Pharmaceutical BIOPROCESSING

Pushing the limits of high-throughput chromatography process development: current state and future directions

"High-throughput scale-down techniques are essential for quickly assessing a wide range of parameter space, which is particularly useful when screening new molecules or chromatography resins where little *a priori* knowledge is available."

Keywords: automation • chromatography • high-throughput process development • micropipette tip • miniature column • monoclonal antibodies • resin slurry

John Welsh
Bioprocess Development, Merck Research Laboratories, Merck & Co., Inc., 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA
john.welsh2@merck.com

With a current monoclonal antibody (mAb) market of over US$50 billion per year and growing [1], high demands are placed on the bioprocess development field to implement robust, scalable processes that meet clinical material demands and provide a line of site for large scale manufacturing. The emergence of antibody drug conjugates and antibody fragments with specialized function [2], in addition to standard mAb programs, will only serve to increase the strain on bioprocessing. In the area of downstream chromatography-based purification, the implementation of automated scale-down techniques and statistically powered process models has become commonplace. High-throughput scale-down techniques are essential for quickly assessing a wide range of parameter space, which is particularly useful when screening new molecules or chromatography resins where little *a priori* knowledge is available. The use of 'platform' purification approaches has also helped companies handle multiple mAb programs when minimal process changes are required [3]. While these advances have undoubtedly assisted in streamlining process development, meeting material demands, and helping to fulfill regulatory filing requirements, key challenges still remain for the high-throughput downstream purification development field. Namely, expanded use of current downstream technologies and better integration with other areas of bioprocessing will serve the field well into the next generation of bioprocess development.

Currently, three standard scale-down chromatography techniques are typically used in bioprocess development: resin slurries in a 96-well plate format, micropipette tips, and miniature columns [4–6]. Particularly for the latter two techniques, automated liquid handlers are required for implementation, and precise robotic setups are required for all three techniques to achieve full automation. Resin slurry plates have long been used as both an industry and academic tool to understand protein and resin interactions, and the technique has been used extensively as part of mAb development pipelines for numerous biotechnology companies [7–11]. Due to differences in the static slurry plate technique and dynamic packed bed interactions at scale, understanding of the transport phenomena at play and having valid scale-down correlations are critical for both experimental setup and data interpretation. Micropipette tips and miniature columns are less flexible, require specialized packing, and provide a higher barrier for implementation than slurry plates, but both techniques utilize packed bed chromatography that can provide more representative results. Micropipette tips use chromatography resin packed in the ends of pipette tips and are easily automated with both complex liquid handlers such as those provided by Tecan Systems as well as more

FUTURE SCIENCE part of fsg

simplified automated multichannel pipette systems. Previous publications in the vaccines process development area at Merck have demonstrated micropipette tips to be a useful tool for quickly assessing the quality of fermentation or cell culture material [12]. Throughput for this technique is typically only 8–12 tips in parallel, but the utilization of 96 tips in parallel, a technology that is currently available, could provide expanded uses for micropipette tips in future applications. Miniature columns are the most nascent scale-down chromatography technique and require a complex liquid handling setup to accurately control flowrate and sample collection. Recent publications have demonstrated the technique useful for process modeling and optimization [13], breakthrough and elution studies [14], and multi-column mAb purifications [15].

> "Expanded utilization of scale-down chromatography will be beneficial for downstream process development, but better integration with other areas of bioprocessing is also required to realize the full potential of scale-down automation."

While scale-down high-throughput techniques typically see the most applications in early-stage development, it is possible that miniature columns could be used in later stage characterizations, potentially even for regulatory filings where validation of precise scale-down models is more critical. The benefits to using miniature columns for late stage characterizations are clear. The smaller column sizes (<1 ml) require much less feed material (approximately tenfold per condition), and operating eight conditions in parallel allows for exploration of more conditions, leading to higher powered statistical process models. It is feasible that miniature columns could provide the necessary levels of precision, but key differences need to be acknowledged compared with traditional lab scale columns typically used for late stage characterization. Namely, the bed heights of miniature columns are much shorter than typical lab scale columns (1 or 3 cm compared with >10 cm). The narrower geometry of miniature columns can also lead to an increase in 'wall effects' observed at column boundaries. Miniature columns also lack a formal flow distributor, and little information on packing and resin compression is currently provided by suppliers. Better communication with vendors and more detailed packing information will be critical for implementing miniature columns for formal characterizations. Nevertheless, recent publications have shown that for many chromatography resins typically used for mAb purifications, the transport mechanisms between miniature and larger packed columns are conserved, and performances are similar when using residence time scaling with the shorter columns [16,17]. For regulatory filings, the International Conference on Harmonization states that, "purification of the scaled-down version should represent as closely as possible the production procedure", and while suggesting factors such as linear flow and bed height remain conserved for scale-down methods, the International Conference on Harmonization only requires that the process, "be representative of commercial-scale manufacturing" [18]. Thus, it is possible that with sufficient testing and supporting data, miniature columns could be used for formal process modeling.

Expanded utilization of scale-down chromatography will be beneficial for downstream process development, but better integration with other areas of bioprocessing is also required to realize the full potential of scale-down automation. Analytics are a common bottleneck for high throughout techniques; assays requiring high sample volumes or lengthy run times can obviously render the benefits of high-throughput chromatography runs moot, but this burden can be reduced through careful experimental planning and implementation of appropriate assays. When available for polishing chromatography steps, absorbance measurements can provide a nearly instantaneous assessment of protein concentration in a 96-well plate. Using ultra performance size exclusion chromatography can greatly reduce run times while maintaining similar quantification of aggregate and fragment content compared with standard HPLC columns [19]. A recent technology from Gyros Biopharma uses microfluidic channels and centrifugal force to perform sandwich assays similar to traditional ELISA assays with higher throughput and greatly reduced material volumes. This technique has already been demonstrated to be effective for detecting host cell protein process residuals [20] and could similarly be utilized for detection of other process residuals like DNA and Protein A.

> "While scale-down high-throughput techniques typically see the most applications in early-stage development, it is possible that miniature columns could be used in later stage characterizations..."

Integrating high-throughput techniques is also critical for cell line development and upstream process development bioprocessing areas. The recent development of single use disposable bioreactors from TAP Biosystems that can operate either 24 bioreactors in parallel at 250 ml volumes or 48 bioreactors at 15 ml has greatly increased the throughput and accuracy with which scale-down bioreactors can be run [21–24]. Resin slurry plates or micropipette tips that can be run in a 96-well format could prove to be crucial techniques for providing rapid quality assessments of material generated in these disposable bioreactors.

Coupled with other high-throughput analytical techniques, this could represent an end-to-end utilization of automated scale-down technologies.

Several key challenges remain for both expanding the use of scale-down chromatography techniques and more fully integrating with other areas of bioprocessing. However, it remains clear that these automated high-throughput techniques will continue to pave the future of downstream process development for years to come.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Dimitrov DS. Therapeutic proteins. In: *Methods in Molecular Biology.* NJ, USA 899, 1–26 (2012).

2 Reichert JM. Which are the antibodies to watch in 2012? *mAbs* 4(1), 1–3 (2012).

3 Shukla AA, Hubbard B, Tressel T, Guhan S, Low D. Downstream processing of monoclonal antibodies – application of platform approaches. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 848(1), 28–39 (2007).

4 Bhambure R, Kumar K, Rathore AS. High-throughput process development for biopharmaceutical drug substances. *Trends Biotechnol.* 29(3), 127–135 (2011).

5 Chhatre S, Titchener-Hooker NJ. Review: microscale methods for high-throughput chromatography development in the pharmaceutical industry. *J. Chem. Technol. Biotechnol.* 84, 927–940 (2009).

6 Lacki KM, Brekkan E. High throughput screening techniques in protein purification. *Methods Biochem. Anal.* 54, 489–506 (2011).

7 Coffman JL, Kramarczyk JF, Kelley BD. High-throughput screening of chromatographic separations: I. Method development and column modeling. *Biotechnol. Bioeng.* 100(4), 605–618 (2008).

8 Gerster A, Rudolph F, Dieterle M. High-throughput chromatography screenings modulating charge-related isoform patterns. *Bioprocess. Int.* 11(5), 46–53 (2013).

9 Hopp J, Pritchett R, Darlucio M, Ma J, Chou JH. Development of a high throughput protein a well-plate purification method for monoclonal antibodies. *Biotechnol. Prog.* 25(5), 1427–1432 (2009).

10 Sisodiya VN, Lequieu J, Rodriguez M, Mcdonald P, Lazzareschi KP. Studying host cell protein interactions with monoclonal antibodies using high throughput protein A chromatography. *Biotechnol. J.* 7(10), 1233–1241 (2012).

11 Toueille M, Uzel A, Depoisier JF, Gantier R. Designing new monoclonal antibody purification processes using mixed-mode chromatography sorbents. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 879(13–14), 836–843 (2011).

12 Wenger MD, Dephillips P, Price CE, Bracewell DG. An automated microscale chromatographic purification of virus-like particles as a strategy for process development. *Biotechnol. Appl. Biochem.* 47(Pt 2), 131–139 (2007).

13 Osberghaus A, Drechsel K, Hansen S *et al.* Model-integrated process development demonstrated on the optimization of a robotic cation exchange step. *Chem. Eng. Sci.* 76, 129–139 (2012).

14 Wiendahl M, Wierling OS, Nielsen J *et al.* High throughput screening for the design and optimization of chromatographic processes - miniaturization, automation, and parallelization of breakthrough and elution studies. *Chem. Eng. Technol.* 31(6), 893–903 (2008).

15 Treier K, Hansen S, Richter C, Diederich P, Hubbuch J, Lester P. High-throughput methods for miniaturization and automation of monoclonal antibody purification processes. *Biotechnol. Prog.* 28(3), 723–732 (2012).

16 Lacki KM. High-throughput process development of chromatography steps: advantages and limitations of different formats used. *Biotechnol. J.* 7(10), 1192–1202 (2012).

17 Welsh JP, Petroff MG, Rowicki P *et al.* A practical strategy for using miniature chromatography columns in a standardized high-throughput workflow for purification development of monoclonal antibodies. *Biotechnol. Prog.* 30(3), 626–635 (2014).

18 ICH Guideline. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. Presented at: *International Conference on Harmonisation Q5A(R1)* 17 July 1997.

19 Mou X, Yang X, Li H, Ambrogelly A, Pollard DJ. A high throughput ultra performance size exclusion chromatography assay for the analysis of aggregates and fragments of monoclonal antibodies. *Pharm. Bioprocess.* 2(2), 141–156 (2014).

20 Heo JH, Mou X, Wang F *et al.* A microfluidic approach to high throughput quantification of host cell protein impurities for bioprocess development. *Pharm. Bioprocess.* 2(2), 129–139 (2014).

21 Bareither R, Bargh N, Oakeshott R, Watts K, Pollard D. Automated disposable small scale reactor for high throughput bioprocess development: a proof of concept study. *Biotechnol. Bioeng.* 110(12), 3126–3138 (2013).

22 Hsu WT, Aulakh RP, Traul DL, Yuk IH. Advanced microscale bioreactor system: a representative scale-down model for bench-top bioreactors. *Cytotechnology* 64(6), 667–678 (2012).

23 Moses S, Manahan M, Ambrogelly A, Ling WLW. Assessment of AMBR as a model for high-throughput cell culture process development strategy. *Adv. Biosci. Biotechnol.* 3(7), 918–927 (2012).

24 Pollard D. Are automated disposable small-scale reactors set to dominate the future of pharmaceutical bioprocess development? *Pharm. Bioprocess.* 2(1), 9–12 (2014).