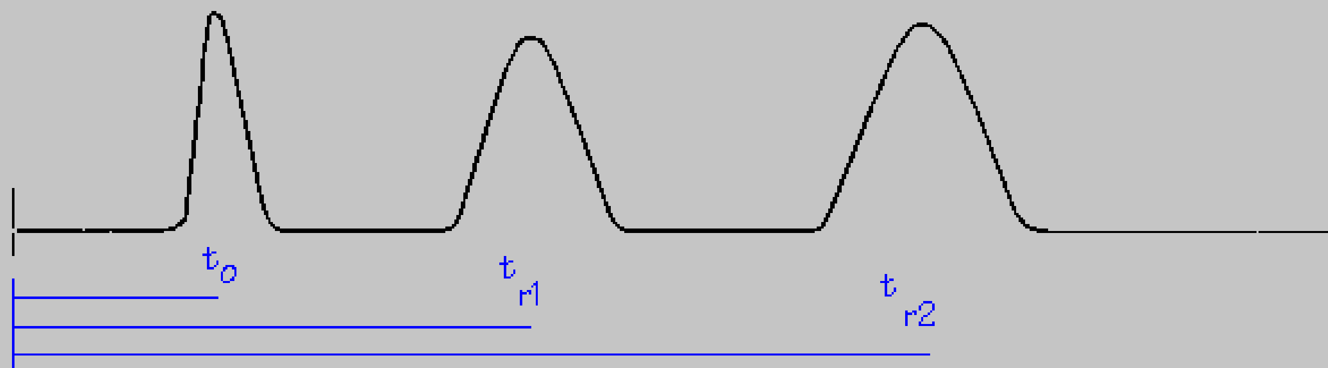
Adsorption isotherm. In adsorption, a plot of the equilibrium concentration of sample in the mobile phase per unit volume vs. the concentration in the stationary phase per unit weight. The shape of the adsorption isotherm can determine the chromatographic behavior of the solute: tailing. fronting, overload, etc.

Asymmetry. Factor describing the shape of a chromatographic peak. Theory assumes a Gaussian shape and that peaks are symmetrical. The peak asymmetry factor is the ratio (at 10% of the peak height) of the distance between the peak apex and the back side of the chromatographic curve to the distance between the peak apex and the front side of the chromatographic curve. A value > 1 is a tailing peak, while a value <1 is a fronting peak.

Bonded-phase chromatography (BPC). The most popular LC mode. A stationary phase chemically bonded to a support is used for the separation. The most popular Support is microparticulate silica gel. and the most popular type of bonded phase is the organosilane, such as octadecyl (for reversed-phase chromatography). Approximately 70% of all HPLC is carried out on chemically bonded phases.

C4, C8, C18, etc. Refers to the alkyl chain length of a reversed bonded phase.

Capacity factor. A chromatographic parameter that measures the degree of retention . See k ' for calculation method.



Channeling. Occurs when voids created in the packing material of a column may cause mobile phase and accompanying solutes to move more rapidly than the average flow velocity, resulting in band broadening. The voids are created by poor packing or by erosion of the packed bed.

Chiral stationary phases (CSP). Stationary phases that are designed to separate enantiomeric compounds. They can be bonded to solid supports or created in situ on the surface of the solid support, or they can be surface cavities that allow specific interactions with one enantiomeric form.

Chromatogram. A plot of detector signal output versus time or elution volume during the chromatographic process.

Column chromatography. Any form of chromatography that uses a column or tube to hold the stationary phase. Open-column chromatography, HPLC, and open-tubular capillary chromatography are all examples .

Column performance. Refers to the efficiency of a column; measured as the number of theoretical plates for a given test compound.

Dead volume (Vd). Also known as Void Volume (V0). The volume outside of the column packing itself. The interstitial volume (intraparticle volume + interparticle volume) plus extra column volume (contributed by injector, detector, connecting tubing, and end fittings) all combine to create the dead volume. This volume can be determined by injecting an inert compound (i.e.. a compound that does not interact with the column packing).

Degassing. The process of removing dissolved gas from the mobile phase before or during use. Dissolved gas may come out of solution in the detector cell and cause baseline spikes and noise. Dissolved air can affect electrochemical detectors (by reaction) or fluorescence detectors (by quenching). Degassing is carried out by heating the solvent or by vacuum (in a vacuum flask), or on-line using evacuation of a tube made from a gas-permeable substance such as PTFE, or by helium sparging.

Effective theoretical plates (N_{eff}). The true number of plates in a column; corrects theoretical plates for dead volume. Neff = 16(t_R -tm)2 , where tm is the void time.

Efficiency (N or H). A measure determined by the number of theoretical plates calculated from the equation shown for H (see HETP).

Eluent. Mobile phase used to carry out a separation.

Elution. The process of passing mobile phase through the column to transport solutes.

Elution chromatography. The most commonly used chromatographic method. The sample is applied to the head of the column, and individual molecules are separated and eluted at the end of the column. Compare Displacement chromatography and Frontal analysis.

Elutriation. A technique used to fractionate packing particles by size. Most often used for the separation of ion-exchange resins, such as amino acid resins. that must have a particularly narrow size range. The technique involves the upward flow of water into a large tube. The unsized beads are added to the moving water, and the particles seek their own level, depending on their density and particle size. They are then removed at certain levels in the tube.

Extract. One of the two product streams which are pumped from the SMB during a separation. The extract corresponds to the most retained compound.

Feed. The feed stock that has been diluted with the eluent and is ready to be injected into the SMB.

Feed Stock. The compound to be separated by SMB chromatography.

Flow rate (F). The volumetric rate of flow of mobile phase through an LC column. For a conventional HPLC column of 4.6-mm i.d.. typical flow rates are 1 to 2 ml/min.

Frit. The porous element at either end of a column that serves to contain the column packing. It is placed at the very ends of the column tube or, more commonly, in the end fitting. Frits are made from stainless steel or other inert metal or plastic, such as porous PTFE or polypropylene.

Fronting. Peak shape in which the front pan of a peak (before the apex) in a chromatogram tapers in advance of the remainder of the peak. There is an asymmetric distribution with a leading edge. The asymmetry factor for a fronting peak has a value < 1. The opposite effect is tailing. Fronting is related to the shape of the sorption isotherm.

Gradient elution. Technique for decreasing separation time by increasing mobile phase strength over time during the chromatographic separation. Also known as solvent programming. Gradients can be continuous or stepwise. Binary, ternary, and quaternary solvent gradients have been used routinely in HPLC .

Irregular packing. Refers to the shape of a silica gel based packing. Irregular silicas are available in microparticulate sizes. The packings are made by grinding silica gel into small particles and then sizing them into narrow fractions using classification machinery. Spherical packings are now used more often than irregular packings in HPLC, but less-expensive irregular packings are still widely used in prep LC.

Isocratic. Use of a constant-composition mobile phase in liquid chromatography.

Lab Study. A series of chromatographic tests run on the feed stock and stationary phase in order to obtain the SMB operating parameters.

k'. Capacity factor; k' = (mass in stationary phase) (mass in mobile phase). Can be calculated from the equation k ' = (tr - t0)/t0, where tr is retention time for the sample peak, and t0 is the retention time for an unretained peak.

Mobile phase. The solvent that moves the solute through the column.

N, The number of theoretical plates. N = 16(tR/Wb)2, where tR is retention time, and Wb is the base width of he peak. A measure of the efficiency of a column. Sometimes measured as N = 5.54(tR/W 1/2)2 where w1/2 is the peak width at half height.

Normal-phase chromatography. A mode of chromatography carried out with a polar stationary phase and a nonpolar mobile phase. Adsorption on silica gel using hexane as a mobile phase would be a typical normal-phase system. Also; refers to the use of polar bonded phases, such as CN or NH2. Sometimes referred to as straight-phase chromatography.

Overload. In preparative chromatography, the overload condition is defined as the mass of sample injected onto the column at which efficiency and resolution begin to be affected if the sample size is further increased. See Sample capacity.

Packing. The adsorbent, gel, or solid Support used in the HPLC column. Most HPLC packings are <10 um in average diameter.

Particle size (dp). The average particle size of the packing in an LC column. A 5-um column would be packed with particles having definite particle size distribution; packings are never monodisperse. See Particle-size distribution.

Particle-size distribution. A measure of the distribution of the particles used to pack the LC column. In HPLC, a narrow particle-size distribution is desirable. A particle-size distribution of dp +/- 10% would mean that 90% of the particles fall between 9 and 11 µm for an average 10-µm dp packing.

Partition coefficient (K). The amount of solute in the stationary phase relative to the amount of solute in the mobile phase. Can be distribution coefficient, KD

Period. Valve switching time.

Pirkle columns. Chiral "brush-type" stationary phases, based on 3 ,5-dinitrobenzoylphenylglycine silica, used in the separation of a wide variety of enantiomers. Named after the developer, Dr. William Pirkle, University of Illinois.

Porosity. For a porous adsorbent, the ratio of the volume of the interstices to the volume of the solid particles. The pore volume is also used as a measure of porosity.

Preparative chromatography. The process of using liquid chromatography to isolate a sufficient amount of material for other experimental or functional purposes. For pharmaceutical or biotechnological purifications,

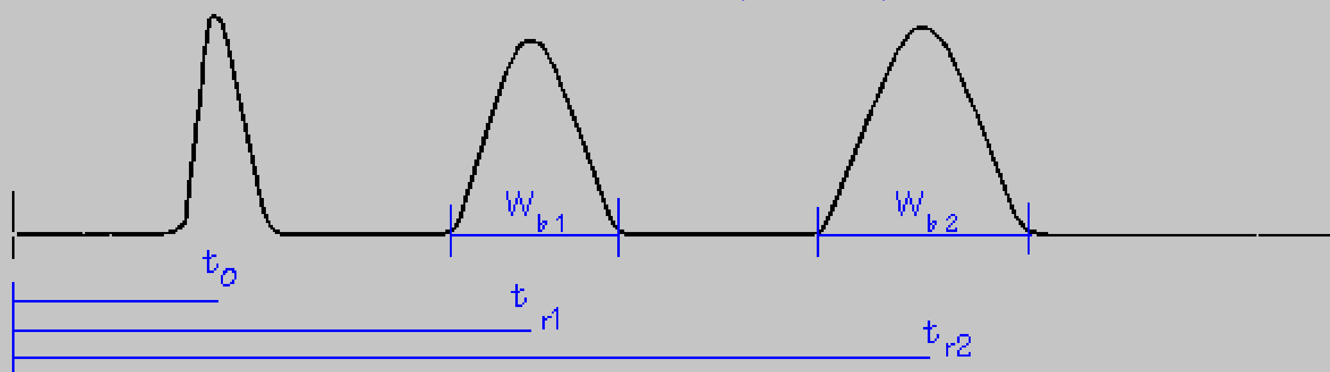
columns several feet in diameter can be used for multiple grams of material. For isolating just a few micrograms of a valuable natural product, an analytical column can be used. Both are preparative chromatographic approaches.

Raffinate. One of the two products streams pumped from the SMB during operation. The raffinate corresponds to the least retained component.

Recovery. The amount of solute (sample) that elutes from a column relative to the amount injected. Most often used with protein separations in which proteins hang up" on active sites of the packing in certain columns .

Recycling. A technique in which the column eluent is recirculated onto the head of the column in an attempt to gain the advantage of extended column length. Can be carried out on a single column by passing the eluent back through the pump. An alternative technique uses two columns connected by a switching valve. Very seldom used in HPLC, and then only in size-exclusion chromatography.

Resolution (Rs). Ability of a column to separate chromatographic peaks. It is usually expressed in terms of the separation of two peaks. One attempts to achieve the best resolution possible. Resolution can be calculated in two ways: Rs = 2(tR.2 - tR.1)/(Wb.1 + Wb.2) or Rs = 1/4(a-1/a) (k'/1+k')N1/2 A value of 1 is considered to be the minimum for a measurable separation to occur and to allow good quantitation . Values of 1.7 or larger are generally desirable for rugged methods



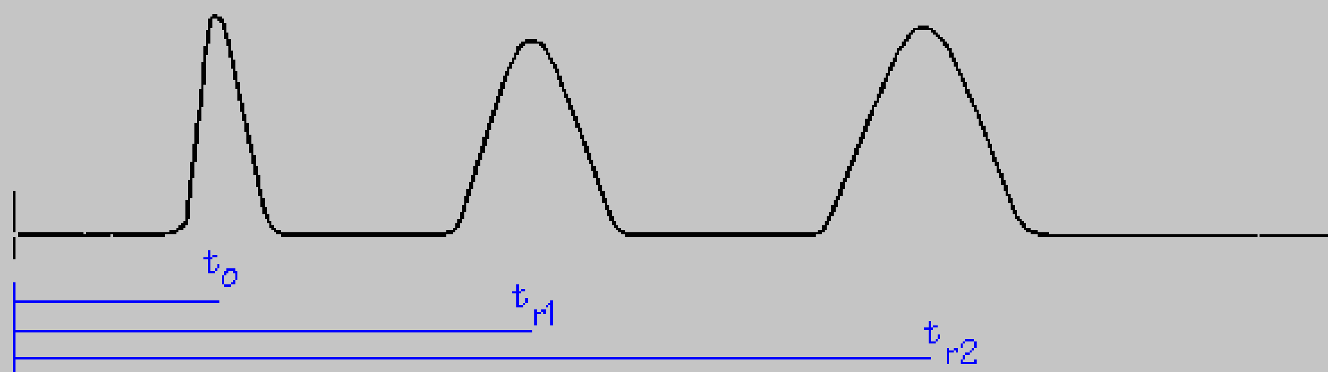
Retention time (tr). The time between injection and the appearance of the peak maximum. The adjusted retention time t'R adjusts for the column void volume. $t_r' = tr - t_0$ (or tm).

Retention time (t_R). The time between injection and the appearance of the peak maximum. The adjusted retention time t_r' adjusts for the column void volume. $t_r' = tr - t_0$ (or tm).

Retention volume (Vr) . The volume of mobile phase required to elute a substance from the column Vr = F x tr or Vr = Vm - KD Vs, where Vm is the void volume, KD the distribution coefficient. and Vs the stationary phase volume.

Reversed-phase chromatography (RPC). The most common HPLC mode. Uses hydrophobic packings such as octadecyl- or octylsilane phases bonded to silica or neutral polymeric beads. Mobile phase is usually water and a water-miscible organic solvent such as methanol or acetonitrile. There are many variations of RPC in which various mobile phase additives are used to impart a different selectivity. For example, for the RPC of anions, the addition of a buffer and tetralkylammonium salt would allow ion pairing to occur and effect separations that rival ion exchange chromatography.

Selectivity (alpha). Same as Separation factor or Relative retention ratio. A thermodynamic factor that is a measure of relative retention of two substances. Fixed by a certain stationary phase and mobile phase composition. a = k'_2/k'_1.



Silica gel. The most commonly used packing in liquid chromatography. It has an amorphous structure, is porous, and consists of siloxane and silanol groups. It is used as a bare packing for adsorption, as the support in liquid-liquid chromatography or for chemically bonded phases, and. with various pore sizes. as packing in size-exclusion chromatography. Microparticulate silicas of 5- and 10-um average particle diameter are used in HPLC.

Slurry packing. The technique most often used to pack HPLC columns with microparticles. The packing is suspended in a slurry (10% wt/vol) and is rapidly pumped into the empty column. Special high pressure pumps are used.

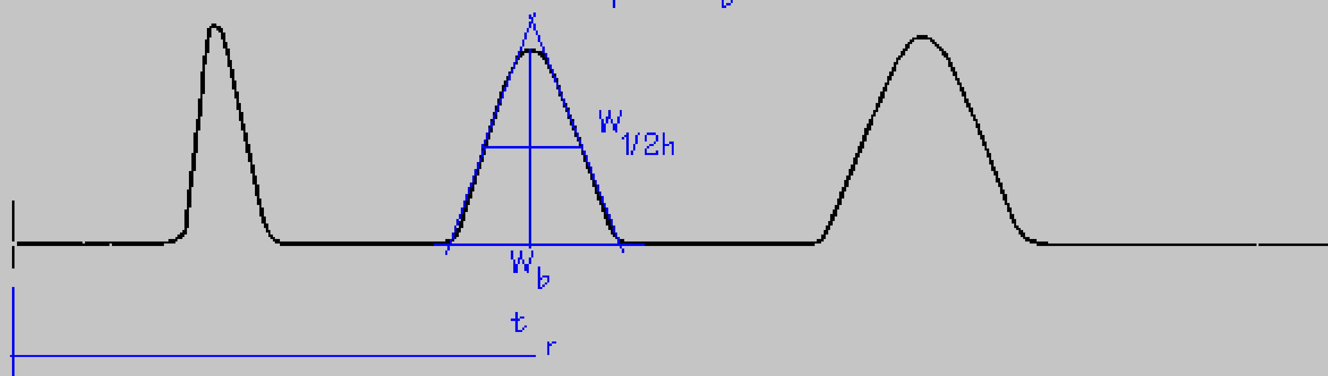
Stationary phase. The immobile phase involved in the chromatographic process. The stationary phase in liquid chromatography can be a solid, a bonded or coated phase on a solid Support, or a wall-coated phase. The stationary phase used often characterizes the LC mode. For example. silica gel is used in adsorption

chromatography, an octadecylsilane bonded phase in reversed-phase chromatography, etc.

Supercritical fluid chromatography (SFC). A technique that uses a supercritical fluid as the mobile phase. The technique has been applied to the separation of substances that cannot be handled effectively by liquid chromatography (because of detection problems) or gas chromatography (because of the lack of volatility). Examples are separations of triglycerides, hydrocarbons, and fatty acids. GC detectors and HPLC pumps have been used together in SFC.

Tailing. The phenomenon in which the normal Gaussian peak has an asymmetry factor > 1. The peak will have a skewed or trailing edge. Tailing is caused by sites on the packing that have a stronger-than normal retention for the solute. A typical example of a tailing phenomenon is the strong adsorption of amines on the residual silanol groups of a low-coverage reversed-phase packing.

Theoretical plate. A concept described by Manin and Synge. Relates chromatographic separation to the theory of distillation. Measure of column efficiency. Length of column relating to this concept is called height equivalent to a theoretical plate (HETP). See HETP.



Void. The formation of a space. usually at the head of the column. caused by a settling or dissolution of the packing. A void in the column leads to decreased efficiency and loss of resolution. Even a small void can be disastrous for small microparticulate columns. The void can sometimes be removed by filling it with glass beads or porous packing

Void time (t_m or t_0). The time for elution of an unretained peak.

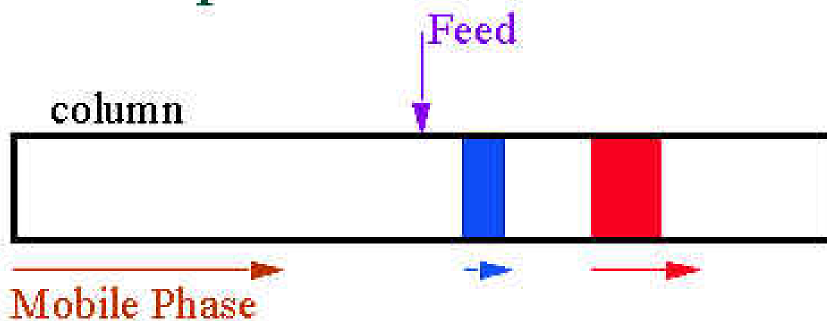
Void volume (V_0). The total volume of mobile phase in the column: the remainder of the column is taken up by packing material. Can be determined by injecting an unretained substance that measures void volume plus extra column volume. Also referred to as interstitial volume. V0 or Vm are sometimes used as symbols.

*Graphics and definitions obtained from http://www.lcsupport.com

Operation**

Continuous Chromatography

Basic Principle



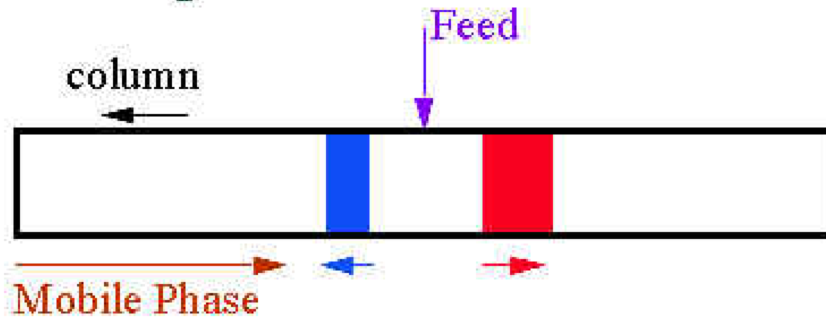
A sample is injected in the centre of a stationary column
The two components move at different speeds and are separated

If we now move the column from right to left, at a speed halfway
between that of the solutes, they now move in different directions ...

G B Cox 1995

Continuous Chromatography

Basic Principle

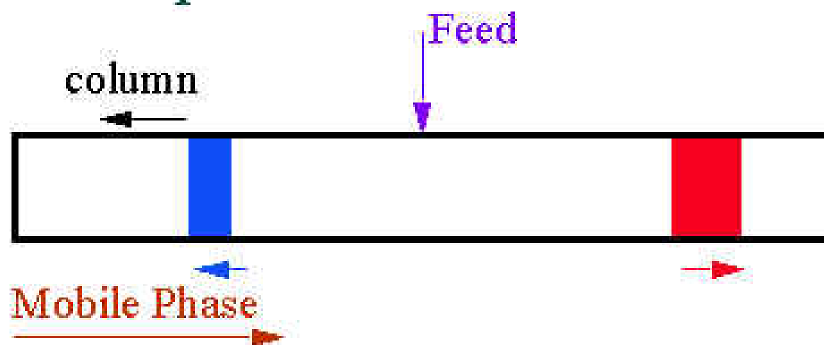


The two solutes now move in different directions relative to a stationary observer. If the column is very long, the bands will continue to separate.

G E Cox 1995

Continuous Chromatography

Basic Principle



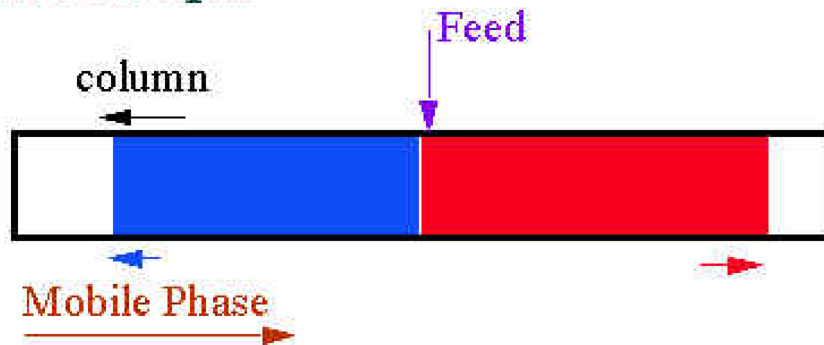
The two solutes now move in different directions relative to a stationary observer. If the column is very long, the bands will continue to separate.

If we continue to add sample at the centre, the components will continue to separate...

G E Cox 1995

Continuous Chromatography

Basic Principle



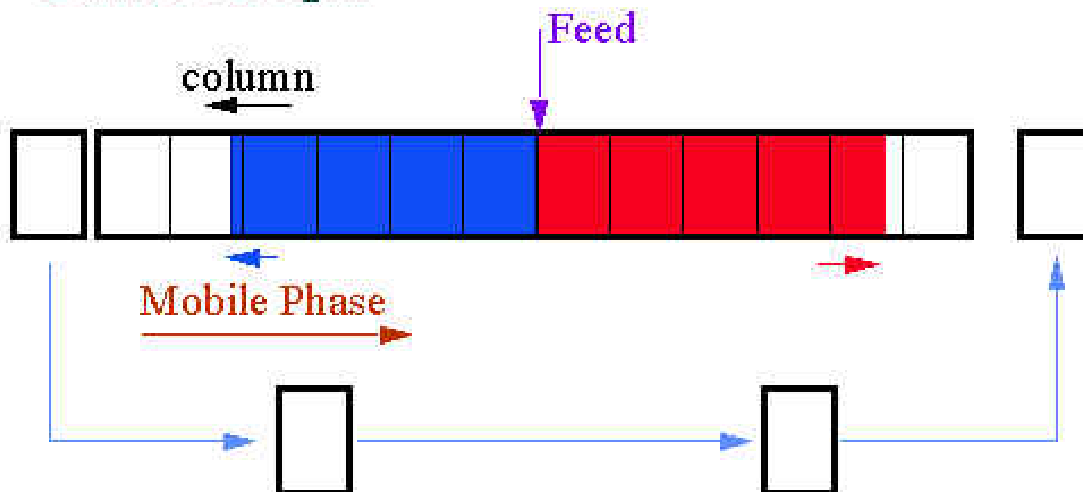
This is clearly a continuous system, but there are problems.
It needs an infinite column length and some way to introduce and remove the sample and the products.

We solve this by cutting the column into small segments and moving them

G E Cox 1995

Continuous Chromatography

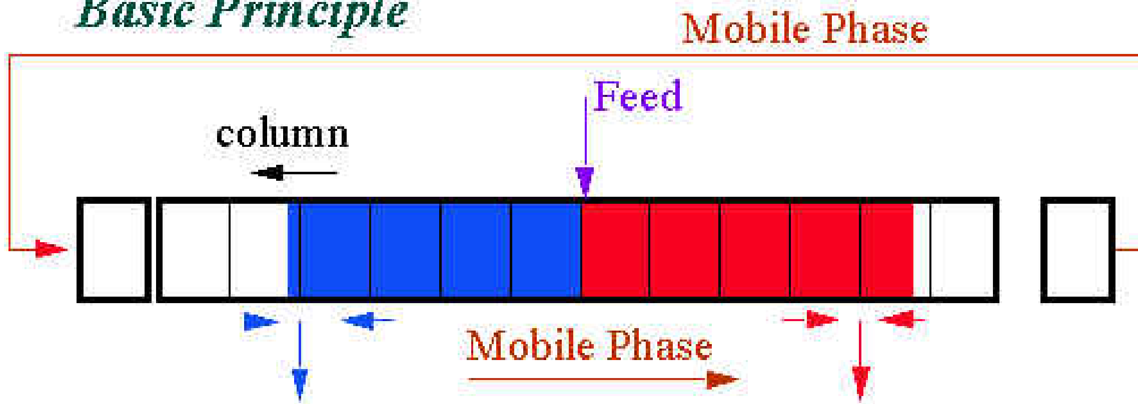
Basic Principle



The feed and solvent inlets are now placed between the segments and are moved each time a segment is moved from one end to the other

G E Cox 1995

Continuous Chromatography

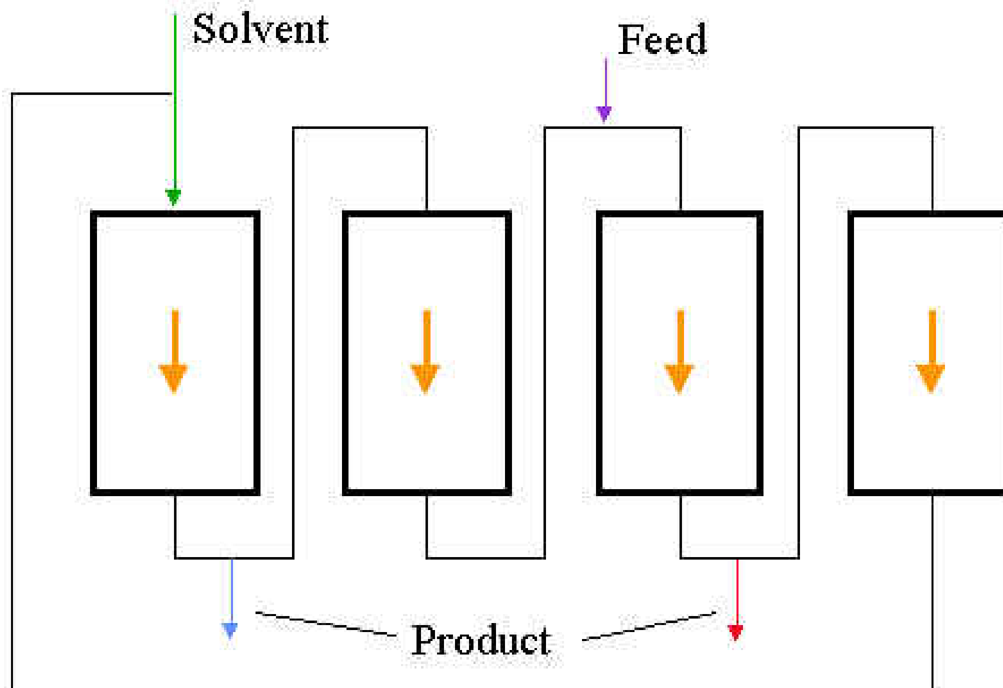


Products are removed by bleeding off a carefully calculated flow at suitable exit points. This changes the velocity of the bands in the column and forces the products to move toward the ports

This ensures that the column segments are clean before they are moved and that the solvent can be recycled directly back through the system

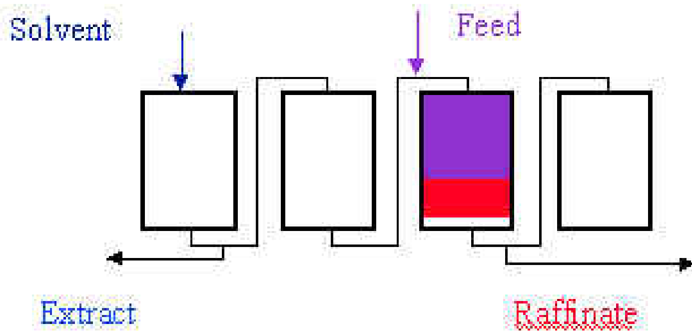
G B Cox 1995

SMB made Simple

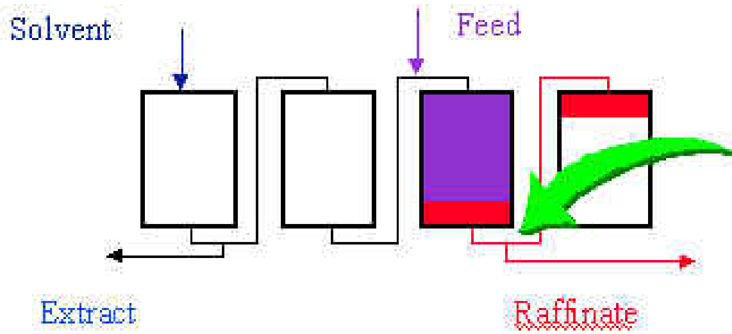


G B Cox & H Colin. 1996

Simplified SMB - 2

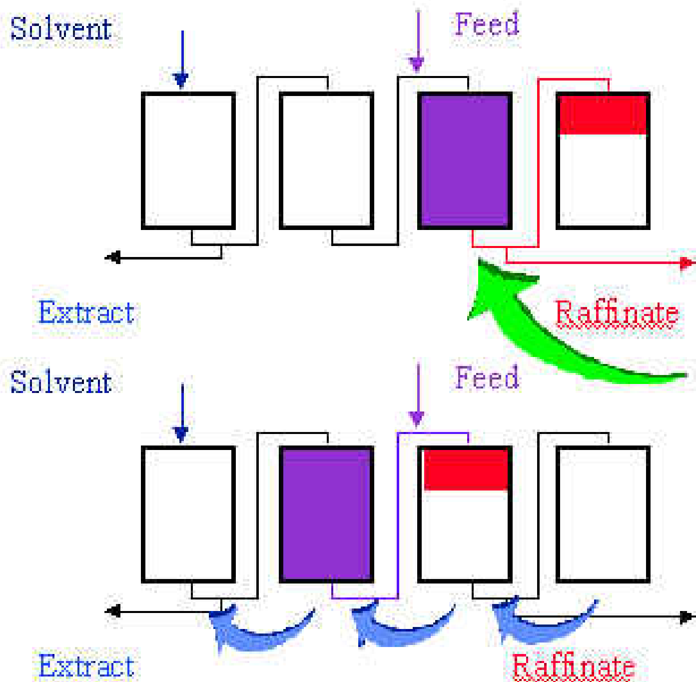


The separation continues.....



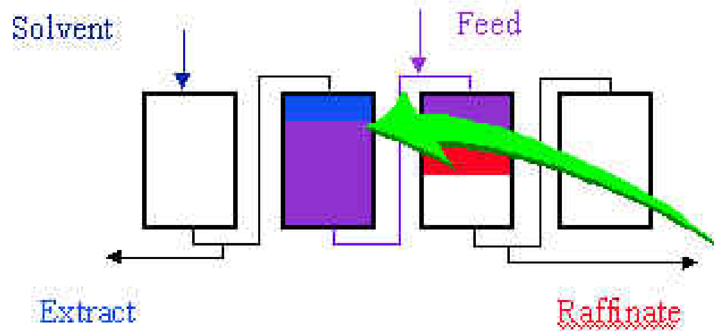
Eventually the front of pure product 1 reaches the outlet. It is distributed between the final Section and the product port.

Simplified SMB - 3

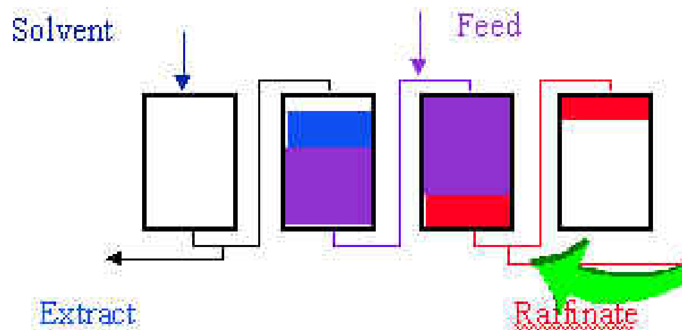


Finally, the mixed product reaches the outlet. To avoid collecting impure material, it is necessary to move the columns 1 position upstream.

Simplified SMB - 4

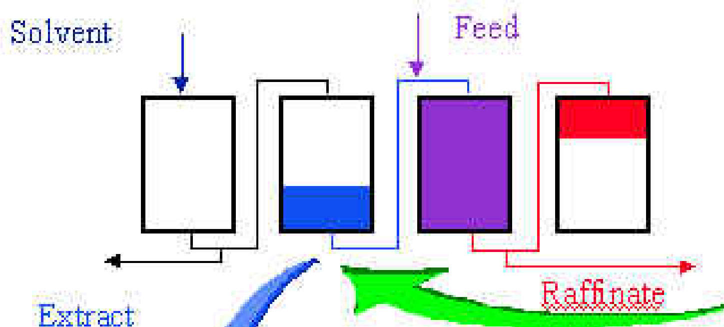


The frontal separation continues; at the same time, the slow moving product starts to separate from the tail of the mixed product band in Section 2

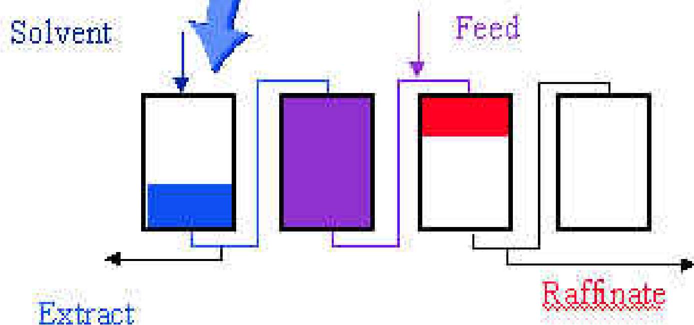


Eventually the fast moving product again reaches the outlet and more pure product is collected.

Simplified SMB - 5

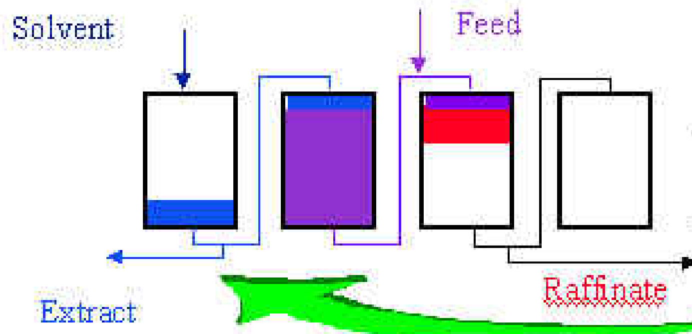


When the mixed band reaches the end of Section 3 its tail has left Section 2 (if the separation has been correctly designed) and only pure product 2 remains in Section 2.

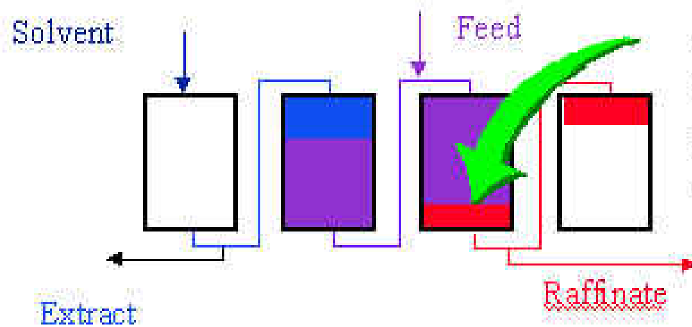


To avoid collecting impure raffinate, the columns are moved once more. Now, the pure component 2 is in Section 1.

Simplified SMB - 6

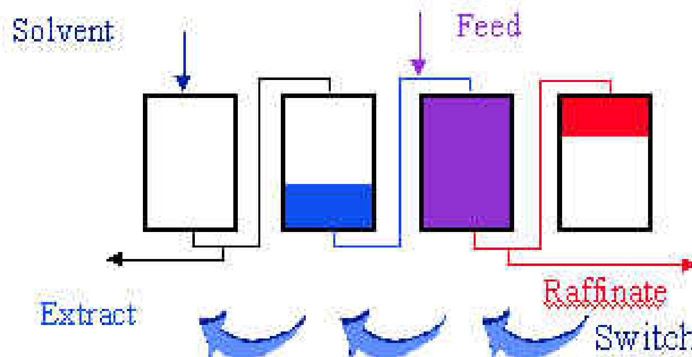


The second component is now collected at the Extract port while the separation continues in Sections 2 and 3.

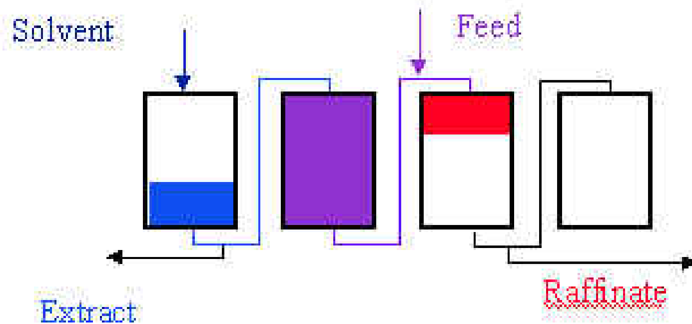


The faster component reaches the Raffinate port and is again collected; note that the outlet concentrations are neither constant nor concurrent.

Simplified SMB - 7

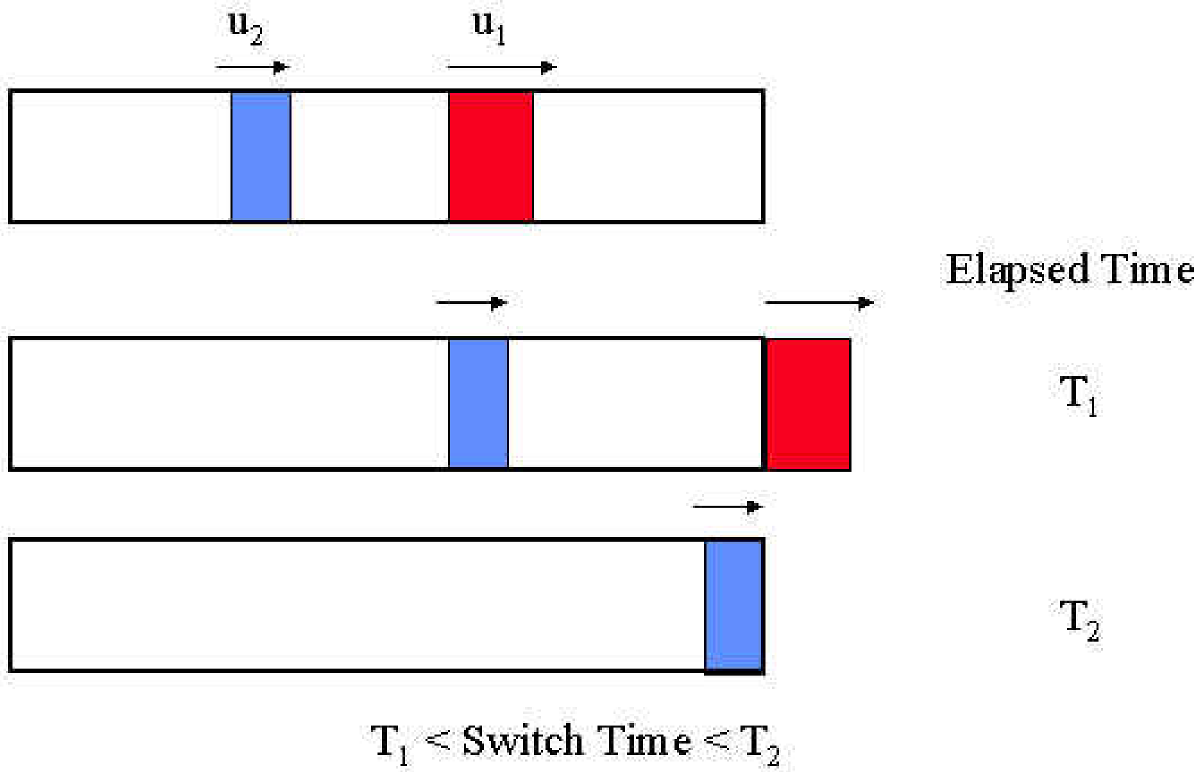


Eventually, the mixed zone reaches the raffinate port and the columns are again switched.

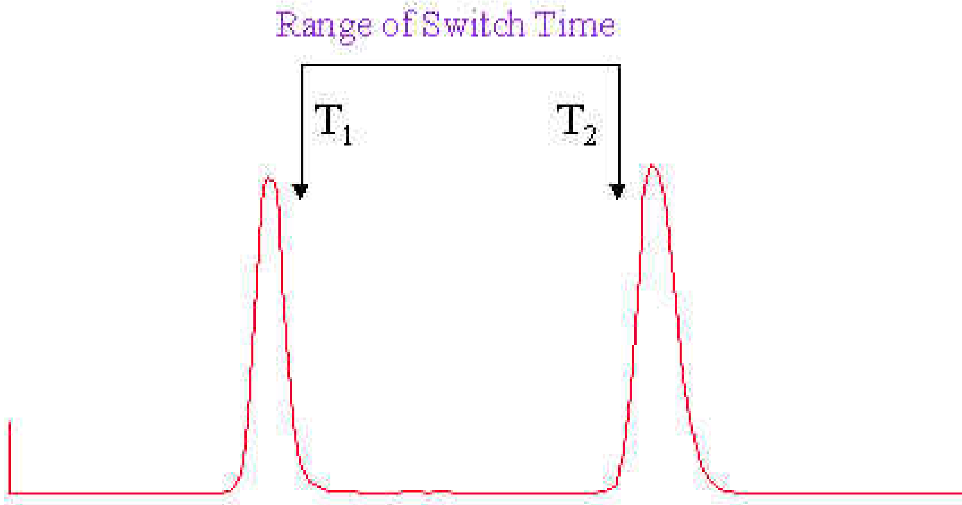


This simplified system is now in a steady state mode and will continue to cycle.

Choice of Switch Time

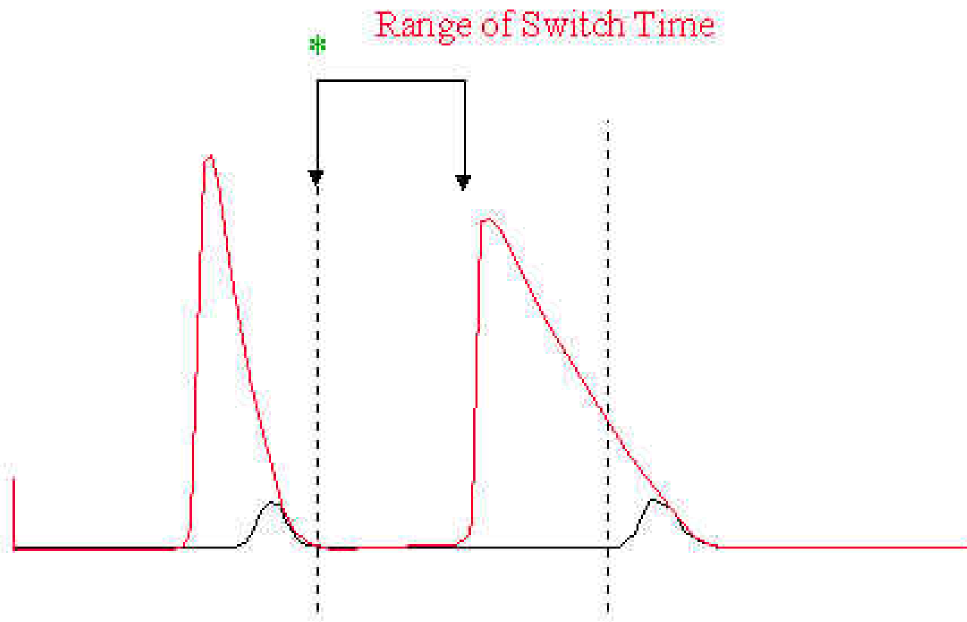


Linear Chromatography



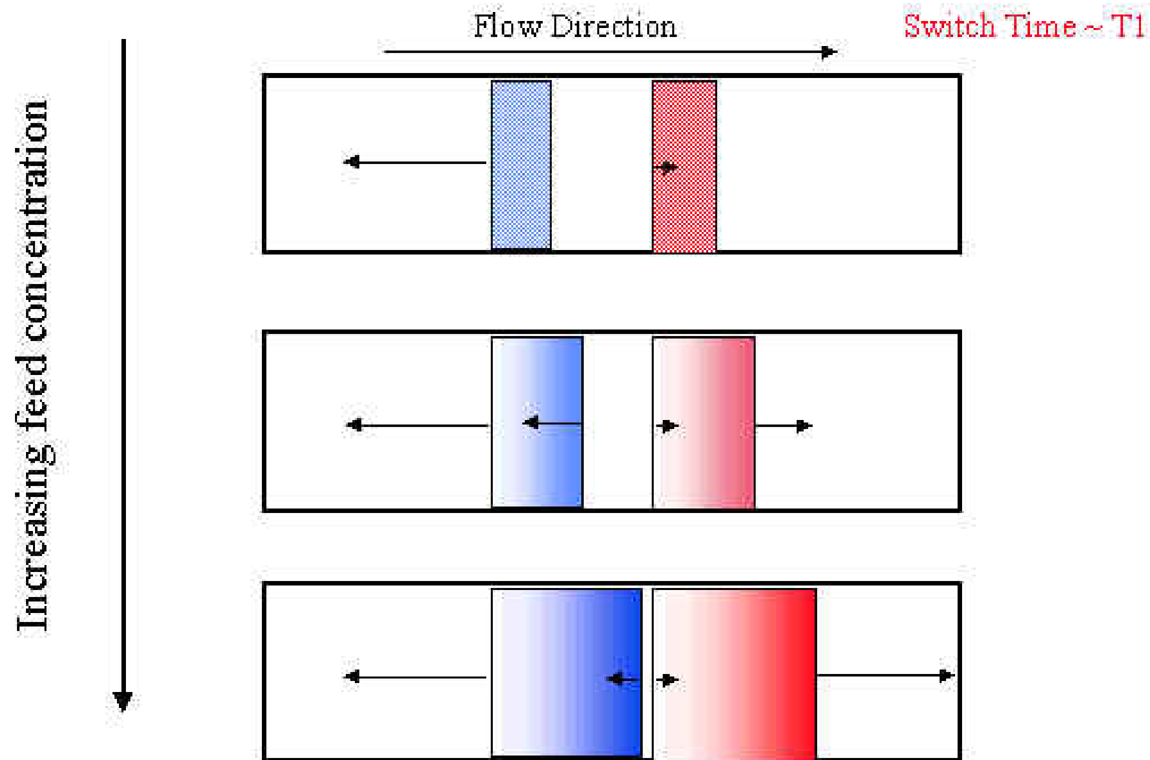
Prochrom R&D 1995

Mass Overload



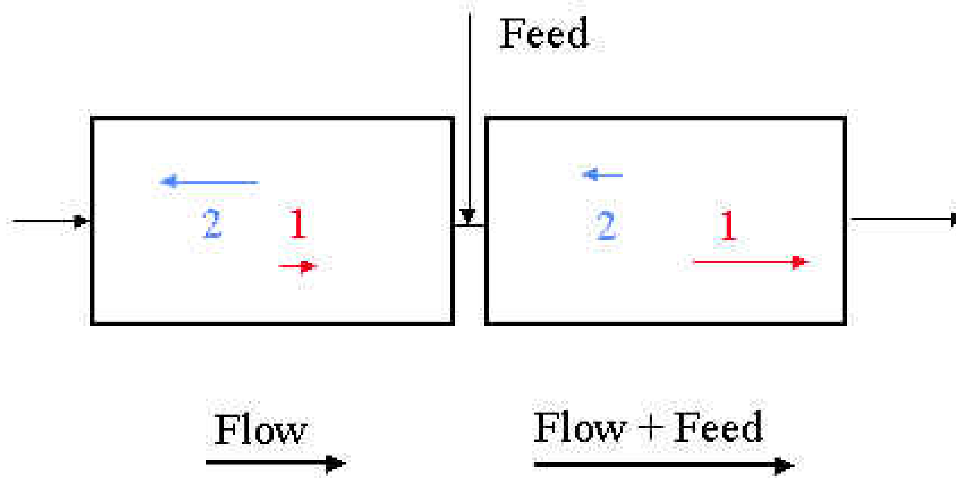
Prochrom R&D 1995

Effect of feed concentration on product velocities



Prochrom R&D 1995

Feed Flow Rate



If the feed flow is too high, product 2 will move in the wrong direction.

Prochrom R&D 1995

How to do it

Switch Time is chosen such that the early eluting product just moves

The feed is introduced at the highest concentration

The feed flow is slowly increased until the later eluting product breaks through in the raffinate stream.

NOTE: Equilibration times are long in SMB. Each change can take several hours to become stable.

Prochrom R&D 1995

**Graphics supplied by G. Cox, H. Colin, T. Lewis, Chiral Technologies Inc.