Affinity Chromatography Methods for Monoclonal Antibody Purification in Downstream Processing

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**Abstract**

This paper reviews the critical role of affinity chromatography in the downstream processing of monoclonal antibodies, highlighting its high selectivity for efficient capture and impurity removal. Predominantly using Protein A, this initial purification step is crucial, followed by polishing methods to meet regulatory standards. Recent advancements include novel ligands and resin technologies, such as nanofiber adsorbents, alongside the development of continuous and automated bioprocessing approaches. Despite its effectiveness in achieving high purity and yield, challenges persist regarding resin cost, scalability, and lifetime. The review concludes that affinity chromatography remains a vital technique for mAb purification, with ongoing research focused on innovative ligands, reduced non-specific binding, improved resin performance, and enhanced process integration to further optimise its application in biopharmaceutical manufacturing.

**1. Introduction**

Monoclonal antibodies (mAbs) have become a cornerstone of modern biopharmaceutical development, revolutionising the treatment of a wide array of diseases. These engineered proteins are designed to specifically target a single epitope on an antigen, offering a high degree of precision in their therapeutic action. Unlike traditional small molecule drugs, mAbs are large, complex molecules produced in living cell systems. The production of mAbs typically involves culturing genetically engineered cells, such as mammalian cell lines, which are designed to secrete the desired antibody. These cellular expression systems are crucial for producing the large quantities of mAbs required for therapeutic applications (Bouvarel et al., 2022). The significance of mAbs in biopharmaceuticals stems from their unique properties. Their high target specificity allows them to selectively interact with disease-related molecules or cells, minimising off-target effects and improving therapeutic outcomes (Bouvarel et al., 2022). Furthermore, mAbs possess distinct pharmacokinetic (PK) and pharmacodynamic (PD) properties, influencing how they are absorbed, distributed, metabolised, and excreted by the body, as well as their mechanism of action (Tran et al., 2024). The Fc region of the mAb, for instance, plays a key role in mediating effector functions and influencing their PK/PD profile through interactions with Fc receptors (Figure 1), (Bouvarel et al., 2022). This allows for the development of mAbs with tailored properties, such as extended serum half-life (Cotham et al., 2023). In essence, monoclonal antibodies represent a vital class of biopharmaceutical agents due to their high specificity, tailored PK/PD properties, and broad therapeutic potential across diseases such as cancer, autoimmune disorders, and infectious diseases (Tran et al., 2024).

A diagram of a protein

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Figure 1.Structural representation of a monoclonal antibody (mAb):  
The antigen-binding sites, known as complementarity-determining regions (CDRs), are located within the Fab region and are responsible for binding specific targets, leading to either inhibition or activation of signalling pathways. The Fc region comprises the hinge segment and the constant domains of the heavy chain (CH2 and CH3), and it plays roles in immune effector functions, such as interacting with Fc receptors and activating the complement system (Aranez and Ambrus, 2019).

However, the production of mAbs is a complex process. As they are produced in biological systems, they are susceptible to various enzymatic and chemical post-translational modifications (PTMs) that can potentially alter their structure, stability, and function (Bouvarel et al., 2022). These modifications can include glycosylation, oxidation, and deamidation, highlighting the importance of robust analytical techniques for their characterisation (Cotham et al., 2023). Their continued development and application underscore their enduring significance in the landscape of modern medicine (Sachio et al., 2024).

Downstream processing (DSP) plays a crucial role in the production of mAbs, as it is responsible for the isolation and purification of the target molecule from the complex mixture of the cell culture, following upstream processing (USP) (Sachio et al., 2024) (Figure2). This purification is essential to remove a variety of process- and product-related impurities, including host cell proteins (HCPs), DNA, endotoxins, and potential aggregates, which can compromise the safety and efficacy of the final therapeutic product (Zollner et al., 2024). Furthermore, DSP can account for a significant portion, potentially up to 80%, of the total manufacturing costs, highlighting its economic importance in mAb production (Sachio et al., 2024). Efficient DSP strategies are therefore vital for achieving the required levels of product purity and quality mandated by regulatory authorities, ultimately ensuring the delivery of safe and effective mAb-based therapies to patients (Sachio et al., 2024). Moreover, the development and optimisation of DSP techniques are critical for the scalability of mAb manufacturing processes to meet increasing demands (Sachio et al., 2024).

A diagram of a baby bed

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Figure 2. Schematic representation of upstream processing (USP) and downstream processing (DSP) in the production of mAbs (Tischler, 2022).

Chromatography constitutes a critical and frequently indispensable step in the purification of mAbs, serving as a versatile suite of techniques essential for achieving the requisite levels of product quality (Tran et al., 2024). Given the inherent complexity of biopharmaceutical production, which yields mAbs within a milieu of host cell components and other potential contaminants, chromatographic methods enable the selective separation of the target antibody based on its distinct physicochemical properties (Cytiva, n.d.). Affinity chromatography, particularly utilising Protein A (a surface protein of the Staphylococcus aureus cell wall), is commonly employed as an initial capture step, leveraging the specific binding interaction between the Fc region of the mAb and the immobilised ligand (Figure 3), to achieve significant enrichment and impurity removal (Tran et al., 2024). Subsequent polishing steps, often employing ion exchange, hydrophobic interaction, or size exclusion chromatography, are then implemented to further eliminate remaining contaminants, aggregates, and product-related variants (Tran et al., 2024). Moreover, the coupling of affinity chromatography with native mass spectrometry has emerged as a powerful analytical tool, providing orthogonal means to study mAb attributes and their biological relevance (Cotham et al., 2023).

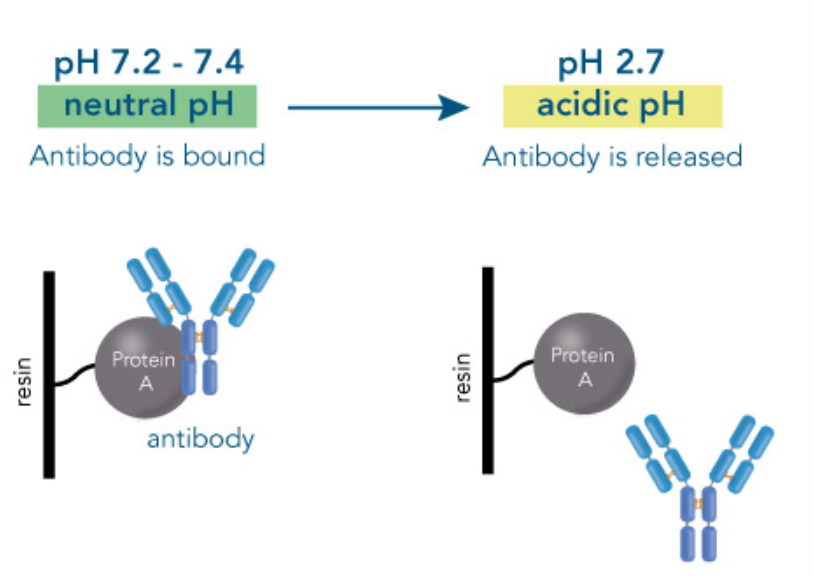


Figure 3. A visual representation of utilizing Protein A binding interaction between the Fc region of the mAb in affinity chromatography. The target mAb is captured and then eluted by altering buffer conditions, such as pH (Neuromics.com, 2023).

Thus, chromatography underpins the entire mAb purification process, from initial capture to final polishing and comprehensive characterisation, ensuring the safety and efficacy of therapeutic antibody products.

This essay aims to critically evaluate the role of affinity chromatography in the downstream processing of monoclonal antibodies, as crucial step in biopharmaceutical production. The scope includes an in-depth analysis of the fundamental principles of affinity chromatography, its integration into large-scale mAb purification, and a review of recent advancements in ligand development, resin technology, and process optimization over the past five years. The objectives are to assess the effectiveness, challenges, and limitations of affinity chromatography in manufacturing-scale DSP, compare it with alternative purification techniques, and highlight case studies showcasing industrial applications. Furthermore, the essay will explore emerging trends, regulatory considerations, and potential future innovations that can enhance the efficiency and sustainability of affinity chromatography in mAb purification.

**2. Basis of Affinity Chromatography in Manufacturing-Scale DSP**

**2.a. Fundamentals of Affinity Chromatography**

Affinity chromatography achieves selective binding of a target molecule, such as a monoclonal antibody by using an immobilised ligand that exhibits highly specific interactions with the target (Cotham et al., 2023). This technique takes advantage of the inherent biological affinities between mAbs and ligands like Protein A or Protein G, Fc receptors (Narges et al., 2022), or antibody fragments like nanobodies (Rezvani and Aspelund, 2024). The selectivity of this separation method arises directly from the unique affinity of the mAb for the chosen ligand, enabling preferential binding while other sample components show minimal interaction (Stern et al., 2024). Following the binding, non-specifically bound impurities are removed through washing steps, and the target mAb is then eluted by altering buffer conditions, such as pH or ionic strength, to disrupt the specific interaction between the mAb and the ligand (Leibiger et al., 2024), (Figure 4).

A diagram of a molecule

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Figure 4. A visual representation of binding and elution of target molecule (step 1-6) in affinity chromatography (SinoBiological, n.d.).

The choice of ligand determines the region or property of the mAb targeted for binding; for example, Protein A typically interacts with the Fc region of antibodies (Narges et al., 2022). In essence, affinity chromatography leverages the molecular recognition capabilities between a specific ligand and the target mAb. By carefully controlling the binding and elution conditions, the technique provides a highly effective means of selectively capturing and purifying mAbs from complex biological samples. The strength and specificity of the affinity interaction directly determine the efficiency and resolution of the separation process.

**2.b. Affinity Ligands**

Protein A and Protein G (Figure 5) are well-established affinity ligands widely used for purifying antibodies and antibody fragments (Narges et al., 2022). Protein A exhibits a strong affinity for the Fc region of many immunoglobulins G (IgG) antibodies from various species, making it a crucial tool in mAb purification (Narges et al., 2022). Similarly, Protein G also interacts with the Fc region of IgGs, but it demonstrates a broader binding affinity across different species and can also bind to certain antibody fragments, including Fab fragments (Stern et al., 2024).

A close-up of several structures

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Figure 5. A three-dimensional structural model depicting the crystalline complex formed between the Fc region of immunoglobulin G (IgG) and either protein A (a) or protein G (b). The ligands, staphylococcal protein A (spA) and streptococcal protein G (spG), are illustrated using orange and yellow ribbon representations, respectively. The two heavy chains comprising the IgG Fc region are represented by green and cyan ribbons (Lee et al., 2021)

Beyond these naturally derived ligands, various alternative synthetic and engineered ligands have been developed to broaden the scope of affinity chromatography. These include immobilised metal ion affinity chromatography (IMAC), targets histidine-tagged proteins (Figure 6), using nickel or other metal ions (Cytiva, n.d.). Another example are strep-tags interacting with streptavidin or its variants; heparin-binding tags utilising electrostatic interactions with heparin matrices (Prabhala and Wood, 2024). For instance, heparin affinity with core-shell flow-through chromatography allows direct step-to-step loading for virus-like particle purification (Zollner et al., 2024).

A diagram of a molecule

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Figure 6. Illustration of a chelator complex involving NTA and Ni²⁺:  
The complex consists of nitrilotriacetic acid (NTA) coordinating a nickel ion (Ni²⁺), which in turn interacts with two imidazole side chains from a histidine tag (His-tag) that has been genetically engineered onto a protein. This specific binding enables the purification of His-tagged proteins, as the histidine residues form stable interactions with the Ni²⁺ ion held in place by the NTA (Cube Biotech, 2017)

Additionally, camelid-derived affinity ligands, like nanobodies (Figure 7), offer high specificity and stability for isolating various biomolecules, including biopharmaceuticals (Rezvani and Aspelund, 2024).

A diagram of a light and heavy chain

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Figure 7. Alpacas (camelid) produce three distinct subclasses of immunoglobulin G (IgG) as part of their immune response. The IgG1 subclass consists of both heavy and light chains, like conventional antibodies. In contrast, IgG2 and IgG3 are classified as heavy-chain-only antibodies (HCAbs), which lack both the light chains and the CH1 domain of the heavy chain. The variable region of these HCAbs, known as VHH, can also exist independently in recombinant form, where it is commonly referred to as a nanobody (Ptglab.com, 2024).

CaptureSelect AAVX ligands demonstrate broad serotype compatibility for Adeno-Associated Virus (AAV) purification (Florea et al., 2023). Titanium dioxide affinity chromatography (TDAC) has been applied to purify small molecules like siderophores based on their iron-chelating groups (Egbers et al., 2022).

These alternative ligands provide versatile options for purifying a wide range of recombinant proteins, antibody formats, and other biological entities, often allowing purification based on specific engineered tags or by exploiting pseudo-affinity interactions (Kadoi et al., 2025).

**2.c. Integration into DSP**

As previously mentioned, affinity chromatography, particularly using Protein A, is often employed as the primary capture step in the purification workflow of monoclonal antibodies due to its high selectivity for the Fc region of IgGs (Tran et al., 2024). This initial step allows for the efficient separation of mAbs from a complex mixture of host cell proteins, DNA, and other contaminants present in the clarified cell culture supernatant (Tran et al., 2024). The high specificity of the interaction between the immobilized ligand (e.g., Protein A) and the target mAb leads to significant enrichment and concentration of the antibody in a single step (Cytiva, n.d.). While affinity chromatography provides high purity in the capture phase, subsequent polishing steps are typically required to remove remaining impurities, such as aggregates, host cell proteins, and other product-related variants (Tran et al., 2024). Ion exchange chromatography (IEC), which separates molecules based on their net charge, hydrophobic interaction chromatography (HIC), which separates based on hydrophobicity and size-exclusion chromatography (SEC) technique that separates molecules based on their size, are commonly used as these downstream polishing steps (Tran et al., 2024). IEC can be particularly effective in removing charged impurities and product variants with different isoelectric points, HIC is also useful for separating aggregates and other species with varying hydrophobic properties while SEC commonly used for removing aggregates and low molecular weight species (Tran et al., 2024). In contrast to the highly selective binding mechanism of affinity chromatography, IEC, HIC and SEC rely on more general physicochemical properties of the proteins, often requiring careful optimisation of mobile phase conditions (e.g., pH, salt concentration) to achieve the desired separation (Cotham et al., 2023). Thus, affinity chromatography serves as a powerful initial purification method, providing high selectivity and capacity for mAbs, which is then complemented by the discriminatory power of techniques like IEC, SEC and HIC in subsequent purification stages to achieve the required purity for therapeutic applications (Cytiva, n.d.).

**2.d.** **Regulatory Considerations**

The purification of monoclonal antibodies must comply with rigorous regulatory standards established under Good Manufacturing Practices (GMP), as mandated by agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Regulatory guidance emphasises the critical role of process validation in establishing that the purification process consistently yields a product meeting predefined quality and purity criteria. Chromatographic operations, including protein A affinity chromatography and ion exchange chromatography, are subject to scrutiny during validation to ensure consistent process performance. FDA guidelines highlight the necessity of process qualification to confirm that the manufacturing process is capable of consistently delivering a commercial product of acceptable quality (FDA, 2011).

Complementing this, EMA guidance outlines detailed data requirements for process characterisation and verification, focusing particularly on the removal of process- and product-related impurities (EMA, 2016). Both agencies underscore the need to demonstrate the effectiveness of the purification process across its lifecycle. Furthermore, ICH Q6B provides expectations on setting scientifically justified specifications, including limits for impurities and critical quality attributes, reinforcing the requirement for robust analytical and process control strategies (ICH, 1999). The relevance of ICH Q5C is also notable, as it addresses the stability testing of biotechnological/biological products, requiring that stability-indicating attributes be defined and controlled throughout the product’s shelf life (ICH, 1995).

ICH Q11 expands on these concepts by requiring that critical parameter in chromatographic steps such as column performance, loading capacity, and elution conditions to be identified and controlled, ensuring consistent performance over the lifecycle of the purification process (ICH, 2012). Regulatory expectations also advocate for the application of a risk-based approach, especially where process modifications (e.g., buffer reuse) could impact multiple unit operations. The EMA encourages the use of prior knowledge and risk assessments to identify material attributes and process parameters that may influence critical quality attributes, advocating for variability management in raw materials and impurity clearance strategies (EMA, 2016). This aligns with the structured risk management framework described in ICH Q9, which supports the identification, evaluation, and mitigation of potential risks to product quality (ICH, 2023). Collectively, the integration of quality risk management, lifecycle validation, and impurity control strategies is essential to meet regulatory expectations and ensure the safety, efficacy, and consistency of biopharmaceutical products.

**3. State of the Art in Affinity Chromatography for mAb Purification**

**3.a. Recent Advances**

Recent advancements in affinity chromatography for monoclonal antibodies are focused on enhancing efficiency, selectivity, and analytical capabilities. Innovations in affinity ligands are expanding the range of targets and conditions for capture and elution. For instance, engineered Protein A variants with improved alkaline stability and elution at milder pH conditions have been developed, alongside the exploration of novel ligands derived from camelid antibodies for highly specific isolation (Rezvani and Aspelund, 2024). Furthermore, resin technology is evolving with the development of materials like nanofiber-based adsorbents that offer increased binding capacity and throughput, potentially overcoming limitations associated with traditional resin beads and monolithic columns (Dewar et al., 2024). In addition to material advancements, a greater emphasis is being placed on process flexibility during resin screening to ensure robustness against feed stream variations (Sachio et al., 2024). The integration of affinity chromatography with other analytical techniques, particularly native mass spectrometry, represents a significant step forward. This online coupling allows for in-depth characterisation of mAbs, including glycoform analysis and the study of interactions with key receptors such as Protein A, FcγRIIIa, and FcRn, providing insights into biological relevance and critical quality attributes. These integrated platforms facilitate simplified experimental setups and the facile exchange of affinity separation modes (Cotham et al., 2023). Moreover, affinity chromatography is being increasingly explored within the context of continuous bioprocessing and for the purification of complex modalities like bispecific antibodies (Figure 8), necessitating tailored strategies and process optimisation (Tran et al., 2024). The drive towards automation in chromatographic purification workflows further contributes to increased efficiency and reproducibility in mAb processing (Prabhala and Wood, 2024).

**A diagram of a cell division

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Figure 8. Structure and general mechanism of action of bispecific antibodies:  
a) Bispecific antibodies are engineered by combining the variable regions of the heavy and light chains from two distinct monoclonal antibodies, each specific to a different antigen.  
b) The illustration demonstrates a typical mechanism whereby bispecific antibodies simultaneously engage two separate targets—one on a cytotoxic T cell and another on a cancer cell—thereby facilitating targeted T cell-mediated cytotoxicity against tumour cells  (Raja et al., 2024).

**3.b. Current Challenges**

Current challenges in affinity chromatography encompass several key areas that impact its broad applicability and cost-effectiveness in biopharmaceutical manufacturing. A significant concern revolves around the economic aspects, as traditional resin processes can incur large costs for purchasing and installing large columns (Qu et al., 2024). The inherent expense of affinity resins represents a considerable operational cost, especially when considering large-scale production and the potential for resin fouling, which can limit their useful lifespan. Scalability presents another hurdle, as translating successful small-scale affinity purification processes to meet the demands of industrial production volumes often encounters limitations. To address throughput in protein A affinity chromatography, a dual-flowrate loading strategy has been explored (Qu et al., 2024), suggesting that maximizing throughput at a large scale is a consideration. The longevity of the affinity resin is crucial for maintaining economic viability and process efficiency; factors such as chemical and physical stability, resistance to fouling from feed stream impurities, and the ability to withstand repeated regeneration cycles are critical but can be challenging to optimise and predict. For example, the impact of photo-oxidation on the long-term storage of affinity chromatography media is being investigated, highlighting concerns about resin lifetime (Rezvani and Aspelund, 2024). Finally, achieving high purification efficiency while maintaining product integrity remains a constant objective, particularly when dealing with complex biological mixtures and closely related impurities that necessitate highly selective binding and elution strategies under near-native conditions. Monitoring purification steps is essential, and various methods are used for sample analysis to determine protein concentration and aggregate concentration, which are indicators of purification efficiency and product integrity (Qu et al., 2024).

**3.c. Knowledge Gaps**

Despite the widespread use of affinity chromatography, several areas require further investigation to enhance its capabilities. One significant knowledge gap lies in the development and application of alternative affinity ligands beyond traditional options like Protein A. While Protein A is widely used for mAb capture (Cotham et al., 2023), exploring and implementing novel ligands, such as Camelid VHH domains (Rezvani and Aspelund, 2024), or those identified for specific targets like extracellular vesicles (Fernandes et al., 2024), could offer advantages in terms of cost, stability, or target specificity. Furthermore, the identification of species-independent and tag-less purification methods using alternatives like Protein G for Fab fragments (Stern et al., 2024), indicates the potential for broadening the scope of affinity purification. Another persistent challenge involves the reduction of non-specific binding, which can compromise product purity. While strategies such as increasing ionic strength or adding detergents are employed to mitigate non-covalent interactions (Cytiva, n.d), a deeper understanding of the mechanisms underlying non-specific retention, including potential interactions with the resin matrix is needed to devise more targeted and effective solutions (Leibiger et al., 2024). Finally, improved resin regeneration methods are crucial for enhancing the economic viability and sustainability of affinity chromatography. Although resin re-use is possible and cleaning-in-place protocols exist, ongoing research into the long-term stability and performance of resins under various conditions, including different storage solutions and repeated alkaline exposures, it remains vital to maximise resin lifetime and ensure consistent purification performance (Florea et al., 2023). Further investigation into the specific sites responsible for resin degradation could inform the development of more resilient affinity media (Rezvani and Aspelund, 2024).

**4. Case Studies and Applications**

**4.a. Industrial Use of Protein A Chromatography for mAb Purification**

Protein A affinity chromatography serves as a cornerstone in the industrial purification of monoclonal antibodies, leveraging the highly specific interaction between the antibody Fc region and the Protein A ligand (Cotham et al., 2023). Recent studies continue to underscore its efficiency as a capture step, often aiming for high yields (≥99%) due to its critical position in mAb manufacturing (Sachio et al., 2024). This method typically achieves significant purity in a single step, facilitating substantial removal of process-related impurities such as host cell proteins and DNA (Prabhala and Wood, 2024). However, challenges persist, including the economic impact of resin costs and the occurrence of fouling, which can diminish resin lifetime. To address these limitations, ongoing research explores various optimisation strategies. These include the development of different elution methodologies, such as rapid screening modes for high throughput and resolving modes for separating variants with differing affinities. The online ProA-MS analysis (Figure 9), facilitates high-throughput screening of mAb molecules by operating in a "bind-and-elute" fashion, enabling swift assessment of various mAb candidates. Additionally, efforts have been made to apply ProA-MS directly to complex feedstocks, such as unclarified cell culture material. This application aims to streamline the analytical process by enabling direct analysis without necessitating extensive sample preparation. Such advancements enhance the efficiency and effectiveness of mAb characterization workflows (Cotham et al., 2023).

Diagram of a diagram of a viper tubing

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Figure 9. Illustration of the native affinity LC-MS platform:  
Affinity-based separation is achieved through a pH gradient utilizing ammonium acetate, tailored to the binding properties of the specific ligand. After separation, a stainless steel post-column tee splits the flow: the higher flow rate is directed to an inline UV detector and pH sensor, while the lower flow rate is sent to a nanospray emitter for mass spectrometric analysis (Cotham et al., 2023).

Furthermore, there is growing interest in alternative process formats, such as nanofiber-based chromatography, and continuous processing approaches to enhance productivity, reduce processing time, and improve overall economic performance compared to traditional resin-based columns (Qu et al., 2024). Investigations into residual impurities, like host cell proteins, post-Protein A purification are also crucial for further downstream process design and ensuring product quality (Leibiger et al., 2024).

**4.b. Alternative Affinity Ligands and Their Performance**

While Protein A affinity chromatography remains a fundamental technique for purifying monoclonal antibodies, the exploration of alternative affinity ligands and separation methods is a significant area of development within the biopharmaceutical industry. Engineered ligands, which can be modified Protein A domains like Fibro PrismA (Figure 10), or non-Protein A scaffolds are entirely new structures, being investigated to overcome some of the limitations of traditional Protein A, such as the necessity for harsh acidic elution conditions that might affect the integrity of certain antibodies (Yang and Zhu, 2025).

A close-up of several images of a structure

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Figure 10. Helium ion microscopy examination of Fibro PrismA before and after 200 cycles. (A, B) Images of unused Fibro PrismA. (C, D) Analysis of the outlet region of Fibro PrismA following 200 cycles at two different magnifications. Red circles highlight polymer beads on the fibres, formed during the electrospinning process (Qu et al., 2024).

The industrial application of these alternative ligands is being explored in various formats, including their immobilisation on innovative chromatography supports like nanofiber devices, and magnetic nanoparticles (Zimmermann et al., 2024), which are noted to be cheaper and can enable a more rapid process (Qu et al., 2024), (Figures 11, 12, 13).

A graph of a cycle and a cycle

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Figure 11. Overlay of 200 cycles of IgG4–3 capture on Fibro PrismA for: A) Chromatograms; B) Delta pressure drop (Qu et al., 2024).

A graph of a number of cells

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Figure 12. IgG binding and release from magnetic nanoparticles@ZCa. (A) Adsorption isotherms of IgG on the nanoparticles in TBSC buffer (50 mM Tris, 150 mM NaCl, 10 mM CaCl₂, pH 7.5). (B) IgG recovery after incubating 110 mg IgG per gram of nanoparticles, followed by washing and elution using 100 mM EDTA (pH 5.5). The amount of bound IgG was determined by comparing the initially loaded and unbound IgG. (C) Reduced SDS-PAGE analysis showing loaded IgG (L) and elution samples collected over three reuse cycles (E1–E3) for the three types of functionalized nanoparticles. The heavy (H; ~50 kDa) and light (L; ~25 kDa) IgG chains are visible. MNP: magnetic nanoparticle, TEOS: tetraethyl orthosilicate, GPTMS: (3-glycidyloxypropyl) trimethoxysilane (Zimmermann et al., 2024).

Performance comparisons between these alternative systems and traditional Protein A often evaluate crucial parameters like dynamic binding capacity, elution efficiency, and selectivity (Yang and Zhu, 2025). Certain alternative ligands, such as calcium-dependent ligands, offer the benefit of elution under milder pH conditions or by manipulating specific buffer components (Yang and Zhu, 2025), potentially leading to enhanced product quality and stability. Moreover, novel separation techniques employing these ligands, for instance, magnetic separation (Figure 12), show promise for faster processing times and the potential for direct capture from unclarified cell culture, which could streamline downstream processing workflows (Zimmermann et al., 2024).

From a cost-benefit analysis perspective, the adoption of alternative affinity ligands and their associated technologies requires considering factors beyond just the initial cost of the ligand itself. These include the lifespan and reusability of the separation media, buffer consumption, process duration, and the potential for process intensification and integration (Qu et al., 2024). Studies indicate that some alternatives, like nanofiber chromatography (Fibro PrismA) utilising Protein A ligands, can offer considerable cost reductions for antibody capture compared to traditional resin columns, especially when considering multiple processing cycles (Figure 13), (Qu et al., 2024). Similarly, magnetic separation with engineered ligands holds the potential to reduce process steps and costs by potentially eliminating the need for clarification in some cases due to the non-porosity of the magnetic nanoparticles preventing clogging (Zimmermann et al., 2024). The economic viability of these alternatives, however, often depends on the specific application, scale of operation, and the ability to ensure their robustness and performance over multiple uses.

**A graph of a manufacturing multiple batch

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Figure 13. A cost analysis comparing MabSelect PrismA and Fibro PrismA at a 2000 L manufacturing scale. (A) Overall cost comparison for a single batch. (B) Cost per gram of monoclonal antibody (mAb) processed over up to 20 harvests, assuming a new membrane device is replaced after each harvest. (C) Cost per gram of mAb when the membrane device is replaced every four harvests (200 cycles (Qu et al., 2024).

**4.c. Continuous and Automated Affinity Chromatography in DSP**

The landscape of downstream processing in biopharmaceutical manufacturing is increasingly witnessing a paradigm shift towards continuous and automated affinity chromatography in downstream processing (DSP). The shift is driven by the need for enhanced efficiency and product quality (Tallvod et al., 2023). Emerging trends in continuous bioprocessing are evident in the exploration of modalities such as periodic counter-current chromatography (PCC**)** (Figure 14). PCC have been developed to support continuous affinity capture, improving resin utilisation and throughput (Narges et al., 2022). High-throughput process development (HTPD) methods using miniature columns and automated screening enable rapid condition optimisation (Fernandes et al., 2024).

Automation plays a crucial role in achieving continuous operation, with the development of automated sample preparation and analysis platforms capable of directly sampling continuous downstream processes. These automated systems can facilitate on-demand analyses and improve process monitoring (Tallvod et al., 2023).

A diagram of a diagram

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Figure 14. Schematic diagram of a periodic counter-current chromatography (PCC). Cycle comprises six distinct steps. The primary loading phases occur in steps 2, 4, and 6, where two columns are actively loaded while a third undergoes elution and regeneration. During these phases, the inlet directs the sample into one column, and any breakthrough from that column is transferred to the next, enhancing resin utilization. A UV detector positioned between the two active columns monitors the breakthrough curve. Steps 1, 3, and 5 serve as intermediate wash phases, ensuring that the wash buffer flows through the fully loaded column and carries any residual product to a fresh column, minimizing product loss. The duration of these wash steps corresponds to the wash phase immediately following the loading phase (Narges et al., 2022).

The adoption of continuous and automated affinity chromatography has a significant impact on process economics and scalability. By reducing manual interventions and streamlining workflows, these approaches can lead to increased throughput and potentially lower costs (Florea et al., 2023). For instance, the reuse of affinity resins like AAVX (Figure 15), for multiple purification cycles without significant loss of efficiency or carry-over contamination demonstrates the potential for cost reduction (Florea et al., 2023).

A graph of different colored bars

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Figure 15. Purification efficiency with repeated AAVX resin use. Vector genomes in lysate, flow-through, and elution (Florea et al., 2023).

Industrial adoption is reflected in the development of versatile automated two-step purification systems for generating preclinical material (Ransdell et al., 2023). The desire for increased diversity and throughput in early drug discovery has driven the deployment of such high-throughput systems leveraging affinity tags (Ransdell et al., 2023). Moreover, the implementation of in-line fibre optical sensors for real-time detection of critical quality attributes like IgG aggregates (Figure 16), signifies a move towards enhanced process control in continuous biomanufacturing (Tran et al., 2024).

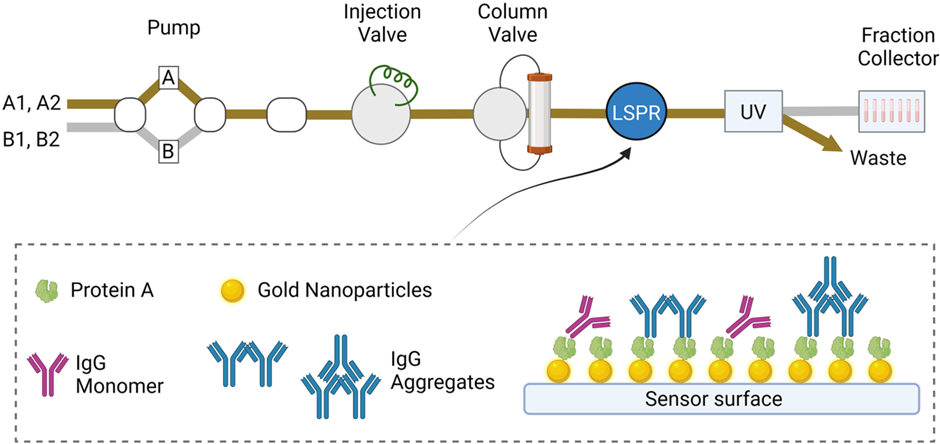


Figure 16. A schematic representation of the in-line fibre optic LSPR sensor integrated with a chromatography system for real-time monitoring of the monoclonal antibody (mAb) capture process. The sensor was tested across three chromatography systems operating at varying scales: ÄKTA pure™ 25, ÄKTA pure™ 150, and ÄKTA pilot™ 600 (Tran et al., 2024).

The coupling of affinity chromatography with other techniques, such as dual column cation exchange is also being explored for potentially higher clearance of impurities (Prabhala and Wood, 2024).

These advancements collectively contribute to more scalable, cost-effective, and efficient DSP workflows in the biopharmaceutical industry.

**5. Conclusion**

This literature review highlights the critical role of affinity chromatography in the downstream processing of monoclonal antibodies due to its inherent high selectivity for the target molecule. Primarily utilising Protein A, affinity chromatography achieves efficient capture and significant impurity removal in a single step. The field is evolving with increasing interest in novel alternative ligands aiming to overcome limitations associated with traditional options. Typically employed as an initial capture step, affinity chromatography is followed by polishing steps to reach required purity levels. Regulatory compliance necessitates adherence to GMP and thorough process validation of these chromatographic steps.

Recent advancements focus on enhancing efficiency and selectivity through novel ligands and resin technologies, including nanofiber-based adsorbents. The integration with analytical techniques like native mass spectrometry and the emergence of continuous bioprocessing and automation represent key trends. Challenges remain in areas like resin cost, scalability, and lifetime, alongside knowledge gaps concerning alternative ligands and resin regeneration.

Based on this review, affinity chromatography is a highly effective technique for mAb purification, particularly in the initial capture phase, providing significant purity and yield. Ongoing innovations are actively addressing existing limitations, further solidifying its importance in mAb production.

For future progress, research should focus on the continued development of novel affinity ligands with enhanced properties, a deeper understanding and reduction of non-specific binding, the improvement of resin longevity and regeneration methods, and the integration with advanced analytical tools. Industrial implementation should prioritise a greater adoption of continuous and automated chromatography systems, comprehensive cost-benefit analyses of new technologies, the implementation of risk-based lifecycle management for chromatography columns, and further exploration of direct capture methods to streamline early purification stages.

In conclusion, affinity chromatography remains an indispensable tool for biomolecule purification, offering exceptional selectivity and the ability to achieve high purity in often a single step. While traditional methods can present throughput limitations, ongoing advancements in continuous chromatography, novel ligand development, and alternative chromatographic formats are actively addressing these challenges The inherent strengths of affinity chromatography make it an indispensable technique across various fields, and continued innovation promises to further enhance its effectiveness in terms of selectivity, purity, and crucially, throughput for a wide range of biopharmaceutical and biotechnological applications.

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