Efficient Purification of Plasmid DNA Using Anion Exchange Chromatography: Comparative Analysis of Different Resin Loadings

Joint Study by Richter Biologics and YMC

Abstract

Plasmid DNA (pDNA) is widely used in biotechnology applications, including gene therapy, DNA vaccination, genome editing, and the production of RNA-based therapeutics. The increasing demand for clinical-grade pDNA requires tight control of critical quality attributes such as the proportion of supercoiled isoforms, residual RNA content, and overall impurity levels. Anion exchange (AEX) chromatography is commonly applied for pDNA purification due to its binding capacity for nucleic acids and its ability to separate pDNA from structurally related impurities such as RNA. Compared to alternative methods, AEX chromatography offers advantages in terms of scalability and process robustness. In this study, the performance of AEX chromatography resins was evaluated with a focus on the influence of loading strategies on yield and RNA removal. The results indicate that optimised loading strategy can improve plasmid purity while maintaining step yields in the range of approximately 65–85%. These findings highlight the relevance of process parameter optimisation for efficient and scalable pDNA purification.

Introduction

pDNA is a key component in many biotechnological and biopharmaceutical applications, including gene and cell therapies as well as nucleic acid-based vaccines. It is also widely used as a template for in vitro transcription processes, for example in mRNA production. These applications require highly pure and well-characterised material. Purification of pDNA from bacterial lysates is challenging due to the presence of structurally similar impurities such as RNA, as well as different plasmid isoforms (supercoiled, open circular, linear). The enrichment of the supercoiled form is particularly relevant, as it is typically associated with higher biological activity.

AEX chromatography is typically employed as a capture step in pDNA purification, enabling the initial isolation and concentration of nucleic acids based on their interaction with positively charged ligands. Differences in charge density and molecular conformation can be exploited to achieve partial resolution between plasmid DNA and RNA. However, due to overlapping elution profiles and the complexity of the feed material, AEX capture is often followed by an additional polishing step, most commonly hydrophobic interaction chromatography (HIC), to further improve purity.

In this context, high loading capacities are of particular importance, as they directly impact process productivity and overall manufacturing efficiency. If sufficient purity can already be achieved during the AEX step, the need for additional downstream processing may be reduced, enabling simplified process designs and, in some cases, single-step purification strategies. Compared to precipitation or density gradient centrifugation, AEX chromatography provides a scalable and robust purification approach. However, chromatographic performance depends strongly on process parameters such as loading, gradient design, and elution pool definition, which influence both yield and impurity clearance. This study investigates the impact of loading conditions on yield and RNA removal during pDNA purification.

Results

To evaluate the impact of loading conditions on process performance and product quality, step yield and residual RNA content were analysed across different loading regimes for two anion exchange resins. For Resin 1, step yield remained relatively stable across the tested loading conditions, showing only minor variation with increasing load. In contrast, residual RNA content decreased with increasing loading, indicating improved impurity removal under these conditions. For MacroSep IEX Q, step yield showed a stronger dependence on loading. While lower yields were observed at intermediate loading, yield increased at higher loading conditions, where the highest recovery was obtained. At the same time, RNA content decreased with increasing load and reached minimal levels under high loading conditions. When comparing both resins, MacroSep IEX Q showed lower residual RNA levels across all tested loading conditions. This difference was most pronounced at high loading, where RNA content was reduced to approximately 0.5% for MacroSep IEX Q, compared to approximately 11% for Resin 1. Under these conditions, MacroSep IEX Q also achieved the highest step yield. Overall, increasing loading improved RNA removal for both resins, while the effect on yield was dependent on the resin.

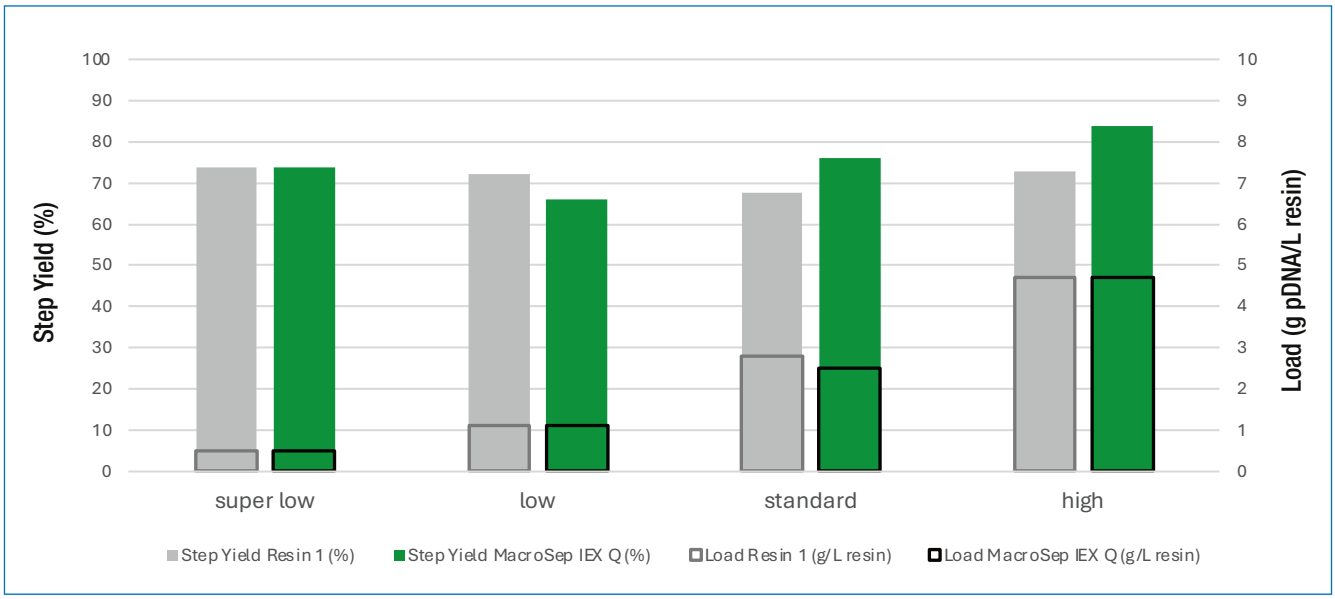

Figure 1: Step yield as a function of loading for two anion exchange chromatography resins (Resin 1 and MacroSep IEX Q). Step yield (%) is shown on the primary y-axis, while the corresponding loading (g pDNA/L resin) is indicated on the secondary y-axis. Increasing load results in stable yields for Resin 1, whereas MacroSep IEX Q shows a more pronounced increase in yield at higher loading conditions.

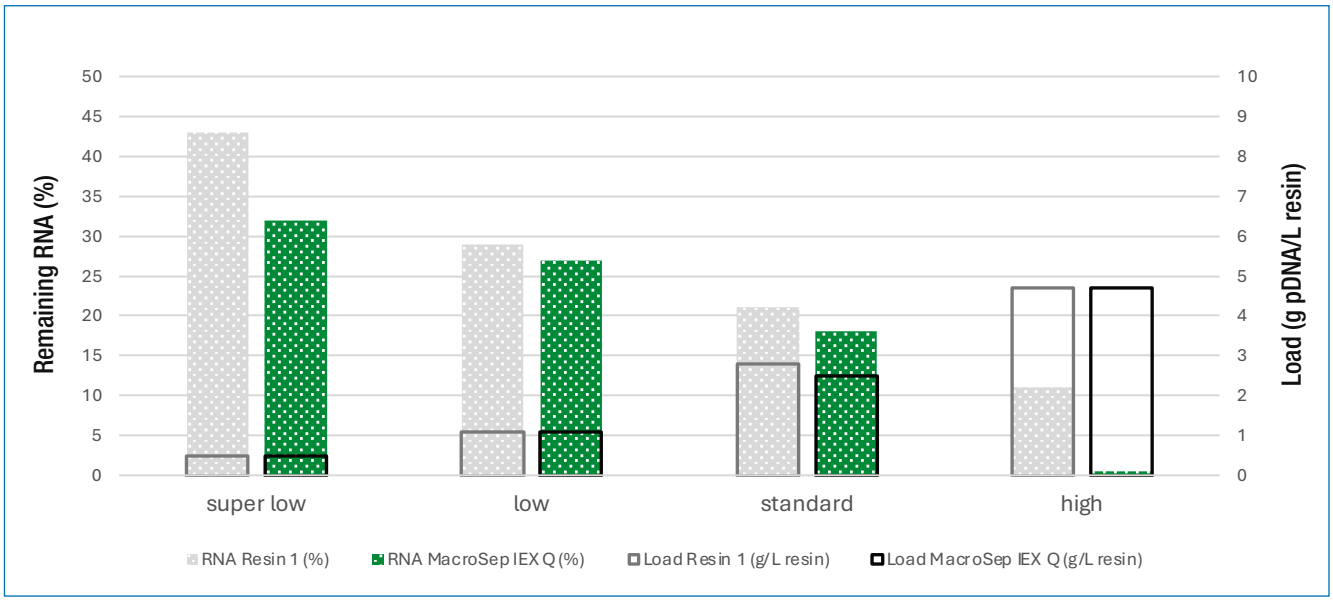

Figure 2: Residual RNA content as a function of loading for two anion exchange chromatography resins (Resin 1 and MacroSep IEX Q). Remaining RNA (%) is shown on the primary y-axis, while the corresponding loading (g pDNA/L resin) is indicated on the secondary y-axis. Increasing load results in a continuous decrease in RNA content for both resins, with MacroSep IEX Q showing consistently lower residual RNA levels and near-complete RNA removal at high loading conditions.

The chromatographic performance of MacroSep IEX Q at high loading was further evaluated by agarose gel electrophoresis of collected fractions. The analysis confirmed a clear separation between flow-through, wash, and elution fractions, with pDNA predominantly detected in the elution fractions. Early fractions showed only minor or no detectable product, indicating efficient binding during sample application. The elution profile revealed a concentrated product peak, consistent with the high step yield observed under these conditions. In addition, the band pattern suggests a high proportion of the supercoiled isoform with only minor contributions from lower molecular weight species. These observations are consistent with the results obtained from the loading study, where higher loading conditions were associated with improved RNA removal and maintained or increased yield for MacroSep IEX Q. The data indicate that increased loading does not negatively affect separation performance under the tested conditions and can be applied without loss of product quality. This behavior is in line with the requirements of capture steps, where high loading is typically applied to maximize throughput.

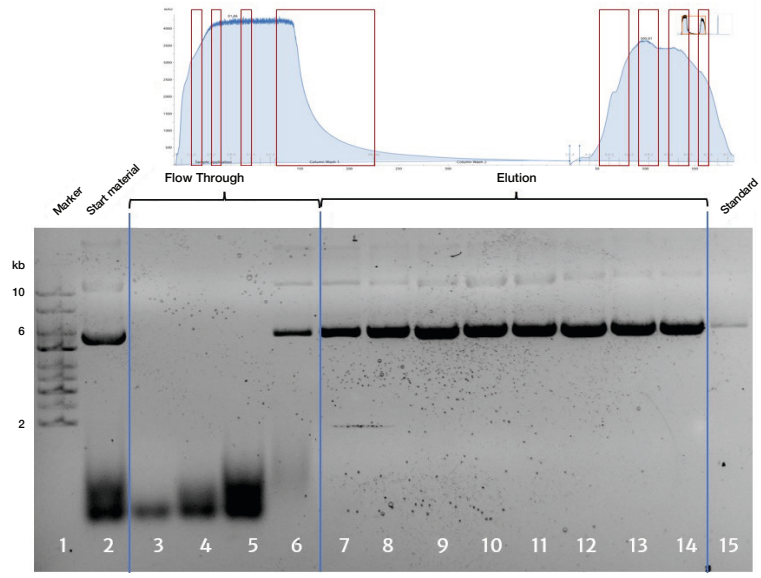

Figure 3: Chromatographic profile and corresponding agarose gel analysis of MacroSep IEX Q AEX purification under high loading conditions (5.2 g pDNA/L resin). The upper panel shows the UV absorbance trace with highlighted fraction collection windows for flow-through and elution. The lower panel presents agarose gel electrophoresis of selected fractions, including starting material, flow-through, wash, and elution fractions. pDNA is predominantly detected in the elution fractions, with a distinct band corresponding to the supercoiled form, while early fractions show minimal product presence, confirming efficient capture and purification.

Discussion

The results indicate that loading conditions influence both step yield and RNA removal, with differences observed between the evaluated resins. For Resin 1, step yield was largely unaffected by loading, suggesting a relatively robust system with respect to recovery. The observed decrease in RNA content with increasing load may indicate improved separation between pDNA and RNA under these conditions. In contrast, MacroSep IEX Q showed a stronger dependence on loading for both yield and purity. The increase in step yield at higher loading may reflect more efficient utilisation of the resin capacity. At the same time, the reduction in RNA content suggests improved separation performance at higher load. These observations may be related to differences in resin structure or ligand accessibility, which could influence the interaction of nucleic acids with the stationary phase and affect selectivity. The improved performance observed for MacroSep IEX Q at higher loading suggests potential advantages for process development, particularly in contexts where high productivity is desired. However, the extent to which these findings can be generalised may depend on additional factors such as feed composition and process conditions. Overall, the results highlight the importance of optimising loading conditions in AEX-based plasmid purification. The relationship between yield and impurity removal appears to be resin-dependent and should be evaluated for each system.

Conclusion

This study shows that loading conditions influence both step yield and RNA removal in anion exchange chromatography-based pDNA purification. While both evaluated resins showed improved RNA clearance with increasing load, their impact on yield differed, indicating resin-specific behavior. MacroSep IEX Q showed improved performance under high loading conditions, combining high step yield with low residual RNA levels in the tested system. These results suggest that high-load operation may provide advantages in terms of productivity and product quality when appropriate resin systems are applied. Further evaluation under process-relevant conditions is required to confirm these findings.