Bioprocess Engineering 21 (1999) 287–292 © Springer-Verlag 1999

The optimal flow rate and column length for maximum production rate of protein A affinity chromatography

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Abstract Production rate is an important parameter in the design of efficient protein A affinity chromatography processes for purifying recombinant monoclonal antibodies. A simple equation was derived that expresses production rate in terms of flow rate and column length. Changes in flow rate and column length will not affect the antibody and are therefore easily varied for bioprocess applications. In the equation, production rate depends on dynamic capacity, which can be expressed as a function of the load flow rate and column length. The only empirical data needed for production rate optimization is the relationship of dynamic capacity to load flow rate and column length, which was quickly determined by using an on-line assay. The optimal production rate was found at a high flow rate, a low column length, and a low dynamic capacity, which has several implications for using high production rate protein A affinity chromatography for antibody manufacturing.

List of symbols

A_c	Column cross-sectional area (cm^2)
C_i	Antibody concentration in the column effluent (g/l)
C_o	Antibody concentration in the load (g/l)
F	Volumetric flow rate (l/h)
F_l	Volumetric flow rate for load (l/h)
F_e	Volumetric flow rate for elution/equilibration/wash (l/h)
L_c	Column length (cm)
L_c/U_l	Superficial residence time (h)
N	Number of column volumes for elution/equilibration/wash
Δ_P	Pressure drop across column (bar)
R_p	Production rate (g h^{-1} cm^{-2}, g of antibody per h per cm^2 of column cross-sectional area)
Q_d	Dynamic capacity (g/l, g of antibody per l of column volume)
t	Time (h)
U	Linear flow rate (superficial mobile phase velocity), $1000 \cdot F/A_c$ (cm/h)
U_l	Linear flow rate (superficial mobile phase velocity) for load, $1000 \cdot F_l/A_c$ (cm/h)
U_e	Linear flow rate (superficial mobile phase velocity) for elution/equilibration/wash, $1000 \cdot F_e/A_c$ (cm/h)
V_c	Column volume (l)

Received: 9 November 1998

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For their generous contributions, we would like to thank Heather Knudsen and Robert Johnson.

1 Introduction

Recombinant monoclonal antibodies have important therapeutic applications, including the treatment of several types of cancer [1–5]. The production of these antibodies at commercial scale has created a need for efficient bioprocessing methods. A common bioprocess method for purifying antibodies is protein A affinity chromatography [6]. It selectively binds antibodies in complex solutions such as cell culture fluid [7–12], and it is often used as the first chromatographic purification step for recombinant antibodies, with clarified cell culture fluid directly loaded onto the column. The recombinant antibody is highly purified and concentrated during protein A affinity chromatography.

The development of a protein A affinity chromatography process typically focuses on several considerations. One common consideration is the dynamic capacity, the amount of antibody that can be loaded before antibody breakthrough occurs. Dynamic capacity is often maximized in order to utilize as completely as possible the expensive protein A affinity chromatography media. Dynamic capacity depends on many factors, including the type of protein A affinity chromatography media, the antibody concentration in the load, the column temperature and column length, the buffer, conductivity, and pH of the load, and the flow rate [13–18]. This paper focuses on flow rate and column length, primarily because they are easily varied in bioprocess applications. While changes in pH, buffer, conductivity, or temperature could denature, precipitate, or otherwise affect the antibody, changing the flow rate and column length to optimize a bioprocess would have little impact on the antibody itself.

Another important consideration in bioprocessing is the production rate [19–22], the amount of product purified per unit of time per unit of column cross-sectional area [23]. Production rate can be especially important for protein A affinity chromatography. Since protein A affinity chromatography media is expensive, rather than using a column large enough to process a batch of antibody in a

single cycle, typical bioprocess applications use a smaller column for several cycles to purify a single batch. This cycling increases the total purification time, which decreases the production rate. Decreases in production rate may adversely affect manufacturing plant capacity and process economics. Also, unstable antibodies may require a high production rate to avoid protein degradation and losses in product yield or product quality. The design of an optimal protein A affinity chromatography process should include a consideration of production rate. Like dynamic capacity, production rate can depend on many factors, including flow rate and column length [13, 21, 24, 25]. This paper focuses on flow rate and column length because they are easily varied to decrease bioprocessing time.

A production rate equation, incorporating dynamic capacity, that can be easily applied to bioprocess operations could aid in developing efficient protein A affinity chromatography methods for bioprocess applications. In this paper, a simple equation relating production rate to flow rate and column length is derived, and this equation is used to find the optimal flow rate and column length for maximum production rate of protein A affinity chromatography of a recombinant monoclonal antibody.

2 Materials and methods

2.1 Materials

PROS columns and BioCAD chromatography instruments were obtained from PerSeptive Biosystems (Framingham, MA). ProSep A chromatography media was obtained from Bioprocessing (Consett, England). The AKTA chromatography instrument was obtained from Pharmacia (Uppsala, Sweden). Load material was clarified cell culture fluid (clarified by tangential flow filtration [26]) containing a recombinant monoclonal antibody with a human constant region, produced in Chinese hamster ovary cells at a concentration of approximately 0.5 g/l. This load material was obtained from Genentech, Inc. (South San Francisco, CA).

2.2 Determination of dynamic capacity

During loading of protein A affinity columns, no antibody is present in the column effluent until the dynamic capacity of the column is reached and antibody begins flowing through. Dynamic capacity was calculated as the amount antibody loaded at antibody breakthrough. Antibody breakthrough was determined when $C_i/C_o = 0.05$, where C_i is the concentration of antibody in the column effluent (g/l) and C_o is the concentration of antibody in the load (g/l). The amount of antibody loaded (g antibody per l column volume) was calculated as $F_l \cdot C_o \cdot t/V_c$, where F_l is the volumetric flow rate for the load (l/h), t is the load time (h), and V_c is the column volume (l). C_i/C_o was found by measuring the amount of antibody in the preparative column effluent using the analytical chromatography method described below. The production rate equation assumes that the column is loaded to the dynamic capacity, determined at $C_i/C_o = 0.05$. Although increasing load beyond $C_i/C_o = 0.05$ can increase production rate [21], protein A affinity chromatography of recombinant antibodies is most often run at maximum yield to prevent loss of expensive product, which would be reduced by loading beyond $C_i/C_o = 0.05$.

2.3 Analytical chromatography

When loading purified antibody, C_i/C_o can be monitored by UV absorbance. However, when loading clarified cell culture fluid containing recombinant antibodies, C_i/C_o cannot be measured using UV absorbance because impurities in the flow-through obscure UV detection of antibody breakthrough. To quickly determine C_i/C_o, an on-line assay was used that directly samples the preparative column effluent. The on-line assay must be rapid and antibody-specific. Chromatographic assays are fast [27] and can be used to quickly assay recombinant proteins [28], and commercial instrumentation specifically designed to run on-line chromatographic assays is available [29]. Perfusion chromatography can produce protein analysis in as little as 20 seconds [30, 31] and protein A is antibody-specific, so the on-line assay used protein A immobilized on perfusion chromatography media, providing a rapid antibody-specific assay [32]. Using an on-line assay makes it possible to gather the necessary empirical data much faster than would be possible otherwise.

Analysis of column effluent for antibody was performed at room temperature by a perfusion affinity assay. The column was a 30 mm length × 2.1 mm inner diameter POROS A/M with a 20 μm particle size. Buffer A was 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2, and buffer B was 10 mM sodium phosphate, 150 mM sodium chloride, pH 2.2. Two flow rates were used: 20 ml/min for purge (column off-line), and 3.5 ml/min for run (column on-line). Injection volume was 20 μl from the process stream using an in-line sampling valve to directly sample the preparative column effluent. Detection was absorbance at 280 nm. The method was: purge 3.5 ml A, run 15 column volumes A, inject, run 12 column volumes A, purge 3.5 ml B, run 20 column volumes B. The total assay time was 2.3 minutes. Chromatography was run on a BioCAD/RPM.

2.4 Preparative chromatography

Preparative protein A affinity chromatography used 0.66 cm diameter columns packed with ProSep A media. Columns were equilibrated with 6 column volumes of 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, washed with 6 column volumes of 25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1, and eluted with 6 column volumes of 0.1 M acetic acid, pH 3.5. Flow rates and column lengths are given in the figure legends. All buffers and load material were filtered through a 0.22 μm filter prior to use. Chromatography was run at room temperature on a BioCAD for dynamic capacity determination, and on an AKTA for running the optimized process.

2.5 Calculation of production rate

The goal of the production rate calculation was to derive a simple expression using a minimum of variables that are easy to control and measure. Production rate was calculated as the mass of antibody purified in one cycle divided by the time it takes to purify the antibody, divided by the column cross-sectional area to make production rate independent of scale. Then:

$$R_p = \frac{\dfrac{V_c Q_d}{\dfrac{V_c Q_d}{\dfrac{C_0}{A_c U_l}} + \dfrac{NV_c}{\dfrac{A_c U_e}{1000}}}}{A_c} \quad (1)$$

where R_p is the production rate (g h^{-1} cm^{-2}), V_c is the column volume (l), Q_d is the dynamic capacity (g/l), C_o is the antibody concentration in the load (g/l), N is the number of column volumes for the elution/equilibration/wash, A_c is the column cross-sectional area (cm^2), U_l is the linear flow rate (superficial mobile phase velocity) for the load (cm/h), and U_e is the linear flow rate (superficial mobile phase velocity) for the elution/equilibration/wash (cm/h). Then $V_c Q_d$ is the mass of antibody purified (g), $V_c Q_d / C_o$ is the volume loaded (l), NV_c is the volume for wash, elution, and equilibration (l), and $A_c U_l/1000$ and $A_c U_e/1000$ are the volumetric flow rates for the load and the elution/equilibration/wash (l/h).

Rearranging terms, Eq. (1) reduces to:

$$R_p = \frac{1}{1000\left(\frac{1}{C_o U_l} + \frac{N}{Q_d U_e}\right)}. \quad (2)$$

3 Results and discussion

The production rate equation, Eq. (2), was used to determine the effect of flow rate and column length on production rate. In Eq. (2), production rate (R_p) depends on five factors: the dynamic capacity (Q_d), the antibody concentration in the load (C_o), the number of column volumes for elution/equilibration/wash (N), the load flow rate (U_l), and the elution/equilibration/wash flow rate (U_e). By substituting functions for Q_d and U_e, Eq. (2) can be reduced to depend only on the variables of the load flow rate (U_l) and the column length (L_c), and the constants C_o and N. A function for dynamic capacity was found by determining the effects of the load flow rate and the column length on dynamic capacity. To express U_e as a function of L_c, U_e is run at the maximum pressure drop (Δp), a function of L_c. Eq. (2) was then used to construct a contour plot to visualize the effect of flow rate and column length on production rate and also to find the optimal flow rate and column length for maximum production rate.

The only empirical data needed for production rate optimization using this approach is the relationship of dynamic capacity to flow rate and column length. The effect of the load flow rate and column length on dynamic capacity was determined experimentally (Fig. 1). In the range studied, dynamic capacity was linearly related to the load flow rate (Fig. 1A), and dynamic capacity was nonlinearly related to the column length (Fig. 1B). The logarithmic fit in Fig. 1B approximates the dynamic capacity approaching the saturation capacity at a sufficiently long column length. A more accurate nonlinear fit could be obtained by generating more data points. For this data set, a logarithmic fit seems to adequately describe the relationship, with $R^2 > 0.9$.

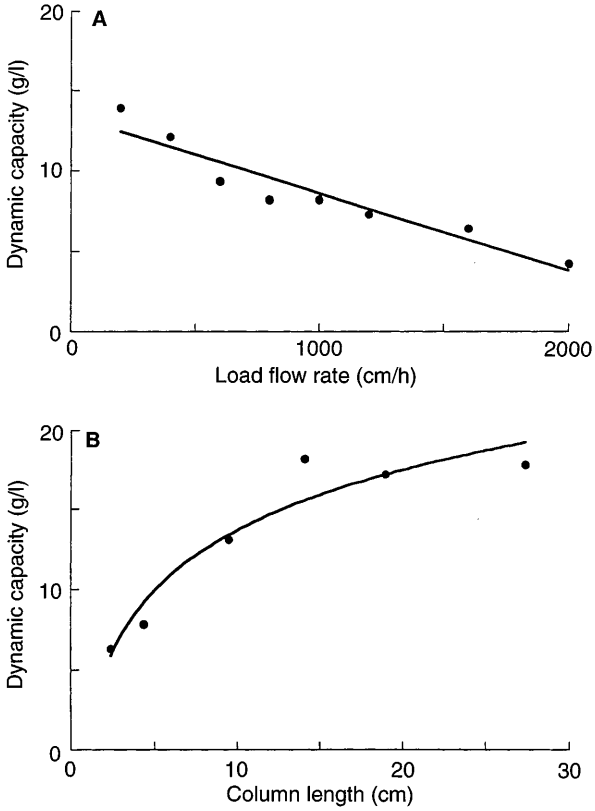

Fig. 1. The effect of (A) load flow rate (L_c = 10 cm, $Q_d = 13 - 0.0048 \cdot U_1$, $R^2 = 0.90$), and (B) column length (U_l = 400 cm/h, $Q_d = 1.1 + 13 \cdot \mathrm{Log}(L_c)$, $R^2 = 0.92$) on dynamic determined at $C_i/C_o = 0.05$

The superficial residence time (L_c/U_l) combines the effects of the load flow rate and the column length into a single expression of dynamic capacity. The superficial residence time is the time required for an element of mobile phase to travel through the column when no chromatography media is present. This is also known as the empty column residence time. An inversely equivalent, commonly used measurement is to express the flow rate in terms of column volumes per hour. Dynamic capacity has a nonlinear relationship to the superficial residence time (Fig. 2). The logarithmic fit in Fig. 2 approximates the dynamic capacity approaching the saturation capacity at large values of superficial residence time, and decreasing quickly as the superficial residence time becomes very small. A more accurate nonlinear fit could be obtained by generating more data points. The superficial residence time seems to be a characteristic parameter that determines the dynamic capacity. Capacity data generated at constant column length correlates well with data generated at constant load flow rate. In Eq. (2), the dynamic capacity

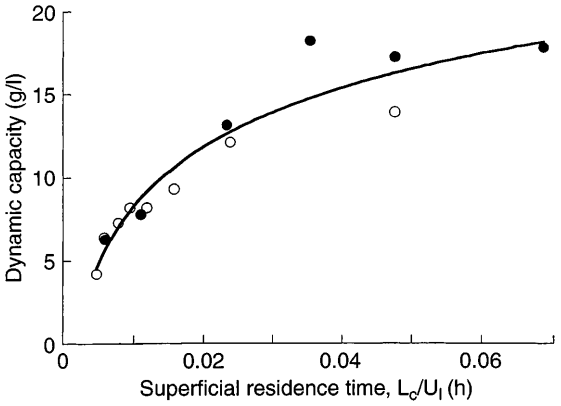

Fig. 2. The effect of the superficial residence time (L_c/U_l) on dynamic capacity (Q_d), combining data from Fig. 1A and Fig. 1B. ● constant load flow rate, ○ constant column length. $Q_d = 32 + 12 \cdot \text{Log}(L_c/U_l)$, $R^2 = 0.91$

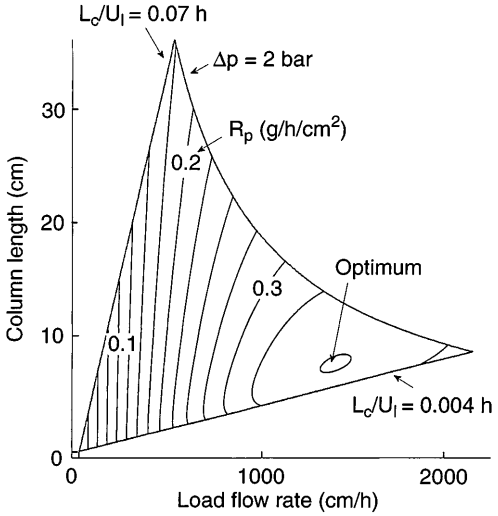

Fig. 3. Contour plot showing the effect of load flow rate and column length on production rate. Contours are constant production rate (R_p), given by Eq. (2) with $Q_d = 32 + 12 \cdot \text{Log}(L_c/U_l)$, $U_e = 2/(1.07 \times 10^{-4} \cdot L_c)$, $C_o = 0.5$ g/l, and $N = 18$. The contour interval is 0.025 g h^{-1} cm^{-2}. The operating area is bounded by the superficial residence time (L_c/U_l) = 0.07 h, L_c/U_l = 0.004 h, and $\Delta p = 2$ bar. The optimum is at a load flow rate of 1400 cm/h and a column length of 7.5 cm

(Q_d) is determined by the superficial residence time (L_c/U_l), using the logarithmic fit in Fig. 2.

The antibody concentration in the load (C_o) depends on cell culture conditions and whether the load is concentrated prior to chromatography. In the load material used for this study, C_o was 0.5 g/l.

The number of column volumes for elution/equilibration/wash (N) is determined by purity and yield constraints. In this study, 6 column volumes of buffer is washed through the column after load to flush out any cell culture fluid, 6 column volumes of elution buffer is used to ensure that the antibody is entirely eluted, and 6 column volumes of equilibration ensures that the column is ready for the next load, for a total of $N = 18$.

The maximum production rate occurs when the flow rate used for the elution/equilibration/wash (U_e) is run at the highest that the pressure drop (Δp) will allow. For ProSep A media, Δp was found experimentally to be $1.07 \times 10^{-4} \cdot U \cdot L_c$. For a maximum $\Delta p = 2$ bar (valid for many acrylic or glass columns), $U_e = 2/(1.07 \times 10^{-4} \cdot L_c)$.

With these constraints ($C_o = 0.5$, $Q_d = 32 + 12 \cdot \text{Log}(L_c/U_l)$, $U_e = 2/(1.07 \times 10^{-4} \cdot L_c)$, and $N = 18$), Eq. (2) is then dependent only on the variables L_c and U_l. Production rate in the form of Eq. (2) has a composite dependence on L_c and U_l (Fig. 3). The contour plot in Fig. 3 includes only the region where data is available, 0.004 h < L_c/U_l < 0.07 h, and is bounded by $\Delta p = 2$ bar. For values of Δp >2 bar the operating area would be extended. For most values of column length, the production rate increases as the load flow rate increases, even though dynamic capacity is decreasing. This is because although more cycles would be required to process a given amount of antibody, each cycle is run very quickly, and the overall production rate increases. The increased number of cycles needed due to the lower dynamic capacity is offset by the higher throughput of each cycle. For some values of column length, increasing the load flow rate above a certain value decreases the production rate because the time needed to process many cycles overcomes the speed of each cycle.

There is a maximum R_p of 0.35 g h^{-1}cm^{-2} at L_c = 7.5 cm and U_l = 1400 cm/h. This gives U_e = 2500 cm/h and Q_d = 5 g/l, for total cycle time of a rapid 6.3 minutes (Fig. 4). For the process shown in Fig. 4, recovery yield was 92% (standard deviation was 2% for three replicate runs). This high yield suggests that little or no antibody flowed through the column during the load, which implies that the superficial residence time was able to accurately predict the dynamic capacity. Using optimized conditions, this 0.66 cm diameter column can purify 0.12 grams of antibody per hour. Although the column is only loaded to 5 g/l, which is about 25% of the saturation capacity of the media, the production rate is maximized because each cycle is run very quickly.

Once the production rate is maximized, the diameter of the bioprocess column can then be chosen. This choice would be based upon several factors. One factor is the total processing time that is acceptable. The total processing

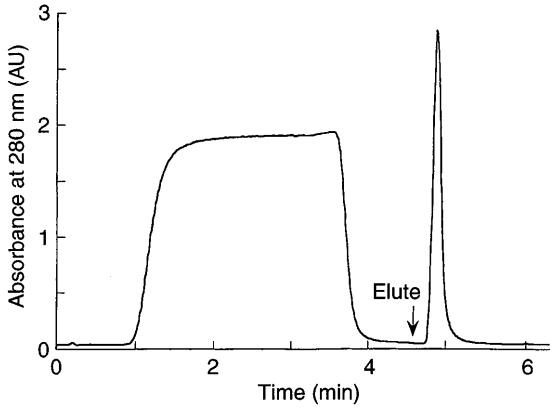

Fig. 4. Chromatogram of protein A affinity chromatography run at optimal conditions for maximum production rate (U_l = 1400 cm/h, U_e = 2500 cm/h, Q_d = 5 g/L, and L_c = 7.5 cm), with C_o = 0.5 g/l, N = 18, and a 0.66 cm diameter column. The eluted peak is antibody

time for a batch of antibody can be determined from the production rate, the column cross-sectional area, and the amount of antibody to be processed. Another factor is the maximum acceptable cost of the protein A affinity column. This cost would be just one consideration in the cost of the process, where the cost of labor, buffers and other raw materials, equipment, and processing time would all need to be considered together. Finally, considerations of manufacturing plant size could put a constraint on the column size and the associated equipment such as pumps, valves, piping, and detectors.

Protein A affinity chromatography run at high flow rate, low column length, and low dynamic capacity has several implications for bioprocess applications. The width of the eluted antibody peak is very small, about 25 seconds, which may pose a problem for large scale production where slow valve switching and a large dead volume to a pool tank may cause difficulties for product collection. For ProSep A chromatography media, even at 2500 cm/h the eluted antibody peak still has a good shape with almost no tailing (Fig. 4), but this may not be true for all protein A affinity chromatography media. For each media, a constraint of maximum flow rate may need to be evaluated. The low dynamic capacity requires an increased number of cycles to process a batch of antibody, which may affect the cost of the process if the media lifetime depends on the number of cycles run. The increased number of cycles and low loading capacity may require more mobile phase and may increase the pool volume of the batch. The increased pool volume may affect the production rate of the next chromatography step by increasing its load time, so for production rate optimization across an entire bioprocess an additional constraint of pool volume may need to be considered.

The production rate equation provides a method not only for maximizing production rate, but also for constructing production scenarios by quantitatively determining the effect of changing process variables on capacity and production rate. For example, the effect of doubling the column length could be readily determined. Also, it is clear that decreasing N would increase production rate, so Eq. (2) provides a simple way to evaluate the impact of decreasing the equilibration volume. Extending the operating region above a 2 bar maximum pressure drop may reveal areas of higher production rate, so Eq. (2) may aid in determining if a column with a higher pressure rating would be cost effective. A more rigorous, calculus-based treatment could differentiate Eq. (2) and use the results to find the maximum production rate and optimal flow rate and column length rate as N, Δp, or C_o are varied.

The low dynamic capacity required for maximum production rate may suggest that using non-porous media or membrane chromatography could further increase production rate.

With only a small amount of empirical information, a bioprocess may be designed for maximum production rate by optimizing the flow rate and column length, factors which have little impact on the antibody itself and thus are easily varied for bioprocess optimization. Using an on-line assay to determine the effect of the superficial residence time on the dynamic capacity allows the required empirical information to be generated quickly. The simple equation used here can quickly and effectively help in determining the most efficient way to operate protein A affinity chromatography for bioprocess applications.

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