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

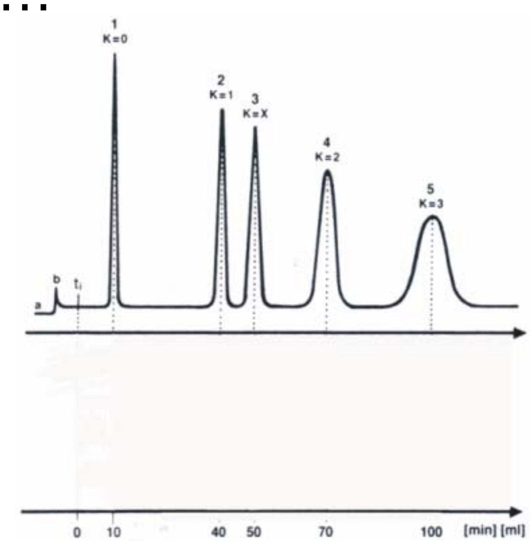
Ito, Y. *High-speed countercurrent chromatography,* Chemical Analysis Series; Ito, Y. & Conway, W. D., Eds.; J. Wiley, New York, 1996; Chapter 1, 3-44.

To help you understand CCC, let's go through a simple HSCCC experiment…

Sample Separation

The figure to the right is a theoretical chromatogram from a HSCCC separation. The details are below.

V_c= 40ml,
f = 1 ml/min
S_F = 0.75



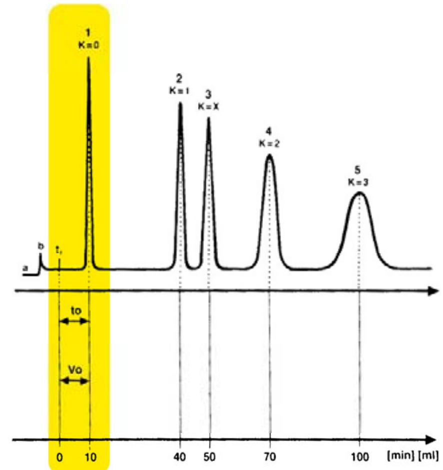
Schaufelberger, D.E. *High-speed countercurrent chromatography,* Chemical Analysis Series; Ito, Y. & Conway, W. D., Eds.; J. Wiley, New York, 1996; Chapter 2, 45-70.

The yellow highlighted area is the first peak.

V_c= 40ml,
f = 1 ml/min
S_F = 0.75

Nonretained solute (**1**) – eluted with MP

K = 0
MP = V_o = 10ml



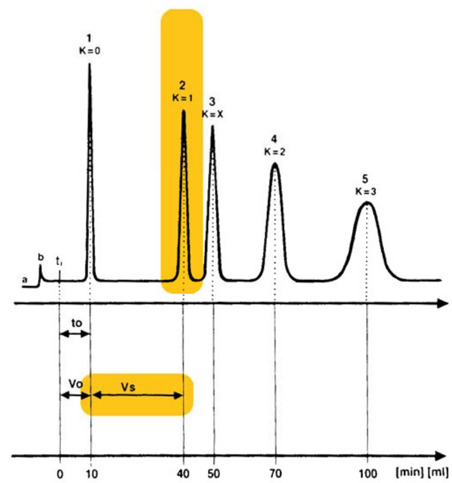
The orange highlighted area is the second peak.

V_c= 40ml,
f = 1 ml/min
S_F = 0.75

K = 1
V_R= 40ml

V_R= V_c if K=1

t_R= V_c/f if K=1
t_R= 40/1 = 40 min.

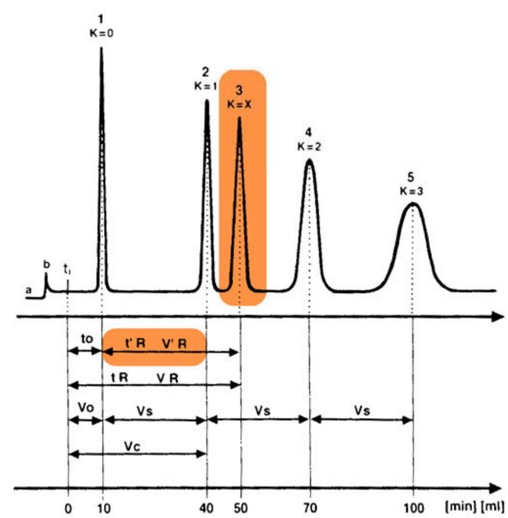


The red highlighted area is the third peak.

Vc= 40ml,
f = 1 ml/min
SF = 0.75

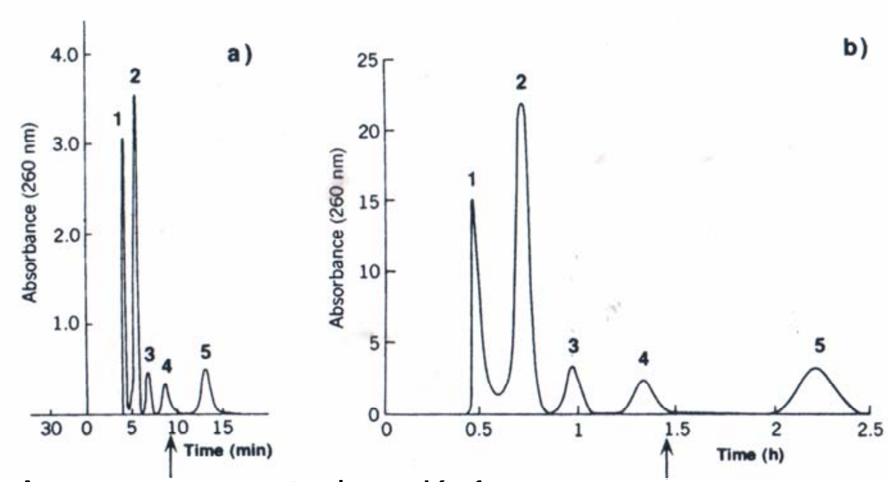
VR= 50ml
K = (VR- Vo/ Vo)(1- SF/ SF)
K = (50-10/10) x
(1-0.75/0.75) = 1.33

tR = Vc/f [1+SF(K-1)]
tR = 40/1[1+0.75(1.33-1)]
tR = 50 min.



Schaufelberger, D.E. *High-speed countercurrent chromatography,* Chemical Analysis Series; Ito, Y. & Conway, W. D., Eds.; J. Wiley, New York, 1996; Chapter 2, 45-70.

The Mathematical Advantage of CCC: Easy Scale-up



Arrows represent where K=1

a) 3mg
V_c = 43ml
i.d. = .85 mm

b) 100mg
V_c = 280ml
i.d. = 1.6 mm

Schaufelberger, D.E. *High-speed countercurrent chromatography,* Chemical Analysis Series; Ito, Y. & Conway, W. D., Eds.; J. Wiley, New York, 1996; Chapter 2, 45-70.