

Comparison of three tentacular strong cation exchange resins that have the same base bead

Introduction

Three tentacular strong cation exchange (CEX) resins, Eshmuno® CP-FT resin, Eshmuno® CPX resin and Eshmuno® CPS resin, share a common 50 µm particle size base bead and have sulfisobutyl ligand groups. However, the resins differ significantly in their ligand density and the structure of their tentacular surface chemistry that were optimized for different applications.

Eshmuno® CP-FT resin has low ligand density that was optimized for the removal of mAb aggregates in flow-through mode using frontal chromatography. Frontal chromatography allows the resin to be loaded to 500-1000 g/L thus significantly reducing the volume of resin and buffer required relative to bind/elute processes. It is loaded at a lower pH and conductivity where the mAb and aggregates both bind strongly to the resin. During loading aggregates displace the bound monomer until the column is completely filled with aggregates.

Eshmuno® CPX resin has an intermediate ligand density that was optimized for the separation of mAb aggregates in the bind/elute mode. The resin provides a higher dynamic binding capacity for mAbs and an excellent selectivity for the separation of mAb aggregates during the higher conductivity elution step.

Eshmuno® CPS resin has a high ligand density that was optimized for the direct capture of recombinant proteins from clarified cell culture. The resin provides high dynamic binding capacities at higher concentrations of salt precluding the need to dilute the feed stream before the CEX capture step. Eshmuno® CPS resin does not have hydrophobic ligands allowing operating conditions to be determined without the need for an extensive DOE study as is often required for salt tolerant mixed mode CEX resins.

Objective

The objective of our investigation was to directly compare the binding properties of these three CEX resins. We hoped that the results could then be instructive for the rational application of the resins beyond their original design. To this end we determined the static binding capacities of the three resins for a monoclonal antibody as the pH and conductivity were varied. We also measured the elution conductivity from the resins for two model proteins. These investigations were designed to demonstrate how the tentacular surface chemistry of the three resins influences their binding properties.

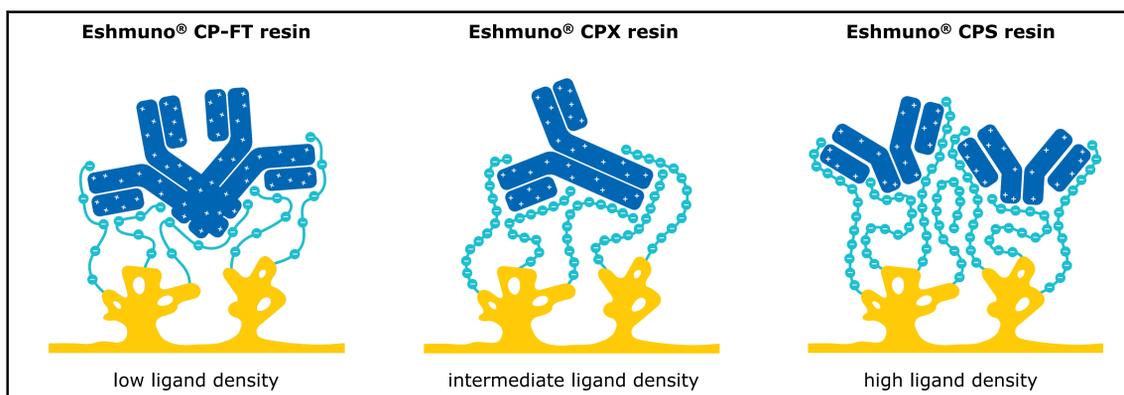
Experimental Plans

1. mAb static binding capacity as a function of pH and conductivity. A clarified cell culture of mAb05 was purified by Protein A chromatography and then dialyzed into 30 different acetate solutions. The solutions had all possible combinations of pH at 4.0, 4.5, 5.0, 5.5, or 6.0 and conductivities at 4, 6, 8, 10, 12, or 14 mS/cm. The solutions were allowed to contact the three resins and the reduction in the concentrations of the resulting solution were determined by measuring the UV absorbance at 280 nm.

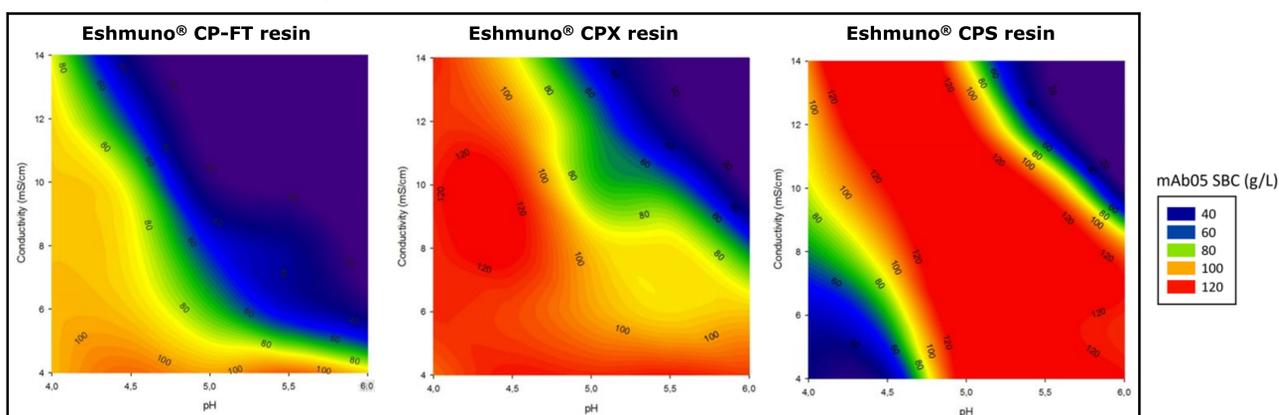
2. Elution conductivities of two model proteins. A solution containing lysozyme (3.5 g/L) and α-chymotrypsinogen A (5.0 g/L) in 50 mM sodium acetate at pH 5 was loaded onto a packed column (0.5 cm I.D., 20 cm bed height, 3.9 mL) of the CEX resin. The proteins were eluted from the column with a gradient elution of 50 mM sodium acetate with 1 M sodium chloride at pH 5 over 20 CV. The final percentage of the high salt buffer in the gradient elution was different for each CEX resin. It was selected as the minimum percentage that allowed full elution of the lysozyme peak.

Resin	Gradient over 20 CV
Eshmuno® CP-FT	0% → 40%
Eshmuno® CPX	0% → 75%
Eshmuno® CPS	0% → 90%

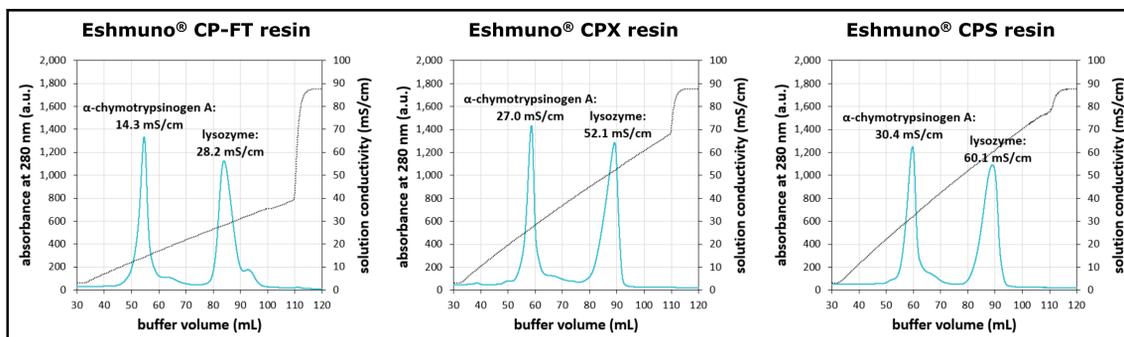
Ligand density and structure



1. mAb Static Binding Capacity as Function of pH and Conductivity



2. Elution conductivities of two model proteins



Results and Discussion

mAb static binding capacity as a function of solution pH and conductivity. The pattern of the mAb static binding capacities for the three resins was consistent with their relative ligand densities. The low ligand density Eshmuno® CP-FT resin was most sensitive to solution pH and conductivity. Eshmuno® CPX resin with an intermediate ligand density maintained higher mAb static binding capacity over a wider pH and conductivity range. The high ligand density Eshmuno® CPS resin had a mAb static binding capacity that was most tolerant of solution pH and conductivity. We also note that Eshmuno® CPS resin had a unusual low capacity area at the lowest pH and solution conductivity.

Elution conductivities of two model proteins. Both proteins eluted from the low ligand density Eshmuno® CP-FT resin, the intermediate ligand density Eshmuno® CPX resin, and the high ligand density Eshmuno® CPS resin at progressively higher solution conductivities.

Resin	α-chymotrypsinogen A	lysozyme
Eshmuno® CP-FT	14.3 mS/cm	28.2 mS/cm
Eshmuno® CPX	27.0 mS/cm	52.1 mS/cm
Eshmuno® CPS	30.4 mS/cm	60.1 mS/cm

Summary

It was found that the binding properties of three Eshmuno® CEX resins were consistent with their relative ligand densities. The mAb static binding capacity of the low ligand density Eshmuno® CP-FT resin was most sensitive to solution pH and conductivity, while the high ligand density Eshmuno® CPS resin was most tolerant. Two model protein were found to elute from the low ligand density Eshmuno® CP-FT resin at the lowest elution conductivities while they required the highest conductivities to elute from the high ligand density Eshmuno® CPS resin. We hope that the results of our investigation into the protein binding properties of these three CEX resins - which share a common base bead - will be instructive for the rational application of the resins beyond their original design.