TOSOH

Efficient mAb Polishing with Low-Salt HIC in FT Mode

Your Challenge

- Traditional HIC struggles with high salt and poor mAb aggregate removal thereby increasing costs.
- Bind-elute mode fails to efficiently remove mAb aggregates thereby limiting productivity

Our Solution

TOYOPEARL® Phenyl FT-750F resin

- Large pore size, high hydrophobicity & high capacity

What was done?

- Process development for a mAb polishing process & comparison with other HIC resins in FT mode

What was the result?

- TOYOPEARL Phenyl FT-750F resin showed high purity & high recovery at 0.15 mol/L NaCl and load levels of 55 to 65 g/L.

TOYOPEARL Phenyl FT-750F resin enables high mAb purity and recovery in FT mode, with low salt use, high loading capacity, and improved productivity over traditional HIC.

Your Benefit

Enhances mAb purity and recovery while reducing salt use and costs.

mAb Polishing

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS.
TOUCHING LIVES.

https://separations.us.tosohbioscience.com/Process_Media/id-8935/TOYOPEARL_Phenyl_FT-750F

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Application Note

Improved mAb polishing efficiency using low-salt hydrophobic interaction chromatography in flow-through mode

Introduction

Monoclonal antibodies (mAbs) are crucial in treating various diseases, including cancers and autoimmune disorders. Therefore, optimizing their purification is paramount to ensuring their efficacy and safety. The biopharmaceutical industry continuously seeks methods to enhance monoclonal antibody purification processes, striving for higher efficiency, cost-effectiveness, and superior product quality. One of the most critical elements in mAb polishing is the removal of antibody aggregates, as these impurities can cause immunological side effects. Due to their structural similarity to the monomers, aggregates are difficult to remove.

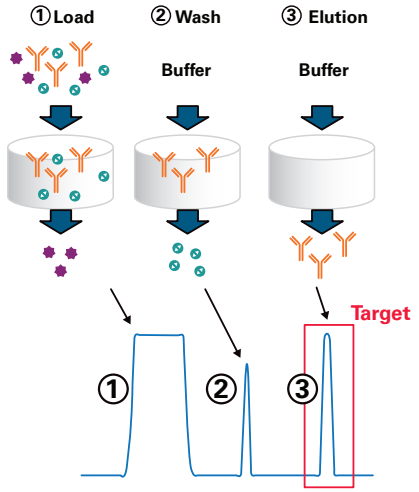
Hydrophobic Interaction Chromatography (HIC) in flow-through (FT) mode has emerged as a promising technique for the polishing step of mAb purification. This study focuses on the use of TOYOPEARL® Phenyl FT-750F resin, which shows high hydrophobicity, allowing the polishing step to be set up as an FT process. FT processes reduce processing time, require fewer consumables such as salts and water, and generate less waste, which simplifies handling and makes the process more economical and efficient. Furthermore, the approach enables high process quality in terms of aggregate removal and recovery under low salt conditions.

Flow-through mode in HIC

Hydrophobic Interaction Chromatography (HIC) is employed to separate mixtures based on differences in hydrophobicity in aqueous solutions and is frequently utilized as a secondary polishing step in monoclonal antibody (mAb) downstream processes. HIC is primarily suitable for the separation of aggregates or steric isomers from the main target molecule. Standard bind and elute HIC processes involve high salt buffers during equilibration and sample loading, followed by a linear reverse salt gradient which results in the elution of bound components according to their respective hydrophobicity.

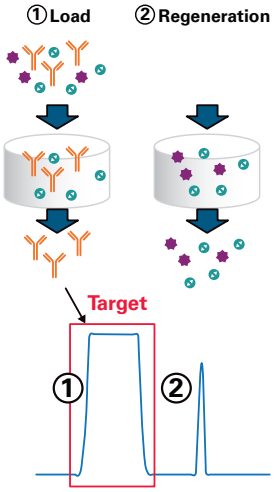
One challenge associated with HIC is the significant amount of salt required for the initial binding of the mixture to the resin. This can pose solubility issues for certain proteins and result in elevated operating costs due to the expense of the salt used. Furthermore, desalting after the process is often required, and the use of large quantities of salt may lead to accelerated corrosion of process equipment.

Figure 1. Process concept: bind & elute chromatography



Converting the process to flow-through (FT) mode addresses some limitations of the bind/elute process. In FT mode, only the impurities are adsorbed by the resin through careful adjustment of ionic strength of the load material. The target product is obtained directly in the loading phase, resulting in optimized process durations and increased productivity. Additionally, the amount of salt required for the process is reduced, as only highly hydrophobic impurities will be bound.

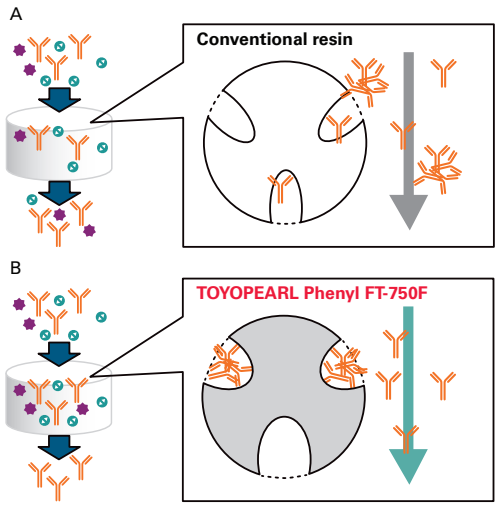
Figure 2. Process concept: flow-through chromatography



While FT processes can significantly increase process productivity, it is important to carefully design parameters such as salt concentration, process pH, and load level. For example, a high salt concentration during loading may cause some of the target mAb to bind to the resin, reducing recovery. On the other hand, if the salt concentration is lower than the optimum, certain hydrophobic impurities might not bind and could flow-through with the target molecule, reducing the final product's purity. Optimal conditions within the loading phase are necessary to recover a highly pure product at a high yield.

TOYOPEARL Phenyl FT-750F resin was designed specifically for FT processing of mAbs, featuring a large pore size of >100 nm, ideally suited for binding of large impurities such as mAb aggregates.

Figure 3. A: Pore size of conventional HIC resins. B: Pore size of Phenyl FT-750F, designed for mAb FT processing



Additionally, it exhibits high hydrophobicity, and thus can employ lower salt concentrations during the loading phase while still binding impurities.

Demonstration of process development for a FT mAb polishing process on TOYOPEARL Phenyl FT-750F resin

Experimental Conditions

In order to find optimum load conditions for a FT mAb polishing process on TOYOPEARL Phenyl FT-750F resin, a high throughput screening (HTS) was performed.

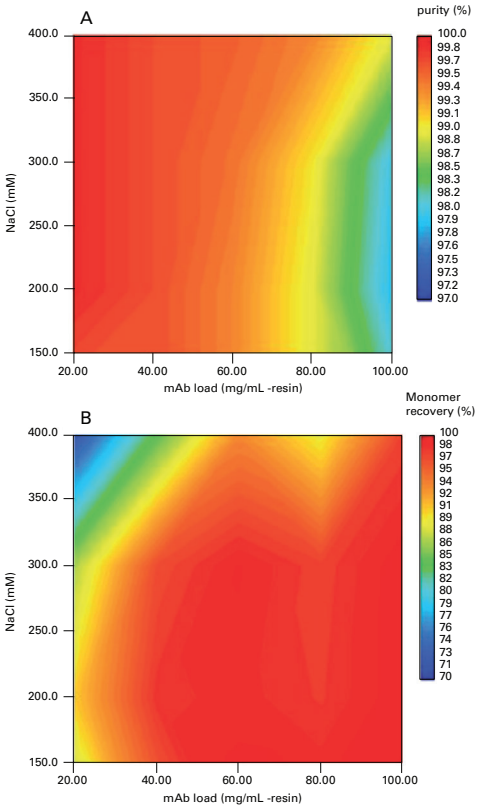
Table 1. Experimental conditions: optimization of load conditions for FT processing of mAbs

System	TECAN Freedom EVO®
Column volume	200 µL
Resin	TOYOPEARL Phenyl FT-750F
Sample	10 mg/mL mAb containing 10% aggregates
Load amount	**20, 40, 60, 80, 100 mg-mAb/mL-resin**
Residence time	2 min
Flow-through buffer	20 mmol/L phosphate buffer (pH 7.2) **+ 0.15, 0.2, 0.3, 0.4 mol/L sodium chloride**

Results and Discussion

When evaluating the HTS screening, both purification purity and recovery were investigated. The results are shown below:

Figure 4. A: Monomer purity in FT fraction at different load amounts and salt concentrations, B: Monomer recovery in FT fraction at different load amounts and salt concentrations



As can be seen in *Figure 4A*, monomer concentration in the flow-though is over 97.5% throughout the investigated conditions. The highest monomer purity is achieved at low load levels and high salt concentration. Monomer purity in the flow-through is not significantly affected by the salt concentration in the loading buffer; at the lowest level tested, 150 mmol/L NaCl, monomer purity was still > 97.8 %. mAb load levels had a larger effect on monomer purity and the effect is pronounced more at low salt concentrations compared to high salt concentrations. This can be explained by impurities breaking through at higher load levels, especially at low salt concentration.

Figure 4B reveals that recovery was found to be highest at low salt concentration and high load levels. At low salt concentration the mAb monomers are least retained, leading to high recovery. An increase in salt concentration leads to adsorption of some of the mAb monomers, lowering recovery. At high load levels, a displacement of potentially bound mAb monomers by stronger adsorbing impurities is to be expected, leading to a higher monomer recovery at high load levels.

Following these results, working at 0.15 mol/L NaCl and load levels of 55 to 65 g/L is recommended in order to maintain high recovery while also ensuring high purity.

Comparison of different HIC resins in FT mode

Experimental Conditions

Performance of TOYOPEARL Phenyl FT-750F resin was evaluated in comparison with commercial resins A (butyl), B (high phenyl) and C (high benzyl). Parameters for the evaluation are listed in *Table 2* below:

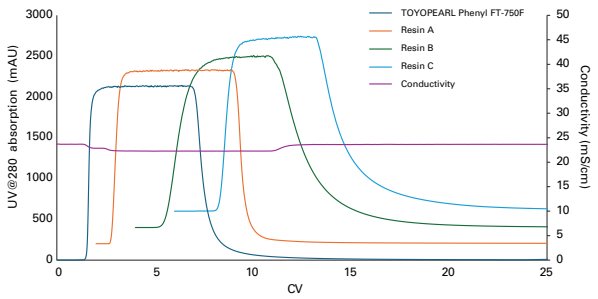
Table 2. Experimental conditions: comparison of multiple HIC resins for FT processing of mAbs

Resins	TOYOPEARL Phenyl FT-750F, Resin A, Resin B, Resin C
Column dimension	4.6 mm ID × 10 cm L CV = 1.66 mL
Sample	10 mg/mL mAb containing 5% aggregates
Load level	60 mg/mL of mAb concentration: 10 mg/mL, 6 CV
Flow rate	0.8 mL/min (Residence time : 2 min)
Buffer	50 mmol/L phosphate buffer(pH 6.8) +150 mmol/L sodium sulfate (22 mS/cm)
Detection	UV @ 280 nm
Flow-through characterization	analytical size exclusion chromatography (TSKgel® G3000SWXL)

Experimental Conditions

An identical process was run on all resins included in this study. The chromatograms are displayed below:

Figure 5. Comparison of TOYOPEARL Phenyl FT-750F and commercial resins for FT processing of mAbs



The flow-through volume was measured, and analytical SEC was used to assess remaining aggregate content. The results are shown in *Table 3* below:

Table 3. Results

	Load	TOYOPEARL Phenyl FT-750 F	Resin A (Butyl)	Resin B (Phenyl)	Resin C (Benzyl)
mAb Aggregate	5.4 %	0.3 %	3.3 %	0.5 %	0.3 %
mAb Monomer	94.6 %	99.7 %	96.7 %	99.5 %	99.7 %
Recovery	-	99.3 %	98.7 %	75.5 %	82.2 %
Volume (CV)	6.0	6.0	6.6	10.8	10.8

The process using resin A resulted in high recovery of 98.7%, but a low monomer purity of 96.7%. This might be due to a low hydrophobicity of the resin, leading to poor binding of mAb aggregates. Using resins B and C resulted in highly pure flow-through pools, but significantly lowered recovery. Additionally, the collected volume containing mAb monomers was significantly higher than the loading volume, which results in an almost 2-fold dilution.

Using TOYOPEARL Phenyl FT-750F resin gave the best results in this study, resulting in a highly pure flow-through fraction with monomer content of 99.7% at a high recovery of 99.3%. Further, the flow-through volume was identical to the load volume, meaning no dilution occurred.

Conclusions

TOYOPEARL Phenyl FT-750F resin was designed specifically for FT processing of monoclonal antibodies. The large pore size allows for high binding capacity for large impurities such as mAb aggregates, while the high hydrophobicity enables low salt concentrations in the HIC FT process. The study presented demonstrates the performance of TOYOPEARL Phenyl FT-750F resin, resulting in high purity mAb flow-through with high recovery. TOYOPEARL Phenyl FT-750F resin addresses several traditional HIC challenges. First, it avoids high salt concentrations in the process and avoids exposing the molecule of interest to high salt conditions. Second, FT mode allows much higher levels of mAb to be loaded onto the column compared to bind and elute mode. This in turn leads to an increase in process productivity and thus overall process costs will tend to be lower.

Featured Products

Part #	Description
0023553	TOYOPEARL Phenyl FT-750F, 25 mL
0023554	TOYOPEARL Phenyl FT-750F, 100 mL
0023555	TOYOPEARL Phenyl FT-750F, 1 L
0023556	TOYOPEARL Phenyl FT-750F, 5 L
0023557	TOYOPEARL Phenyl FT-750F, 50 L
0023556	TSKgel G3000SWXL, 7.8 mmID × 30 cm
0045299	SkillPak 5 Phenyl FT-750F, 5mL col.
0045558	SkillPak 50 Phenyl FT-750F, 50 mL col.
0045559	SkillPak 200 Phenyl FT-750F, 200 mL col.
0045075	RoboColumn Phenyl FT-750F 200 µL × 8
0045076	RoboColumn Phenyl FT-750F 600 µL × 8
0045514	Resin Seeker Phenyl FT-750F