The Role of More than 40 Years of Improvement in Protein A Chromatography in the Growth of the Therapeutic Antibody Industry

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DOI 10.1002/btpr.2324
Published online July 23, 2016 in Wiley Online Library (wileyonlinelibrary.com)

Protein A chromatography has been used as the mAb capture step in the majority of FDA submissions. In this study, the performance of protein A chromatography, as indicated by capacity, operational flow rate, and productivity (rate of mAb production per liter of resin) was examined over its full history to gain insights into the reasons for its consistent use. Protein A productivity and capacity have increased 4.3 and 5.5% a year, respectively, since 1978. In contrast, protein A operational flow rate increased between 1978 and 2001 and then remained constant or declined as further improvements provided only marginal benefits. The productivity of protein A resin and also the mAb bioreactor titer (14% growth) rapidly improved starting in about 1990 to economically provide material for clinical trials. Technology improvement is typically driven by product sales. The sales of protein A resin, as indicated by sales of protein A ligand (21% growth), have closely paralleled the sales of mAbs (20% growth). Both increased rapidly in 2000 after the first major mAb therapeutics were approved and the markets were developed. It is likely that alternatives to protein A chromatography have not been implemented because of the order of magnitude improvement in protein A performance. Protein A membrane adsorbers and monoliths have higher productivity than packed columns due to their short bed heights and high operational flow rates. These devices are not currently practical for large-scale manufacturing but may represent a format for future improvements in protein A productivity. © 2016 American Institute of Chemical Engineers *Biotechnol. Prog.*, 32:1193–1202, 2016
Keywords: protein A, SpA, mAb, monoclonal antibody, affinity chromatography, purification

Introduction

The standard process for the purification of a mAb from harvested cell culture fluid typically involves at least two chromatography steps: protein A affinity and ion-exchange chromatography. Protein A chromatography separates proteins from impurities based on a reversible interaction between the Fc portion of a molecule and a protein A ligand immobilized on a chromatography matrix.[1] Impurities flow through the matrix while the mAb stays bound to the column. The column is subsequently washed to achieve additional impurity removal.[2] Product is eluted from the matrix by a reduction in pH. Subsequently, the mAb is typically either bound to a cation-exchange chromatography resin or flowed through an anion-exchange resin to remove additional host-cell proteins, DNA, leached protein A, media derived impurities, potential viruses, and aggregated species.[3] This is often followed by an additional polishing ion-exchange or hydrophobic interaction chromatography step.[4]

The binding of protein A to a wide range of human and rabbit serum antibodies was first observed by Jensen,[5] who attributed the binding to natural antibody specificity for a bacterial antigen (antigen A). Subsequently, the antigen was incorrectly described as a bacterial polysaccharide[6] and then correctly shown to be a bacterial cell wall protein.[7] This protein was first called protein A in 1964 to distinguish it from other polysaccharides and proteins.[8]

Subsequently, the theory of natural antibody specificity for a bacterial antigen was disproven. Protein A was shown to bind to antibodies lacking bacterial antigen specificity and also to bind to Fc portions of antibodies.[9] In addition, it was shown that protein A bound more strongly to Fc fragments of antibodies than Fab regions or light chains. Subsequently, use of protein A immobilized on a solid support for the affinity purification of antibodies was developed[10] and patented.[11] The first protein A resin was commercialized in 1978.[12]

Protein A will bind to a range of antibody isotypes derived from different species.[13] The first therapeutic antibody, Orthoclone, was approved in 1986 and utilized protein A chromatography as a capture step in the purification process.[14] Protein A was subsequently used as the mAb capture step in 86% of FDA submissions.[15] Early protein A resins were expensive and had limited capacity, flow rate, and lifetime. A number of studies have described replacing the

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protein A chromatography step with alternative purification methods. Ion-exchange chromatography is a frequently discussed alternative mAb capture strategy.[15–18] Antibody or impurity precipitation[4,14,19,20] or crystallization[21] have also been suggested. Alternative protein[22] or synthetic molecule[23] affinity resins have also been proposed to replace protein A.

However, since 1978, both the performance and availability of protein A resins have steadily increased. Newer beads are more rigid yet highly porous, allowing fast flow rates, low pressure drops, and high mAb capacities.[24] The sizes of both beads and bead pores have been optimized to improve performance. A number of base bead chemistries including cross-linked agarose,[25] controlled pore glass,[26] polystyrene-divinylbenzene,[24] ceramic material,[27] polymethacrylate,[28] cross-linked cellulose,[29] and polyvinylether[30] have been developed to improve protein A performance.

Protein A resins have benefited from the removal of animal derived production materials.[31] Improvements in how protein A ligands are immobilization on beads, e.g., via a single or multipoint linkage,[24] has helped improve ligand accessibility and increase capacity. Changes to the number of protein A domains and the amino acids in those domains has led to improved protein A capacity, lifetime, caustic stability, elution pH, and mAb Fc specificity.[32–34] These improvements have led to an improvement in the amount of mAb that can be produced per liter of protein A resin per hour (productivity).

In this study, the growth of protein A productivity, capacity, and operational flow rate is tracked using available literature data. This comparison provides insights into how fast the performance of protein A chromatography has improved and how this improvement compares to the rate of improvement in bioreactor titers. In order to get insights into the timing and reasons for current and potential future improvements in protein A chromatography, the growth of protein A performance is then compared to the growth of protein A resin sales and therapeutic mAb sales.

Review of Protein A Evolution

Data from citable studies that provide the protein A column bed height, flow rate, and capacity at 10% breakthrough were compiled and used to evaluate growth in protein A productivity, capacity, and operational flow rate. The resin launch date was obtained from the resin vendors or estimated from the earliest date of publication of resin information.

The flow rate used to obtain the reported capacity data (operational flow rate) was compiled. The maximum flow rate is not summarized here because in some studies only maximum pressure is reported, and in other studies maximum flow rate values are provided without also providing the corresponding capacity data.

There is limited data available for some of the key attributes of protein A media. Unfortunately, pricing information is rarely published by vendors or customers[35] and therefore was not evaluated in this study. The global protein A media market was estimated to generate annual revenues between 350 and 400 million USD annually in the 2015 Repligen annual report. In addition, limited data are available for resin lifetime, nonspecific product and impurity adsorption,[2] ligand leaching, and reproducibility

The high pH stability of protein A ligands has improved through selective mutation of the protein A ligand.[33] The chemical stability data provided in the references are listed in Table 1. However, very few references provide the expected loss in performance based on exposure to a specific pH value for a fixed duration. This makes it difficult to measure the rate at which chemical stability has improved.

Productivity is defined as the grams of mAb that can be purified per hour on a liter of protein A resin. Most, but not all studies provided the antibody load concentration. Therefore, a mAb titer of 1 g/L was assumed during loading. The wash, elution, and regeneration steps were assumed to be 10, 3, and 5 column volumes (CV), respectively. The total CVs per run were determined from the reported resin capacity, the bioreactor titer, the number of wash CV, the number of elution CV, and the number regeneration CV according to Eq. 1:

$$Total\ CV = \frac{Resin\ Capacity}{Bioreactor\ Titer} + Wash\ CV + Elution\ CV + Regen\ CV \quad (1)$$

The productivity was then calculated from the total CV using the reported resin capacity, linear flow rate, and bed height according to Eq. 2:

$$Productivity = \frac{Resin\ Capacity * Linear\ Flow\ Rate}{Total\ CV * Bed\ Height} \quad (2)$$

The amount and the quality of protein A performance data varied. Data for early protein A resins typically had only one or a few low capacity and/or low flow rate data points. Therefore, it was not possible to compare all resins at a single reference flow rate, or to compare flow rates at a single reference capacity. Therefore, data from all flow rates reported in each study are used to plot and evaluate the growth in productivity, capacity, and operational flow rate. Some of the vendor literature provided limited experimental details and may have utilized conditions favorable to their products. One study utilized an IgG3, which may have impaired the capacity of some resins.[25] The load antibody concentration ranged from 0.33 to 10 mg/mL and was not reported in some studies. Higher concentration has been reported to improve[49] or not affect protein A capacity.[43,54,58] A load concentration of 1 mg/mL was assumed to calculate productivity. The bed heights used in the studies ranged from 2 to 30 cm, with lower bed heights typically allowing higher flow rates and productivity. Lower bed heights however are less representative of a mAb manufacture process and can result in lower capacities.

Protein A productivity growth

The resin productivity is plotted vs. resin launch year in Figure 1. A compounded annual productivity growth rate r of 4.3 ± 1.4% and an initial value Y_0 of 5.8 ± 2.4 g/L/h was determined by fitting all data points except the membrane adsorber and monolith data to Eq. 3, where t is the number of years:

$$Y = Y_0(1+r)^t \quad (3)$$

The growth rate was somewhat sensitive to the assumed titer but not the assumed CV for the various process steps. Assuming a load titer of 0.1 or 10 g/L, instead of 1 g/L, changed the growth rate to either 2.4 ± 1.2 or 7.7 ± 1.8%. Doubling the assumed wash, elution, and regeneration CV did not alter the productivity growth rate as all data was changed to a similar degree.

Table 1. Protein A Launch Date, Bed Height, Operational Flow Rate, Capacity at 10% Breakthrough, Productivity, Feed Composition, and Caustic Stability*

Protein A Resin	Manufacturer	Launch Year	Capacity (g/L) at 10% BT	Flow Rate (cm/h)	Productivity (g/L/h)	Bed Height (cm)	Feed Information	Caustic Stability	Reference
Protein A Sepharose™ CL 4B	GEHC	1978	5.0	100	0.7	30	Mouse Serum IgG		12
Protein A Sepharose™ CL 4B	GEHC	1978	10.0	60	4.3	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
Protein A Ultrogel	Pall/IBF	1982	0.7	60	0.4	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
Protein A Sepharose™ 6 FF	GEHC	1983	20.1	60	6.3	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
Affi-Gel® Protein A	Bio-Rad	1984	12.8	60	5.0	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
ProSep®-A	Millipore	1988	10.0	60	4.3	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
Eupergit® C Protein A	Rohm Pharma	1989	0.5	60	0.3	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
Protein A Sepharose™ 4 FF	GEHC	1989	18.5	60	6.1	5	0.5 mg/mL Monoclonal mouse IgG3 at pH 8.9		25
Affi-Prep® Protein A	Bio-Rad	1990	11.0	543	48.8	4.2	1 mg/mL Humanized mAb		36
nProtein A Sepharose™ 4FF	GEHC	1991	6.0	300	15.0	5	0.33 mg/mL Human IgG	Short-term pH 2–10	37
nProtein A Sepharose™ 4FF	GEHC	1991	10.0	200	14.3	5	0.33 mg/mL Human IgG	Short-term pH 2–10	37
nProtein A Sepharose™ 4FF	GEHC	1991	17.0	100	9.7	5	0.33 mg/mL Human IgG	Short-term pH 2–10	37
nProtein A Sepharose™ 4FF	GEHC	1991	24.0	30	3.4	5	0.33 mg/mL Human IgG	Short-term t pH 2–10	37
ProSep®-A	Millipore	1993	16.0	1,000	29.4	16	0.8 mg/mL Chimeric antibody		26
rProtein A Sepharose™ (4) FF	GEHC	1995	50.0	100	7.4	10	0.5 mg/mL Human IgG pH 7.0	Cleaning in place pH 2–11	38
rProtein A Sepharose™ (4) FF	GEHC	1995	42.0	200	14.0	10	0.5 mg/mL Human IgG pH 7.0	Cleaning in place pH 2–11	38
rProtein A Sepharose™ (4) FF	GEHC	1995	31.0	300	19.0	10	0.5 mg/mL Human IgG pH 7.0	Cleaning in place pH 2–11	38
StreamLine™ A	GEHC	1998	11.0	500	19.0	10	0.35 mg/mL Humanized mAb		24
Poros® LP	Thermo Fisher	1998	16.0	500	23.5	10	0.35 mg/mL Humanized mAb		24
Poros® 50A	Thermo Fisher	1998	18.0	500	25.0	10	0.35 mg/mL Humanized mAb		24
rmp Protein A Sepharose™ 4FF	GEHC	2000	20.0	200	25.7	4.1	5 mg/mL Human IgG pH 7.4	Cleaning in place 2–11	31
rmp Protein A Sepharose™ 4FF	GEHC	2000	32.0	100	15.6	4.1	5 mg/mL Human IgG pH 7.4	Cleaning in place 2–11	31
rmp Protein A Sepharose™ 4FF	GEHC	2000	41.0	60	10.2	4.1	5 mg/mL Human IgG pH 7.4	Cleaning in place 2–11	31
Protein A Ceramic HyperD® F	Pall	2000	30.0	100	5.3	12	2.55 mg/mL Human IgG	Cleaning pH 2–13	27
MabSelect™	GEHC	2001	29.0	171	5.3	20	3 mg/mL IgG		39
MabSelect™	GEHC	2001	28.0	300	9.1	20	3 mg/mL IgG		39
MabSelect™	GEHC	2001	20.0	600	15.8	20	3 mg/mL IgG		39
MabSelect™	GEHC	2001	10.0	1,500	26.8	20	3 mg/mL IgG		39
MabSelect™	GEHC	2001	50.0	167	12.3	10	1.8 mg/mL mAb		40
Prosep®-VA HiCap	Millipore	2003	30.0	500	16.4	19	1 mg/mL Polyclonal human IgG		41
Prosep®-VA Ultra	Millipore	2004	18.0	1,200	30.0	20	2 mg/mL mAb		42

TABLE 1. *Continued*

Protein A Resin	Manufacturer	Launch Year	Capacity (g/L) at 10% BT	Flow Rate (cm/h)	Productivity (g/L/h)	Bed Height (cm)	Feed Information	Caustic Stability	Reference
Prosep®-VA Ultra	Millipore	2004	26.0	600	17.7	20	2 mg/mL mAb		42
Prosep®-VA Ultra	Millipore	2004	34.0	300	9.8	20	2 mg/mL mAb		42
Prosep®-VA Ultra	Millipore	2004	37.0	200	6.7	20	2 mg/mL mAb		42
MabSelect SuRe™	GEHC	2005	20.0	600	31.6	10	Human IgG		43
MabSelect SuRe™	GEHC	2005	39.0	250	17.1	10	Human IgG		43
MabSelect SuRe™	GEHC	2005	49.0	100	7.3	10	Human IgG		43
MabSelect SuRe™	GEHC	2005	50.0	75	5.5	10	Human IgG		43
MabSelect Xtra™	GEHC	2005	40.0	171	5.9	20	3 mg/mL IgG		39
MabSelect Xtra™	GEHC	2005	40.0	300	10.3	20	3 mg/mL IgG		39
MabSelect Xtra™	GEHC	2005	34.0	600	19.6	20	3 mg/mL IgG		39
MabSelect Xtra™	GEHC	2005	20.0	1,500	39.5	20	3 mg/mL IgG		39
Sartobind® Protein A (Membrane Adsorber)	Sartorius	2006	7.5	120	88.2	0.4	1 mg/mL Polyclonal human IgG	Short term pH 2-10	44
Poros® MabCapture™ A	Thermo Fisher	2007	43.0	700	24.7	20	5 mg/mL Polyclonal human IgG in PBS		45
Poros® MabCapture™ A	Thermo Fisher	2007	45.0	500	17.9	20	5 mg/mL Polyclonal human IgG in PBS		45
Poros® MabCapture™ A	Thermo Fisher	2007	46.0	400	14.4	20	5 mg/mL Polyclonal human IgG in PBS		45
Poros® MabCapture™ A	Thermo Fisher	2007	49.0	200	7.3	20	5 mg/mL Polyclonal human IgG in PBS		45
Bio-Monolith Protein A (Monolith)	Agilent Technologies	2008	8.0	297	184.6	0.5	0.5 mg/mL hIgG in PBS	Cleaning in place pH 2 to 13	46
ProSep® Ultra Plus	Millipore	2008	38.0	1,200	40.7	20	2 mg/mL mAb		42
ProSep® Ultra Plus	Millipore	2008	45.0	600	21.4	20	2 mg/mL mAb		42
ProSep® Ultra Plus	Millipore	2008	50.0	300	11.0	20	2 mg/mL mAb		42
ProSep® Ultra Plus	Millipore	2008	52.0	200	7.4	20	2 mg/mL mAb		42
UNOsphere SUPrA™	Bio-Rad	2008	26.0	150	17.7	5	1 mg/mL Polyclonal human IgG	Working pH range 3-11	47
UNOsphere SUPrA™	Bio-Rad	2008	23.0	300	33.7	5	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	18.0	400	40.0	5	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	29.0	150	9.3	10	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	27.0	300	18.0	10	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	24.0	400	22.9	10	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	32.0	150	4.8	20	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	31.0	300	9.5	20	1 mg/mL Polyclonal human IgG		47
UNOsphere SUPrA™	Bio-Rad	2008	27.0	400	12.0	20	1 mg/mL Polyclonal human IgG		47
CaptivA™ PriMAB	Repligen	2010	10.0	600	42.9	5	2 mg/mL Polyclonal human IgG in PBS	Cleaning in place pH 2-11	48
CaptivA™ PriMAB	Repligen	2010	17.0	300	29.1	5	2 mg/mL Polyclonal human IgG in PBS		48
CaptivA™ PriMAB	Repligen	2010	38.0	100	13.6	5	2 mg/mL Polyclonal human IgG in PBS		48

TABLE 1. *Continued*

Protein A Resin	Manufacturer	Launch Year	Capacity (g/L) at 10% BT	Flow Rate (cm/h)	Productivity (g/L/h)	Bed Height (cm)	Feed Information	Caustic Stability	Reference
CaptivA™ PriMAB	Repligen	2010	45.0	50	7.1	5	2 mg/mL Polyclonal human IgG in PBS		48
Toyopearl® AF-rProtein A-650F	Tosoh	2010	30.5	100	12.6	5	10 mg/mL Human polyclonal IgG in PBS		28
Toyopearl® AF-rProtein A-650F	Tosoh	2010	24.0	200	22.9	5	10 mg/mL Human polyclonal IgG in PBS;	80% of initial binding capacity after 50 cycles with 17 min of 0.5 M NaOH	28
Monofinity A Resin™	Genscript	2010	52.0	231	11.1	15.4	5 mg/mL hIgG	80% of initial binding capacity after 200 cycles with 0.5 M NaOH	49
MabSelect SuRe™ LX	GEHC	2011	18.3	600	30.2	10	Human polyclonal IgG		43
MabSelect SuRe™ LX	GEHC	2011	37.1	250	16.8	10	Human polyclonal IgG		43
MabSelect SuRe™ LX	GEHC	2011	60.5	100	7.7	10	Human polyclonal IgG		43
MabSelect SuRe™ LX	GEHC	2011	67.7	75	5.9	10	Human polyclonal IgG		43
AbSolute® High Cap	Novasep	2011	44.0	200	28.4	5	1.5 mg/mL IgG	150 cycles with 20 min of 0.05 M NaOH	50
AbSolute® High Cap	Novasep	2011	42.0	300	42.0	5	1.5 mg/mL IgG	Working pH 1.5–9.0	50
AbSolute® High Cap	Novasep	2011	32.0	600	76.8	5	1.5 mg/mL IgG	Working pH 1.5–9.0	50
Amsphere™ Protein A	JSR	2012	20.0	750	39.5	10	3.8 mg/mL IgG1 from CHO at pH 7.3		51
Amsphere™ Protein A	JSR	2012	30.0	600	37.5	10	3.8 mg/mL IgG1 from CHO at pH 7.3		51
Amsphere™ Protein A	JSR	2012	40.0	188	12.9	10	3.8 mg/mL IgG1 from CHO at pH 7.3		51
KANEKA KanCapA™	Kaneka	2012	24.0	667	19.0	20	Not provided	100 cycles with 15 min of 0.5 M NaOH	29
KANEKA KanCapA™	Kaneka	2012	42.0	400	14.0	20	Not provided	100 cycles with 15 min of 0.5 M NaOH	29
KANEKA KanCapA™	Kaneka	2012	51.0	240	8.9	20	Not provided	100 cycles with 15 min of 0.5 M NaOH	29
Eshmuno® A	Millipore	2013	45.0	400	14.3	20	mAb	Cleaning stability pH 1.5–13.5	30
Toyopreal® AF-rProtein A HC-650F	Tosoh	2013	102.3	120	51.0	2	10 mg/mL mAb		52
Toyopreal® AF-rProtein A HC-650F	Tosoh	2013	124.2	60	26.2	2	10 mg/mL mAb		52
Toyopreal® AF-rProtein A HC-650F	Tosoh	2013	143.3	24	10.7	2	10 mg/mL mAb		52
Toyopreal® AF-rProtein A HC-650F	Tosoh	2013	58.7	400	15.7	19.5	3 mg/mL mAb	15 min with 0.1 M NaOH	53
Toyopreal® AF-rProtein A HC-650F	Tosoh	2013	52.2	400	35.4	8.4	3 mg/mL mAb	15 min with 0.1 M NaOH	53
Toyopreal® AF-rProtein A HC-650F	Tosoh	2013	36.2	400	76.3	3.5	3 mg/mL mAb	15 min with 0.1 M NaOH	53
Provance™ Protein A	Grace	2014	28.0	600	36.5	10	Not provided		54
Provance™ Protein A	Grace	2014	38.0	300	20.4	10	Not provided		54
CIM® n or r-Protein A-1 TUBE MONOLITHIC COLUMN (Monolith)	BIA Separations	2014	10.0	357	107.1	1.2	1.0 mg/mL human IgG 20 mM Tris-HCl buffer, pH 7.4	Cleaning in place pH 1–13	55

TABLE 1. *Continued*

Protein A Resin	Manufacturer	Launch Year	Capacity (g/L) at 10% BT	Flow Rate (cm/h)	Productivity (g/L/h)	Bed Height (cm)	Feed Information	Caustic Stability	Reference
CIM® r-Protein A-1 TUBE MONOLITHIC COLUMN (Monolith)	BIA Separations	2014	4.2	8	5.0	0.3	Human polyclonal IgG		56
CIM® r-Protein A-1 TUBE MONOLITHIC COLUMN (Monolith)	BIA Separations	2014	3.7	30	17.2	0.3	Human polyclonal IgG		56
CIM® r-Protein A-1 TUBE MONOLITHIC COLUMN (Monolith)	BIA Separations	2014	3.2	80	39.9	0.3	Human polyclonal IgG		56
CIM® r-Protein A-1 TUBE MONOLITHIC COLUMN (Monolith)	BIA Separations	2014	2.7	160	70.4	0.3	Human polyclonal IgG		56
MabSelect SuRe™ pcc	GEHC	2015	60.0	250	19.2	10.0	Human polyclonal IgG	Short-term pH 2–13.7	57

*Productivity is defined as the grams of mAb that can be purified per hour per liter of protein A resin included loading, wash, elution, and regeneration. Productivity was calculated with Eq. 2 using the resin capacity and flow rate data assuming a mAb titer of 1 g/L and 10, 3, and 5 column volumes of wash, elution, and regeneration, respectively.

Figure 1. Protein A resin productivity as a function of product launch date.

Productivity was determined by the amount of grams of antibody that could be purified on a liter of protein A resin within the time required for column loading (assuming a 1 g/L titer), washing (10 CV), elution (3 CV), and regeneration (5 CV). Data are connected by solid (solid and dotted when there are two studies in the same year) lines for studies providing capacities at multiple flow rates. A compounded annual productivity growth rate of 4.3 ± 1.4% and an initial value Y_0 of 5.8 ± 2.4 g/L/h was determined by fitting all data except the membrane adsorber and monolith data to Eq. 3. Resins are depicted with squares and membrane adsorbers and monoliths are depicted by crosses.

Protein A capacity growth

The improvement in protein A capacity vs. year is plotted in Figure 2. A compounded annual capacity growth rate of 5.5 ± 1.2% and an initial value Y_0 of 7.2 ± 2.6 g/L was determined by fitting all data points except the membrane adsorber and monolith data to Eq. 3.

Operational flow rate growth

The improvement in operational flow rate vs. year is plotted in Figure 3. In contrast to protein A capacity and productivity, operational flow rate increased between 1978 and 2001, as new rigid and highly porous beads were introduced,[24] and then remained constant or declined.

Protein A sales growth

There is limited data describing the growth in protein A production and sales in the literature or in the annual reports of the major protein A resin producers: GE Healthcare, Millipore, and Applied Biosystems. However, Repligen Incorporated, the largest supplier of protein A ligand to the resin manufacturers, provided the percent of revenue from sales to GE healthcare, the largest protein A producer, and Applied Biosystems in annual reports. In addition, Repligen provided data for total protein A ligand sales from 2003 to 2011. These sales included protein A ligands used for production resins and small-scale research and diagnostic purposes. Sales to GE Healthcare grew from $310,800 in 1999 to $24,148,373 in 2014. A compounded annual sales growth rate of 21 ± 2% and an initial value Y_0 of

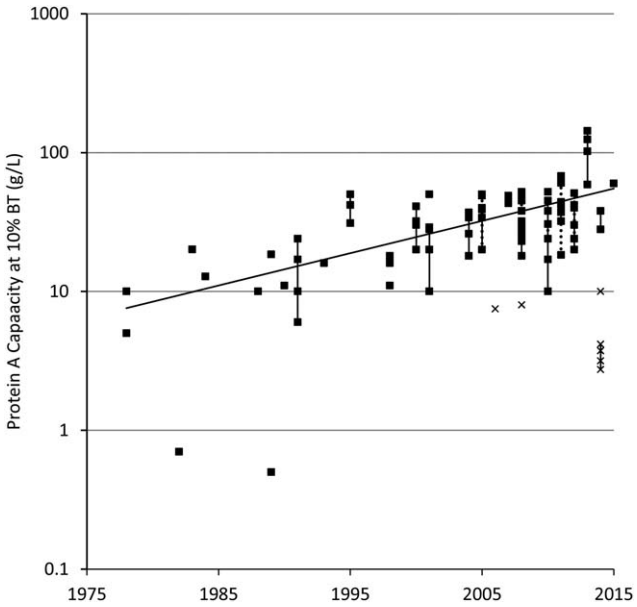

Figure 2. Protein A capacity at 10% breakthrough as a function of protein A launch date.

Data are connected by solid (solid and dotted when there are two studies in the same year) lines for studies providing capacities at multiple flow rates. A compounded annual capacity growth rate of 5.5 ± 1.2% and an initial value Y_0 of 7.2 ± 2.6 g/L was determined by fitting all data except the membrane adsorber and monolith data to Eq. 3. Resins are depicted with squares and membrane adsorbers and monoliths are depicted by crosses.

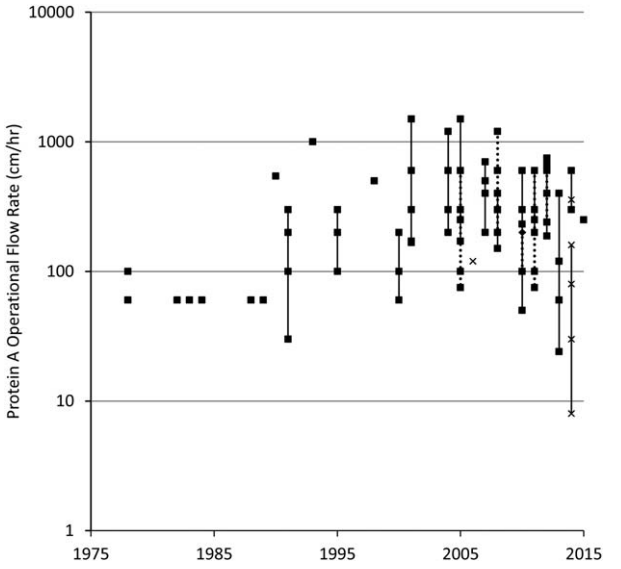

Figure 3. Protein A operational flow rate (the flow rate used for the reported capacity data) as a function of protein A launch date.

Data are connected by solid (solid and dotted when there are two studies in the same year) lines for studies providing capacities at multiple flow rates. Resins are depicted with squares and membrane adsorbers and monoliths are depicted by crosses.

$1,300,000 ± 390,000 was determined by fitting the GE Healthcare sales data to Eq. 3 and is plotted in Figure 4. This was similar to the over 20% ligand sales growth rate reported by Repligen in their 2015 annual report.

Repligen's annual reports indicated that GE Healthcare, their biggest protein A ligand customer by a large margin, primarily purchased protein A ligand for resin production.

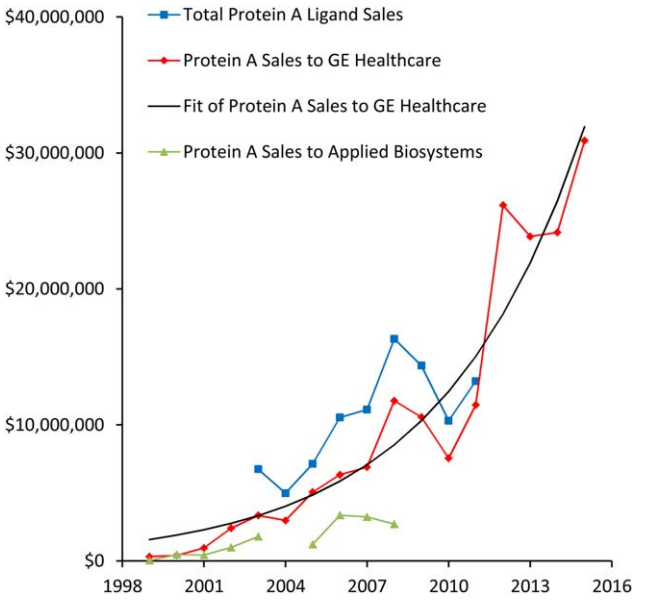

Figure 4. The growth of Repligen protein A ligand sales.

A compounded annual sales growth rate of 21 ± 2% and an initial value Y_0 of $1,300,000 ± 390,000 was determined by fitting the GE Healthcare sales data to Eq. 3. Though these sales included protein A ligands used for small-scale research and diagnostic purposes, Repligen's annual reports indicated that GE Healthcare, primarily purchased protein A ligand for resin production.

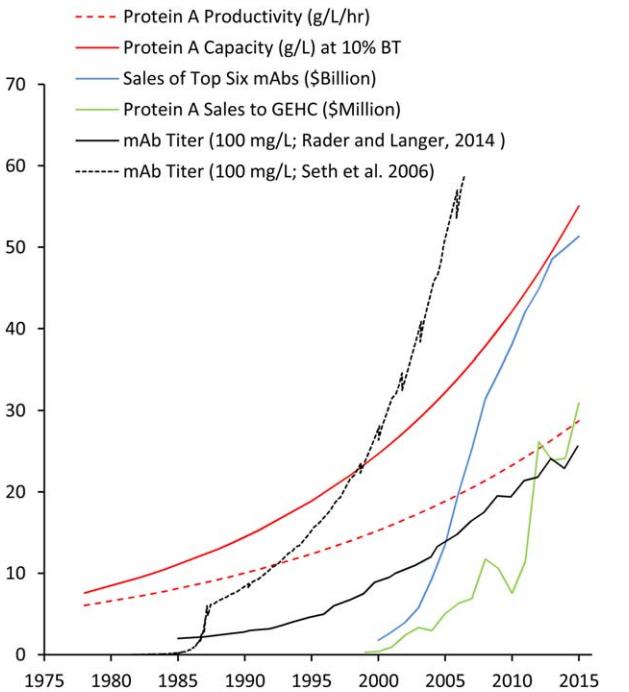

Figure 5. The growth of protein A productivity, protein A capacity, mAb titer, protein A sales, and mAb sales vs. year.

mAb sales were determined from the sum of the sales of the top five mAbs (Avastin®, Humira®, Rituxan®, Remicade®, Herceptin®) and Enbrel® in each year. The growth curves that best fit the protein A productivity and capacity data in Figures 1 and 2, respectively, are plotted here. The sales of protein A ligand from Repligen to GE Healthcare in Figure 4 are plotted here.

Based on this, it is estimated here that total sales of protein A resin grew at the same rate as sales of Repligen protein A ligand to GE Healthcare: 21% between 1999 and 2014.

The growth of protein A productivity, protein A capacity, bioreactor mAb titer,[59,60] protein A sales and mAb sales were compared vs. year in Figure 5 . The growth curves that best fit the protein A productivity and capacity data in Figures 1 and 2, respectively, are plotted in Figure 5. The sales of the top five mAbs (Avastin®, Humira®, Rituxan®, Remicade®, Herceptin®) and Enbrel®[61] in each year were obtained from annual reports and then added and plotted. The sales of protein A ligand from Repligen to GE Healthcare in Figure 4 are plotted in Figure 5.

Discussion

Growth in productivity, capacity, and operational flow rate

Protein A productivity (Figure 1) and capacity (Figure 2) have increased steadily since 1978 and have improved by an order of magnitude. It is believed that protein A productivity and capacity are still improving. One indicator of this is the fact that two of the highest performing resins, MabSelect SuRe™ LX and Toyopreal® AF-rProtein A HC-650F, have been launched recently (2011 and 2013, respectively).

Surprisingly, the operational flow rate increased from 1978 to 2001 and then remained constant or declined (Figure 3). Operation at or above 1000 cm/h was first reported in 1993 and last reported in the Table 1 data in 2008. It is possible that this is because operating above 1000 cm/h provides a marginal improvement in the total time required for a protein A step. For example, at high flow rate it takes 70 min to do one protein A cycle (including the load, wash, elution, and regeneration steps), assuming a 20 cm bed height, 1000 cm/h flow rate, 40 g/L capacity, and 1 g/L titer. Given that the harvest and low pH viral inactivation steps that occur before and after the protein A step typically take more than an hour, and the antibody titers are now typically above 1 g/L, there is limited incentive to reduce the protein A cycle time below 70 min. The protein A flow rate can also be limited by pumping capacity.[42]

The highest productivity for a protein A resin always occurred at the highest flow rate in cases where data for multiple flow rates are reported. For example, the capacity of one resin was reported to be 10 g/L at 1500 cm/h and 20 g/L at 600 cm/h.[39] The higher flow rate condition is more productive on a g/L/h basis, but it is unlikely a capacity of 10 g/L would be acceptable in a manufacturing process.

It is interesting in Figure 2 to speculate on the theoretical limits of protein A capacity. Cation-exchange chromatography capacities approaching 200 g/L have been reported.[18,62] It is far easier to produce a cation-exchange resin with a high density of properly orientated ligands than a protein A resin. However, it is likely that the capacity of protein A resins will continue to increase. It is likely that the size of the protein A ligand will be further reduced by eliminating all amino acids not required for structure, expression, or binding. This would allow for an increase in ligand density on the beads and an increase in space available for mAb binding. More porous but structurally rigid beads will also increase the space available for ligand immobilization and mAb binding. The number of protein A domains per ligand can be altered to optimize resin capacity. Optimal ligand immobilization, using more selective reactions and spacer arms, can improve capacity.

Protein A monoliths and membrane adsorbers can be operated at far higher flow rates and shorter bed heights than packed columns and can therefore provide very high productivity.[63,64] They have a number of disadvantages however that have prevented their use in manufacturing. They are currently only available up to the 8 mL scale and have low capacities (2.7–10 g/L in this study) and therefore dilute elution peaks.[65] For example, if three column volumes are used for elution, the elution pool concentration would range between 1 and 3 g/L. Breakthrough of mAb has been reported during loading of protein A membrane adsorbers.[66] Despite these disadvantages, they are included in this evaluation because of their potential. They may represent a format for future improvements in protein A productivity. The residence times, and therefore also the cycle times of protein A membrane adsorbers and monoliths are dramatically lower than those of columns, which would be beneficial for multicolumn chromatography operations.[67]

Growth of protein A performance in the context of the mAb industry

It is interesting to consider the reasons for protein A productivity growth and to compare it to other industries. The technology improvement in 62 different technology industries has been described to be associated mainly with cumulative production growth in that industry.[68] Since protein A resin is mainly used for the production of mAbs, the growth of protein A production will be associated with the growth of the mAb industry. The average compound annual revenue growth rate for the top five mAb products and Enbrel over the 2004–2013 period was 20%.[61] The growth rate of protein A sales (estimated here to be 21%) is higher than that reported for other advanced chromatography resins (5.8%[69]), but similar to the sales growth rate of the mAb industry (20%), which is not surprising since protein A resin is used for mAb production.

Complicating the comparison of antibody and protein A resin sales is the fact that mAb revenues have increased in part due to higher drug prices, which would not be associated with increased protein A use. This is possibly compensated for by the growing use of protein A resin to make clinical phase antibodies which do not generate mAb revenue.

The mAb titer in bioreactors has increased from about 500 mg/L in 1987 to 5 g/L in 2006 in one study,[60] an annual growth rate of 14%. In another study,[59] the titer increased from 0.2 g/L in 1985 to 2.64 g/L in 2015, also an annual growth rate of 14%, which is higher than the rate of growth of protein A productivity (4.3%). This difference is not surprising because limitations in titer and bioreactor capacity were a significant threat to the early growth of the therapeutic mAb industry.[70,71] Limitations in protein A productivity never threatened mAb industry growth and required large development investments. In addition, since roughly 2000, revenue from protein A sales have been far lower than revenue from mAb sales (Figure 5). Because of this, the financial resources available to protein A vendors for product improvements are far below the resources available to mAb producers to improve bioreactor titers.

It is notable in Figure 5 that protein A productivity and mAb titer both increased rapidly starting in about 1990, with the mAb titer increasing faster than protein A productivity. This coincides with the beginning of early clinical development of the highest selling mAbs. The sales of protein A and therapeutic mAbs started to increase rapidly about ten

years later in 2000. This gap roughly coincides with the time required to perform all clinical testing, obtain regulatory approval, and develop the market for a mAb. The fact that growth in bioreactor titer and growth in protein A performance happened before growth in sales indicates that investments in development were occurring at risk assuming that future sales would be strong.

Conclusions

While the sequence of steps used for mAb purification has not changed since the first therapeutic antibody was licensed, the performance of protein A chromatography, as indicated by capacity and productivity has steadily improved. Protein A productivity and capacity have increased 4.3 and 5.5% a year, respectively, since 1978 and are likely to continue to increase. The improvements have been caused in part by improvements in bead rigidity, size, polymers, and pore structure. In addition, changes in ligand immobilization, the number of protein A domains, and the protein A amino acid sequence have led to improvements.

In contrast, protein A operational flow rate increased between 1978 and 2001 and then remained constant or declined as further improvements provided only marginal benefits. The productivity of protein A resin rapidly improved starting in about 1990, in parallel with improvements in bioreactor titer, to economically provide material for clinical trials. The sales of protein A resin, as indicated by sales of the protein A ligand (21% growth), have closely paralleled the sales of mAbs (20% growth). Both increased rapidly in 2000 after the first major mAbs were approved and the markets were developed. It is likely that the many alternatives to protein A chromatography that have been explored have not been implemented because of the steady improvement in protein A performance.[35] Protein A membrane adsorbers and monoliths have higher productivity than packed columns due to their short bed heights and high operational flow rates. These devices are not currently practical for large-scale manufacturing, but may represent a format for future improvements in protein A productivity.

Acknowledgments

The authors acknowledge the help and assistance of Ganesh Vedantham, Paul O'Neill, John Royce, and Alejandro Becerra-Arteaga for their contributions to this work.

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Manuscript received May 24, 2016, and revision received July 3, 2016.