

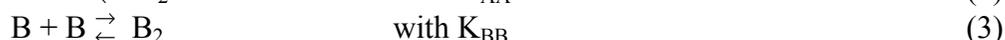
## HPLC Simulator

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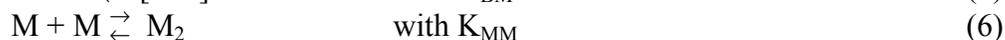
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### Introduction

The simulation of chromatographic experiments can take several forms. This simulator uses an equilibrium plate model to simulate HPLC<sup>1</sup>. This model is useful for exploring the effects of column length, packing, and injection volume on the separation process. This simulation is primarily designed to study the effects of simultaneous equilibria on the separation process. The equilibria can involve both analytes or dimerization. For analytes A and B, consider:

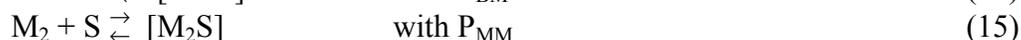
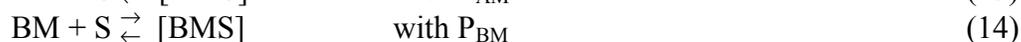
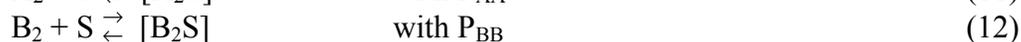
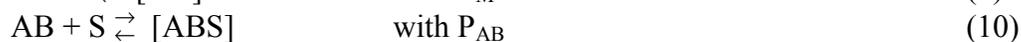
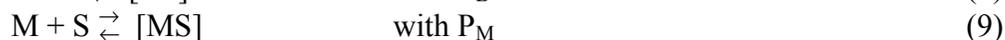
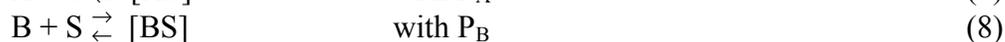


Mobile phase additives often are used to enhance resolution. These additives complex with the analytes and enhance their mobility. Cyclodextrins are commonly used for this purpose. Ion pairing reagents can also be considered in this category. Equilibria with mobile phase additives include, for M the additive:



In fact HPLC is an ideal method for the determination of binding constants for guest-host complexes<sup>2-4</sup>. In binding constant determinations the host is added to the mobile phase and the retention time of the guest-analyte is determined as a function of the host concentration. HPLC binding constant determinations work well when other methods fail. This program can be used to simulate these binding constant experiments.

The chromatography progresses because of the interaction of the solutes with the stationary phase, S. These interactions are also characterized by equilibrium constants, which are called partition coefficients:



This program allows the chromatographic behavior of all these equilibria to be handled simultaneously.

This simulation program can be used to model many types of separations: reversed phase (RP), normal phase, ion exchange (IEX), hydrophobic interaction (HIC), and hydrophilic interaction chromatography (HILIC) for example. All that is necessary is that the analyte-stationary phase interaction is characterized by a partition coefficient. The partition equilibrium can either be based on simple partitioning or competitive partitioning. Competitive mode corresponds to non-linear chromatography, which is characterized by a Langmuir absorption isotherm type interaction (see the Competitive section below). Preparative chromatography is modeled using competitive mode.

This simulation also works for ion-pairing chromatography and with dynamic coatings. For many mobile phase additives, the mobile phase additive has a small partition coefficient with the stationary phase. For example, cyclodextrins don't interact strongly with reversed phase columns. For ion-pairing chromatography, the ion-pairing reagent can have a strong interaction with the stationary phase. For example, long chain quaternary amines have large partition coefficients on reversed phase columns. Dynamic coatings take this mode to the extreme, where the dynamic coating-mobile phase additive has a very large partition coefficient.

One unique aspect of this program is the flexibility in dealing with mobile phase additives. They can be added at a constant level, which corresponds to adding the additive to the eluent solution. Or the mobile phase additive can be pulsed. In a chromatograph, this could be done with a second, large-loop injector or with rapid changes in solvent programming from a binary pump.

## Theory

### Partition Mode<sup>5</sup>

The partition coefficient for interaction of a solute with the stationary phase is

$$P = \frac{C_s}{C_m} = \frac{[A]_s}{[A]} \quad (16)$$

$C_s$  and  $[A]_s$  are the concentration of a solute in the stationary phase.  $C_m$  and  $[A]$  are the concentration of a solute in the mobile phase. The larger  $P$  the slower the analyte moves through the column and the longer the retention time. In this program the retention time,  $t_r$ , is measured at the maximum of the peak. The retention time of a non-retained solute is given as  $t_m$ . Uracil is often used to determine this time. Assuming the volume of the connections between the injector and the column and the column and the detector are negligible,  $t_m$  is also the void volume, which is the volume of the mobile phase in the column. The retention time,  $t_r$ , of a component is determined by its capacity factor

$$k' = \frac{t_r - t_m}{t_m} \quad (17)$$

and the capacity factor of the analyte is related to the partition coefficient through

$$k' = P \frac{V_s}{V_m} \quad (18)$$

where  $V_s/V_m$  is the ratio of the volume of the stationary phase to the volume of the mobile phase in the column. This ratio is often given by specifying the packing fraction

$$pkf = \frac{V_s}{V_s + V_m} \quad (19)$$

The column length,  $L$ , and the internal diameter,  $D$ , determine the total column volume

$$V_{tot} = \pi \left(\frac{D}{2}\right)^2 L = V_s + V_m \quad (20)$$

The packing fraction rarely exceeds 30% even in the most efficient columns.

The efficiency of the column is measured by the number of theoretical plates,  $N$ , which is the ratio of the variance of the time on the column and the variance of the eluting band

$$N = \frac{t_r^2}{\sigma^2} = \frac{16 t_r^2}{w^2} \quad (21)$$

Chromatographers usually choose to determine the width of the band by the time spread at the base of the peak, which is approximately  $w = 4\sigma$ . If the peak is not Gaussian in shape, it is better to actually calculate the variance using a second moment calculation. The first moment corresponds to the average. The peak profile,  $g(t)$ , is the probability distribution function for the average, with normalization

$$N = \int_{-\infty}^{\infty} g(t) dt \quad (22)$$

The first moment is the average of the time

$$\langle t \rangle = \frac{1}{N} \int_{-\infty}^{\infty} t g(t) dt \quad (23)$$

For a symmetrical peak, the first moment is the retention time. The variance is the square of the standard deviation. The variance is the second central moment

$$\sigma^2 = \langle (t - t_r)^2 \rangle \quad (24)$$

$$\sigma^2 = \langle (t - \langle t \rangle)^2 \rangle = \langle t^2 \rangle - \langle t \rangle^2 \quad (25)$$

For a Gaussian peak Eq 24 and 25 are equivalent. For skewed peaks, there is a subtle difference. Eq 25 is used in the program.

The second moment is calculated using the average of  $t^2$

$$\langle t^2 \rangle = \frac{1}{N} \int_{-\infty}^{\infty} t^2 g(t) dt \quad (26)$$

Equations 16-26 are used by the program to characterize each separation. In effect, the program treats the calculated chromatogram just like a real chromatogram and determines the coefficients and efficiency just like an experimental run. Note that equations 16-18 can be used to determine the effective partition coefficient for each solute. The effective partition coefficient will only be equal to the true partition coefficients, Eq 7-9, if there are no other simultaneous equilibria. In other words, don't expect the calculated effective  $P$ 's to be the same as those you input, unless there are no equilibria involving those analytes.

### Competitive Mode and Nonlinear Chromatography

In partition mode, the stationary phase is assumed to have a large active surface area and  $P=C_s/C_m$ . In competitive mode, the stationary phase has a limited number of active sites and

$$P = \frac{C_s}{C_m S} \quad (27)$$

$S$  is the concentration of unoccupied active sites on the stationary phase. This is equivalent to a Langmuir adsorption isotherm. To show this, let  $S_0$  be the total concentration of stationary binding sites. Then,  $S = S_0 - C_s$ ,

$$P = \frac{C_s}{C_m (S_0 - C_s)} \quad (28)$$

Cross-multiplying gives  $PC_m(S_0 - C_s) = C_s$  and solving for  $C_s$  gives

$$C_s = \frac{PC_m S_0}{(1 + PC_m)} \quad (29)$$

Eq. 29 is the normal way to express the Langmuir adsorption isotherm. Competitive mode is necessary for modeling ion exchange equilibria, when the ion exchange resin has a limited exchange capacity relative to the concentration of the analyte. But, the competitive mode is also useful for overloaded reversed phase and normal phase columns. Such overloading is common for LC/MS separations. Separations that involve the competitive mode are called non-linear.

The equilibrium plate model is particularly well suited to studies of non-linear chromatography<sup>1</sup>. Non-linear models are most often used to optimize preparative scale separations. Preparative chromatography often produces shock-fronts instead of well-resolved, symmetric bands. Simulations are very useful for optimizing such separations.

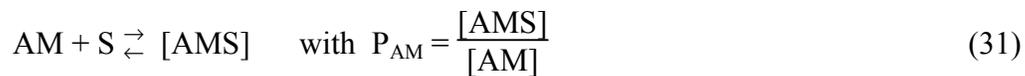
To get comparable retention between a partitioning run and a competitive run, divide the partition coefficient for the partitioning run by the concentration of stationary sites. For example, if the partitioning partition coefficient for A is 0.1 and the concentration of stationary sites is 10 equiv/L, then divide the partition coefficient by 10 equiv/L to get the competitive partition coefficient,  $P_A=0.01$ .

## Ion Pairing and Dynamic Coatings

This simulation also works for ion-pairing chromatography and dynamic coatings. For ion-pairing chromatography and dynamic coatings, the mobile phase additive has a strong interaction with the stationary phase as well as the analyte. The only requirement to use this simulation program under these circumstances is that the interaction of the mobile phase additive with the analyte is given by Eqs. 4 and 5. To be explicit, we do not consider (in partition mode)



distinctly from



Instead, the coupled equilibria require that

$$P_A^i = \frac{[AMS]}{[A]} = \frac{[AMS]}{[AM]} \frac{[AM]}{[A][M]} [M] = P_{AM} K_{AM} [M] \quad (32)$$

If this were not true, detailed balancing would be violated.

If you prefer to model the partitioning interaction using Eq. 30, there are two choices. The best option is to use Eq. 32 to calculate  $P_{AM}$ . The second option is to consider that the coating is static over the course of the separation; therefore the dynamic coating is just considered part of the stationary phase and the normal Eqs. 7 and 8 are used for partitioning. In this second case you would not use a mobile phase additive explicitly.

## Equilibrium Plate Model for Chromatography

The algorithm for simulating a chromatographic separation is quite easy to visualize. The column is divided into theoretical plates of equal length. Each theoretical plate can be considered as a separate beaker. The beakers all contain equal amounts of mobile phase and stationary phase. Consider just one analyte, A. The injection introduces a given number of moles into the first plate,  $n_{A,m}(0)$ . On the first plate, the analyte will partition between the mobile phase and the stationary phase

$$P = \frac{[A]_s}{[A]} \quad \text{or} \quad [A] = \frac{[A]_s}{P} \quad (33)$$

Or in terms of molar amounts

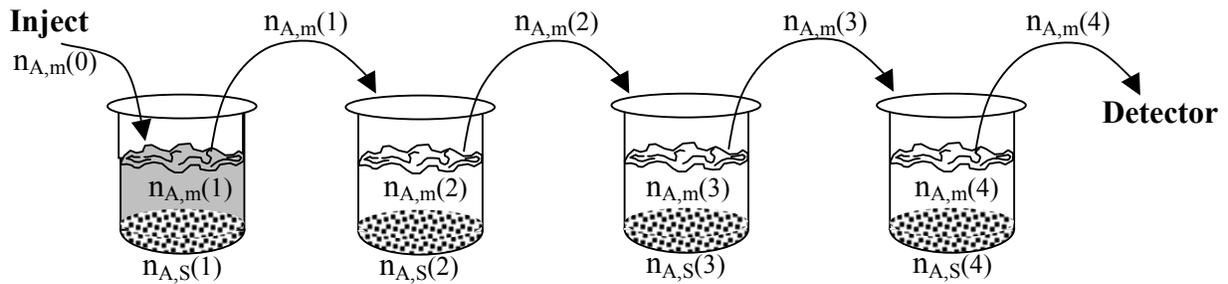
$$n_{A,m}(1) = V_m [A] = V_m \frac{[A]_s}{P} \quad (34)$$

and for the stationary phase

$$n_{A,s}(1) = V_s P [A] \quad (35)$$

At the next time step, the A in the mobile phase is passed to the next plate. The A adsorbed on the stationary phase, of course, stays behind. At each time step the mobile phase is passed on to the next plate, then the new equilibrium is established.

**Time Step 1:**



**Time Step 2:**

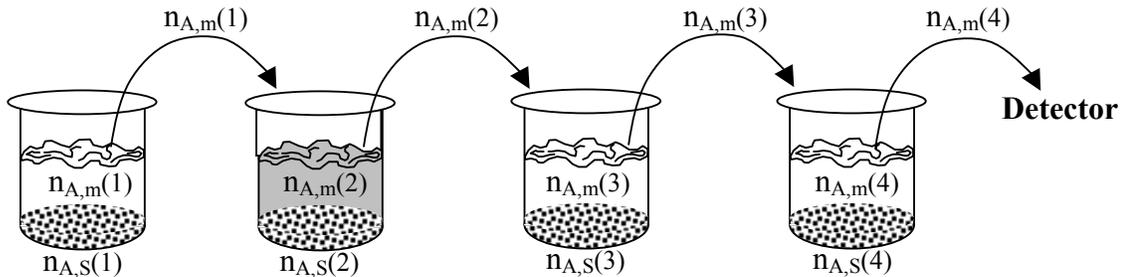


Figure 1. At each time step the mobile phase is passed along to the next theoretical plate. The theoretical plate number is in ( ).

The equilibrium plate model corresponds to ideal chromatography. Rate processes are assumed to be so rapid that all the equilibria are attained at each point along the column for each time increment. The equations describing the operation of chromatography are continuous. This simulation corresponds to a finite difference approximation to these differential equations.

There are no dispersive processes directly included in this model. In other words, diffusion of the analyte is not part of the model. In the limit that the number of theoretical plates approaches infinity, the chromatographic bands would become infinity thin. However, the finite plate height that results from the finite difference approximation does cause band broadening. In effect, the concentrations in each plate are homogeneous. In other words the beakers are well mixed, so that the left and right side of the beaker have the same concentrations. This uniform finite plate has the effect of causing band dispersion. You can imagine that the effective diffusion distance of the analyte during the time step is approximately the size of the beaker. This is the reason that the

measured efficiency of the column, as calculated from the eluting band profile and Eq. 21, is very different from the number of theoretical plates that is chosen for running the simulation.

To set the time step for the simulation from the input column diameter,  $D$ , length,  $L$ , packing fraction,  $pkf$ , number of theoretical plates,  $N$ , flow rate,  $F$ , and Eqs. 19 and 20

$$dt = \frac{V_m}{FN} \quad (36)$$

For example, for a 4.5x100-mm column with a packing fraction of 30% the mobile phase volume is 1.11 mL. If the column has 500 plates for a 1 mL/min flow rate the time step is 0.00223 min and the mobile phase volume per step is 1.11 mL/500 = 2.23  $\mu$ L. The void volume of the column is  $V_m$ , and therefore the number of steps required to elute a non-retained solute is equal to the number of plates,  $N$ . The void time is then  $N dt$ . Actually, the time only enters the calculation when the data are plotted. Each data point is separated from the next by the time  $dt$ . Time never enters the equations of the simulation explicitly.

### Equilibrium Calculations

Each theoretical plate in the column is treated as a separate beaker. The distribution of analytes is allowed to come to equilibrium with the stationary phase and with each other at each step of the simulation for each plate. The equilibrium calculations are done using standard techniques<sup>5</sup>. Several comments may be helpful, however. There are three thermodynamic components in this equilibrium system, A, B, and M. The other species are all related to A, B, and M by equilibrium equations, so they don't count as components. The number of degrees of freedom are  $f = c - \tilde{n} - p + 2 = 3 - 2 + 2 = 3$ . Therefore, the system is completely specified with only three variables per plate. All the other species are fixed by the equilibria. Therefore, the program never explicitly calculates the concentrations of [AB], [AM], [BM], etc. Instead, the main variables are the number of moles of each component at each plate. Since only the material in the mobile phase passes from one plate to the next, it is necessary, however, to calculate the number of moles of each component in the mobile phase. These values are not really new variables because the mass balance on each plate constrains the partitioning:

$$n_{A,tot} = n_{A,mobile} + n_{A,stationary} = n_A + n_{A,s} \quad (33)$$

and the mobile and stationary amounts are fixed by the partition equilibria. So even though there are many species possible, there are really just six variables necessary per plate that need to be calculated.

The calculations use the distribution coefficients for the free species,  $\alpha_i$ , for  $i = A, B, \text{ and } M$ . For each plate then using A as an example and  $n_A$  the number of moles of free A,

$$\alpha = \frac{n_A}{n_{A,tot}} \quad \text{or} \quad 1/\alpha = \frac{n_{A,tot}}{n_A} \quad (34)$$

$$1/\alpha = \frac{1}{V_m[A]} ( V_m[A] + V_s[A]_s + V_m[AB] + V_s[AB]_s + \ddot{O} ) \quad (35)$$

plus terms for AM and  $A_2$ .

The corresponding equilibrium expressions give

$$[A]_s = P_A [A] S \quad (36)$$

$$[AB] = K_{AB} [A][B] \quad (37)$$

$$[AB]_s = P_{AB} [AB] S \quad (38)$$

with S the concentration of free stationary phase sites. For partitioning mode, S is set to 1. Substitution of Eq 37 into Eq 38 eliminates the [AB] concentration

$$[AB]_s = P_{AB} S K_{AB} [A][B] \quad (39)$$

The total amount of  $n_{AB,tot} = V_m[AB] + V_s[AB]_s$  from Eqs. 37 and 39 gives

$$V_m[AB] + V_s[AB]_s = K_{AB} [A][B](V_m + V_s P_{AB} S) \quad (40)$$

The phase ratio is given as

$$\phi = \frac{V_s}{V_m} \quad (41)$$

Substitution of Eqs. 36, 37, 40, and 41 into Eq 35 gives

$$1/\alpha = 1 + \phi P_A S + K_{AB} [B] (1 + \phi P_{AB} S) + \ddot{O} \quad (42)$$

plus additional, similar terms for AM and A<sub>2</sub>.

In general then, considering all equilibria

$$1/\alpha_i = 1 + \phi P_i S + \sum_j K_{ij} [X]_j (1 + \phi P_{ij} S) \ddot{O} \quad (43)$$

The corresponding sum for the  $\alpha$  for the free stationary phase sites, when using competitive mode, is

$$1/\alpha_s = 1 + \sum_i P_i [X]_i + \sum_i \sum_j P_{ij} K_{ij} [X]_i [X]_j \ddot{O} \quad (44)$$

The  $\alpha$ 's can then be used to determine the amounts of free component in the mobile and stationary phases

$$n_i = \alpha_i n_{i,tot} \quad [X]_i = \frac{n_i}{V_m} \quad (45)$$

These free species concentrations are not known at the beginning of the calculation, so Eqs. 43-45 must be iterated starting with initial guesses for the free concentrations  $[X]_i$ .

It is not necessary to keep track of the free concentrations; the total moles are the only necessary variables. None-the-less the program does store the  $[X]_i$  values and passes the values

to the next plate to provide good guesses for the iteration of Eqs. 43-45. This expedient speeds the convergence of the equations, but the free concentrations are not used to maintain the mass balance for each plate.

## The Components

### *Injector*

A and B are handled differently from M in the injected sample. The simulation is based on the number of moles of A and B that you inject. For example, for a 4.5x100-mm column with a packing fraction of 30% the mobile phase volume is 1.11 mL. If the column has 500 plates for a 1 mL/min flow rate the time step is 0.00223 min and the mobile phase volume per step is  $1.11 \text{ mL}/500 = 2.23 \text{ }\mu\text{L}$ . Let the injection volume of a 1 mM sample be 10  $\mu\text{L}$ , that is  $10 \text{ }\mu\text{L} \times 1 \text{ mM} = 1 \times 10^{-8}$  moles total will be injected. The injection will require  $10 \text{ }\mu\text{L}/2.23 \text{ }\mu\text{L} = 4.48$  steps. Since the injection process must take an integer number of steps, then we must round down to 4 steps. These 4 steps will have the same concentration of sample, but calculated to ensure that  $1 \times 10^{-8}$  moles total will be injected, or  $1 \times 10^{-8}$  moles/(4\*2.23 $\mu\text{L}$ ) .

Because M is often used as the mobile phase additive, M must be handled differently to avoid unphysical baseline disturbances at the detector. The concentration of M in each injection step is kept at the selected value.

There is no extra-column volume in this simulation. In essence, the injector is ìconnectedî directly to the column. In other words, the proper number of moles of A, B, and M are placed in the top plate of the column during each injection step. Likewise, there is no extra volume between the column and the detector.

### *Column*

The column length and diameter are used to get the total volume of the column. With the packing fraction, the volume of the mobile phase in the column and the stationary phase are calculated. These geometrical constants and the flow rate do not have an effect on the separation efficiency. The separation efficiency is completely determined by the number of theoretical plates that you choose. In other words, the mobile phase velocity does not change the column efficiency, as it would in a real column.

As the number of theoretical plates and the packing fraction increase, the retention times will increase. As the column volume increases, the retention times will also increase, for a fixed flow rate. As the number of theoretical plates increases the separation will become more efficient.

### *Mobile Phase*

Mobile phase additives are often added directly to the eluant. This addition provides a constant level of additive during the separation. The determination of binding constants using HPLC uses this scheme<sup>2-4</sup>. This facility may also be used to study the effect of ion-pairing reagents. If you have guesses for the equilibrium constants, this simulation program can be used to optimize the separation.

More intriguing is the ability to program the addition of the mobile phase additive. Programmed or pulsed addition might be useful if the mobile phase additive is very expensive

and you want to minimize the amount used. Programmed addition may also be useful since you don't need to flush the eluant reservoirs between users. For example, an ion-pairing reagent or cyclodextrin may take a long time to flush from the solvent handling system, before another type of separation is attempted. Another possibility is to control the amount of mobile phase additive that is injected into a mass spectrometer. For example, if the mobile phase additive can be designed to elute before the sample, then a valve before the mass spectrometer can be used to divert the additive to waste before the analysis. This type of programming is usually done with a super-loop, which is a second injector with an extra large loop, Figure 2.

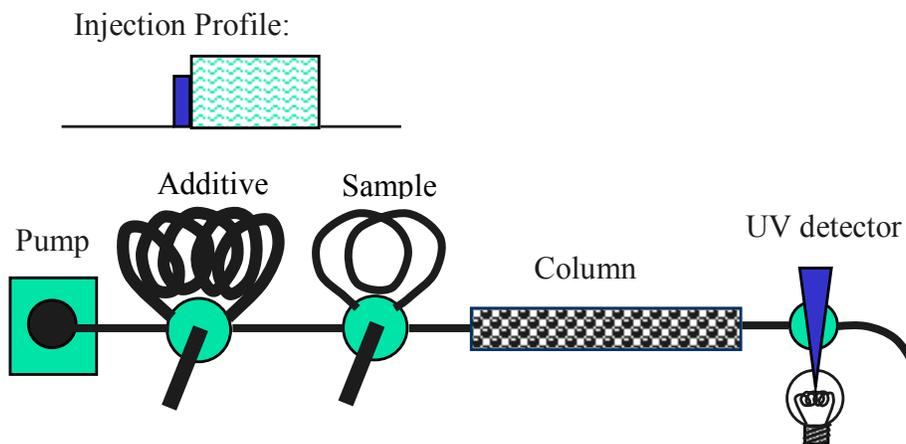


Figure 2. Mobile phase additive programming with a super-loop. The additive may be pulsed before, after (shown), or during injection.

The timing in simulations of this type is best expressed as multiples of the void, or mobile phase, volume. For example, the length of time that is required for an unretained analyte to pass through the column is one void volume. Therefore, a mobile phase program that runs from injection to  $1 \times V(\text{void})$  will keep the fastest analytes in the mobile-phase additive as long as the mobile phase additive has a partition coefficient that is smaller than the fastest analyte. On the other hand, a mobile phase program started before injection is necessary if the mobile phase additive has a larger partition coefficient than the analyte.

Like injection, the mobile phase program must be in integer steps. The actual number of steps before and after injection is listed in the printout. Keep in mind that, just like a real injector, if you don't add the mobile phase additive to the sample that there will be a gap in the mobile phase concentration upon injection.

### *Detector*

The detector simply plots the total moles of A, B and M. That is,  $\text{signal}(A) = n_A + n_{AB} + n_{A^2} + n_A$ . No attempt is made to determine the speciation. The plot begins at the void volume. That is, an unretained component will elute in the first plotted time increment. Not every time point is plotted. The plot will contain a maximum of 200 points, however the plot stops automatically when A and B have eluted.

To determine when A and B have eluted, the program maintains the total mass balance. When 98.5% of the last analyte elutes the run ends. The program does not monitor the total mass balance for M. The reason is that M is often a constant mobile phase additive. The signal scale

for A and B runs from zero to the maximum value indicated. The plot scale for M runs from the minimum to the maximum value. This plot scaling allows the program to easily simulate indirect detection.

When using indirect detection, remember that the plotted value is the sum of all species that contain M, not just the free M concentration.

The printed retention time is based on the maximum of the eluted band. This value may be in-between the plotted points. The time uncertainty is the time step of the simulation. The statistics are based on the detector output. So for example, the effective number of plates is expected to be different from the value you chose for input to the program.

## Running hplc

The hplc program may be run from a Web-cgi interface or from the command line and an input file. A typical input file is given in Table I.

Table I. Input file.

```
0.225 // rad: column radius in cm
25.0 // len: column length in cm
0.70726 // pkf: packing fraction, Vs/(Vs+Vm)
2800 // nplate: number of theoretical plates
10.0 // So: conc. of stationary phase sites; So=1.0 for partitioning
0.0 // Mo: mobile phase additive concentration
1.90 // flow: in ml/min
30 // runV: run time (volume) as runV*V(void)
0.0 // cmpulse: additive programming concentration in molar(M)
0.0 // add1: additive programming start time as add1*V(void)
0.0 // add2: additive programming stop time as add2*V(void)
10.0e-6 // Vinj: injector volume in liters eg. 10.0e-6 typical
0.00333 // ntotA: sample concentration of A, B, and M in molar(M)
0.01 // ntotB
0.000 // ntotM
.false. // partition: .true.-partitioning .false.-competition
0.238 // P[0]: partition coefficient for A, PA
0.256 // P[1]: PB
0.000 // P[2]: PM
0.0 // Pij[0]: PAA [j>=i]
0.0 // Pij[1]: PAB
0.0 // Pij[2]: PBB
0.0 // Pij[3]: PAM
0.0 // Pij[4]: PBM
0.0 // Pij[5]: PMM
0.0 // K[0]: equilibrium constant for AA, KAA
0.0 // K[1]: KAB
0.0 // K[2]: KBB
0.0 // K[3]: KAM
0.0 // K[4]: KBM
0.0 // K[5]: KMM
0.00001 // rtol: tolerance for skipping equilibration=rtol*(est. [X]m)
.false. // skip: .false. start plot at V(void), .true. start at first peak
```

This input file corresponds roughly to Figure 2 in Ref. 1. You can edit these files to produce your runs. Note that the comments are ignored when the file is read in, so it is best to leave them in the file to enhance readability. The values must be read in the given order and the data types must be maintained. For example, keep the number of theoretical plates an integer. The *rtol* parameter is discussed below.

## Accuracy

An option to save time is available. A tolerance value, *ntol*, is set. If the mobile phase concentration drops below this value in a particular plate, then equilibration with the stationary phase is skipped. This approximation is particularly helpful when small amounts of solute remain adsorbed on the stationary phase after the bulk of the peak passes. If the equilibration step is skipped, we can't just forget about the residual solute, however. The residual solute is treated differently depending on whether the skipped plate is behind or in front of the bulk of the main peak.

If equilibration is skipped after the peak maximum passes through then all of the solute in this plate is placed in the mobile phase. The new mobile phase amount then includes any solute passed in from the previous plate and any solute adsorbed on the stationary phase in this plate. The result is to clean up, so small amounts don't get stuck on column. The reason that we just don't forget about the residual adsorbed solute is for the total mass balance.

If equilibration is skipped before the peak maximum passes through then all of the solute in this plate is placed onto the stationary phase. This approximation may seem strange at first. However, if all of the solute is not held back, then very small amounts of solute will rapidly pass on through the rest of the column without interacting with the stationary phase at all. This would be observed as small amounts of solute eluting well before the main peak, which is particularly unphysical.

These approximations can have a large effect on the column efficiency. The column appears more efficient than it should be since residual solute is "squeezed" towards the bulk of the peak.

The tolerance *ntol* is the minimum number of moles of solute in the mobile phase before skipping the equilibration calculation. The value of *ntol* is controlled by an input parameter, *rtol*, which is used in the following way. The maximum possible number of moles of solute on a given plate is the number of moles of solute injected, *ntotA*. The mass balance for this extreme would be:

$$ntotA = n_{A,m} + n_{A,s} = C_{A,m} V_m + C_{A,s} V_s \quad (46)$$

Using Eq. 27 to find  $C_{A,s}$  with the approximation that  $S = S_0 - C_{A,s} \approx S_0$  gives

$$ntotA = C_{A,m} V_m + P C_{A,m} S_0 V_s \quad (47)$$

Eq. 47 can then be solved for  $n_{A,m}$ :

$$n_{A,m} = C_{A,m} V_m = \frac{ntotA}{\frac{P S_0 V_s}{V_m} + 1} \quad (48)$$

This result gives a rough approximation for the number of moles of A in the mobile phase. The actual value will be smaller, since the injection usually over a number of plates and dispersion

decreases the moles per plate as the solute spreads out. The input value of *rtol* is the ratio of the minimum value to this estimated value. The value of *ntol* is then calculated from:

$$ntol = rtol \frac{ntotA}{\frac{P_{max}S_oV_s}{V_m} + 1} \quad (49)$$

where *S<sub>o</sub>* is the concentration of stationary sites, *V<sub>s</sub>* is the volume of the stationary phase, and *V<sub>m</sub>* is the volume of the mobile phase, and *P<sub>max</sub>* is the largest partition coefficient for any solute. The value of *ntol* is calculated using both *ntotA* and *ntotB* and the smaller value is chosen for the run. Accuracy and speed are tradeoffs when choosing the *rtol* value. Larger values of *rtol* give faster calculations and smaller values give more accurate calculations. A value of *rtol* = 1.0x10<sup>-15</sup> essentially turns the option off to give the maximum accuracy.

An example of the speed savings and accuracy are given in Table II.

Table II. Effect of *rtol* on accuracy. *T<sub>r</sub>(A)* = retention time for A, *N(A)* = number of theoretical plates for A.

**Conditions:**

```
0.225 // rad: column radius in cm, i.e. 4.5 mm ID
25.0 // len: column length in cm
0.70726 // pkf: packing fraction, Vs/(Vs+Vm)
2800 // nplate: number of theoretical plates
10.0 // So: conc. of stationary phase sites; So=1.0 for partitioning
0.0 // Mo: mobile phase additive concentration
1.90 // flow: in ml/min
30 // runV: run time (volume) as runV*V(void)
10.0e-6 // Vinj: injector volume in liters eg. 10.0e-6 typical
0.00333 // ntotA: sample concentration of A, B, and M in molar(M)
0.01 // ntotB
.false. // partition: .true.-partitioning .false.-competition
0.238 // P[0]: partition coefficient for A, PA
0.256 // P[1]: PB
```

<i>rtol</i>	Time/sec	<i>T<sub>r</sub>(A)</i> /min	<i>N(A)</i>	<i>T<sub>r</sub>(B)</i> /min	<i>N(B)</i>
1.0x10 <sup>-15</sup>	109	4.137	3286	4.403	3588
0.00001	83	4.137	3298	4.403	3590
0.0001	78	4.137	3597	4.403	3709
0.001	71	4.136	8812	4.403	3704

Table II shows that the retention times are little effected by the approximations, but the column efficiency and peak shape are strongly dependent on the value of *rtol*. The timesavings is not particularly great, so for best results turn the option off if you are concerned about the column efficiency. For routine calculations choose *rtol* = 0.0001, which gives ~30% time savings.

**Examples**

**References**

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