Theory of CCC

In order to understand Counter-current Chromatography, it is necessary to understand some parameters of CCC…

Partition Coefficient $K = C_s/C_m$
- Good $K$ values – between 0.5 and 2
  - If $K < 0.5$ - loss of peak resolution
  - If $K > 2$ - long retention time, peak broadening (though application of EECCC methodology raises the $K$ value for a good separation to much higher)

$$t_R = \frac{V_c}{f} \{1 + S_F (K - 1)\}$$

- $f$ – flow rate
- $V_c$ – coil volume
- $t_R$ – retention time
- $S_F$ – Stationary phase fraction $S_F = V_s/V_c$
  - $S_F$ is dependent on solvent system, flow-rate, speed of revolution, etc.
  - The higher the $S_F$, the higher the resolution

Ito, Y. High-speed countercurrent chromatography, Chemical Analysis Series; Ito, Y. & Conway, W. D., Eds.; J. Wiley, New York, 1996; Chapter 1, 3-44.

To help you understand CCC, let’s go through a simple HSCCCC experiment…

Sample Separation

The figure to the right is a theoretical chromatogram from a HSCCCC separation. The details are below.
- $V_c = 40$ ml,
- $f = 1$ ml/min
- $S_F = 0.75$

The yellow highlighted area is the first peak.

\[ V_c = 40 \text{ml}, \]
\[ f = 1 \text{ml/min} \]
\[ SF = 0.75 \]

Nonretained solute (1) – eluted with MP

\[ K = 0 \]
\[ MP = V_o = 10 \text{ml} \]

The orange highlighted area is the second peak.

\[ V_c = 40 \text{ml}, \]
\[ f = 1 \text{ml/min} \]
\[ SF = 0.75 \]

\[ K = 1 \]
\[ V_R = 40 \text{ml} \]

\[ V_R = V_c \text{ if } K=1 \]

\[ t_R = V_c / f \text{ if } K=1 \]
\[ t_R = 40 / 1 = 40 \text{ min.} \]

The red highlighted area is the third peak.

\[ V_c = 40 \text{ml}, \]
\[ f = 1 \text{ml/min} \]
\[ SF = 0.75 \]

\[ V_R = 50 \text{ml} \]
\[ K = (V_R - V_o / V_o)(1 - SF / SF) \]
\[ K = (50 - 10 / 10)(1 - 0.75 / 0.75) = 1.33 \]

\[ t_R = V_c / f [1 + SF(K-1)] \]
\[ t_R = 40 / 1[1 + 0.75(1.33 - 1)] \]
\[ t_R = 50 \text{ min.} \]
The Mathematical Advantage of CCC: Easy Scale-up

Arrows represent where K=1

a) 3mg
   \[ V_c = 43\text{ml} \]
   i.d. = .85 mm

b) 100mg
   \[ V_c = 280\text{ml} \]
   i.d. = 1.6 mm