Screening and optimization of the loading conditions on Capto S

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Introduction

Increasing expression levels of recombinant proteins and the need for improved productivity and overall process economy puts extra demand on the next generation of chromatography media for the bio-pharmaceutical industry. A technique for significantly improving the capacity and mass transfer properties of ion exchange media is modification of the porous base matrix with a surface extender such as dextran. This has recently been used in the development of a new cation exchange medium, Capto™ S, and gives a substantial increase in binding capacity compared to the corresponding media without dextran.

Capto S is a chromatography medium based on the novel high flow agarose platform. The medium combines high rigidity with high dynamic binding capacity (DBC) and fast mass transfer to allow faster purification, more flexible process design and maximum cost efficiency [1]. Previously launched products on this platform include Capto Q, Capto MMC and the MabSelect™ family. Capto S is a strong cation exchanger designed for capture and intermediate purification of recombinant proteins and as a second step in MAb purification.

To use the full potential of Capto S, we show how to thoroughly screen the loading parameters, conductivity and pH. In addition, recommended screening strategies are presented where a sequential approach and Design of Experiments are used and discussed.

Design of Capto S

The design of Capto S is shown schematically in Figure 1. The high flow agarose base matrix is significantly more rigid than the base matrix of the Sepharose™ platform. The dextran surface extender increases the DBC by increasing the equilibrium capacity and the mass transfer rate. The ligand, sulfoethyl, is a strong cation exchange ligand. The high flow agarose base matrix gives Capto S excellent pressure-flow properties as shown in Figure 2.

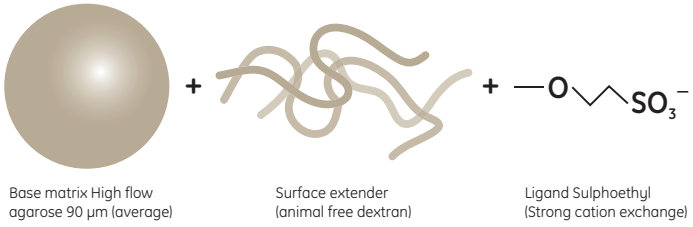


Fig 1. Capto S is based on a high flow agarose base matrix modified with dextran and strong cation exchange groups for optimal rigidity, capacity and mass transfer.

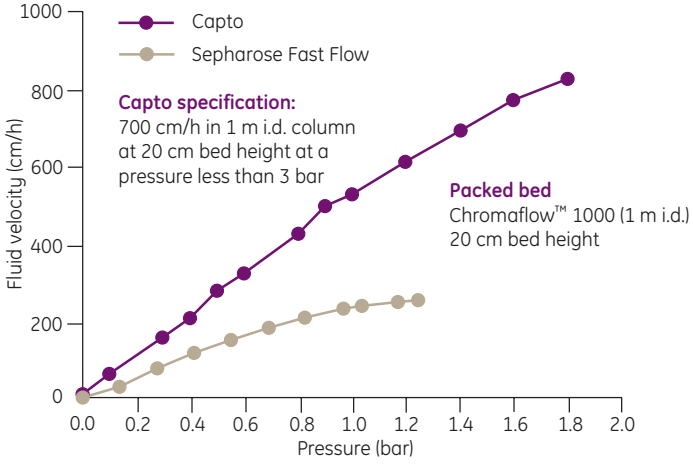


Fig 2. Operating fluid velocity vs. pressure drop in 1 m ID column for Capto and Sepharose Fast Flow packed to 20 cm bed height.

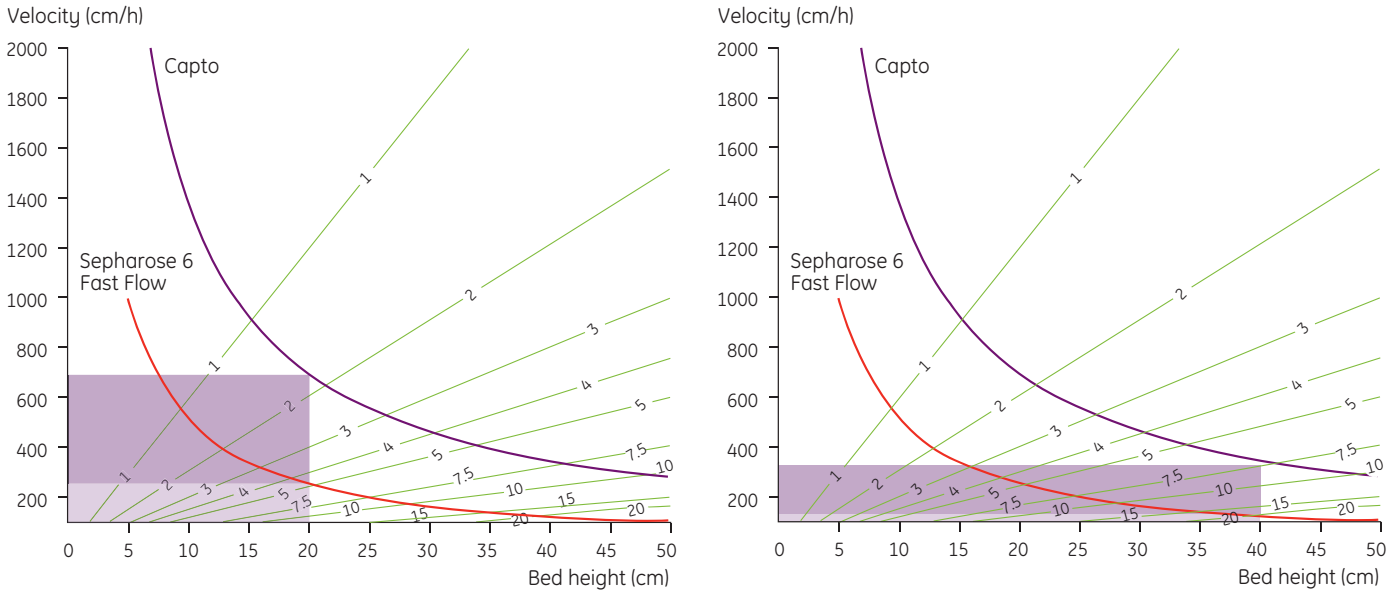


Fig 3. Operating fluid velocity vs. bed height for Capto and Sepharose Fast Flow. Blue line: Pressure limits for Capto S base matrix (set by the column and system pressure rating). Red line: Pressure limits for Sepharose 6 Fast Flow base matrix (related to compressibility of the base matrix). Green lines: Residence times obtained at the given velocities and bed heights. Boxes: Much larger window of operation for Capto than for Sepharose 6 Fast Flow is illustrated for bed heights of 20 cm and 40 cm.

One of the main benefits of Capto S is that it increases flexibility of design for large scale processes. As illustrated by the shaded boxes in Figure 3, the "window of operation" for Capto media is much larger than for Sepharose media. Although bead size and pore size of the Capto and Sepharose media are similar, Capto S has wider working ranges with respect to fluid velocities, bed heights and sample viscosities. At a 20 cm bed height in a 1 m diameter column Capto media can be run with a linear fluid velocity of up to 700 cm/h while the Sepharose media are restricted to approximately 250 cm/h at this scale. Even at a bed height of 40 cm, the Capto media allow for fluid velocities above 300 cm/h.

The dextran surface extender creates a three-dimensional lattice in which proteins can bind, thus increasing the equilibrium capacity. In addition, dextran also enhances mass transfer in ion exchange chromatography. Figure 4 shows that DBC on Capto S with a dextran extender is greater than on SP Sepharose Fast Flow, which lacks a surface extender.

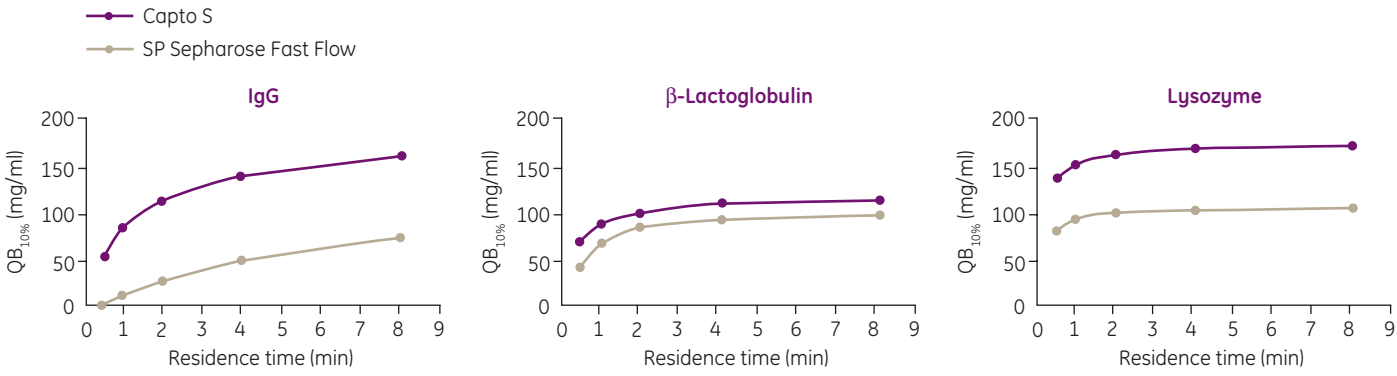


Fig 4. The dynamic binding capacity at 10% breakthrough ($QB_{10\%}$) for IgG, β-lactoglobulin and lysozyme vs. residence time.

Screening and optimization of loading conditions

Effect of pH and conductivity on dynamic capacity

The highest DBC's on ion exchangers are typically obtained at low conductivities and as the conductivity of a sample increases the DBC decreases (Fig 5, red curve). With Capto S, this traditional type of behavior should also be expected for most proteins but at a higher capacity than SP Sepharose Fast Flow (Fig 5, blue curve). However, some proteins purified with Capto S demonstrate non-traditional behavior (Fig 5, green curve). The non-traditional behavior is characterized by a DBC peak as the conductivity is increased. This means that it is necessary to screen across a range of conductivities to find the interval that can deliver the full potential of the Capto S with respect to DBC.

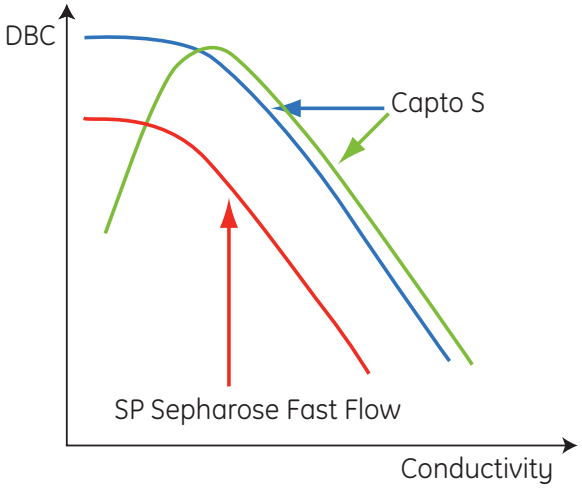


Fig 5. DBC vs. conductivity curves for Capto S and SP Sepharose Fast Flow. Illustration of traditional (red and blue curves for SP Sepharose Fast Flow and Capto S, respectively) and non-traditional behavior (green for Capto S).

In addition to conductivity, solution pH during loading will also affect the results, since the protein net charge is dependent on the pH as illustrated by titration curve in (Fig 6A). At low pH, the protein will have a large positive net charge, while at higher pH values (closer to the isoelectric point of the protein) the positive net charge will be smaller. For a protein behaving in the traditional manner, the salt tolerance of the DBC will be greater at low pH than at high pH (Fig 6B). For a protein behaving in the non-traditional manner, at low pH (higher protein net charge) the optimal DBC is obtained at higher conductivity than at high pH (lower protein net charge) (Fig 6C).

α-chymotrypsin and conalbumin are examples of two proteins that behave in the traditional and non-traditional manner, respectively (Fig 7).

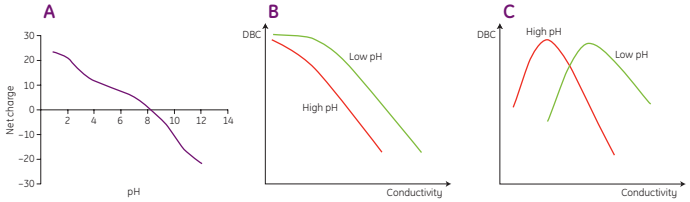


Fig 6. Hypothetical protein titration curve (A) and the effect of pH on the shape of the DBC vs. conductivity curves for traditional (B) and non-traditional behavior (C).

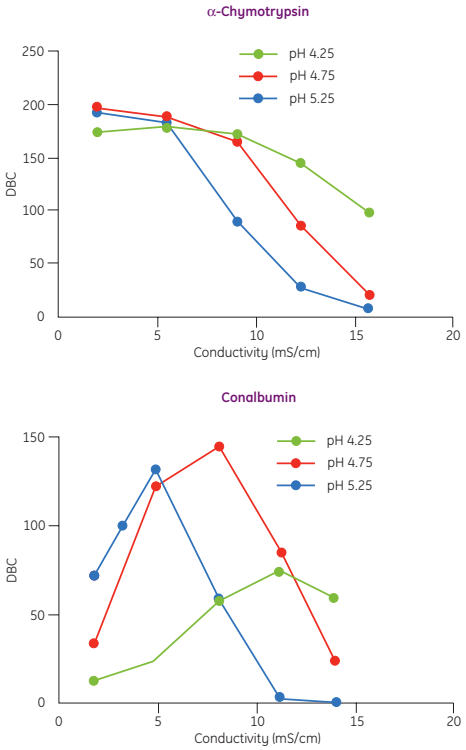


Fig. 7. DBC vs. conductivity curves for α-chymotrypsin and conalbumin at different pH values. α-chymotrypsin behaves in the traditional manner where DBC decreases with increased conductivity, while for conalbumin the DBC goes through a maximum as conductivity is increased.

Screening strategy

The importance of doing a thorough screening in conductivity is illustrated in Fig 8. If only one point at low conductivity is tested as in Figure 8A, it is possible to conclude that the capacity is low. By adding another point at high conductivity the conclusion can be that traditional behavior is observed and that the capacity is even lower (Fig 8B). It is only when thorough screening of conductivity is done (Fig 8C), that non-traditional behavior is revealed and high capacity is obtained at intermediate conductivity.

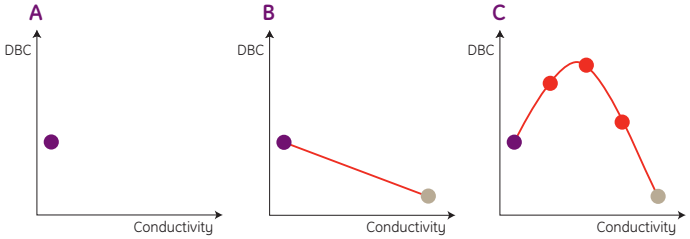


Fig 8. Illustration of how the maximum DBC can be missed without thorough screening of conductivity.

Similarly, pH should also be screened across a range of conductivities since it also affects DBC (Fig 9). Since net protein charge decreases with increased pH and the lower the net protein charge is the lower conductivity is needed to reach the optimum dynamic capacity. The maximum dynamic capacity may not be the same at different pH values which further illustrates the necessity of screening both conductivity and pH.

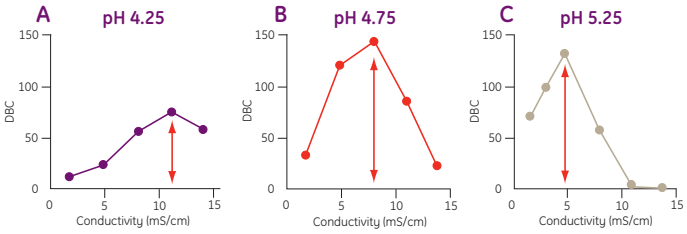


Fig 9. DBC of conalbumin vs. conductivity at three different pH values. The maximum DBC is obtained at different conductivities at different pH values. The maximum DBC also varies with pH.

The sequential approach

When evaluating Capto S for binding capacity it is preferable to use a rapid screening strategy. By using a sequential approach it is possible to reveal the behavior type (traditional vs. non-traditional) of a target protein and find what DBC's can be expected, in three to six experiments. The sequential approach has three steps:

1. Select a pH 1-2 pH units below the isoelectric point of the target protein.
2. Measure the DBC at three conductivities, for example, 3, 6 and 9 mS/cm.
3. Repeat the conductivity screening for another pH.

In the first step a relevant pH is selected. In the second step DBC is determined at three conductivities, starting with low conductivity and then increasing the conductivity in intervals that are small enough to prevent missing trends between the different conductivities. In this example, 3, 6 and 9 mS/cm are examined, but other conductivities can be used. By plotting the DBC vs. conductivity for the three runs, different scenarios can be obtained (Fig 10). These scenarios can be divided into two groups. The scenarios in Figure 10A show that the target protein behaves in a traditional manner, (highest DBC is found at low conductivity), and

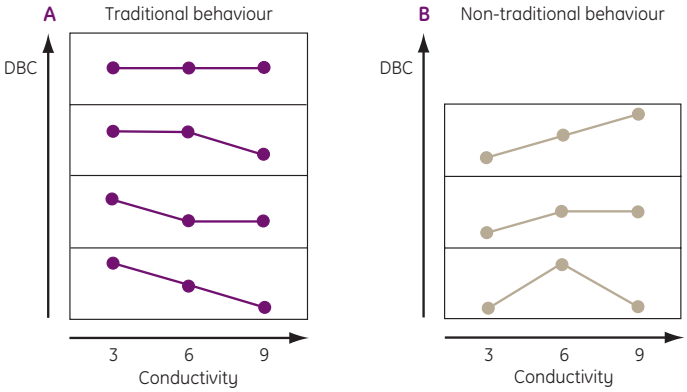


Fig 10. Different scenarios that can be obtained after the DBC at three conductivities have been screened. The scenarios in panel A show that the protein behaves in the traditional manner and the scenarios in panel B show that the protein behaves in the non-traditional manner.

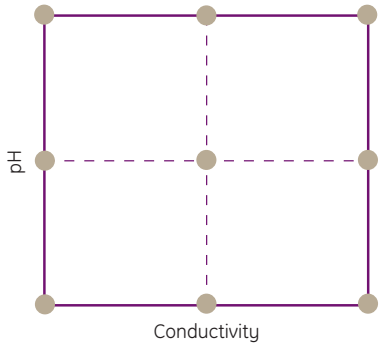
the scenarios in the Fig 10B show that the target protein behaves in the non-traditional manner (the highest capacity is not found at low conductivity). The three experiments revealed the behavior type where maximum DBC can be found. If the DBC is less than expected or desired, it is recommended that additional pH values are screened as in the example of Conalbumin (Fig 9).

Note that the desired conductivities for screening can be achieved by dilution or by adding salt; however, dilution will also change the feed concentration. Since feed concentration itself can affect DBC the results may not only reflect the effect of conductivity, but also the effect of feed concentration. The type of salt used can also affect optimum conductivity. Therefore, it is wise to keep the feed concentration as constant as possible and avoid mixing different types of salts during conductivity screening. Finally, use relevant flow rates and keep temperatures constant during screening unless they are experimental parameters.

The Design of Experiments approach

The use of Design of Experiments (DoE) has become a standard tool in process design. This approach is an alternative to the sequential approach or can compliment experiments done with the sequential approach. With DoE, all relevant factors are varied simultaneously over a set of planned experiments. The results are then interpreted by a mathematical model that can be used for prediction, screening, optimization and robustness testing.

A



B

Exp no	Run order	Cond	pH	DBC
1	4	5	4.5	96
2	10	15	4.5	102
3	3	5	5.5	137
4	11	15	5.5	4
5	7	5	5.0	139
6	1	15	5.0	54
7	5	10	4.5	119
8	9	10	5.5	84
9	2	10	5.0	121
10	6	10	5.0	137
11	8	10	5.0	127

C

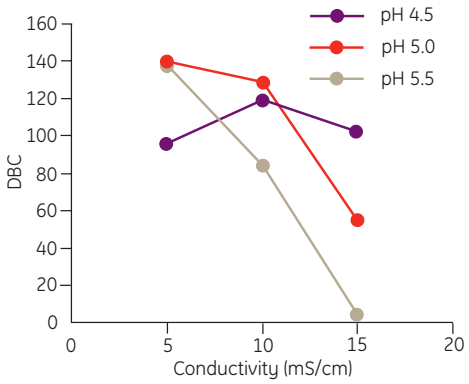
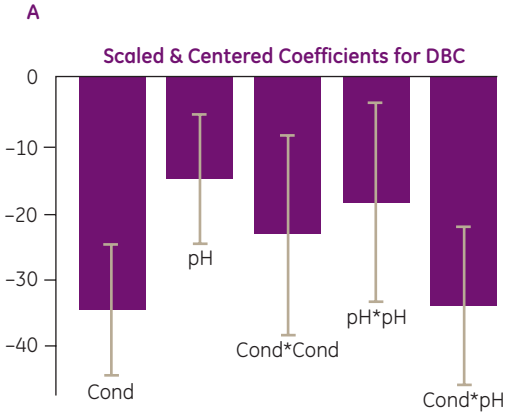


Fig 11. (A) Face centered composite design used for experimental setup. **(B)** Data table. **(C)** The DBC vs. conductivity plots obtained at the three pH values. The residence time was 4 min and the feed concentration was kept constant at 5 mg/ml.

A



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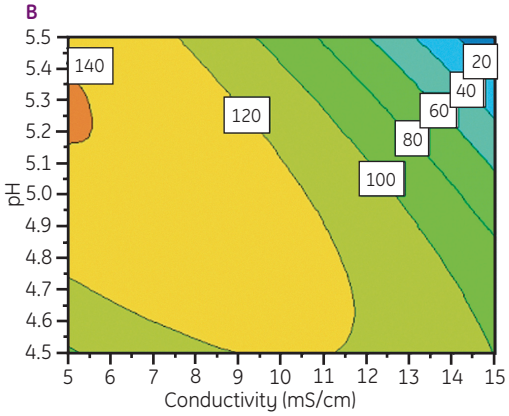

Fig. 12 The coefficient plot that shows the terms that significantly affect the response **(A)**. The response surface plot that shows the predicted DBC as a function of pH and conductivity **(B)**.

The following example shows how DoE can be used to evaluate the effect of pH and conductivity on the DBC of a monoclonal antibody on Capto S (see also Ref 2). A face centered composite design with pH and conductivity was setup using MODDE 7 software (Umetrics) (Fig 11A). In total, 11 experiments were conducted with three center points (Fig 11B), The raw data plot in Fig 11C showed that, depending on pH, a combination of traditional (pH 5.0, 5.5) and non-traditional (pH 4.5) behavior was observed. Note that the lowest conductivity tested was 5 mS/cm and if lower conductivity had been used, non-traditional behavior may have also been revealed at the higher pH values (5.0, 5.5).

The data analysis in MODDE generated a well-explained model (R^2=0.98) with good stability according to cross validation (Q^2=0.83). The coefficient plot (Fig 12A) showed that both pH and conductivity were significant factors. Curvature effects were found in both pH and conductivity and an interaction effect between pH and conductivity was also found. The coefficient plot therefore showed that:

- DBC is reduced by high Conductivity (Cond) with curvature (Cond*Cond)
- DBC is reduced by high pH with curvature (pH*pH)
- The effect of Conductivity depends on pH (Cond *pH)
  - Traditional behavior at high pH (low net charge of the target protein)
  - Non-traditional behavior at low pH (high net charge of the target protein)

A response surface plot (Fig. 12B) that graphically illustrates the results of the coefficient plot shows that the highest DBC is predicted at low conductivity and at approximately pH 5.25.

Why the Non-traditional behavior?

Low conductivity

At constant pH and low conductivity, lower DBC is caused by a slow mass transfer inside the ion exchange particle. The slow mass transfer can be attributed, yet not restricted, to the following effects:

- Steric hindrance
  - The protein molecules binding strongly at the pore entrance may reduce the pore accessibility for subsequent protein molecules entering the pore. The effect should thus be more pronounced with larger proteins.
  - The surface extender, dextran, is less flexible than at higher conductivities, which decreases the accessible pore volume.
- Charge repulsion
  - Positively charged proteins binding strongly at the pore entrance may present their positive charges to the subsequent protein molecules approaching the pore. This situation can be summarized as a local charge reversal, resulting in electrostatic repulsion that reduces the flux of protein into the pore network. At low conductivity this effect will be more pronounced because of the thicker electric double layer around protein molecules.

These effects will result in the protein adsorption occurring only in the regions close to the bead surface as shown in Figure 13 causing very slow mass transfer into the interior of ion exchange bead.

Optimal conductivity

Increased conductivity will result in a lower equilibrium capacity but a faster mass transfer. At the optimum conductivity the effect of increased mass transfer on the DBC is larger than the effect of decreased equilibrium capacity (Fig 14).

At higher conductivity the equilibrium is shifted to the unbound state, which facilitates rapid transfer of the the protein molecules into the pores. Consequently, the overall steric exclusion and charge repulsion effects are weaker. In addition, the mobility of the dextran chains increases with the increase in ionic strength of the solution. All these effects contribute to a fast mass transfer of unbound molecules throughout the porous network resulting in a fast saturation of all available binding sites as shown in Figure 14. Overall the mass of adsorbed protein in a single bead is higher than that at low conductivities (optimum in DBC values).

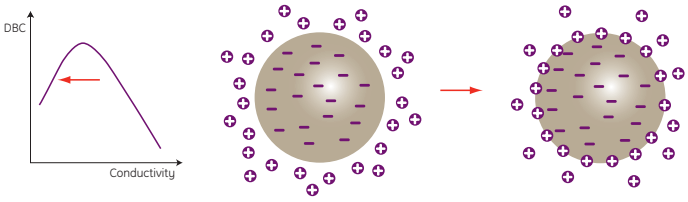

Fig 13. Binding at low conductivity. As a result of slow mass transfer, protein adsorption will occur only at the surface of the particles even after a prolonged incubation time.

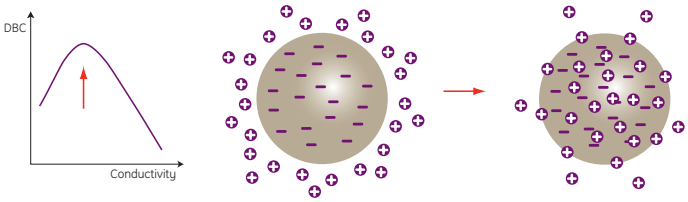

Fig 14. Binding at optimal conductivity. Rapid protein transfer into the pores resulting in higher DBC. The same arbitrary incubation time as in Fig. 13.

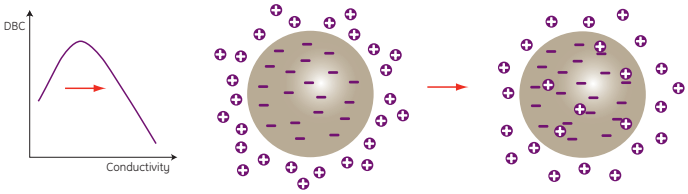

Fig 15. Binding at high conductivity. Protein binding to the medium decreases with increased conductivity due to loss of equilibrium capacity. The same arbitrary incubation time is used as in Fig. 13.

High conductivity

At high conductivity the effect of decreased equilibrium capacity predominates. The mass transfer is fast but the levels of DBC obtained are governed by the total available capacity (Fig. 15). As the conductivity is increased the conditions approach “elution mode”.

Different pH values

Considering the electrostatic nature of the effects described above and that at different pH values the net charge of the protein will vary, it is clear that pH of the solution may have a significant impact on DBC and on the optimum value of conductivity at which the DBC shows maxima. At low pH values (higher positive charge of the proteins) the effects related to protein charge such as strength of interaction, protein-protein repulsion will be more pronounced. The ionic strength of solutions necessary to bring the point to where any electrostatic process will occur at certain magnitude will be higher. This is why at the lower pH optimum conductivity increases in comparison to optimum conductivities measured at higher pH.

Summary

Capto S is a strong cation exchanger for capture and intermediate purification of recombinant proteins and as a second step in MAb purification. The medium is designed for high capacity and high-throughput operations that allows improved process economy. The high capacity and fast mass transfer characteristics are attributed to the use of a dextran surface extender and the rigidity of the high-flow agarose base matrix.

Since it is difficult to predict whether a protein will behave in a traditional or a non-traditional manner, it is important to examine both pH and conductivity during screening and optimization of loading conditions on Capto S. Different approaches to screening are suggested to exploit the full potential of Capto S. The sequential approach will, with a few experiments, reveal traditional or non-traditional behavior and indicate at which conductivity and pH the best capacity is obtained. The DoE approach gives similar information but with more detail. By using DoE it is easier to keep track of many factors and responses to reveal interaction and quadratic terms that are difficult to extract with a sequential approach.

References

1. Application note 28-4078-15: High-productivity capture of α–chymotrypsin on Capto S cation exchanger.
2. Application note 28-4078-17: Capto S cation exchanger for post-Protein A purification of monoclonal antibodies.

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