TECHNICAL BRIEF

Determination of Dynamic Binding Capacity for ProSep®-vA Media

Introduction

The dynamic binding capacity of a chromatography media is the amount of target protein the media will bind under actual flow conditions before significant breakthrough of unbound protein occurs. As this parameter reflects the impact of mass transfer limitations that may occur as flow rate is increased, it is much more useful in predicting real process performance than a simple determination of saturated or static capacity.

General Methodology

The dynamic binding capacity can be determined using either partially purified monoclonal antibody (MAb), or feedstock.

If partially purified MAb is used, the breakthrough curve can be seen directly by monitoring UV absorbance. In most cases, MAb, which has already been purified using protein A affinity chromatography media, can be used for this with the following notes:

1. The pH of the purified MAb must be adjusted to reflect that of the crude cell culture supernatant.
2. The partially purified MAb will most probably be at a higher concentration than that in the cell culture supernatant. In such cases, it is advisable to dilute the MAb to better reflect the feedstock concentration. To some extent, dynamic binding capacity will be affected by the concentration of the MAb, especially at low titers of <100 mg/liter. More dilute feedstock will require longer loading times. If only limited time is available, it is possible to perform a dynamic binding capacity using higher concentration MAb, but this should be noted when presenting the final data.
3. Some antibodies can experience partial denaturation on passage though protein A media. For these antibodies, a second passage through the same media may result in a lower binding capacity.
4. Using MAb, which has already been partially purified on a protein A column, means that the eluted MAb from the dynamic binding capacity experiment cannot be used to give a reliable estimate of protein A leakage.
5. Using eluted antibody from dynamic binding experiments where the column is effectively overloaded for the determination of purity will impact recovery and may impact purity.

If clarified cell culture supernatant is used, the intrinsic UV absorbance of the feedstock will mask breakthrough of the MAb. In this case, further analysis of the breakthrough fractions will be required to determine the breakthrough characteristics.

MILLIPORE

Method

Pack a suitable column (e.g. Omnifit 0.66 cm diameter column or Vantage VL11 1.1 cm diameter column) to a bed height of at least 10 cm (3.4 mL or 10 mL respectively) with ProSep-vA Ultra media or ProSep-vA High Capacity media. Ensure the column is well packed using the column tapping method (as detailed in the ProSep-vA Operating Instructions).

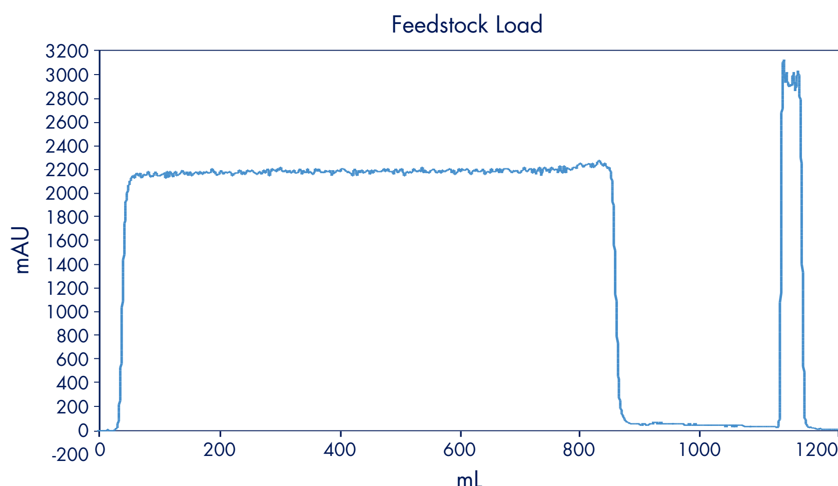
a. Use clarified cell culture supernatant freshly passed through a 0.22 or 0.1 µm filter, or

b. Prepare a solution of partially purified MAb in a loading buffer at a concentration and pH that reflects the feedstock conditions (in most cases the equilibration buffer is appropriate). This feedstock should also be freshly filtered as above.

1. Equilibrate the ProSep-vA column with equilibration buffer.
2. Isolate the column and pass MAb feedstock through UV detector, to determine the UV absorbance for 100% breakthrough.
3. Flush the system lines and put protein A column on line.
4. Start loading the MAb feedstock at the desired flow rate or residence time.
5. Monitor the UV absorbance of the column eluate.

Figure 1.

Chromatograph with Clarified Monoclonal Antibody Cell Culture Supernatant



For an initial screen, it is recommended that a residence time of three minutes be used. Residence time is the time a nominal non-binding molecule would take to pass through the packed bed.

When using clarified cell culture supernatant, it is normal to observe a significant amount of UV absorbing material passing through the column after approximately one column volume.

When using partially purified MAb, a UV plateau may also be observed. This represents some impurities, but mainly non-binding or weakly binding antibodies or aggregates resulting from the previous passage through a protein A column. The level of this plateau will depend upon the individual MAb being used.

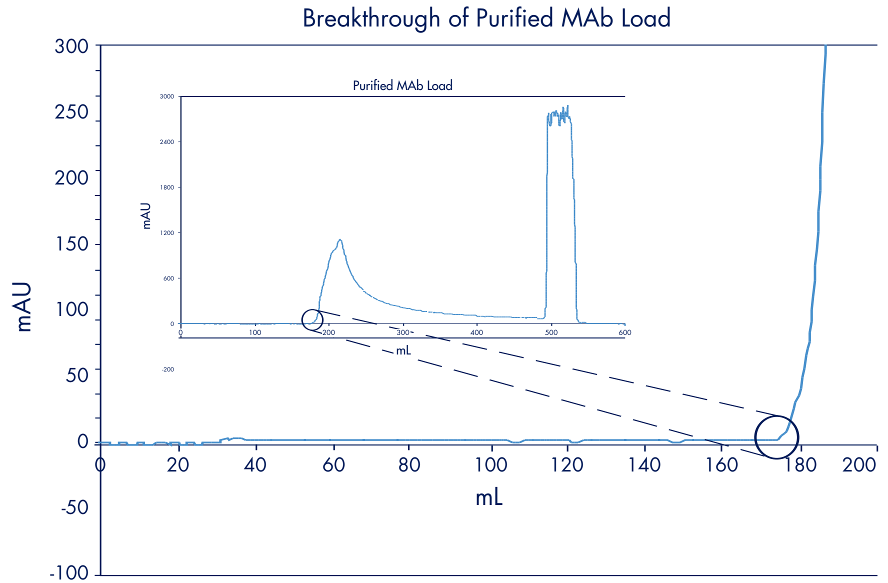
Table 1.

Suggested Fraction Sizes for a Range of Antibody Feedstock Titers

MAb Titer (g/Liter)	3 mL Column (Omnifit)	10 mL Column (Vantage VL11)
0.10	22 mL	75 mL
0.25	9 mL	30 mL
0.50	4.5 mL	15 mL
0.75	3 mL	10 mL
1.0	2.3 mL	7.5 mL
1.5	1.5 mL	5 mL
2.0	1.1 mL	3.8 mL
3.0	0.8 mL	2.5 mL
4.0	0.6 mL	1.9 mL

Figure 2.

Breakthrough Curve with Partially Purified Monoclonal Antibody



Using Clarified Cell Culture Supernatant

For cell culture supernatant, the high amount of UV absorbing material will mask MAb breakthrough. It is therefore necessary to collect fractions throughout the loading phase. The amount of MAb loaded should be calculated so that it will exceed the anticipated capacity of the media, in the case of ProSep-vA Ultra media, this should be loaded to a capacity of 45 – 50 mg MAb/mL media.

The collected fractions can then be analyzed for the presence of MAb. The early fractions should not contain MAb. Initially, fractions at infrequent intervals (e.g. every 10th fraction) can be tested to find the approximate volume in which breakthrough occurs. Once this region has been identified, all the surrounding fractions can be tested. The smaller the fraction volume, the more fractions there will be and the greater the resolution of the breakthrough curve. Typical fraction sizes are given in Table 1.

Secondary analysis of the fractions can be conducted using protein A chromatography (pH adjustment to neutral required). SDS-PAGE or ELISA can be run as a semi-quantitative step to screen many fractions to quickly identify the fractions of interest.

Once the titer of MAb in each of the relevant fractions has been determined, the data can be plotted to give a breakthrough curve similar to that shown in Figure 2.

Using Partially Purified Monoclonal Antibody

Continue to load MAb until breakthrough of IgG occurs. Breakthrough will be indicated by an increase in the UV absorbance of the eluate (Figure 2). Loading is stopped once the UV absorbance of the eluting flow stream reaches approximately 10% of the UV absorbance of the pure feedstock (10% breakthrough). From the chromatogram, the total mass of MAb, which was bound before breakthrough occurred, can be calculated by multiplying the concentration of MAb in the feedstock by the total volume loaded up to the desired breakthrough point (usually 5 or 10%) less the column void volume (usually this can be determined by inspection of the chromatogram – a slight shift in the UV signal occurs after approximately 0.7 x the bed volume). For dilute feedstocks, this value can usually be ignored.

Post Load Wash and Elution

Following each load, any remaining unbound material is washed off the column with 7 – 10 column volumes of equilibration buffer. The addition of 0.5 M NaCl to the wash buffer may reduce any non-specific binding. The bound antibody can then be eluted by a step change to low pH buffer (typical buffers are 100 mM Citrate, 100 mM glycine, 100 mM acetate pH 3 – 4). After each run, any remaining tightly bound antibodies can be removed and the column regenerated using 5 column volumes of a pH 1.5 solution of phosphoric acid (150 mM) or HCl (10 mM) before re-equilibration in loading buffer.

Breakthrough Formula

$$\text{Mass bound} = C_o \times (V_L - V_o)$$

Where:

V_L = volume loaded up to the breakthrough point

V_o = void volume of the column

C_o = concentration of MAb in the feedstock.

Note: this is an approximation assuming complete capture of MAb prior to breakthrough.

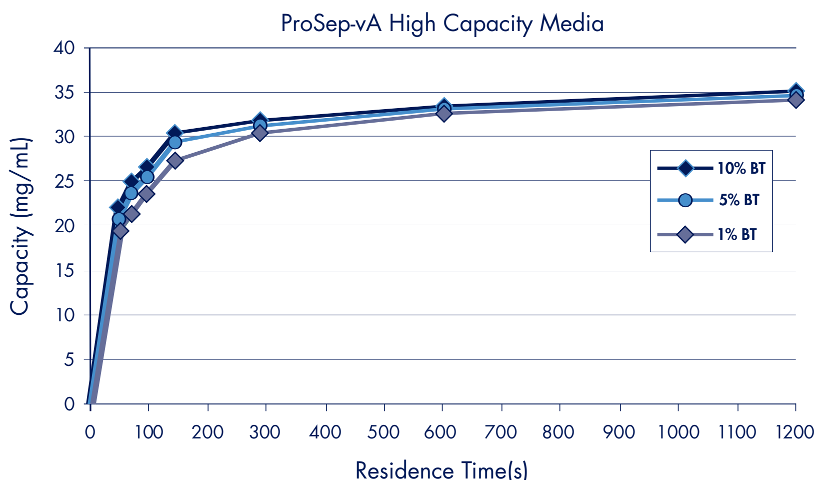
Effect of Residence Time

If time is limited, the above experiment should be performed at the recommended operating flow rate for the media. In the case of ProSep-vA Ultra media, this should correspond to a residence time of 3 minutes, or 1.14 mL/min in a 0.66 x 10 cm bed. However, for a better understanding of the process characteristics, the dynamic binding capacity over a range of residence times can be considered. The efficient mass transfer characteristics of ProSep-vA media allow faster flow rates to be used, decreasing cycle time and increasing throughput.

In general, dynamic capacity will decrease as the residence time decreases, however the rate at which the dynamic (i.e. usable) capacity decreases can vary greatly from media to media. An ideal media would have efficient mass transfer properties across the range of flow rates being used. In practice, there is an upper limit to the flow rate that is determined by the mechanical strength of the media, which is the case for compressible media such as agarose, or by the mechanical capabilities of the equipment being used (for example, the pressure rating of a process scale column) or by the degree of loss of capacity at very short residence times. An example curve of residence time vs. dynamic capacity for ProSep-vA High Capacity media is given in Figure 3.

Figure 3.

Effect of Residence Time and Breakthrough Point on Dynamic Binding Capacity



Shape of Breakthrough Curve

The relative sharpness of the breakthrough curve is indicative of the efficiency of mass transfer within the pores of the chromatography media. The higher the efficiency, the sharper the breakthrough curve. Ideally, under process conditions, the column would be loaded to a point just prior to breakthrough. In practice, a safety margin is included and the column is typically loaded to 80 – 90% of the breakthrough point. The sharper the breakthrough curve, the nearer to 100% utilization of binding capacity can be achieved. As can be seen in Figure 2, ProSep-vA media has a very sharp breakthrough curve.

Recovery

From the above experiments, the dynamic binding capacity can be determined for a given residence time. Using this data, a column can be loaded to a working capacity of 80 – 90% of the theoretical binding capacity. The loaded column can then be eluted at low pH (e.g. 100 mM acetate buffer pH 3.5). The amount of IgG eluted is determined and compared to the amount loaded to obtain the % recovery. Typically a protein A column should produce >95% recovery. It is important to note that the column conditions used to determine the dynamic binding capacity will result in overloading the column; therefore these data cannot be used directly to determine recovery of MAb, as they will always be low.

For optimum binding, the following buffers are recommended (for buffer recipes, refer to ProSep-vA Operating Instructions):

1. PBS (phosphate buffered saline) pH 7.4
2. 50 mM phosphate and 0.15 M NaCl pH 7.5
3. 50 mM phosphate, 0.5 M glycine, 0.15 M NaCl pH 7.5
4. 50 mM Tris HCl, 0.15 M NaCl, pH 7.5
5. 1M glycine/NaOH + 0.15 M NaCl pH 8.6
6. 0.1 M borate + 0.15 M NaCl pH 8.5

Binding buffers with pH greater than pH 8.6 should not be used. Recommended elution buffers are 0.1 M glycine/HCl, 0.1 M Na acetate buffer, 0.1 M citrate buffer, all pH 3.0 – 3.5.

Appendix 1.

Suggested Process Steps

Step	Buffer	Column Volumes (CV)	Flow Rate (Based on 0.66 cm x 10 cm Column)
Equilibration	PBS pH 7.4	10	2.0 mL/min
Load	Direct clarified feed	Dependent on sample volume and concentration	1.14 mL/min (Residence time 3 min)
Wash	PBS pH 7.4	10 – 15	2.0 mL/min
Elute	0.1 M glycine pH 3.5	7 – 10	2.0 mL/min
Regenerate	Phosphoric acid pH 1.5	5	2.0 mL/min
Re-equilibrate	PBS pH 7.4	10	2.0 mL/min

Glossary

Breakthrough
The point at which some MAb passing through a packed bed is no longer able to find an available protein A binding site. This is indicated by an increase in the UV absorbance of the process stream leaving the column.

Clarified feedstock/cell culture supernatant
The fermentation liquor from which the cells and large cell debris have been removed. This is normally clarified to the extent that it can be passed through a 0.22 – 0.1 µm filter before chromatography.

Dynamic binding capacity
The amount of antibody that can bind to a chromatography media under flow conditions. This value is always lower than the static or saturation capacity

Feedstock
Any solution passed through the column that contains the target protein to be purified.

Overloaded
A column which continues to be loaded after product breakthrough occurs. Overloading the column can still allow extra binding sites to be utilized and the capacity of an overloaded column may be higher than the same column loaded just to the point of breakthrough. However, the efficiency in capturing the target protein is greatly reduced and there will be loss of product as some passes straight through the column.

Partially purified MAb
Monoclonal antibody which has already been partially purified by passage through another chromatography media before use in the capacity determination. Purification is usually on a protein A media. In this case, the pH of the feedstock should be adjusted to ensure it reflects the pH of the cell culture supernatant. Using partially purified MAb allows direct determination of breakthrough and eliminates the need to perform secondary assays on the collected fractions. However, caution must be taken that prior purification of the antibody does not affect its performance on the protein A media.

Recovery
The recovery is the amount of antibody eluted from the column compared to the amount of antibody loaded. Recovery is usually expressed as a percentage with complete recovery being 100%. In dynamic binding capacity studies, the column is overloaded. As a result, recovery will always be below 100% and should not be used as an indication of the column performance in the final process.

Static binding capacity
The static or saturated capacity of a column is the amount of target protein the column can bind if every available binding site is utilized. This is determined by loading a large excess of target protein either at very slow flow rates or after prolonged incubation in a closed system. In practice, the static capacity is never available under process conditions using packed columns.

Throughput
The throughput of a process is the mass of antibody purified per unit of time. To optimize a separation, it is important to consider the throughput rather than the binding capacity, since the highest capacities will usually be achieved at the lowest flow rates and therefore the slowest cycle times. It is only by considering capacity and flow rate (i.e. cycle time) together that the optimum throughput can be determined.

Discover the More in Millipore™

In every application, every step and every scale, count on Millipore to be everywhere for you—from monoclonals to vaccines, from clinical through pilot to full-scale manufacturing. Our technologies are used by most of the world's major biopharmaceutical companies. But we deliver more than advanced separation, purification, sterilization and quality control products. With Millipore, you get services to optimize and validate your processes, comprehensive resources to streamline and enhance your operation, unmatched know how forged from 50 years' experience—and solutions that integrate it all. For higher yields, improved process economics and faster speed to market, discover the more in Millipore.

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:
In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**
In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**
Outside of North America contact your local office. To find the office nearest you visit **www.millipore.com/offices.**
Internet: **www.millipore.com**
Technical Service: **www.millipore.com/techservice**

MILLIPORE

Millipore, ProSep and Vantage are registered trademarks of Millipore Corporation.
Discover the More in Millipore is a trademark of Millipore Corporation.
Omnifit is a registered trademark of Omnifit Limited.
Lit. No. TB1175EN00 Rev. - 03/05 Printed in U.S.A. 05-083
© 2005 Millipore Corporation Billerica, MA 01821 U.S.A. All rights reserved.