Application of Cation Exchange Chromatography with Linear Gradient Elution for Charge Variants Optimization in mAbdi2 Product

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1. INTRODUCTION

- ✓ Monoclonal antibodies (mAbs) play a significant role in the biopharmaceutical industry.
- ✓ Differences occur in antibodies when producing mAbs during the upstream and downstream process periods. One common form of heterogeneity[1] is the formation of charge variants, which can significantly impact the quality, safety, and efficacy of the final product.
- ✓ The separation of acidic and basic variants becomes a critical bottleneck in downstream process development.

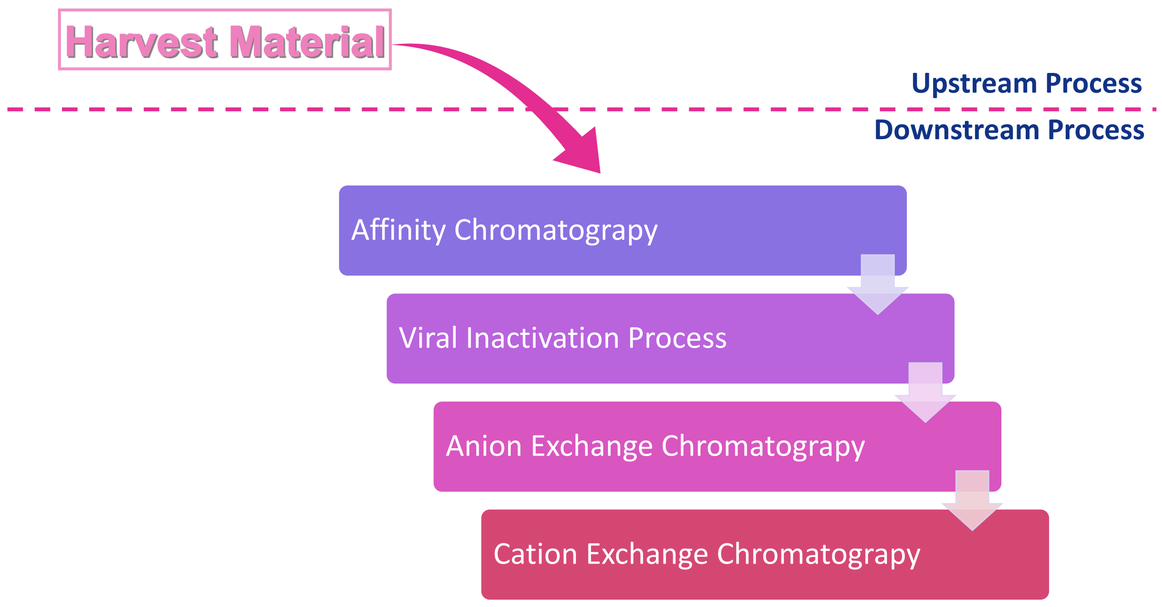
AIM

The purpose of our study is to develop and optimize a feasible Cation Exchange Chromatography step for producing a functional and highly purified mAbdi2 product that is suitable for biopharmaceutical use.

2. CATION EXCHANGE CHROMATOGRAPHY (CEX)

- ✓ Cation Exchange Chromatograpy (CEX), is a powerful tool, to be utilized as an intermediate or a final (polishing) downstream process step for removing product and process-related impurities. [2]
- ✓ CEX resin which has negatively charged ligands, binds positively charged surface parts of mAbs.[3]
- ✓ Various elution types and techniques have been evaluated to optimize CEX method in our study. [4]
- ✓ Bind and elute mode is a common strategy which we also use in our CEX Method. [5]

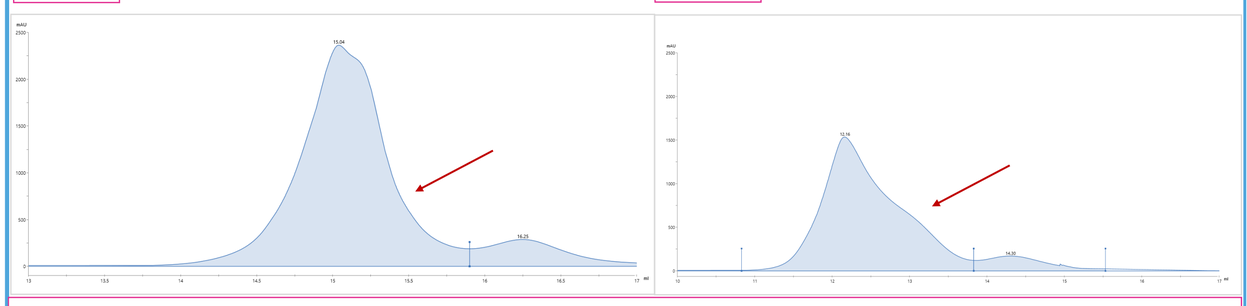
3. PROCESS FLOWCHART



4. CHROMATOGRAPHIC RESULTS

I.Resin Selection

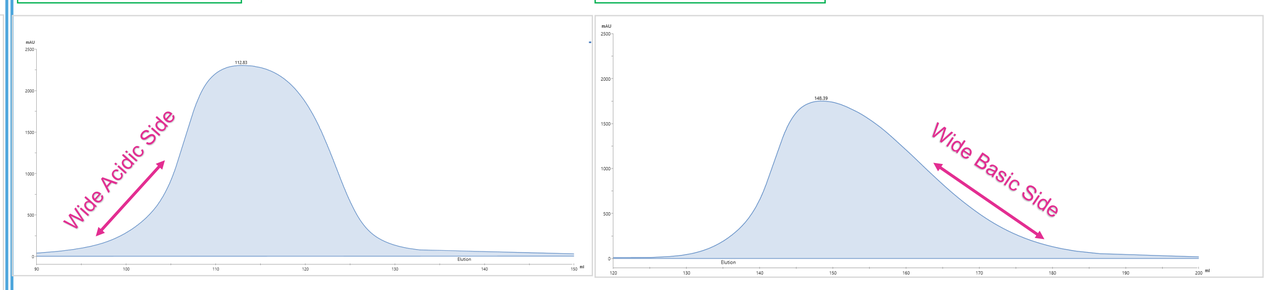
Resin 1 | Resin 2 ✓



As Resin 2 gave better resolution compared to Resin 1 under same conditions, it was selected for further studies.

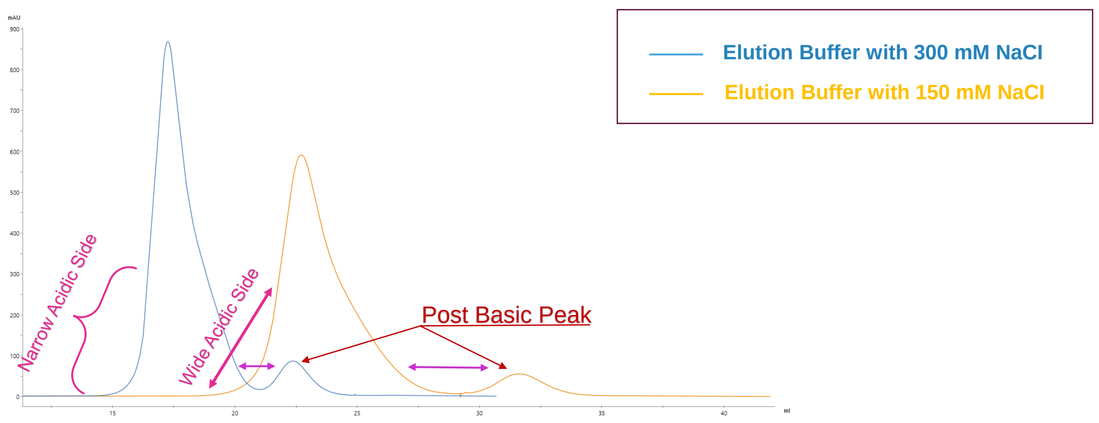
III. Buffer pH Selection

6.0 pH Buffer Sets ✓ | 5.5 pH Buffer Sets



Buffer sets pH was selected as 6.0 for the next studies since this pH value resulted in wider acidic side.

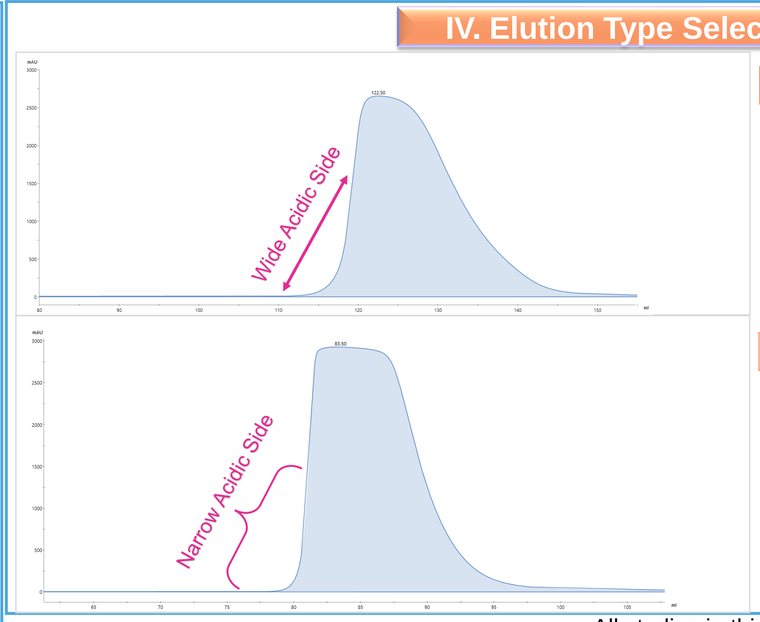
II. Buffer Conductivity Selection



- ✓ **Resolution at acidic side improved when elution buffer conductivity decreased.**
- ✓ **We observed post impurity peak far later at less ionic buffer with same conditions.**

Further studies were performed with less ionic buffer contents and as a result of these, elution buffer with 100 mM NaCl was chosen (data not shown).

IV. Elution Type Selection



Linear Gradient ✓

Step Gradient

In the acidic side of the elution peak better seperation was observed in linear gradient compared to step gradient.

All studies in this poster were performed in salt gradient techique.

5. BIOANALYTICAL RESULTS

1) Amount of acidic variants

Sample ID	Charge Variant Result: Acidic (%Area)	Main (%Area)	Basic (%Area)	Process Recovery Between Steps (%)
mAbdi2 Batch 1 Harvest	20,40	67,00	12,60	89
mAbdi2 Batch 1 AEX-FT	20,19	67,60	12,22	
mAbdi2 Batch 2 Harvest	16,85	65,70	17,45	91
mAbdi2 Batch 2 AEX-FT	16,00	65,30	18,71	
mAbdi2 Batch 3 Harvest	22,35	58,69	18,97	80
mAbdi2 Batch 3 AEX-FT	20,83	59,07	20,11	

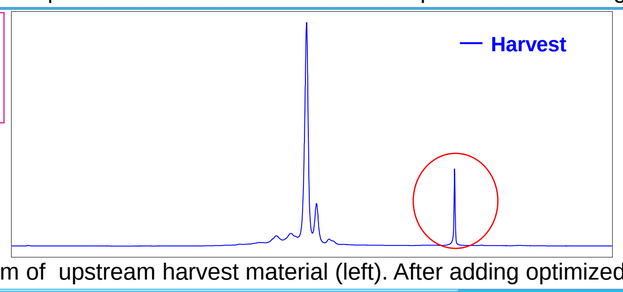
Acidic variants were decreased by 2-5%.

Sample ID	Charge Variant Result: Acidic (%Area)	Main (%Area)	Basic (%Area)	Process Recovery Between Steps (%)
mAbdi2 Batch 4 Harvest	18,80	68,60	12,60	80
mAbdi2 Batch 4 CEX POOL	**14,60**	68,70	16,80	
mAbdi2 Batch 5 Harvest	22,30	71,80	5,90	72
mAbdi2 Batch 5 CEX-POOL	**16,00**	74,70	9,30	
mAbdi2 Batch 6 Harvest	19,40	73,60	7,10	87
mAbdi2 Batch 6 CEX POOL	**16,74**	71,45	11,81	

Mabdi2 batches from 1 to 3 were purified without CEX step. Mabdi2 batches from 4 to 6 were purified after adding optimized CEX step into downstream process.

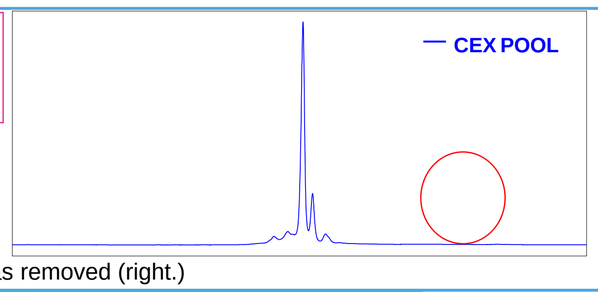
2) Post-peak observation

Charge Variant Chromatogram of Mabdi2 Harvest by CEX-HPLC



Undesired basic post-peak was removed.

Charge Variant Chromatogram of Mabdi2 Cex Pool by CEX-HPLC



Post impurity peak was observed in the chromatogram of upstream harvest material (left). After adding optimized CEX step into downstream process, this impurity peak was removed (right.)

6. CONCLUSION

- ✓ In this study, we have added in-house developed and optimized CEX step into mAbdi2 purification flow for separating acidic and basic variants.
- ✓ With this method, we achieved a reduction in acidic variant by 2-5%, and the removal of undesired post basic variant.
- ✓ As a result, we produced highly purified product with better-quality attributes.

7. REFERENCES

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2. Development Strategy for a Cation Exchange (CEX) Chromatography Step in a Monoclonal Antibody (mAb) Process, Technical Note. (2021) Millipore, Merck.
3. Biopharmaceutical Processing. (2018)
4. Ion Exchange Chromatography, Principles and Methods. (2021) Cytiva
5. Masuda et al. Cation Exchange Chromatography Performed in Overloaded Mode is Effective in Removing Viruses During the Manufacturing of Monoclonal Antibodies. (2019)

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