Rapid scale up on proteins purification with bench and pilot scale CPC

G.Audo*, F.Couillard*, I.Sutherland+
*Armen instrument application laboratory
+ Advanced Bioprocessing Centre, Brunel Institute for Bioengineering, Brunel University, Uxbridge, UB8 3PH, UK

Introduction

The main objective of this application note is to report on the successfull rapid optimisation and scale up from an Armen SCPC-1000 to a pilot scale Armen SCPC-12.5L CPC [1]. A simple mixture of two proteins lysozyme and myoglobin was chosen for this study using an ATPS system comprising 12.5% w/w PEG-1000: 12.5% w/w K_2HPO_4.

Separation of both proteins was linearly scaled up from one of the 500 ml column of a 1 litre CPC to one of the 6,25 litre column of a 12.5 litre Pilot Scale CPC unit by increasing mobile phase flow rate, fraction size and sample loading in the ratio of the system capacities (ie 6.25:0.5). Flow rate was therefore increased from 10ml/min to 125ml/min, fraction size from 10ml to 125ml and sample loading from 43ml to 500ml. Rotor speed however was reduced from 2000rpm on the SCPC-1000 to 1293rpm on the SCPC-12.5L on scale-up to maintain the same 224 g field in each chamber, as the 12.5 litre CPC unit has a larger rotor radius than the 1 litre CPC.

Results and discussion

The results of the HPLC analysis of peak area for fractions from the laboratory scale run are plotted in Figure 1 against fraction number. Myoglobin is detected between fractions 37 to 48 while lysozyme is detected between fractions 52 and 70. The resolution between the peaks is 1.28. This result has to be compared to industrial scale run [Fig. 2] where myoglobin elution peaks are between fractions 32 and 46 and lysozyme elution peaks are between fractions 55 to 72 with a resolution of 1.88.

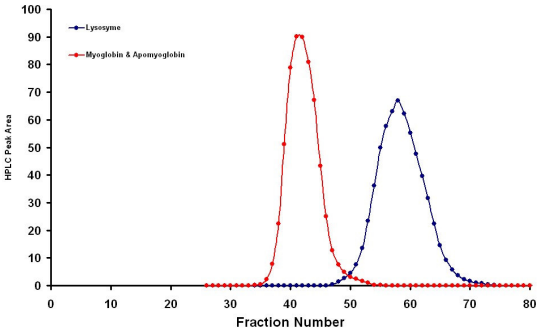

Figure 1: Variation of HPLC Peak Area of 1 min Fractions for a CPC separation of Lysozyme and Myoglobin using a 500ml rotor. Separation Conditions: Rotor speed 2000rpm, Mobile phase flow 10ml/min, fraction volume 10ml, sample loading 40ml (10%CV), sample concentration 2.2+2.2 mg/ml, phase system 12.5% w/w PEG-1000: 12.5% w/w K_2HPO_4 ATPS system.

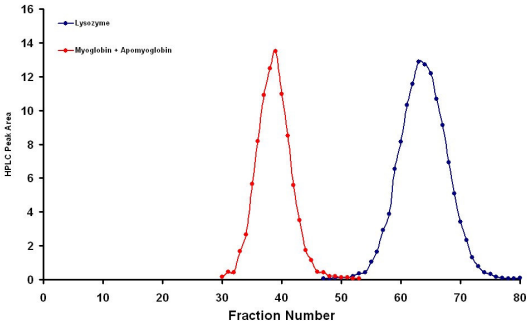

Figure 2: Variation of HPLC Peak Area of 1 min Fractions for a CPC separation of Lysozyme and Myoglobin using a 6.25 litre rotor. Separation Conditions: Rotor speed 1293rpm, Mobile phase flow 125ml/min, fraction volume 125ml, sample loading 500ml (10%CV), sample concentration 2.2+2.2 mg/ml, phase system 12.5% w/w PEG-1000: 12.5% w/w K_2HPO_4 ATPS system

	Masse injected* (g)	Flow rate (mL)	Rs
CPC 0.5L	0.176 g	10 ml/mn	1,28
CPC 6.25L	2.2 g	125 ml/mn	1.88

Conclusion

Low cost optimisation at the laboratory scale has been demonstrated and rapid scale-up to pilot scale was done with success on the first industrial scale run. The increased resolution at pilot scale suggests that even higher sample loading (ie >10%Column volume) may be feasible. As it is throughputs of 1.65g/hour (40g/day) have been achieved in batch mode and it is possible that this could double with even higher column loading beyond linear scale-up. The next step will be to see if continuous extraction with throughputs of up to 0.5kg/day will be feasible on our True Moving Bed (TMB CPC) systems.

[1] I.A. Sutherland, G. Audo. Journal of Chromatography A, 1190 (2008) 57-62. "Rapid linear scale-up of a protein separation by centrifugal partition chromatography"

Armen instrument : ZI de Kermelin-16, rue Ampère-56890 St-AVE-France
Phone : +33(0)2 97 61 84 00 – Fax : +33 (0)2 97 61 85 00
contact@armen-instrument.com; www.armen-instrument.com