

Continuous purification of proteins using True Moving Bed CPC/CCC system

G.Audo^{1*}, F.Couillard¹, D.Fisher², I.Sutherland²

¹ Armen Instrument – application laboratory – ZI Kermelin – 16, rue Ampère - 56890 Saint Ave, France Tel : +33 (2) 97 61 84 00; Fax : +33 (2) 97 61 85 00; e-mail : gregoire.audo@armen-instrument.com

² Advanced Bioprocessing Centre, Brunel Institute for Bioengineering, Brunel University, Uxbridge, UB8 3PH, UK

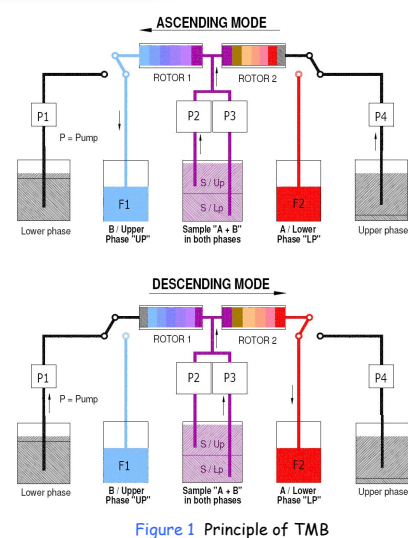
The continuous separation of two proteins with an Armen TMB¹ CPC 1L system is presented. A simple mixture of lysozyme and myoglobin already purified in batch mode [1] was chosen for this study using the same ATPS system comprising 12.5% w/w PEG-1000: 12.5% w/w K₂HPO₄. This mixture solubilised in both lower and upper phase is continuously injected into the TMB CPC system [2] according to the process described in Figure 1.

¹ True Moving Bed Centrifugal Partition Chromatography



Keywords: Gradient, software, standard mixture, SCPC

TMB principle



Principle of True moving Bed process [Fig. 1]:

- The Armen TMB is equipped with two columns (rotor 1 and 2), two elution pumps: two injection pumps, two fraction collectors. All peripherals are using alternatively in ascending and descending mode
- Both rotors are filled with 50% of each phase.
- The sample, dissolved into both phases is continuously injected between the two rotors. In descending mode as the mobile phase is the lower phase and in ascending mode as the mobile phase is the upper phase.
- The collections of the molecules A and B is done alternatively during each cycle.
- Cycle numbers are determined according to the volume of sample to purify, injection flow-rate and elution flow-rate
- Molecules which have more affinity for upper phase ($K_d[\text{upper}]/[\text{lower}] > 1$) will be eluted in ascending outlet and molecules which have more affinity for lower phase will be eluted in descending direction ($K_d[\text{upper}]/[\text{lower}] < 1$)

Solvent system determination.

In this case solvent system is an ATPS (Aqueous Two Phase System) made of 12.5% w/w PEG-1000 and 12.5% w/w K₂HPO₄ already used in batch mode [1]. In this system myoglobin has a partition coefficient K_d of 0,57 and lysosyme 1,46 which means that myoglobin has more affinity for lower phase and lysosyme has more affinity for upper phase.

TMB run and results

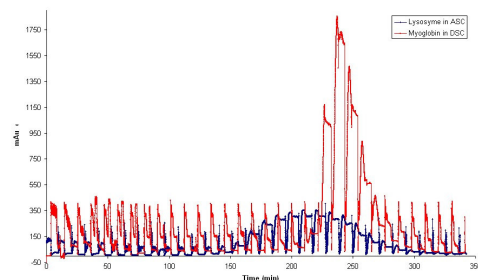


Figure 4: UV chromatograms, 280 nm obtained with

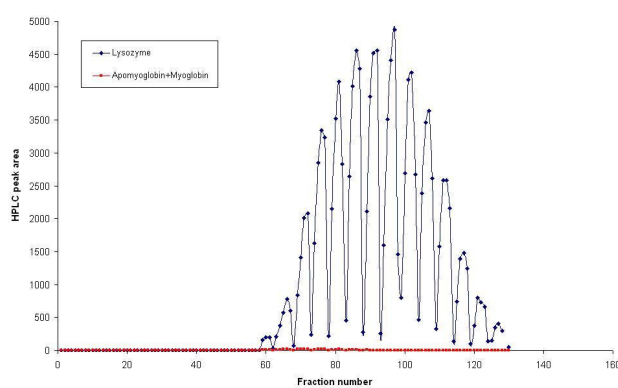


Figure 2: Variation of HPLC Peak Area of 1 min fractions collected in ascending fraction collector (F1) for a CPC separation of Lysozyme and Myoglobin using the TMB CPC 1L. Separation Conditions: Rotor speed 2000rpm, Elution flow 5ml/min, Injection flow 3ml/min, fraction volume 8ml, sample concentration 2.2+2.2 mg/ml, phase system 12.5% w/w PEG-1000: 12.5% w/w K₂HPO₄ ATPS system.

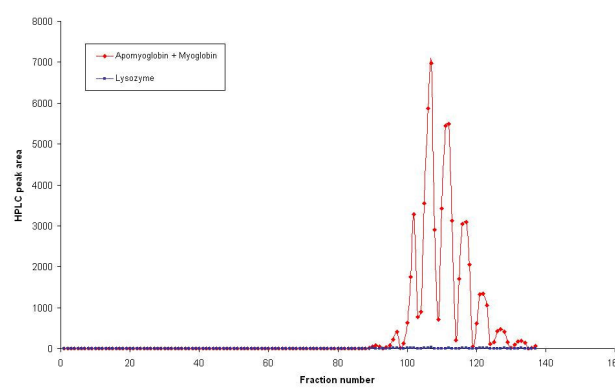


Figure 3: Variation of HPLC Peak Area of 1 min fractions collected in descending fraction collector (F2) for a CPC separation of Lysozyme and Myoglobin using the TMB CPC 1L. Separation Conditions: Rotor speed 2000rpm, Elution flow 5ml/min, Injection flow 3ml/min, fraction volume 8ml, sample concentration 2.2+2.2 mg/ml, phase system 12.5% w/w PEG-1000: 12.5% w/w K₂HPO₄ ATPS system.

Peak area of HPLC analysis for the fractions coming from ascending direction and descending direction are plotted against fraction number [Fig. 2 and 3]. Note that the myoglobin elution peaks in red are coming in descending mode with a trace of lysozyme in blue [Fig. 3] and the lysozyme elution peak are coming in ascending mode with a trace of myoglobin in red [Fig. 2] as expected according to the partition coefficient of each protein in the solvent system.

HPLC analysis of each fraction recovered in ascending and descending directions shows that both proteins are continuously purified with more than 99% HPLC purity and high recovery without denaturation

It can also be highlighted that the UV traces of both detectors in ascending and descending mode [Fig. 4] allows one to follow the elution of both proteins and that the general form is similar to each fractogram.

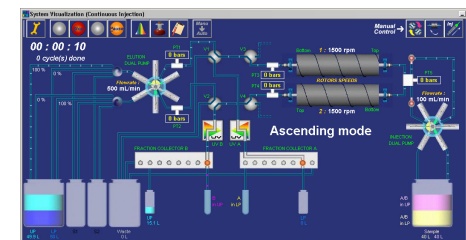


Figure 4: Armen TMB software for complete automation

Conclusion

A continuous injection of a binary mixture of myoglobin and lysosyme on an Armen TMB CPC 1L is reported. HPLC analysis of each fraction recovered in ascending direction and descending direction shows that both proteins are purified to more than 99% purity with high recovery. The next step will be to scale up this separation on a 12,5L TMB CPC to increase the productivity and to demonstrate the ease of the scale up process in continuous mode

References

[1] SUTHERLAND IA, AUDDO G., BOURTON E., COUILLARD F., FISHER D., GARRARD I., HEWITSON P., INTES O., Rapid linear scale-up of a protein separation by centrifugal partition chromatography, *Journal of Chromatography A*, 2008, 1190, 57–62.

[2] TMB patented process IFP, François Couillard