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The Effect of Conductivity on Dynamic Binding Capacity on CEX Resins

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Abstract

In an effort to improve the antibody manufacturing platform, newer Cation Exchange Resins were evaluated. Dynamic binding studies were conducted using 2 different monoclonal antibodies at a stable pH and varying conductivities across nine resins to determine the differences in binding capacity. DBC Studies were performed at a pH of 5 and conductivities of 5, 10 and 15 mS/cm for the nine cation exchange resins with breakthrough criteria set at 10%. While most of the resins provided excellent binding capacity (>50 g/L), the impact of conductivity on the dynamic binding capacity was shown to be very molecule dependent and varied significantly with the resin type.

Introduction

A Typical Purification Manufacturing process consists of a product capture step as well as a fine purification step. For many manufacturers the fine purification process consists of a two step polishing column operation, each designed to tackle different impurities. In utilizing this approach, manufacturing is faced with the costs associated with running two columns, i.e. time, buffer, man power. By developing an approach for a second generation process which employs a single column as a polishing column, one can cut down on all costs associated with the two column process.

As a first step towards realizing the one column fine purification process, dynamic binding capacity studies were performed in an attempt to evaluate the resins. During these studies it was soon observed that the CEX resins behaved quite differently with respect to conductivity and molecule variations.

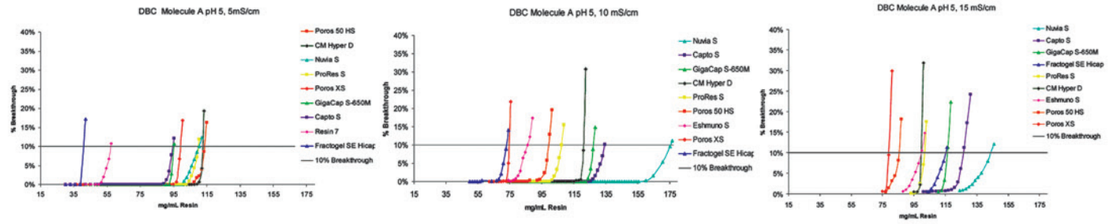
Materials and Methods

To perform the dynamic binding studies, nine CEX resins were acquired. 10 cm columns were packed using water and qualified by HETP before use.

Two monoclonal antibodies were used as load materials, Molecule A and Molecule B. To make the field as level as possible among the resins, one CEX buffer system was chosen, which consisted of a Sodium Phosphate, Sodium Chloride process. High salt regeneration was employed as well as NaOH cleaning and finally storage in 20% ETOH.

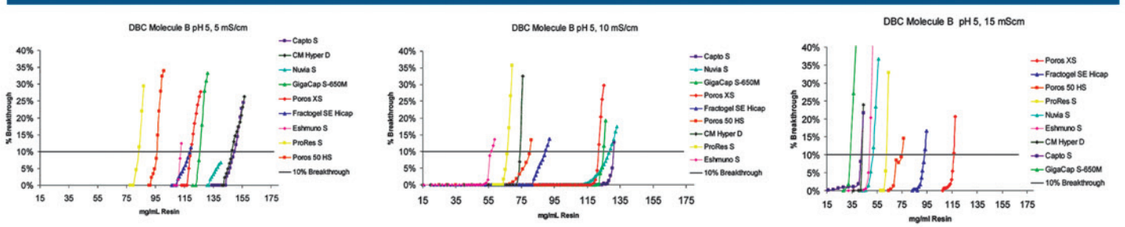
Each column was loaded to reach 10% breakthrough using an AKTA explorer. Fractions were collected through out the load and measured on a UV spectrophotomer to confirm loading and breakthrough.

Molecule A Dynamic Binding Studies



At a pH of 5, Conductivity 5 mS/cm, the breakthrough curves had a large grouping over 85mg/ml resin, with only a couple reins that preformed under 65 mg/ml resin. When the conductivity was changed to 10 mS/cm, the range spread out with the majority of resins lying between 60 mg/ml and 100 mg /ml. For the last condition of pH 5 and conductivity 15 mS/cm, all resins rose to above 60mg/ml resin, averaging at greater then 100 mg/ml resin. The groupings for Molecule A showed that the performance as based on capacity increased with the increasing conductivity.

Molecule B Dynamic Binding Studies



At a pH of 5, Conductivity 5 mS/cm, the breakthrough curves had a large range, with the majority lying between 110mg/ml resin and 130mg/ml resin. When the conductivity was changed to 10 mS/cm, two groups were seen to emerge, those below 90mg/ml resin and those above 100mg/ml resin. For the last condition of pH 5 and conductivity 15 mS/cm, most resins dropped off to below 60mg/ml resin. The groupings for Molecule B showed that the performance as based on capacity generally decreased with the increasing conductivity.

Summary of the Dynamic Binding Studies

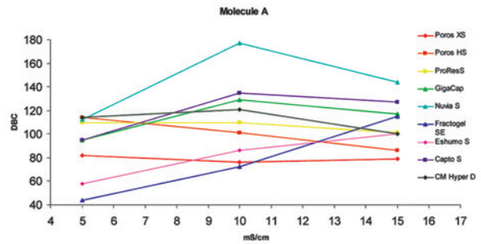
Molecule A 5 mS/cm	Molecule A 10 mS/cm	Molecule A 15 mS/cm	Molecule B 5 mS/cm	Molecule B 10 mS/cm	Molecule B 15 mS/cm
Poros 50 HS	Nuvia S	Nuvia S	Capto S	Capto S	Poros XS
CM Hyper D	Capto S	Capto S	CM Hyper D	Nuvia S	Poros 50 HS
Nuvia S	GigaCap S-650M	Giga Cap	Nuvia S	GigaCap S-650M	Fractogel SE Hicap
ProResS	CM Hyper D	Fractogel	GigaCap S-650M	Poros XS	ProResS
GigaCap S-650M	ProResS	ProResS	Poros XS	Fractogel SE Hicap	Nuvia S
Capto S	Poros 50 HS	Eshmuno S	Fractogel SE Hicap	Poros 50 HS	Eshmuno S
Poros XS	Eshmuno S S	CM Hyper D	Eshmuno S S	CM Hyper D	Capto S
Eshmuno S S	Poros XS	Poros 50 HS	Poros 50 HS	ProResS	CM Hyper D
Fractogel SE Hicap	Fractogel SE Hicap	Poros XS	ProResS	Eshmuno S S	GigaCap S-650M

In a ranking from best to worst across all resins and molecules, no clear winner was seen when based solely on dynamic binging capacity. When comparing resins within individual molecule groups, certain resins were seen consistently in the top performing half. Further studies assessing other attributes will have to be performed in order to narrow the resins down.

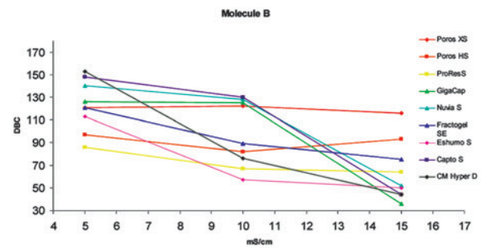
Conclusion

In performing Cation Exchange Resins evaluations, dynamic binding studies were conducted using 2 different monoclonal antibodies at a pH of 5 and varying conductivities across nine resins to determine the differences in binding capacity. Using the same load and buffer conditions, and just varying the conductivities, differences emerged between Molecule A and Molecule B.

Molecule A was quite conductivity tolerant and even increased DBC at a conductivity of 10 mS/cm for most of the resins evaluated.



Molecule B was found to have a lower conductivity tolerance and had better DBC at 5 mS/cm.



The comparisons of the two molecules shows that there is a big difference between how the molecules behave with different resins and at different conductivities. Selecting a single resin based on DBC as a first pass criteria, will have to be expanded to measuring product quality attributes as well.

Therefore designing a platform process for these two molecules will take further studies to aid in determining optimal ranges that will function across the differences discovered as no one resin performed consistently for both molecules under the same conductivity.

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