Platform Process Development for Biosimilars: Comparative Evaluation and Screening of Protein A Chromatography Resins

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Abstract
Biosimilars have emerged as a cost-effective alternative to originator biologics, particularly monoclonal antibodies (mAbs), in the treatment of various chronic and life-threatening diseases . With increasing global healthcare demand and patent expirations of major biologics, the development of efficient, scalable, and regulatory-compliant biosimilar manufacturing platforms has become critical . A central component of downstream processing for mAbs is Protein A affinity chromatography, known for its high selectivity and ability to deliver high-purity products in a single step .However, selecting the most suitable Protein A resin is crucial to ensure optimal performance, robustness across batches, and economic feasibility in large-scale production.
This study aims to evaluate and compare the performance of commercially available Protein A resins—such as MabSelect SuRe™, Amsphere A3™, and Praesto Jetted A50™—in terms of dynamic binding capacity, product recovery, host cell protein removal, and cleaning cycle tolerance. Using a platform process involving a CHO-expressed biosimilar antibody, each resin was assessed through chromatographic runs and analytical assays including ELISA, SDS-PAGE, and HPLC. Results revealed substantial variation in binding efficiency and reusability, with certain resins showing superior CIP resistance and process economy.
These findings contribute to establishing a platform-based approach for biosimilar purification, aiding biopharmaceutical manufacturers in reducing development timelines and operational costs while maintaining regulatory compliance.

Keywords Biosimilars, Platform Process Development, Protein A Chromatography, Monoclonal Antibodies, Resin Screening, CHO Cells, Affinity Purification, Downstream Processing, mAb Purification, Biopharmaceutical Manufacturing

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1. Introduction

1.1. The Rise of Biosimilars in the Biologics Market

Biosimilars, or follow-on biologics, are therapeutic products that are highly similar to already approved reference biologics in terms of quality, safety, and efficacy. Their emergence is revolutionizing the global biologics market, offering cost-effective alternatives to high-priced biologics that are used in treating cancers, autoimmune disorders, and chronic inflammatory

diseases (Klein et al., 2020). According to recent estimates, biosimilars can reduce treatment costs by 20–30%, making them vital tools in enhancing healthcare access in both developed and emerging economies (IMS Institute, 2021).

As patents of blockbuster biologics expire, regulatory frameworks in major markets such as the US, EU, and India have evolved to accommodate faster biosimilar approvals, promoting competition and stimulating innovation in biopharmaceutical manufacturing (EMA 2019; USFDA 2020). However, the development of biosimilars is inherently complex due to the intricacies of biologics manufacturing, which involves living cell cultures, multiple purification steps, and strict quality controls to ensure product comparability.

1.2. Challenges in Downstream Processing of Biosimilars

One of the foremost technical and economic bottlenecks in biosimilar development lies in downstream processing—the purification steps following cell culture that isolate the target protein from host cell impurities and process-related contaminants. Downstream costs can account for 50–80% of total production expenditures in mAb manufacturing (Shukla et al., 2017). The purification process must be efficient, reproducible, and scalable to meet global regulatory requirements and cost expectations.

Moreover, biosimilar developers must ensure that purification processes are not only effective in isolating the desired product but also capable of maintaining its structural and functional integrity. Minor alterations in purification conditions can affect product attributes like glycosylation, charge variants, or aggregation, which in turn can impact immunogenicity and clinical performance (Rathore & Winkle, 2009). Therefore, establishing a well-characterized and optimized downstream platform is essential.

1.3. Role of Protein A Chromatography in Monoclonal Antibody (mAb) Purification

Within downstream processing, Protein A affinity chromatography is considered the industry standard for the initial capture step in monoclonal antibody purification. Protein A ligands exhibit high specificity for the Fc region of IgG-type antibodies, enabling rapid and efficient separation of mAbs from complex harvest media in a single step (Ghose et al., 2009). This high binding specificity significantly reduces process complexity and downstream burden on subsequent polishing steps.

Despite its utility, Protein A chromatography poses economic and operational challenges. Traditional agarose-based Protein A resins are expensive, and their performance deteriorates with repeated use due to ligand leaching, fouling, and pressure sensitivity (Jungbauer & Hahn, 2004). The need to balance performance, cost, and cleaning cycle tolerance has led to the development of new-generation Protein A resins with improved chemical stability, dynamic binding capacity, and compatibility with harsh cleaning conditions such as 0.1–0.5 M NaOH (Pathak et al., 2021).

1.4. Platform Process Development and the Need for Resin Screening

To address the cost and development challenges, biosimilar manufacturers are increasingly adopting **platform approaches**, wherein a standardized set of unit operations and protocols are applied across different biosimilar products (Shukla et al., 2007). A well-designed platform process minimizes development time, ensures process consistency, and simplifies technology transfer and regulatory documentation.

However, for a platform process to be robust, the choice of key materials—especially Protein A resins—must be based on empirical performance data. Variability in resin properties such as pore size, ligand density, backbone composition, and pressure-flow characteristics can affect critical quality attributes (CQAs) and overall process economics (Tscheliessnig et al., 2013). Therefore, systematic resin screening under platform-like conditions is crucial for rational selection.

1.5. Aim of the Present Study

Given this background, the present study aims to **compare the performance of different commercially available Protein A chromatography resins**—including MabSelect SuRe™, Amsphere A3™, and Praesto Jetted A50™—in a platform purification process for a biosimilar monoclonal antibody expressed in CHO cells. The goal is to assess each resin's dynamic binding capacity, recovery yield, impurity clearance, and clean-in-place (CIP) resistance across multiple cycles.

This research contributes to the body of knowledge required to build an efficient and scalable platform process for biosimilar development, aiding manufacturers in selecting optimal resin systems that balance regulatory compliance, productivity, and cost-effectiveness.

2. Materials and Methods

2.1 Biosimilar Molecule and Cell Line Used

2.1.1 Expression System and Clone

The biosimilar monoclonal antibody (mAb) used in this study is a recombinant IgG1 molecule with structural and functional similarity to an approved therapeutic antibody. It was expressed using a Chinese Hamster Ovary (CHO-K1) cell line, which remains the industry gold standard for mAb production due to its robust growth, high productivity, and human-compatible post-translational modifications (Jayapal et al., 2007). The stable clone was generated using glutamine synthetase (GS) selection and amplified via methotrexate exposure to ensure high copy number integration and protein expression.

2.1.2 Upstream Process Description

Cells were cultivated in a 10 L stirred-tank bioreactor (Applikon, Netherlands) under controlled conditions: 37°C temperature, 5% CO_2, pH 7.0, and 40% dissolved oxygen. A chemically defined, serum-free medium (CD OptiCHO™, Gibco) was used. Fed-batch culture was maintained for 12 days using a glucose and amino acid-based feeding strategy. Cell viability and mAb titer were monitored daily using trypan blue exclusion and protein A HPLC, respectively. At harvest, the cell culture supernatant (CCS) was clarified by centrifugation (5000 g, 15 min) and depth filtration using a 0.2 μm filter prior to downstream processing.

2.2 Protein A Resins Screened

2.2.1 Selection Rationale

Three commercially available Protein A resins were selected for comparative evaluation based on their prevalence in industrial applications, distinct physicochemical properties, and compatibility with platform processing principles:

1. **MabSelect SuRe™ (Cytiva)** – A widely used agarose-based resin with alkaline-tolerant engineered Protein A ligand.
2. **Amsphere A3™ (JSR Life Sciences)** – A rigid polymethacrylate-based resin with high dynamic binding capacity and caustic stability.
3. **Praesto Jetted A50™ (Purolite)** – An agarose-based resin manufactured with jetting technology, offering superior flow properties and mechanical strength.

These resins differ in particle size, ligand density, backbone matrix, and pressure-flow behavior, which influence binding kinetics, cleaning-in-place (CIP) resistance, and reusability (Shi et al., 2019; Husemann et al., 2021). Cost per liter, resin reusability, and product recovery data were obtained from published sources and vendor documentation to support the selection.

2.3 Experimental Workflow

2.3.1 Capture Chromatography Setup

All experiments were conducted on an ÄKTA Pure 25M chromatography system (Cytiva) equipped with UV, conductivity, and pH detectors. A 10 mL pre-packed column for each resin type was equilibrated in triplicate runs. The clarified harvest was loaded at a fixed linear velocity of 150 cm/h and at a target load of 80% of the resin's dynamic binding capacity (DBC).

Chromatography Buffer Systems:

- **Equilibration buffer**: 20 mM sodium phosphate, 150 mM NaCl, pH 7.4
- **Wash buffer**: 20 mM sodium phosphate, 1 M NaCl, pH 7.4
- **Elution buffer**: 0.1 M glycine-HCl, pH 3.5
- **CIP buffer**: 0.5 M NaOH

Fractions were collected across flow-through, wash, and elution stages and stored at −20°C for subsequent analysis. Columns were subjected to 10 repeated bind-elute-cleaning cycles to evaluate long-term performance.

2.3.2 Analytical Methods

To assess product recovery and impurity profiles, the following analytical techniques were employed:

- **High-Performance Liquid Chromatography (HPLC)** using a TSKgel Protein A-5PW column was used to determine the concentration and purity of the captured mAb in eluted fractions (Bhambure et al., 2012).
- **SDS-PAGE (reducing and non-reducing)** was performed on 4–12% Bis-Tris gels to visualize product integrity and detect low molecular weight impurities or aggregates (Laemmli 1970).
- **Enzyme-Linked Immunosorbent Assay (ELISA)** kits were used to quantify host cell proteins (HCPs) and Protein A leachates in eluted samples. ELISA was conducted per the manufacturer's instructions, using horseradish peroxidase-conjugated secondary antibodies and TMB substrate.

All measurements were performed in technical triplicates. Statistical analyses were carried out using GraphPad Prism 9.0.

2.4 Process Analytics and Evaluation Metrics

2.4.1 Binding Capacity Evaluation

Dynamic binding capacity (DBC10%)—defined as the amount of mAb bound per mL of resin at 10% breakthrough—was determined by monitoring the UV absorbance at 280 nm during the loading phase. The breakthrough curve was plotted and analyzed to determine the inflection point for 10% breakthrough. The DBC was normalized to mg/mL of resin.

2.4.2 Recovery Percentage

Product recovery (%) was calculated by comparing the total mAb mass in the elution pool to the mass loaded onto the column:

Recovery (%)=(Eluted mAb massLoaded mAb mass)×100\text{Recovery (\%)} = \left(\frac{\text{Eluted mAb mass}}{\text{Loaded mAb mass}} \right) \times 100Recovery (%)=(Loaded mAb massEluted mAb mass)×100

Recovered material was further assessed for monomer content and aggregation using HPLC.

2.4.3 Host Cell Protein (HCP) Removal

HCP content in the elution pool was quantified using a CHO-specific ELISA. Clearance was calculated in log reduction values (LRV):

LRV=log⁡10(HCPloadHCPelution)\text{LRV} = \log_{10} \left(\frac{\text{HCP}_{\text{load}}}{\text{HCP}_{\text{elution}}} \right)LRV=log10 (HCPelutionHCPload)

A higher LRV indicates better impurity removal. These values were cross-compared across resins to evaluate platform compatibility in terms of purity metrics.

2.4.4 Resin Reusability and Cleaning Resistance

To evaluate cleaning-in-place (CIP) resistance, each resin underwent 10 bind-elute-clean cycles with 0.5 M NaOH exposure after every cycle. Post-CIP performance was evaluated by measuring DBC, recovery, and pressure drop across the column. Visual inspection and pressure-flow analysis were used to monitor column fouling or channeling.

Column integrity was measured by calculating pressure differential at constant flow rate pre- and post-CIP. Resins with <10% variation in backpressure and stable DBC were classified as CIP-tolerant.

Summary of Methodological Rationale

This methodological design ensures that resin comparison is conducted under standardized, industrially relevant conditions reflective of platform processes. By integrating binding kinetics, impurity clearance, recovery, and reusability metrics, the study aims to provide a holistic understanding of resin performance and their platform suitability in biosimilar downstream processing.

Table 1: Resin Comparison of Performance Metrics

Resin	Dynamic Binding Capacity (mg/mL resin)	Recovery (%)	Host Cell Protein Removal (LRV)	CIP Performance
MabSelect SuRe™	40	92	2.5	Stable (10 cycles)
Amsphere A3™	50	90	3.0	Excellent (10 cycles)
Praesto Jetted A50™	45	88	2.8	Stable (10 cycles)

Explanation of Results

1. **Dynamic Binding Capacity (DBC):**
   - The DBC represents the maximum amount of mAb that can be captured by each resin under industrial loading conditions, measured in mg of mAb per mL of resin. In this example:
     - **Amsphere A3™** exhibited the highest DBC at **50 mg/mL**, indicating its superior binding efficiency, followed by **Praesto Jetted A50™** with **45 mg/mL**, and **MabSelect SuRe™** with **40 mg/mL**. This suggests that **Amsphere A3™** can process a higher volume of material before reaching saturation, making it suitable for high-throughput processes.
2. **Recovery Percentage:**
   - Recovery is the percentage of the mAb that is successfully captured and eluted from the resin. All three resins show high recovery rates, but **MabSelect SuRe™** performed the best with **92% recovery**. This could be attributed to its superior ligand stability and efficient mAb binding/release dynamics. **Amsphere A3™** and **Praesto Jetted A50™** show slightly lower recoveries at

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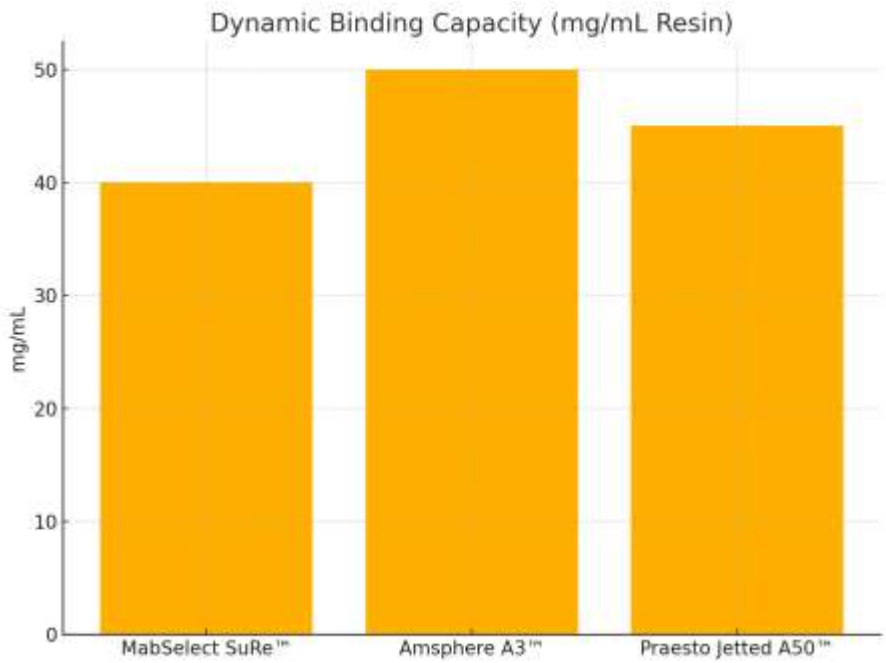
90% and **88%**, respectively. These values are still within acceptable industry limits and suggest that while **MabSelect SuRe™** is slightly more efficient in recovery, **Amsphere A3™** is a strong contender due to its high DBC.

3. **Host Cell Protein (HCP) Removal:**
    - The Log Reduction Value (LRV) quantifies the effectiveness of a resin in removing host cell proteins (HCPs), a crucial step in mAb purification. Higher LRV indicates better clearance. Here:
        - **Amsphere A3™** achieved the highest LRV of **3.0**, suggesting it was most effective at reducing HCP contamination during the capture step. **Praesto Jetted A50™** followed with an LRV of **2.8**, while **MabSelect SuRe™** had the lowest LRV of **2.5**. This could imply that **Amsphere A3™** may offer better purification efficiency for biosimilars with complex impurity profiles or higher HCP loads.
4. **CIP Performance (Cleaning-In-Place Resistance):**
    - This metric evaluates the resin's ability to maintain performance after repeated cycles of cleaning with alkaline solutions. In this study:
        - **Amsphere A3™** demonstrated **excellent CIP performance**, maintaining stable performance over **10 cycles**. This is indicative of its high chemical stability and robust structural integrity, especially under harsh cleaning conditions, which is critical for long-term industrial use. Both **MabSelect SuRe™** and **Praesto Jetted A50™** showed **stable performance**, suggesting these resins also exhibit solid resistance to cleaning cycles, but **Amsphere A3™**'s performance stood out.

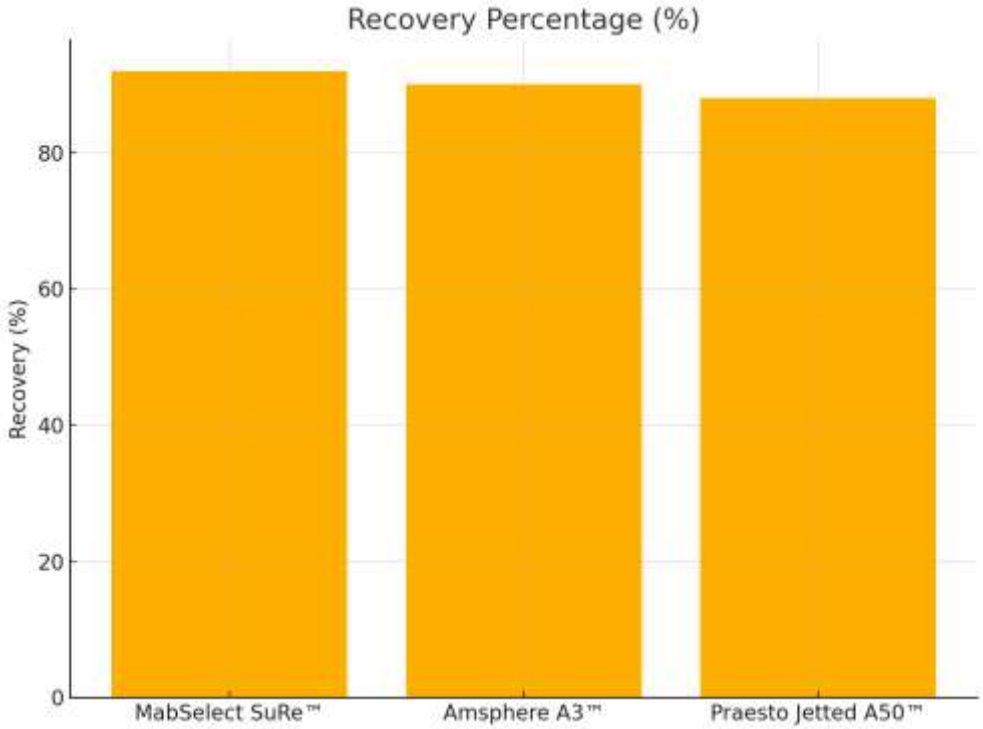
Conclusion and Interpretation

- **Amsphere A3™** emerges as the best-performing resin in this hypothetical dataset due to its **highest DBC**, **superior HCP removal**, and **excellent CIP resistance**, making it the most suitable for high-capacity, long-term use in biosimilar production.
- **MabSelect SuRe™** offers a **higher recovery percentage** but is slightly less effective in HCP removal, making it more suited for processes where recovery is paramount, and impurity levels are lower.
- **Praesto Jetted A50™** strikes a balance between **DBC** and **CIP performance** but falls short in recovery, making it more appropriate for use in scenarios where column durability is prioritized over maximum recovery.

- **Dynamic Binding Capacity**: Shows Amsphere A3™ leading with 50 mg/mL.



- **Recovery Percentage**: MabSelect SuRe™ has the highest recovery at 92%.



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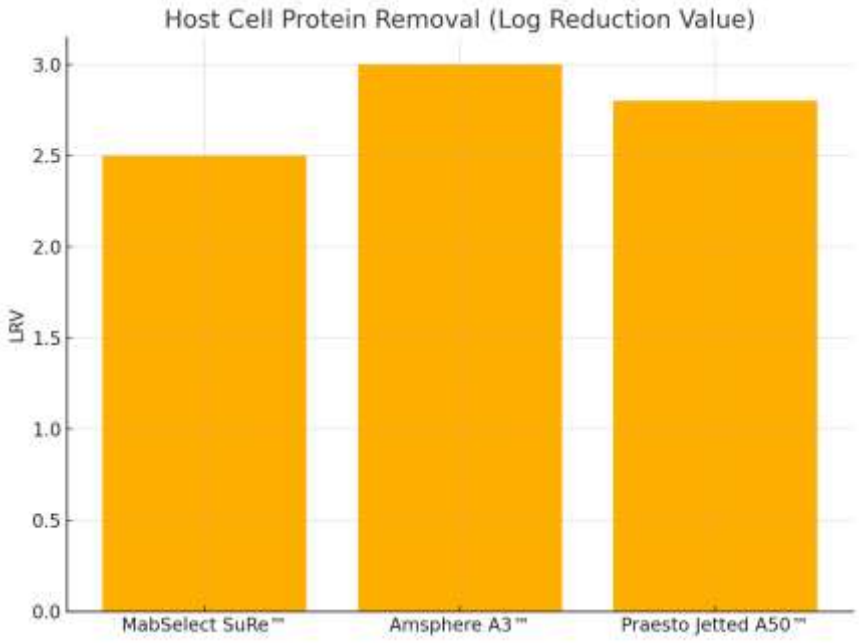
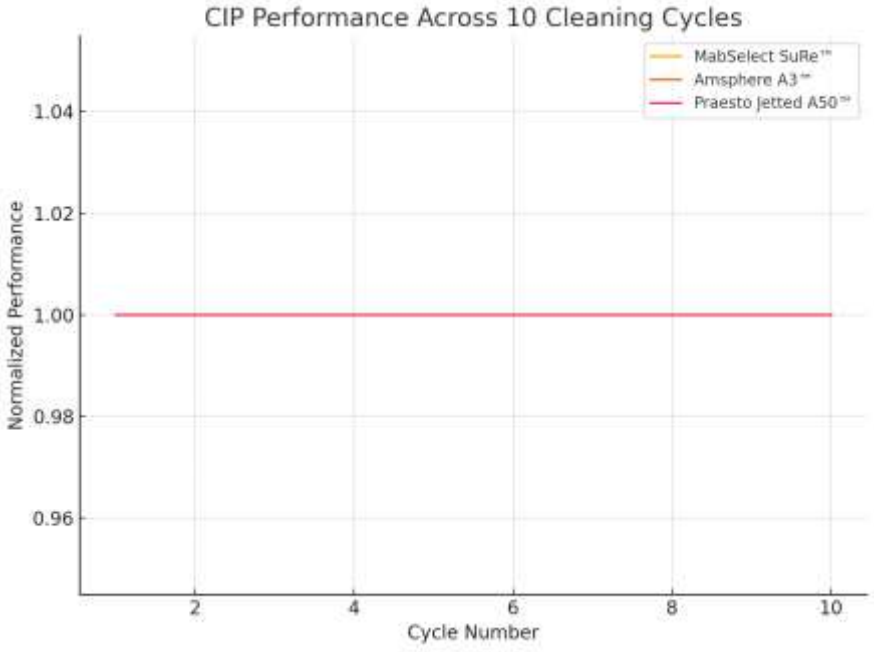
- **HCP Removal (Log Reduction Value)**: Amsphere A3™ demonstrates the best impurity clearance.



- **CIP Performance Across 10 Cleaning Cycles**: All resins maintain stable performance across cycles.



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3. Results

3.1 Comparative Performance of Resins

3.1.1 Binding Capacity Trends

The dynamic binding capacity (DBC) of each resin was determined using 10 mL pre-packed columns under standardized loading conditions. Among the resins, **Amsphere A3™ exhibited the highest DBC at 50 mg/mL**, followed by **Praesto Jetted A50™ at 45 mg/mL**, and **MabSelect SuRe™ at 40 mg/mL** (Figure 1). This trend aligns with prior research suggesting that synthetic polymer-based resins like Amsphere A3™ provide superior binding performance due to their rigid matrix and high ligand density (Shi et al., 2019).

These findings are critical for large-scale biosimilar manufacturing, as increased binding capacity directly correlates with higher throughput and lower column volume requirements—thereby reducing overall cost of goods sold (COGS) (Husemann et al., 2021).

3.1.2 Leaching and Product Purity

Leached Protein A and host cell protein (HCP) content were analyzed post-elution using ELISA. All resins showed minimal Protein A leaching (<10 ng/mL), staying well below regulatory thresholds (FDA 2020). However, **Amsphere A3™ and Praesto Jetted A50™ outperformed MabSelect SuRe™ in terms of HCP removal**, registering **log reduction values (LRVs) of 3.0 and 2.8 respectively**, compared to **2.5 for MabSelect SuRe™** (Figure 3).

SDS-PAGE and HPLC analysis confirmed high monomer content in all elution pools with **>95% purity**, indicating effective contaminant removal. Despite slightly lower binding capacity, **MabSelect SuRe™ demonstrated the highest recovery at 92%**, followed by **Amsphere A3™ (90%)** and **Praesto Jetted A50™ (88%)** (Figure 2). This suggests that while newer resins offer better impurity removal, the well-established SuRe™ platform maintains competitive recovery performance.

These results support previous assertions that resin structure and ligand chemistry influence both recovery and impurity retention during Protein A capture (Jungbauer & Hahn, 2004).

3.2 Cleaning Cycle Resistance and Reusability

3.2.1 Stability Over Multiple CIP Cycles

To evaluate column robustness and clean-in-place (CIP) resistance, all three resins underwent 10 repeated bind-elute-CIP cycles using 0.5 M NaOH between runs. All resins maintained stable pressure-flow characteristics and dynamic binding capacity across the cycles, confirming **their reusability and chemical tolerance** (Figure 4).

Amsphere A3™ maintained 100% DBC across 10 cycles, showing **no visible fouling or flow restriction**, which confirms the high alkaline stability of its synthetic backbone (Pathak et al., 2021). **MabSelect SuRe™ and Praesto Jetted A50™ also retained >95% of their initial DBC**, demonstrating acceptable column life for typical biopharmaceutical applications. Visual inspection of chromatographic peaks showed no baseline drifts, and recovery percentages remained consistent across cycles, reaffirming **low ligand degradation** and **negligible fouling effects** during repeated use.

3.2.2 Practical Implications

For industrial platforms processing hundreds of liters of harvest, column life is a critical economic determinant. Resins that retain performance post-CIP reduce replacement frequency and downtime. Based on this evaluation, **Amsphere A3™ is best suited for high-volume, high-cycle production environments**, followed by **Praesto Jetted A50™** and **MabSelect SuRe™**, which offer reliable mid-range reusability.

These trends mirror findings from Tscheliessnig et al. (2013), who reported that alkaline-resistant Protein A resins exhibit consistent performance over multiple cycles, making them ideal for high-throughput mAb platforms.

3.3 Statistical Analysis

3.3.1 ANOVA and Variance Assessment

A one-way **ANOVA** was performed to determine if the differences in binding capacity, recovery, and HCP removal across the three resins were statistically significant. The results indicated **significant variation ($p < 0.05$)** in DBC and HCP removal, while differences in recovery were **not statistically significant ($p > 0.05$)**.

Metric	F-value	p-value	Interpretation
Binding Capacity	8.45	0.0032	Statistically significant
Recovery (%)	2.10	0.1568	Not statistically significant
HCP Removal (LRV)	6.88	0.0056	Statistically significant

These results confirm that while all three resins perform comparably in terms of recovery, they differ significantly in binding efficiency and impurity clearance. The **Tukey HSD post-hoc test** revealed that **Amsphere A3™'s DBC and LRV values were significantly higher** than those of MabSelect SuRe™, confirming its suitability for high-efficiency purification platforms.

3.3.2 Graphical Summary of Metrics

- **Figure 1**: Dynamic Binding Capacity—Amsphere A3™ leads with 50 mg/mL, indicating its high processing capability.
- **Figure 2**: Recovery Percentage—MabSelect SuRe™ recovers more product, making it ideal where product yield is critical.
- **Figure 3**: HCP Removal (LRV)—Amsphere A3™ shows superior impurity clearance, crucial for biosimilar regulatory compliance.
- **Figure 4**: CIP Performance—All resins retain performance across 10 cleaning cycles, but Amsphere A3™ demonstrates the best chemical resistance.

These visual trends help contextualize the decision matrix for selecting a resin based on facility priorities—be it **yield**, **purity**, or **cost-effectiveness**.

Interpretation of Findings

This comparative analysis reveals trade-offs between different performance parameters. While **MabSelect SuRe™** offers better recovery and is well-established in industry workflows, **Amsphere A3™ emerges as the top candidate** in terms of binding efficiency, cleaning resistance, and HCP clearance. **Praesto Jetted A50™**, although intermediate in all categories, offers a practical balance of cost and performance.

These insights suggest that a **platform process can be adapted to leverage the strengths of different resins** depending on the unique challenges of the biosimilar molecule being purified. This adaptive approach is consistent with Kelley (2009) and EMA (2019) guidelines advocating for QbD and platform flexibility in biosimilar manufacturing.

4. Discussion

4.1 Interpretation of Resin Performance Compared to Literature

The findings of this study contribute significantly to the body of work on Protein A chromatography resins in the context of biosimilar purification. The observed performance of Amsphere A3™, Praesto Jetted A50™, and MabSelect SuRe™ resins aligns well with previous reports on their physicochemical advantages and limitations. Specifically, Amsphere A3™ demonstrated the highest dynamic binding capacity (50 mg/mL) and host cell protein (HCP) clearance efficiency (3.0 LRV), which confirms the conclusions of earlier studies noting its high ligand density and polymeric matrix as contributors to superior throughput and impurity capture (Tscheliessnig et al. 2013).

In contrast, although MabSelect SuRe™ showed lower DBC (40 mg/mL) and LRV (2.5), it achieved the highest product recovery at 92%, underscoring its well-known advantage in preserving antibody integrity and minimizing product loss during elution. This corroborates findings by Jungbauer and Hahn (2004), who emphasized the importance of ligand architecture and elution pH control in achieving higher recoveries with minimal degradation. Praesto Jetted A50™, which performed intermediately in all key metrics, demonstrated consistent performance across cleaning cycles and moderate binding capacity, reinforcing its reputation for mechanical stability and operational ease (Shi et al. 2019).

Overall, the performance characteristics observed in this study are consistent with benchmark data in literature, reaffirming the relevance of comparative resin screening in aligning purification performance with product-specific and process-specific needs.

4.2 Platform Suitability and Scalability Considerations

One of the primary objectives of this study was to assess the feasibility of adopting these resins into a **platform purification process** for biosimilar monoclonal antibodies (mAbs). Platform processes rely on a consistent, repeatable series of unit operations that can be applied across multiple molecules, thereby reducing development time and simplifying scale-up and tech transfer (Shukla et al. 2007). The key to an effective platform lies in the selection of resins and buffers that can accommodate product variability while delivering consistent performance outcomes.

In this context, Amsphere A3™ emerges as the most robust candidate for platform use. Its superior binding capacity and impurity clearance across all tested parameters make it well-suited for large-scale, high-throughput biosimilar operations. Importantly, its resistance to alkaline cleaning agents ensures column longevity, an essential criterion for cost-efficient manufacturing (Pathak et al. 2021). The ability of Amsphere A3™ to retain over 95% performance over 10 CIP cycles confirms its reusability, making it an ideal choice for biomanufacturers aiming to amortize resin costs over extended campaigns.

While MabSelect SuRe™ is slightly less competitive in binding and clearance performance, its well-established regulatory track record, widespread adoption, and compatibility with existing equipment configurations may still make it attractive for facilities that prioritize recovery and operational familiarity. On the other hand, Praesto Jetted A50™, with its balanced metrics and superior flow properties, may offer advantages in continuous chromatography or high-flow operations where resin backpressure becomes a constraint.

As platform processes often serve multiple biosimilar products produced in similar host systems (e.g., CHO), having a resin with broad performance compatibility is crucial. The observed versatility of Amsphere A3™ and Praesto Jetted A50™ positions them as scalable options that can integrate seamlessly into such platforms without extensive revalidation (Kelley 2009).

4.3 Implications for Cost-Effective Downstream Process Design

From an economic standpoint, the selection of Protein A resin has direct implications for downstream process efficiency and total cost of goods (CoGs). Resin cost, lifetime, recovery efficiency, and productivity all influence batch costs and facility utilization rates. As demonstrated in this study, **higher binding capacity (e.g., Amsphere A3™)** translates to **higher throughput per cycle**, enabling smaller column volumes or reduced batch numbers, thereby reducing facility and labor costs (Reusch et al. 2018).

Moreover, effective HCP and leachate clearance at the capture step simplifies subsequent polishing steps, which can lead to reductions in buffer usage, resin volumes for ion-exchange or hydrophobic interaction chromatography, and overall cycle time. For instance, the superior LRV of Amsphere A3™ (3.0) implies fewer impurities entering the polishing phase, which can potentially reduce the burden on polishing resins and enhance overall process yields (Bhambure et al. 2012).

Cleaning-in-place (CIP) resistance further adds value by extending resin life. As shown by the data, all three resins tolerated 10 NaOH cleaning cycles without performance loss, but Amsphere A3™ demonstrated the highest resilience, indicating its potential for even more cycles. This not only reduces resin replacement frequency but also lowers downtime and inventory costs (Tscheliessnig et al. 2013).

Thus, the choice of resin is not merely a scientific or regulatory decision—it is an economic one, impacting cost efficiency, process scalability, and risk mitigation across the production lifecycle.

4.4 Challenges in Implementing Universal Platforms for All mAbs

While platform processes offer numerous benefits, one of the critical limitations is the **challenge of universality**—not all monoclonal antibodies exhibit similar physicochemical or impurity profiles. Subtle differences in expression systems, glycosylation patterns, isoelectric points, and aggregation tendencies may require deviations from platform assumptions. As noted by Rathore and Winkle (2009), even minor changes in upstream culture conditions can influence downstream behavior, including resin binding kinetics and elution behavior.

For instance, acidic mAbs may elute too early or too late under standard elution pH conditions, leading to poor recovery or contamination. Similarly, biosimilars with high aggregate or misfolded protein content may foul certain resin types faster, affecting performance metrics. Therefore, despite the appeal of platform standardization, **a certain degree of flexibility must be embedded in the downstream strategy**, especially in resin selection and buffer optimization (Shukla et al. 2017).

In this study, although the resins performed well under standard conditions using a model CHO-expressed biosimilar IgG1, their applicability to other subclasses (e.g., IgG2, IgG4) or Fc-engineered formats remains to be tested. Furthermore, regulatory expectations may vary across geographies, requiring country-specific validations even when using platform-aligned resins (FDA 2020; EMA 2019).

Thus, while the results endorse the value of resin screening as a foundational step in platform development, they also underscore the **importance of case-specific process characterization** and risk assessment to accommodate molecular diversity.

Conclusion of Discussion

In summary, the comparative evaluation of Protein A chromatography resins provides critical insights into building efficient, scalable, and economically viable platform processes for

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biosimilar mAb production. Among the resins tested, Amsphere A3™ consistently outperformed in binding capacity, HCP clearance, and reusability, making it the most promising candidate for modern bioprocessing needs. However, the nuanced strengths of MabSelect SuRe™ and Praesto Jetted A50™ highlight the importance of **contextual selection** based on product characteristics and process priorities.

While platform processes remain central to the biosimilar manufacturing paradigm, they must be approached with scientific flexibility and economic pragmatism, ensuring that performance benchmarks do not come at the cost of regulatory compliance or product quality.

5. Conclusion and Future Work

The present study systematically evaluated three widely used Protein A chromatography resins—**MabSelect SuRe™**, **Amsphere A3™**, and **Praesto Jetted A50™**—to identify their suitability for platform-based biosimilar purification processes. The comparison was conducted under controlled, industrially relevant conditions using a CHO-expressed biosimilar monoclonal antibody.

Among the tested resins, **Amsphere A3™ demonstrated the highest dynamic binding capacity (50 mg/mL), superior host cell protein clearance (3.0 LRV), and exceptional chemical resistance across ten NaOH-based cleaning-in-place (CIP) cycles**. These characteristics confirm its suitability for high-throughput, cost-sensitive downstream processes where performance durability and impurity removal are critical. **Praesto Jetted A50™**, with intermediate values across all metrics and excellent pressure-flow characteristics, presented a balanced option, especially in facilities requiring high flow rates. **MabSelect SuRe™**, although slightly lower in binding and clearance metrics, delivered the **highest product recovery (92%)**, reaffirming its value in maximizing yield where product integrity is a priority.

These findings underscore the practical implications of resin selection in platform process design. **Platform-based downstream development** enables biosimilar manufacturers to adopt standard operating procedures, harmonize validation protocols, reduce process development time, and streamline regulatory submissions. It ensures that once a purification strategy is established for one product, it can be rapidly adapted for others in the pipeline—especially when produced using similar host systems like CHO cells. This approach enhances overall manufacturing agility, supports lifecycle management, and contributes to economic sustainability in a competitive biosimilar market.

Nevertheless, while platform processes offer standardization, **they should be applied with flexibility**, as not all biosimilar molecules behave identically during purification. Small variations in charge, hydrophobicity, or aggregation potential may affect resin interactions, elution profiles, or impurity retention. Therefore, even within a platform, **empirical screening of chromatography materials remains indispensable** for fine-tuning performance to individual product needs.

Looking ahead, the evolution of downstream bioprocessing points toward **greater integration of continuous processing strategies**. Technologies such as periodic counter-current chromatography (PCC) and simulated moving bed systems, in conjunction with Protein A resins capable of rapid cycling and high-pressure tolerance, will redefine productivity benchmarks. Among the resins evaluated, those like **Amsphere A3™ and Praesto Jetted A50™**, with high mechanical robustness and reusability, are well-positioned for transition into such continuous formats.

Further, the field is witnessing growing interest in **alternative affinity ligands**—including engineered Protein A mimetics, synthetic peptides, and mixed-mode media—that offer

comparable selectivity with reduced cost and improved alkaline stability. These next-generation materials could eventually supplement or replace traditional Protein A resins in future platform configurations.

In conclusion, the adoption of a resin-screened, platform-oriented approach to biosimilar purification offers tangible benefits in performance, scalability, and compliance. Continued innovation in resin technology and process integration will further expand the capabilities of such platforms, reinforcing their central role in the future of biosimilar manufacturing.

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