Fall 11-2-2015

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Recommended Citation

Broly, H., Mitchell-Logean, C., Costioli, M. D. & Guillemot-Potelle, C., 2010. Cost of Goods Modeling and Quality by Design for Developing Cost-Effective Processes. BioPharm International. Konstantinov, K. B. & Cooney, C. L., 2014. White Paper on Continuous Bioprocessing. May 20–21, 2014 Continuous Manufacturing Symposium. Journal of Pharmaceutical Sciences, pp. 813-820. Pollock, J. et al., 2013. Optimising the design and operation of semi-continuous affinity chromatography for clinical and commercial manufacture.. Journal of Chromatography A,, Volume 1284, pp. 17-27.

Factors Affecting the Productivity of 4-Column Periodic Counter Current Chromatography (4C-PCC)

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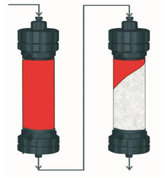
Introduction

Figure 1: *Periodic counter current chromatography*



(Ulmer et al., 2015)

It has been shown, through cost of goods modelling, that the cost of protein A resin is one of the most significant costs in a MAb process, and that decreasing the cost of protein A resin by maximising loading or increasing lifetime will have a significant effect on consumable costs (Broly, et al., 2010).

Consequently, the application of continuous or semi continuous chromatography to primary capture of MAbs is of great interest for reducing overall product cost. The advantages of continuous bioprocessing include; steady state operation, reduced equipment footprint, reduced buffer consumption, streamlined process flow, and reduced capital cost. These benefits have led to considerable interest in evaluating these technologies for the purposes of bioprocess intensification (Konstantinov & Cooney, 2014).

A key factor affecting the productivity of periodic counter current chromatography is the loading time (figure 2). This must be closely matched to the regeneration time in order to maximize productivity (Pollock, et al., 2013).

Figure 2: *Optimisation of 4C-PCC through matching loading time to regeneration time*

4C PCC	Low titre	High titre	(optimisation)
Load time			
W/E/R time			
	Can be operated as 4C-PCC with more complex wash strategies	The addition of a fourth column splits the W/E/R steps over two columns	Increased productivity

In order to take full advantage of the benefits of continuous chromatography for primary capture of MAbs, a good understanding of the factors affecting productivity is required. This presentation will aim to highlight those factors and assess their relative importance in maximising the productivity of this downstream processing unit operation.

Materials and Methods

A fractional factorial Design of Experiments (DoE) was performed at lab scale on single columns, where a non-compressible protein A resin was loaded up to 100% breakthrough to determine the shape of the breakthrough curves (figure 4). Load mAb concentration (0.5-2.0 mg/ml), linear velocity (150-600 cm/h) and residence time (0.2-2.0 minutes) were varied to assess their effect on productivity and run time (table 1). Combinations of these variables which resulted in impractical column dimensions were disallowed from the DoE.

Productivity (amount of MAb purified (mg) per volume resin (ml) per hour (h)) calculations assumed one cycle on a 4C-PCC system which includes 5 load cycles and was calculated using the following equation.

$$Productivity = \frac{M_t}{4Vt}$$

Where M_t is the total mass of mAb loaded during a cycle, V is the column volume and t is the total run time. The total mass of mAb loaded on all columns was calculated from single column breakthrough curves by integrating them using SigmaPlot (Systat Software Inc., London, UK) to calculate bound and unbound protein at a range of percentage breakthroughs (25%, 40%, 50%, 75%). The following equations were used to calculate the total mass of mAb loaded.

$$M_t = 5M_b + M_{ub} \qquad M_b = M_l - M_{ub}$$

Where, for a single column at a given percentage breakthrough, M_b is the mass of bound mAb, M_{ub} is the mass of unbound mAb and M_l is the mass of mAb loaded. The total run time was calculated using the following equation.

$$t = \frac{M_t}{CQ} + t_r$$

Where C is the concentration of mAb in the load, Q is the volumetric flowrate and t_r is the time taken for the post cycle regeneration steps. All regeneration steps where assumed to have a 0.5 minute residence time (based on previous data); where the load time for a single column was less than the time taken for the simultaneous regeneration of other columns regeneration time was used to calculate productivity. Results were analysed using JMP (SAS, Marlow, UK).

Results

The productivities obtained for all experiments within the DoE ranged from 4.43mg mL^{-1} hr^{-1} to 153mg mL^{-1} hr^{-1} (table 1). This is a very wide range and demonstrates the importance of selecting the appropriate operating conditions in order to maximise the productivity of 4C-PCC. For all breakthrough curves (25-75%) the top 5 productivities (40-150 mg MAb/ml resin/hr) all had the lowest residence time (0.2 minutes) but varying load velocities and load concentrations (table 1). Figure 5 shows the prediction profiler for 50% breakthrough, similar trends were observed for all breakthrough percentages.

Figure 4: *DBC curve (experiment 6)*

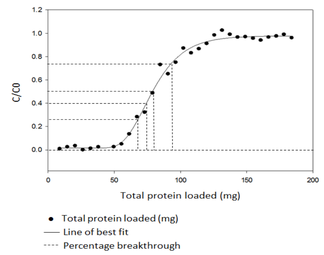
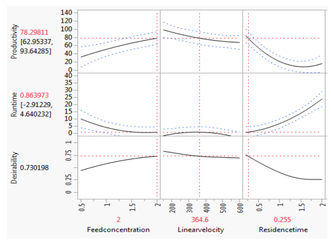


Figure 5: *Prediction profiler for 50% breakthrough*



Residence time

For breakthroughs from 25%-50% productivity is most significantly effected by residence time. For all breakthrough curves run time is most significantly effected by residence time.

Feed concentration

Feed concentration has a statistically significant effect on productivity for breakthroughs from 25%-40%. For all breakthrough curves feed concentration has a statistically significant effect on run time. These effects are less pronounced than for residence time.

Linear Velocity

Linear velocity has a statistically significant effect on run time at 40% breakthrough.

Conclusions

The highest productivity observed within this DoE was 153 mg MAb/ml resin/hour for 75% breakthrough in experiment 2. This experiment used a low residence time, a low linear velocity, and a high feed concentration.

In all instances productivity increased as higher percentage breakthroughs were achieved.

Productivity can be increased and run times can be decreased by reducing the residence time and increasing the feed concentration.

The Design of Experiments model shows that residence time is the most significant factor affecting productivity of those investigated in this study.

Aspect ratio of the column hardware also appears to have an effect on productivity with wider column diameters and shorter bed heights (experiment 1, 2 and 5) having an increased productivity.

It is recommended that a residence time of 0.2 minutes and a load concentration of >1.5 mg/ml is used in order to maximise productivity and reduce run time.

Acknowledgments

The authors would like to thank colleagues in the Process Sciences team in particular Verity Nancollis, Andrew Brown, Linda Randall and Robert Olliver for their input and constructive suggestions.

References

Broly, H., Mitchell-Logean, C., Costioli, M. D. & Guillemot-Potelle, C., 2010. Cost of Goods Modeling and Quality by Design for Developing Cost-Effective Processes. BioPharm International.

Konstantinov, K. B. & Cooney, C. L., 2014. White Paper on Continuous Bioprocessing. May 20–21, 2014 Continuous Manufacturing Symposium. Journal of Pharmaceutical Sciences, pp. 813-820.

Pollock, J. et al., 2013. Optimising the design and operation of semi-continuous affinity chromatography for clinical and commercial manufacture. Journal of Chromatography A, Volume 1284, pp. 17-27.

Ulmer, J. et al., 2015. Affinity Capture of $F(ab')_2$ Fragments: Using Twin-Column Countercurrent Chromatography. Bioprocess International.

Figure 3: *GE Healthcare's 4C-PCC chromatography system*

Table 1: *Productivity and runtime results for Design of Experiments (DoE)*

Experiment No.	Load Conc. (mg/ml)	Linear Velocity (cm/h)	Residence Time (mins)	25% breakthrough		40% breakthrough		50% breakthrough		75% breakthrough	
				Productivity (mg MAb/ml resin/hr)	Runtime (hours)	Productivity (mg MAb/ml resin/hr)	Runtime (hours)	Productivity (mg MAb/ml resin/hr)	Runtime (hours)	Productivity (mg MAb/ml resin/hr)	Runtime (hours)
11	1.74	375.0	1.09	16.21	7.57	16.81	7.95	17.24	8.05	19.46	7.85
3	1.73	397.5	0.17	65.66	1.24	77.78	1.27	84.53	1.29	101.65	1.33
1	0.73	150.0	0.19	39.59	1.80	45.81	2.07	51.06	2.17	94.72	1.65
16	2.73	297.3	2.00	12.81	21.50	12.99	22.05	13.18	22.27	14.11	22.06
8	1.61	330.0	0.80	20.00	6.08	21.02	6.49	21.92	6.62	41.20	5.00
14	0.73	285.0	1.96	4.43	31.70	4.71	34.13	5.53	35.74	No data	No data
12	2.32	600.0	0.92	23.22	4.45	24.74	4.92	25.93	5.07	33.12	4.82
5	2.73	600.0	0.18	46.32	1.15	65.29	1.17	78.12	1.19	119.98	1.24
9	2.61	352.5	0.83	28.58	3.56	30.20	3.88	31.38	3.98	36.61	3.91
10	1.74	150.0	1.96	9.38	12.77	9.72	13.49	10.00	13.69	11.33	13.31
6	2.92	150.0	1.28	19.01	6.85	19.58	7.17	20.02	7.27	22.31	7.19
13	0.73	600.0	0.92	9.14	11.55	11.51	14.97	No data	No data	No data	No data
4	0.73	600.0	0.21	37.05	1.91	43.07	2.26	47.47	2.34	76.11	1.93
15	1.75	600.0	0.99	17.46	4.82	18.80	6.57	19.13	6.63	21.27	6.49
2	2.40	150.0	0.19	86.50	1.22	110.57	1.26	123.33	1.28	153.05	1.33
7	0.73	150.0	1.28	6.55	17.06	6.76	17.68	6.93	17.82	7.85	17.11