avantor™

Adopting novel technologies to improve monoclonal antibody processes

As therapeutic monoclonal antibodies (mAbs) continue to dominate the biotherapeutics market, there is growing demand for structurally diverse mAbs, namely bispecific antibodies. As of late 2022, there were nearly 140 monoclonal antibodies approved or in regulatory review; representing approximately 20% growth in the late-stage commercial clinical pipeline. Of the 23 marketing application submittals expected in 2023, five are bispecfic[1].

Bispecific antibodies can cross-link two different antigens delivering more effective cancer treatment[2]. While mAbs can only bind to a single epitope, bispecific antibodies can bind two antigens at one time[3, 4]. One arm of the path will bind to one part of a tumor cell, the other will bind with the T-cell — bringing them together to kill cancer cells more efficiently.

Though extremely promising, this therapy does have a downside — bispecific antibodies are more challenging to manufacture as they are unstable, prone to aggregation and difficult to define due to mispairing of molecules. This instability can result in lower product yields, reduced therapeutic efficacy and patient immune reactions or complications[5].

Given their enhanced functionality in cancer treatment as compared to traditional mAbs, overcoming the following production challenges is critical to meet growing demand and patient need:

Upstream

- Lower target product yield; typical overall yield of bispecific antibodies is 40%
- Complex composition (three - four chains) results in higher product-related impurities
- Stability of the product

Downstream

- Separation of mispaired product, aggregates, fragments and monospecific antibodies
- Product degradation/aggregation at low pH during low pH viral inactivation
- Removal of highly toxic impurities (MEA, GHS, etc.)

Fill/finish formulation

- Shear stress-induced impurities
- Low solubility

Detergent-based viral inactivation

Traditionally, viral inactivation is done using a low pH process after the protein A capture step. This is not a workable approach for bispecific antibodies, as low pH has been shown to cause aggregation of the antibodies and Fc-fusion proteins as well as decreasing protein stability[5].

Instead, consider implementing detergent-based viral inactivation right before the protein A column, removing the issue of pH-mediated aggregation. This can reduce the viral load without contaminating future process steps. In addition, removing a significant proportion of aggregates and unstable material generated in the upstream process enhances the efficiency of the protein A step.

Critical parameters of an effective detergent-based viral inactivated solution include:

- > log 4.0 viral clearance
- Compatibility with protein A column purification and subsequent processing steps
- Compatibility with mAb (low protein reactive impurities), with minimal impact on $hIgG_1$ purification process
- Addresses environmental concerns with a readily biodegradable solution; Triton X-100, an industry standard, is at risk in the European Union (REACH)

Maintaining protein integrity is crucial to the viral inactivation step for process yield and product quality. The approach should not impact in the downstream process and should provide consistency in yield, purity and binding capacity.

To show proof of efficacy, we tested a novel detergent-based viral inactivation solution to determine compatibility with affinity column purification steps. After the harvest, the cell culture media was treated with 0.1% of the solution.

Results showed that the viral inactivation solution had no significant impact on dynamic binding capacity, SEC purity and yield; in fact, recovery was slightly better with detergent use (Fig. 1).

Next, we incubated the purified media for up to two hours to examine the detergent's impact on aggregation.

This impact assessment demonstrated comparable compatibility with the mAb (IgG_1) and Fab, and stability of the protein (Fig. 2). This data shows that using an appropriate viral inactivation solution can eliminate pH mediated aggregation.

It is crucial that the viral inactivation process used protect the column of Protein A, with no impact on downstream processes and the integrity of the protein of interest to maintain process yield and product quality.

Design chromatography affinity ligands to improve functionality

Protein A-based affinity chromatography is the most commonly used capture step when purifying antibodies; but there are improvements to be made to enhance protein function, such as higher binding capacity and better elution behavior[6]. The cost of the resin is also substantial, so engineering a solution to maximize efficiency is key.

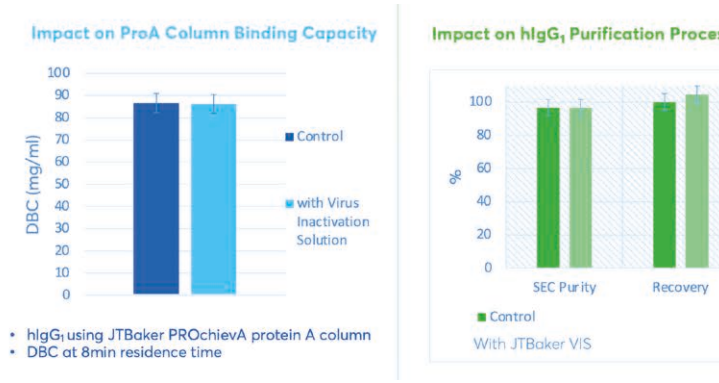
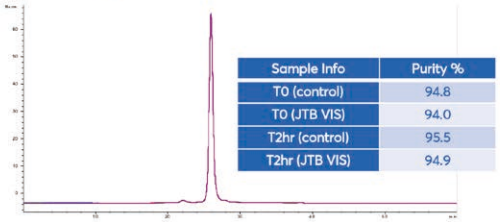


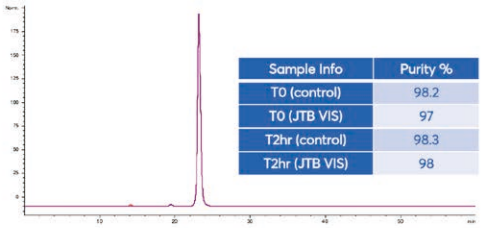
FIGURE 1: Measurements in the protein A column: impact on dynamic binding capacity, recovery of material in hIgG$_1$ purification process, and whether the detergent was present in the ProA step.

Impat Assessment on Purity of IgG (1 mg/ml) using J.T.Baker® Viral Inactivation Solution



Sample Info	Purity %
T0 (control)	94.8
T0 (JTB VIS)	94.0
T2hr (control)	95.5
T2hr (JTB VIS)	94.9

Impat Assessment on Purity of Fab (1 mg/ml) using J.T.Baker® Viral Inactivation Solution



Sample Info	Purity %
T0 (control)	98.2
T0 (JTB VIS)	97
T2hr (control)	98.3
T2hr (JTB VIS)	98

FIGURE 2: Purity impact assessment to determine protein stability with IgG$_1$ and Fab and controlled protein reactive impurities.

Several approaches exist for designing proteins: change the Fc domain to create low or high binding capacity to specific protein A, or bind the Fab domain, like the protein L, and create a molecule that will specifically bind the antibody. That way, if you make a mispaired molecule it can be selectively removed to improve clearance.

Proper evaluation and understanding of proteins prior to wet lab experimentation can lead to more efficient engineering of the protein with enhancement of desired properties. Our own research has found that optimizing the amino acid, using a computational model to review amino acid interactions for effective protein bindings, can determine the right sequence to achieve the proper Kd value.

Through optimization of amino acids responsible for IgG binding, Avantor developed a ligand with high affinity for target antibodies. SPR analysis demonstrated picomolar ligand affinity.

Enhanced impurity clearance using additives in wash step

If the protein ligand is properly designed with high dynamic binding capacity, can the process be improved further by removing host cell protein (HCP) and DNA from the protein A column?

To answer this question, we studied several additives in the PBS wash buffer to determine their effectiveness at cleaning the protein A itself.

Using clarified cell culture harvest (mAb concentration of 4.33 mg/ml) in a pre-packed 1 ml FPLC column, with sample load at four-minute residence time. Additives were added to an EQ buffer of 10 mM Na_2HPO_4, 2mM Na_2HPO_4, 137 mM NaCl, pH 7.4 for four minutes.

Test results showed that all additives were successful in removing HCP as compared to control; however, the addition of IPA, arginine and urea to the PBS wash buffer showed the greatest enhancements (Fig. 3). This novel approach to cleaning the protein A increases impurity clearance to reduce the burden on future steps (eliminating a step?) and enables process intensification.

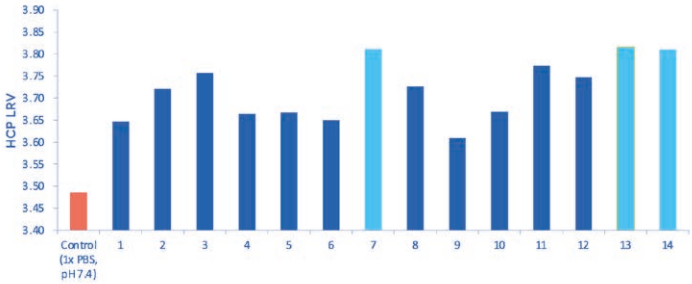
Improving the mAb capture step, using an enhanced resin with superior binding capacity and a proven additive to the wash step successfully reduces aggregation, removal of HCP/HCDNA and increases yield.

Use appropriate excipients to reduce protein-protein interactions

One of the biggest challenges to antibody stability, particularly at final fill, is aggregation due to protein-protein attractive interactions. Though excipients have been shown to greatly influence the stability of antibodies and their derivatives [4], typical excipients like buffer or arginine do not reduce protein-protein interactions and deamidation substantially.

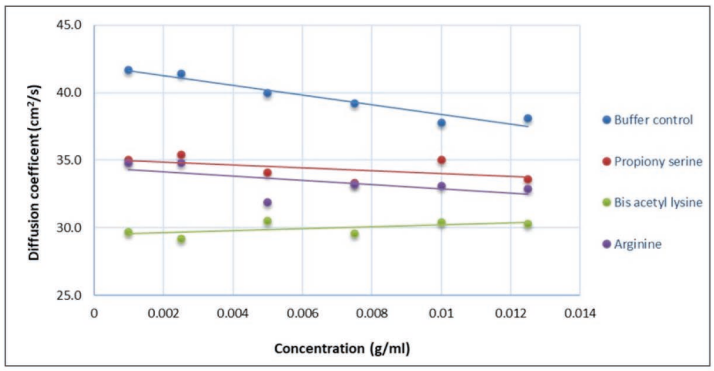
To that end, we evaluated several amino acid derivatives to determine if another formulation could outperform the more

INCREASE OF HCP LRV BY ADDITION OF ADDITIVES IN WASH STEP



	Buffer	Additives to the buffer	pH
1	1x PBS	10% propylene glycol, 1M Arg, 1% tween 20	7.4
2		1M GuHCl,	
3		10% propylene glycol, 1M GuHCl	
4		10% propylene glycol, 1M Arg,	
5		0.5M Arg,	
6		1M Arg	
7		10% isopropanol, 1M Arg	
8		10% isopropanol, 1M Arg, 1% Tween20	
9		1M Urea	
10		10% isopropanol, 1M urea	
11		10% isopropanol, 2M urea	
12		110% isopropanol, 2M urea, 1% Tween20	
13		10% isopropanol, 3M urea	
14		10% isopropanol, 3M urea, 1% tween20	

FIGURE 3: Multiple additives of different strengths and formulations added to the buffer wash step to determine HCP clearance.



Formulations	k_D (ml/g)
Buffer (10 mM phosphate, pH 8.0)	-8.53
Buffer w/arginine	-4.54
Buffer w/propionyl serine	-3.11
Buffer w/bis acetyl lysine	2.39

FIGURE 4: The apparent diffusion coefficient (D_m) of multiple excipient formulations was measured as a function of antibody concentration, ranging from 1 mg/ml to 12.5 mg/ml, in order to analyze the interaction parameter (k_D).

broadly used excipient arginine (Fig. 4). In the study[7], antibody protein-protein interactions were measured in buffer alone, and buffer containing arginine, bis acetyl lysine and propionyl serine as follows:

$$D_m=D_s(1+k_Dc)$$

Where D_m is apparent diffusion coefficient, D_s is diffusion coefficient at zero concentration, c is concentration and k_D is interaction parameter. The D_m was measured as a function of antibody concentration, ranging from 1 mg/ml to 12.5 mg/ml.

As shown in Figure 4, the negative D_m slopes suggest attractive interaction was weakest in propionyl serine, followed by arginine and buffer. The formulation for bis acetyl lysine, with a positive D_m slope, suggests protein-protein repulsive interactions, which would reduce aggregation.

The effect of bis acetyl lysine and propionyl serine on deamidation was also analyzed, using LD/MS-MS. After eight weeks of storage, there was 8% deamidation in the control sample stored at 4 °C, and samples stored at 40 °C showed deamidation of 23% in the buffer, 8% in bis acetyl lysine and 7% in propionyl serine formulations. These results clearly demonstrate that bis acetyl lysine and propionyl serine can control asparagine deamidation of mAbs.

Overall, bis acetyl lysine and propionyl serine outperformed arginine in stabilization of the antibody and were shown to be efficient at reducing solution viscosity and aggregation[7]. Adding these excipients into your formulation buffer, even in the downstream purification process during the polishing step, will reduce aggregation of protein A, improving stability for a longer period of time.

Conclusion

Emerging multi-specific antibody and antibody fragment challenges can be addressed by adopting new approaches and novel technologies:

- Viral inactivation using an environmentally friendly and low protein reactive detergent
- Designing specific affinity chromatography ligands based on Fc and Fab binding domains
- Utilizing additives for removing product-related impurities
- Using excipients to reduce protein-protein interactions and improve stability

Our results show a final SEC purity of 99%, with overall process yield greater than 90%.

REFERENCES

1. Hélène Kaplon, Silvia Crescioli, Alicia Chenoweth, Jyothsna Visweswaraiah, Janice M. Reichert, Antibodies to watch in 2023, mAbs, 15(1), 2023, DOI: 10.1080/19420862.2022.2153410.
2. Qiong Wang, Yiqun Chen, Jaeyoung Park, Xiao Liu, Yifeng Hu, Tiexin Wang, Kevin McFarland, Michael J. Betenbaugh, Design and Production of Bispecific Antibodies, Antibodies (Basel), 8(3): pp43, 2019, doi: 10.3390/antib8030043.
3. Ulrich Brinkmann, Roland E. Kontermann, The making of bispecific antibodies, mAbs, 9(2): pp182-212, 2017, doi: 10.1080/19420862.2016.1268307.
4. Sergey E Sedykh, Victor V Prinz, Valentina N Buneva, Georgy A Nevinsky, Bispecific antibodies: Design, therapy, perspectives, Drug Des. Dev. Ther., 12: pp195–208, 2018, doi: 10.2147/DDDT.S151282.
5. Hui Ma, Ciarán Ó'Fágáin, Richard O'Kennedy, Antibody stability: A key to performance - Analysis, influences and improvement, Biochimie, 177: pp213-225, 2020, doi.org/10.1016/j.biochi.2020.08.019.
6. Sara Kanje, Julia Scheffel, Johan Nilvebrant, Sophia Hober. Chapter 2 - Engineering of Protein A for improved purification of antibodies and Fc-fused proteins, Editor(s): Allan Matte, Approaches to the Purification, Analysis and Characterization of Antibody-Based Therapeutics, Book: pp35-54, 2020, doi: 10.1016/B978-0-08-103019-6.00002-3.
7. Arvind Srivastava, Courtney O'Dell, Evon Bolessa, Suman McLinden, Lori Fortin, Nandkumar Deorkar, Viscosity Reduction and Stability Enhancement of Monoclonal Antibody Formulations Using Derivatives of Amino Acids, Journal of Pharmaceutical Sciences, 10: pp2848-2856, 2022, doi: 10.1016/j.xphs.2022.05.011. Retrieved from https://www.biophorum.com/biophorum-blazes-a-trail-tackling-your-buffer-preparation-bottlenecks/

ABOUT THE AUTHOR:

Dr. Nandu Deorkar
Senior Vice President, Research & Development
Biopharma Production at Avantor

Nandu Deorkar is Senior Vice President, Biopharma Production Research & Development for Avantor. He is responsible for innovation strategy and planning, and execution of new products and technology development. During his more than 25-year career in research & development, Dr. Deorkar has been leading teams working on various aspects of upstream and downstream bioprocessing, single use systems, chemical/polymer R&D, drug development, formulation, drug delivery technologies, process development, and technology transfer. He has published more than 30 articles and holds more than 20 patents. Dr. Deorkar received Ph.D. from Indian Institute of Technology, Mumbai, India and MBA in Marketing from Fairleigh Dickinson University, Madison, NJ, USA.

This article was being published in: European Biopharmaceutical Review, Autumn 2023, pages 88-91. © Samedan Ltd.
https://www.calameo.com/read/0061133853e46b9213e70?page=88