HPLC 2012 – June 16-21, 2012 – Anaheim CA – Poster **274**

Efficient small volume hydrostatic countercurrent chromatography columns

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What is countercurrent chromatography?

Working with a support-free LIQUID stationary phase

CCC is a PREPARATIVE technique since solutes can access to the volume of the liquid stationary phase and not just its surface.

Any one of the two liquid phases can be the stationary phase.

The retention equation is simply:

$$V_R = V_M + K_D V_S$$

V_R retention volume

V_M mobile phase volume

V_S stationary phase volume

K_D solute distribution ratio

$V_M + V_S = V_C$ column volume

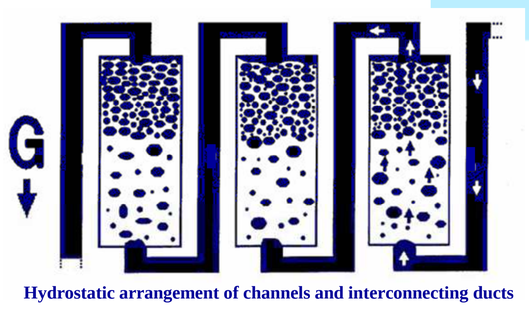
The two MAJOR problems of CCC:

1- Find the appropriate biphasic liquid system

The two liquid phases are related. Changing anything in one phase changes the other phase as well. The best way to evaluate a given liquid system is to make a try with the sample. It can be time consuming.

2- Retain enough stationary phase (V_S)

In CCC, the resolution factor Rs is directly proportional to V_S, the stationary phase volume retained inside the CCC column. Some liquid systems (especially the most polar ones) are poorly retained by hydrodynamic CCC columns.



Hydrostatic arrangement of channels and interconnecting ducts

Hydrostatic CCC design

One rotor axis

Constant centrifugal field

Rotating seals

Good liquid phase retention

Dead volumes in connecting ducts

Significant pressure (10-80 bars)

Hydrodynamic CCC design

Two rotor axes

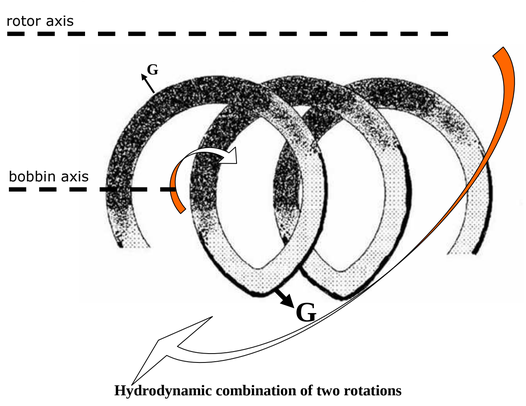
Variable centrifugal field

No rotating seal

Possible problem with liquid retention

Good efficiency (reduced dead volume)

Low pressure (1-5 bars)



Hydrodynamic combination of two rotations

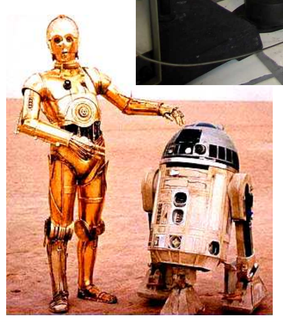
Proposed solution:

1- Design a small volume CCC column

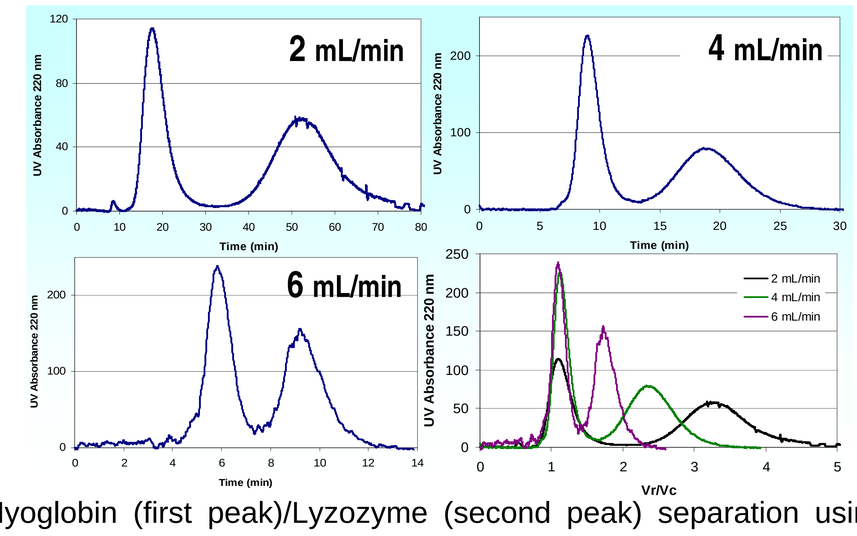
As evidenced by the CCC retention equation, if the column volume is small, the mobile and stationary phase volume will also be small and consequently the solute retention volumes will be reduced.

2- Use the hydrostatic scheme for the CCC column

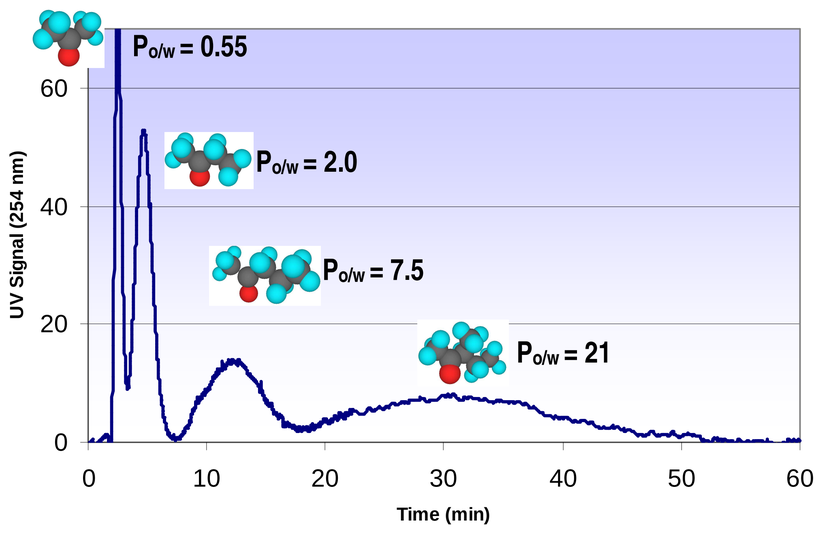
Hydrostatic CCC columns contain channel interconnected by ducts, engraved in disks. Stacked disks are placed in a single axis rotor producing a constant centrifugal field. Hydrostatic CCC columns are less efficient than hydrodynamic CCC columns but they have a better liquid phase retention capability.



Two hydrostatic CCC columns: left, the Kromaton FCPC 250 that can hold different rotors including a 36 mL rotor; right, the Rousselet-Robatel-Decisive-Design prototype (R2D2), a 32 mL hydrostatic column. The small inset picture gives the prototype scale.



Myoglobin (first peak)/Lyzozyme (second peak) separation using an aqueous two phase system (K_2HPO_4/PEG 1000/H_2O 14/14/72 w/w/w). 32 mL hydrostatic CCC column prototype R2D2. 2000 rpm at different flow rates as indicated on figures of the lower phosphate-rich aqueous phase in the descending mode. The lower right figure shows the same three chromatograms in a relative volume scale (V_R/V_C). Injection volume: 1 mL; sample lyzozyme 8.3 g/L, myoglobin 4.3 g/L



Direct measurement of octanol/water partition coefficient, $P_{o/w}$, of four ketones. Chromatograms of acetone (115 mg), methyl ethyl ketone (170 mg), methyl propyl ketone (170 mg) and methyl isobutyl ketone (345 mg), 1 mL injected. Hydrostatic CCC column FCPC 38 mL rotating at 1800 rpm. Liquid stationary phase: 16.5 mL octanol saturated by water, Sf = 43%. Mobile phase: aqueous lower phase at 12 mL/min in the descending direction. Pressure 42 bars (600 p.s.i.).

Conclusion

Small hydrostatic CCC columns are able to retain any liquid system including the most polar ones and allow for fast mobile phase flow rates.

The quick separations obtained allow for rapid finding of the most appropriate liquid system for the preparative purification that will be done on a larger volume CCC column.

Thanks to Rousselet-Robatel, Annonay, France, for the loan of the R2D2 prototype presented in this poster