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Comparison of protein A affinity sorbents

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Abstract

Protein A is a popular generic ligand for purification of monoclonal and recombinant antibodies. The performance of 15 commercially available protein A media was studied. Equilibrium and dynamic binding capacity for human IgG was determined and the capture of IgG from a crude feed-stock was investigated. For initial screening the dynamic binding capacity was determined at small scale. Media with good performance were further tested with increased column height. Comparing the data from the two different column heights it could be shown that the dynamic capacity strongly depends on the residence time. Agarose based media exhibited higher binding capacity at residence times longer than 3 min whereas polymeric media or media based on porous glass showed a lesser dependence on the flow velocity and the residence time. A quantitative description of this behavior was derived by determination of the adsorption isotherms and fitting the breakthrough profiles with the Thomas solution. Agarose based media exhibited higher maximum equilibrium binding capacities and the dissociation constants derived from adsorption isotherms were smaller. The other media exhibited higher apparent rate constants, indicating a faster mass transfer. This can be explained by the smaller particle diameter of these media and it can be assumed that constant pattern conditions are thereby obtained more quickly. Selectivity was tested by performing antibody purification under standardized conditions. Polyclonal human IgG in cell culture supernatant containing 2.5% fetal calf serum was used as a representative feed-stock. Under the applied conditions several sorbents showed very tight binding of IgG and in some cases most of the sample remained on the sorbent. The study can be useful as a guide for optimization of large-scale purification processes.

Keywords: Affinity sorbents; Binding studies; Dynamic binding capacity; Breakthrough curves; Thomas solution; Protein A; Proteins

1. Introduction

Protein A, a protein anchored in the cell wall of *Staphylococcus aureus* [1], has the ability to selectively interact with immunoglobulins [2]. The architecture of the full-length protein A molecule is characterized by five homologous domains and one cell wall associated domain [3,4]. Immunoglobulin (Ig) G binds to protein A via its Fc region [5,6]. The interaction appears to be characterized by hydrophobic interaction together with some hydrogen bonds and two salt bridges [7]. High selectivity and high physicochemical stability make it a preferred generic ligand for IgG purification. The selectivity for IgG varies with species and subclasses [8]. Human IgG is bound with high affinity except for IgG3, which is weakly bound [2]. Sample loading can be affected in the presence of sodium chloride, avoiding excessive conditioning of the feed stream

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by dilution or diafiltration [9]. Salt ions even promote binding of IgG to protein A through its hydrophobic nature. Elution conditions may vary with subclasses and species. Especially for monoclonal and recombinant antibodies, desorption conditions have to be individually optimized. Protein A releases most IgG molecules at low pH, using a typical elution buffer such as glycine–HCl at pH 3.0 [10]. The conformational integrity of antibodies may be partially compromised under such conditions. For many antibodies it has been shown that they can be desorbed at pH 4.0 or even higher pH [9].

Initially, protein A was produced by the *KowanI* strain of *S. aureus* [11]. The protein had to be extracted from the cell wall. Later a strain was found which secreted protein A into the culture supernatant [12]. With advance of recombinant DNA technology, protein A was expressed as a fragment without the cell wall domain using *E. coli* as host [13–16]. Protein A has been immobilized to all chromatographic supports suited for protein chromatography [17]. Most popular initially was protein A immobilized on CNBr activated Sepharose CL 4B. This medium exhibited high selectivity and low non-specific adsorption but due to the nature of the support the packed bed was soft and did not allow high flow rates. This medium has been largely replaced by more highly cross-linked Sepharose for large-scale applications. Nowadays, protein A sorbents based on porous glass, coated porous polystyrene materials [18], gel-filled mineral materials and other types are available [19–21]. These materials are rigid and can be operated at high flow velocities. Sepharose materials have recently been further improved to obtain a highly porous material that is also mechanically stable. Highly porous materials exert a low mass transfer resistance, low pressure drop and high dynamic binding capacity [22]. These various features are mutually exclusive to some extent. A highly porous medium could have good mass transfer characteristics but low equilibrium capacity due to limited surface area, and bad flow properties due to its softness. Media with high equilibrium capacity might have increased mass transfer resistance.

Recombinant DNA technology allows the industrial production of chimeric and humanized antibodies for a range of clinical applications. Thus a generic purification strategy is desirable. Despite the numerous reports on purification of antibodies of various kinds and species, there are only limited comparative studies in the literature. Fuglistaller [23], Fahrner et al. [18] and Godfrey et al. [20] compared various materials. Such reports may originate from internal screening and optimization studies of chromatography materials for a specific purification scenario. Here we report on a comparative study on all commercially available protein A matrices manufactured for lab scale and process chromatography.

2. Materials and methods

2.1. Buffers and proteins

All buffer ingredients were from Merck (Merck, Vienna, Austria). Human polyclonal IgG was obtained from Octapharma, Vienna, Austria.

2.2. Instrumentation and stationary phases

All chromatography experiments were performed on a fast protein liquid chromatography (FPLC) system (Amersham Biosciences, Uppsala, Sweden). The UV signal and the conductivity signal were acquired by a chromatography data acquisition system with automatic A/D conversion (Analytical Series 900 from PE Nelson System, Cupertino, CA, USA). The digital data were transformed into an ASCII format and further processed with the computer program Sigma Plot 2001 (SPSS, Erkrath, Germany). The stationary phases used in this study are listed in Table 1.

2.3. Dynamic binding capacity

For all experiments HR 5 columns (Amersham Biosciences, I.D. 5 mm) were used. Approximately 0.5 ml of each sorbent was filled into the column and allowed to settle overnight. For the longer columns the amount of each sorbent was ~2.0 ml. Finally columns were packed at a flow velocity of 750 cm/h. As equilibration buffer PBS incomplete (0.05 *M* sodium phosphate, 0.15 *M* NaCl; buffer A), pH 7.5 was used. As elution buffer 0.1 *M* glycine–HCl,

Table 1
Properties of protein A affinity media investigated in this study

Sorbent	Lot No.	Manufacturer	Matrix	Mean particle diameter (μm)	Ligand density (mg/ml)
rProtein A Sepharose Fast Flow	256997	Amersham Biosciences, Uppsala, Sweden	Crosslinked agarose	90	4–6
Protein A Sepharose 4 Fast Flow	255240	Amersham Biosciences	Crosslinked agarose	90	4–6
MabSelect	288113	Amersham Biosciences	Crosslinked agarose	85	n.a.
IPA-500	DG032498-1	RepliGen Corp., Waltham, MA, USA	Crosslinked agarose	90	n.a.
Protein A Ceramic HyperD F	7270	Biosepra, Villeneuve la Garenne Cedex, France	Polyacrylamide gel in ceramic macrobead	50	4–5
Prosep-A High Capacity	0180001	Bioprocessing, distributed by Millipore, Watford, UK	Porous glass	75, irregular shape	n.a.
Prosep-rA High Capacity	107009	Bioprocessing	Porous glass	75, irregular shape	n.a.
Poros 50 A High Capacity	A250-021	PerSeptive Biosystems, Framingham, MA, USA	Polystyrene-divenylbenzene	50	n.a.
UltraLink Immobilized Protein A Plus	98040762	Pierce, Rockford, IL, USA	Polymeric	60	n.a.
UltraLink Immobilized Protein A	98061064	Pierce	Polymeric	60	n.a.
Affi-Gel Protein A Gel	59072B	Bio-Rad, Hercules, CA, USA	Crosslinked agarose	n.a.	2
Affi-Prep Protein A Support	60163E	Bio-Rad	Polymeric	n.a.	2
Protein A Agarose 4XL	FA0287	Affinity Chromatography Ltd., Freeport, Isle of Man, UK	Crosslinked agarose	n.a.	n.a.
Protein A Cellthru 300	98AB45	Sterogen Bioseparations, Carlsbad, CA, USA	n.a.	200–300	n.a.
AF-Protein A Toyopearl 650 M	65PRM82Y	Tosoh Biosep, Stuttgart, Germany	Polymethacrylate	80	n.a.

n.a., not available.

pH 3.5 (buffer B) and as regeneration buffer 0.1 *M* glycine–HCl, pH 2.5 (buffer C) were used. All buffers were filtered (0.2-μm filter) and degassed prior to chromatography. The column was regenerated with buffer C before the first sample was applied. Then the column was equilibrated with 6 column volumes (CVs) of buffer A. After sample loading the gel was immediately regenerated with 6 CVs of buffer C and then equilibrated with another 6 CVs of buffer A. Polyclonal human IgG stock solution was diluted to a concentration of 0.4 mg/ml with PBS. At each run 75 ml (150 CVs) were loaded onto the gel, which corresponds to 60 mg IgG loaded per ml gel. For the longer columns the corresponding feed volume was 250 ml (125 CVs). The operating flow velocities were 50, 100, 200, 300, 400, 500, 600 and 700 cm/h. All experiments were performed at room temperature.

2.4. Adsorption isotherm

A 0.5-ml amount of each sorbent was packed into an HR 5 column and breakthrough curves (BTCs) were developed with six different concentrations (3.0, 2.5, 2.0, 1.5, 1.0 and 0.4 mg IgG/ml) at a flow velocity of 100 cm/h. The chromatography set-up and data handling were identical to the experiments for dynamic binding capacity. Breakthrough was determined at 50% and the equilibrium binding concentration was extrapolated from this value assuming a symmetrical BTC.

2.5. Selectivity

A 0.5-ml sample of each regenerated and equilibrated sorbent was loaded with 5 ml spiked culture medium (RPMI 1640, Biochrom, Germany) con-

taining 1 mg IgG/ml and 2.5% fetal calf serum. After washing with 4 CVs of buffer A, elution and regeneration with 8 CVs of buffer B and C was performed. The fractions were analyzed by sodium dodecylsulfate–polyacrylamide gel elecrophoresis (SDS–PAGE) with 4–20% acrylamide gradient gels in a Xcell Mini-Cell (Novex Experimental Technology, San Diego, CA, USA). Samples were boiled with 2% SDS for 10 min and 20 μl were loaded on the electrophoresis gel. After separation with 125 V for 90 min the proteins were silver stained [24].

3. Theory

Adsorption equilibrium was described by a Langmuir type adsorption isotherm [25]:

$$q = \frac{q_{\max} C}{K_d + C}$$

with q being the stationary phase concentration of adsorbate (IgG) in equilibrium with the mobile phase concentration of adsorbate (IgG), $q_{\max}$ the maximal equilibrium binding capacity and K_d the equilibrium dissociation constant. Breakthrough curves were approximated by the Thomas solution originally developed for ion exchange [26]. A Langmuir rate expression is combined with convection and accumulation terms, neglecting axial dispersion. The boundary conditions are that the column is initially devoid of adsorbate and that the concentration of the applied adsorbate is constant. The model accounts for total mass transfer effects by empirically including them in apparent rate constants. An analytical solution adopted by Chase [27] to describe affinity separations can be written in the following form:

$$\frac{C}{C_0} = \frac{J(n/r, n\mathrm{T})}{J(n/r, n\mathrm{T}) + [1 - J(n, n\mathrm{T}/r)] \exp[(1 - r^{-1})(n - n\mathrm{T})]}$$

where

$$r = 1 + C_0/K_d$$

with C_0 being the feed concentration,

$$n = q_{\max} k_{on} h A_c / f$$

with $q_{\max}$ being the maximum equilibrium binding capacity, k_{on} the apparent association rate constant, h the column length, A_c the cross-sectional area and f the volumetric flow rate,

$$T = (K_d + C_0/q_{\max})[(ft/A_c h) - \epsilon]$$

with K_d being the equilibrium dissociation constant, t time and ϵ the void fraction of the column.

According to Thomas [26] values of $J(\alpha,\beta)$ can be calculated more easily from an asymptotic series, when α and β are large. The first two terms of the series are:

$$J(\alpha,\beta) = \frac{1}{2} \cdot \left\{ 1 - \mathrm{erf}(\sqrt{\alpha} - \sqrt{\beta}) + \frac{\exp[-(\sqrt{\alpha} - \sqrt{\beta})^2]}{\sqrt{\pi}[\sqrt{\beta} + (\alpha\beta)^{1/4}]} \right\}$$

The error of this function is 1% accuracy when $\alpha \cdot \beta > 36$. BTCs were approximated with this function and apparent k_{on} calculated.

4. Results and discussion

The objective of the study was the comparison of the most commonly used protein A affinity sorbents. Godfrey et al. [20], Fahrner et al. [18] and Fugistaller [23] have previously reported comparative studies of protein A sorbents. These studies did not include the most recently developed materials, nor mass transfer characteristics and selectivity. For economic reasons we decided to use polyclonal human IgG as model protein. The whole study consumed ~15 g IgG. Scientific arguments were also taken into consideration. A single monoclonal antibody does not represent the broad spectrum of IgG binding properties of the various antibodies to protein A. We decided to use human polyclonal IgG from conventional blood plasma fractionation.

Initially, for rapid screening, we decided to carry out the column experiments on a very small scale, which saved time and material, and allowed us to repeat the experiments. A 0.5-ml sample of each medium was packed into an HR 5 column and connected to an FPLC system. We are aware that at this scale the dynamic capacity may be dependent on the column geometry. The total running time was 600 h for this first set of experiments. Using a high

feed concentration would have accelerated the experiments, but the precision would also have decreased, since the breakthrough volume would have become very low. If we increased the column size by a factor of ten, we would not be able to carry out the study within a reasonable time frame. Therefore we have standardized our experimental set-up to focus on direct comparison of the different gels; this included column packing, extra column space, flow rate, sample concentration and data handling. A total of 15 different protein A sorbents have been compared (Table 1). The BTCs at different velocities have been normalized according to initial feed concentration (C_0) and superimposed for each sorbent. The BTCs of all tested gels are shown in Fig. 1. Polyclonal IgG is composed of four IgG subclasses: 56% IgG1, 34% IgG2, 6% IgG3 and 4% IgG4. IgG3 is not bound by protein A. In order to compensate for the early breakthrough of IgG3, dynamic binding capacity (DBC) was determined by neglecting the first plateau of the BTCs. The dynamic capacity was estimated manually by determination of the volume corresponding to 2.5% breakthrough (V2.5). By multiplying the V2.5 with the initial feed concentration the dynamic capacity is obtained. The dynamic capacity has been plotted versus the linear flow velocity and these plots are shown in Fig. 2.

MabSelect showed the highest dynamic capacity among all tested sorbents at 50 and 100 cm/h, corresponding to a residence time greater than 1.5 min. At higher flow rates, Poros 50 A exhibited a significantly higher dynamic capacity compared to all other sorbents. Ultralink Protein A and Ultralink Protein A Plus had a significantly lower DBC than media shown in Fig. 2A,B. Affi-Gel Protein A, Affi-Prep Protein A, Protein A Agarose 4XL, Protein A Cellthru 300 and Protein A Toyopearl showed an extremely low dynamic capacity. At a flow rate of 200 cm/h and higher these sorbents did not exhibit a significant DBC at all (Fig. 2C).

After this initial screening, only sorbents with a reasonable DBC were selected for further studies. At a column height of 2.5 cm it can be assumed that constant pattern conditions are not reached for those media which exhibit slower mass transfer; whether caused by small pore size or large particle diameter. Once constant pattern conditions are reached, the shape of the BTC will be independent of the column length [22]. Thus the media were packed into columns with 10-cm bed height and BTCs were performed (Fig. 3). From these curves DBCs were calculated and plotted against linear flow velocity. Agarose based media showed higher DBCs along a broader velocity range compared to the small column data (Fig. 4). The other materials exhibited almost identical performance compared with the small column data. At flow velocities higher than 300 cm/h DBCs were higher for those media. This suggests that the design of a large-scale separation has to be carefully adjusted regarding residence time. Either column length or velocity can be varied. In Fig. 5A two examples demonstrate that the residence time (t) is an important criterion. At $t<3$ min Poros 50 A has a higher DBC, whereas when $t>3$ min rPrA Sepharose FF has a higher DBC. t was calculated from the experiments with different column heights and velocities. In Fig. 5B DBC versus residence time has been plotted for the eight different media which had been selected for the further investigations. At residence times higher than 3–5 min MabSelect and rPrA Sepharose FF exhibit superior DBCs over all other media. This can be explained by the high equilibrium binding capacity and the low dissociation constants (Table 2 and Fig. 6), and on the other hand, by the larger particle diameter, which requires longer residence times for mass transfer. The productivity of agarose based media was higher at low flow velocities due to higher DBCs whereas the non-agarose based media exhibited higher productivity at high flow velocities. The same column life time has been assumed in this reasoning.

To extract quantitative data from the BTCs we approximated them with the well known Thomas solution. This model requires the maximum binding capacities and the equilibrium dissociation constants. Therefore adsorption isotherms have been determined and fitted with the Langmuir isotherms. The isotherms are shown in Fig. 6. Maximum binding capacities at equilibrium for agarose based media are 55–67 mg/ml whereas the corresponding values for the other media are in the range of 40 mg/ml. MabSelect, rPrA Sepharose FF and Prosep-rA exhibited the steepest isotherms. The presentation of the ligand by oriented immobilization through a C-terminal cystein or a polylysine tail results in a

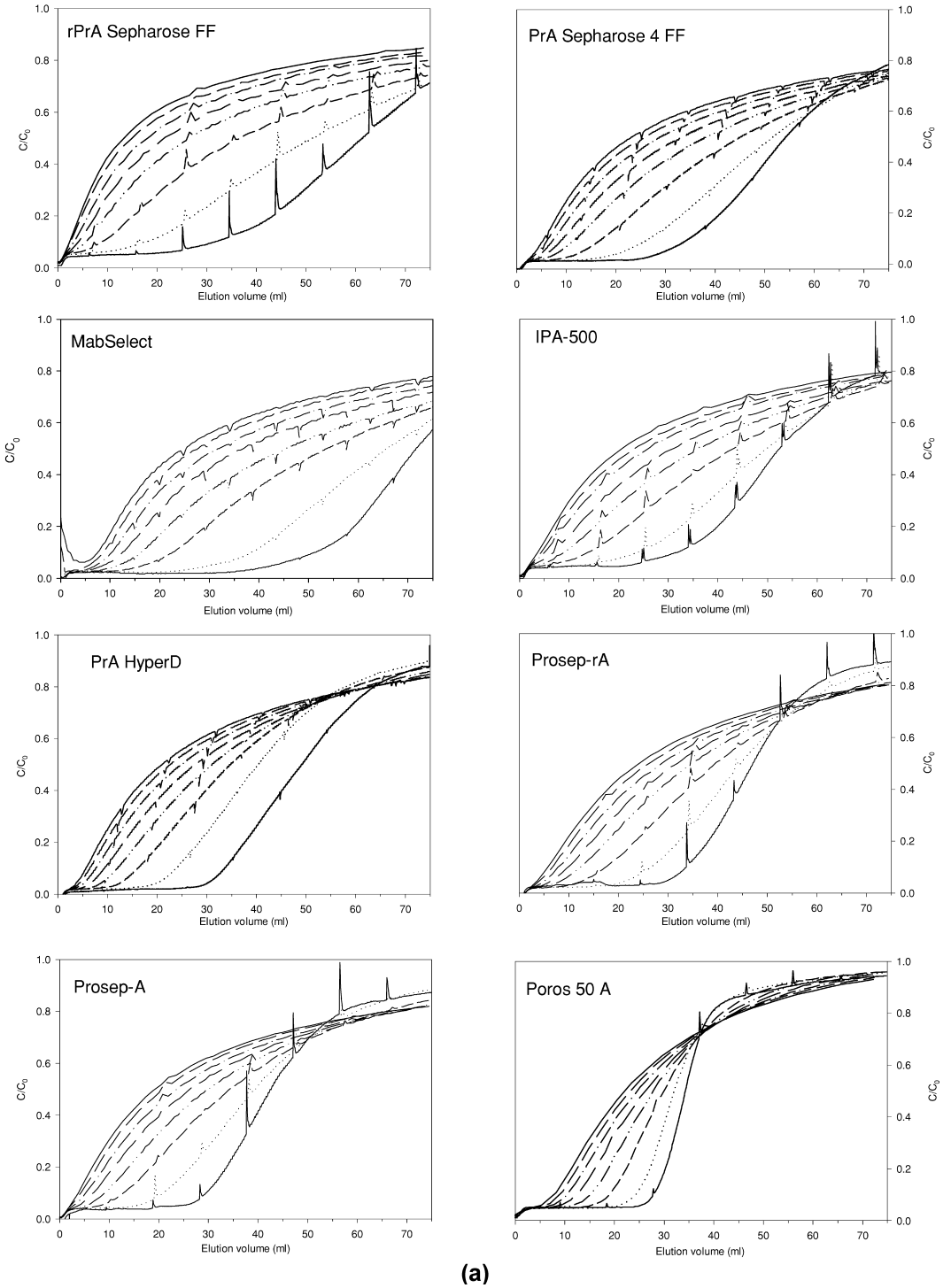
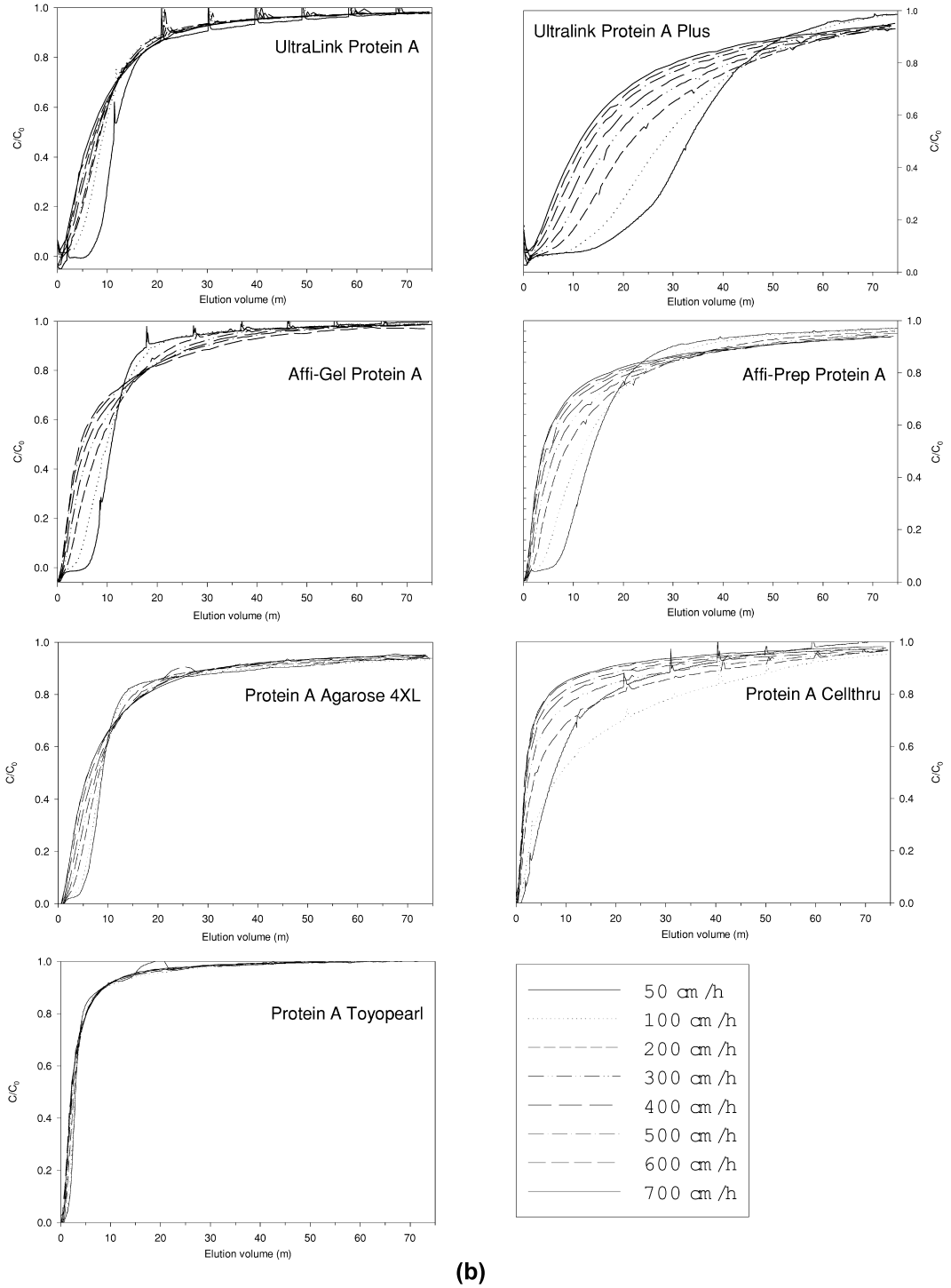
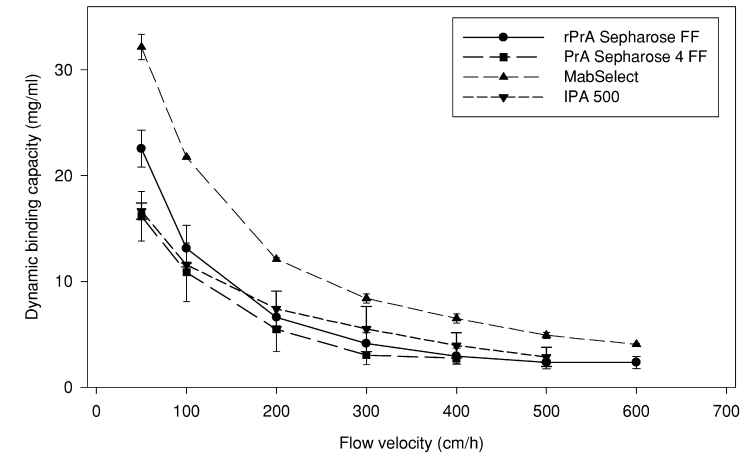


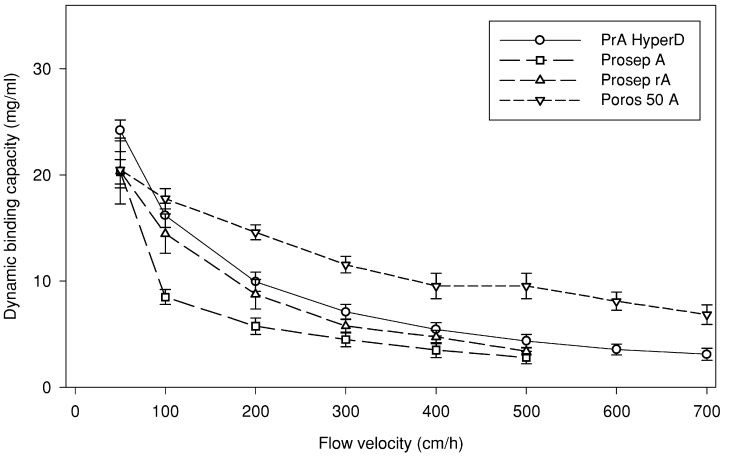
Fig. 1. Breakthrough curves of protein A affinity media at a column height of 2.5 cm. IgG was loaded at a concentration of 0.4 mg/ml. PBS buffer at pH 7.5 was used as running buffer. All chromatographic runs were performed at room temperature. The flow velocity ranged from 50 to 700 cm/h, corresponding to residence times between 0.2 and 3 min.



(b)

Fig. 1. (*continued*)





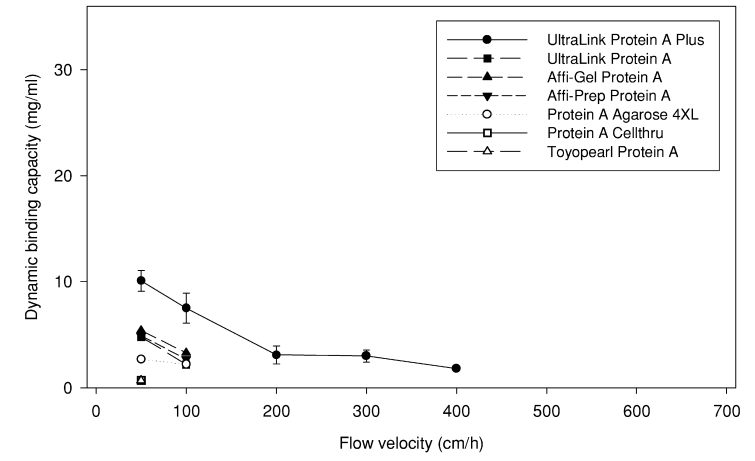


Fig. 2. Dynamic binding capacities for IgG determined at 2.5% breakthrough. Data were determined from breakthrough curves at a column height of 2.5 cm (Fig. 1).

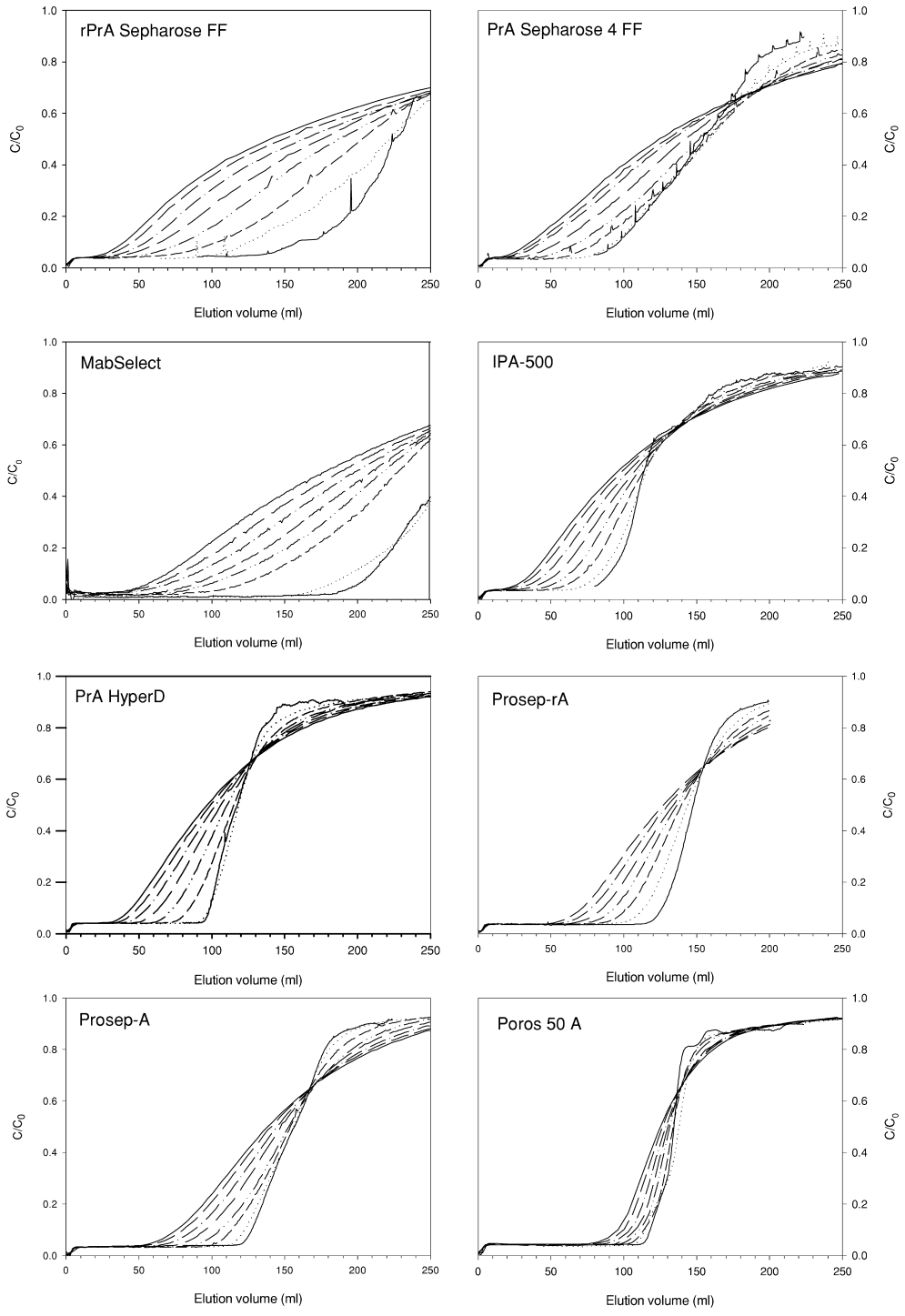


Fig. 3. Breakthrough curves of protein A affinity media at a column height of 10 cm. IgG was loaded at a concentration of 0.4 mg/ml. PBS buffer at pH 7.5 was used as running buffer. All chromatographic runs were performed at room temperature. The flow velocity ranged from 50 to 700 cm/h, corresponding to residence times between 0.86 and 12 min.

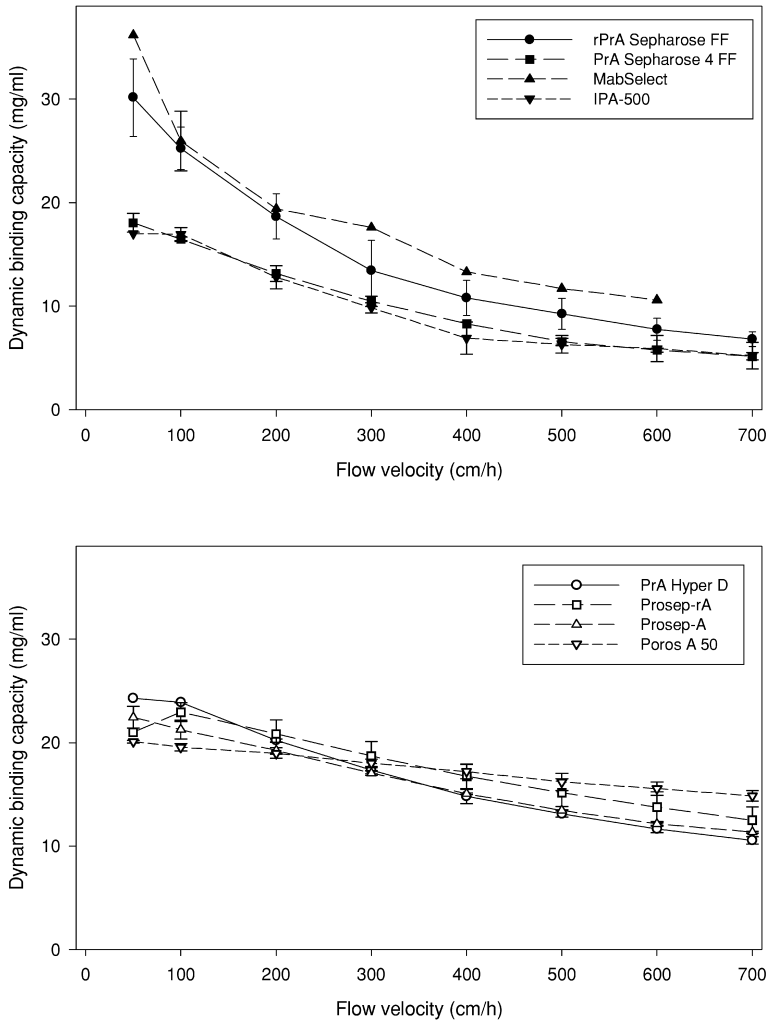


Fig. 4. Dynamic binding capacities for IgG determined at 2.5% breakthrough. Data were determined from breakthrough curves at a column height of 10 cm (Fig. 3).

very low dissociation constant (K_d), derived from the isotherm. Furthermore, from the approximation with the Thomas solution an apparent k_{on} value was obtained. This lumped parameter includes both mass transfer resistance and kinetics of adsorption. The sharper breakthrough of the non-agarose based media is reflected by the high apparent k_{on} rates. These media had smaller particle diameters or in the case of the irregular shaped Prosep material, shorter diffusional pathways. It can be assumed that for a large molecule such as IgG, pore diffusion is the most relevant mass transfer resistance. Since the effective pore diffusivity depends on the square of the particle diameter, the overall mass transfer rate is higher for media with small particle diameter. On the other hand, small particles give rise to a higher pressure drop. In Table 2 all parameters obtained from the adsorption isotherm and the approximation with the Thomas solution are listed. Apparent k_{on} values as a function of the flow velocity are shown in Fig. 7. Poros 50 A showed significantly higher k_{on} values compared to all other media. It has been shown by Chase [27] that with these parameters the performance of an affinity column can be excellently pre-

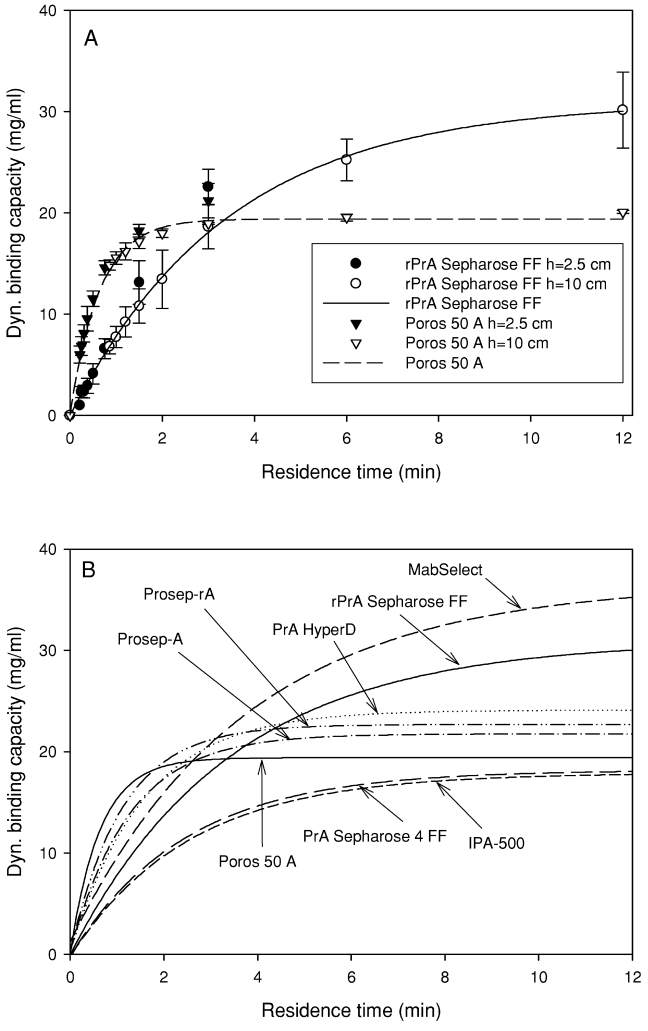


Fig. 5. Dynamic binding capacity for IgG dependent on the residence time determined at two column heights (2.5 and 10.0 cm) and different velocities (50–700 cm/h). (A) Data points and regression for rPrA Sepharose FF and Poros A 50. (B) Regression for all sorbents.

Table 2
Equilibrium and kinetic constants for protein A affinity media

	q_{max} (mg/ml)	K_d (mg/ml)	K_d ($M\times10^{-6}$)	k_{on} (M^{-1} s^{-1}) at 100 cm/h	k_{on} (M^{-1} s^{-1}) at 500 cm/h
rPrA Sepharose FF	55.1±1.5	0.037±0.007	0.25	35	110
PrA Sepharose 4 FF	61.6±1.7	0.11±0.09	0.73	72	214
MabSelect	67.3±1.6	0.032±0.007	0.21	66	174
IPA-500	64.8±3.3	0.31±0.70	2.1	101	186
PrA Hyper D	41.7±1.0	0.15±0.02	1.0	165	375
Prosep-A	37.7±0.7	0.091±0.012	0.61	156	332
Prosep-rA	38.8±1.0	0.044±0.012	0.29	200	401
Poros 50 A	42.2±1.1	0.16±0.03	1.1	240	810

q_{max} and K_d were determined from the Langmuir isotherm, k_{on} from the approximation using the Thomas solution.

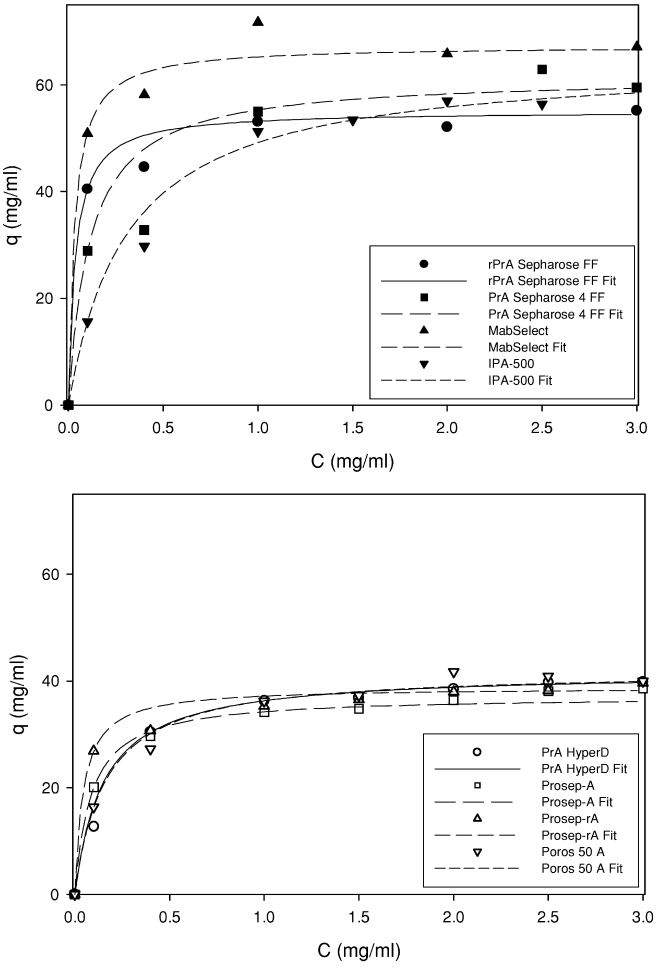


Fig. 6. Adsorption isotherms for IgG on protein A affinity media. The data points were approximated with the Langmuir isotherm.

dicted, and thus they can be used to simulate scale-up scenarios. Further modeling and additional experiments are required to quantitatively distinguish the different mass transfer mechanisms and the kinetics of adsorption.

For the comparison of the selectivity of the media, an operational approach was used. Cell culture medium was spiked with polyclonal IgG. This material was loaded onto the different sorbents and after excessive washing the bound material was eluted with a defined buffer. The column was again equilibrated and the residual material was desorbed by a regeneration buffer. The regenerated gel was boiled with SDS buffer and the supernatant was analyzed together with the other peak fractions by SDS–PAGE. From these experiments the non-specific adsorption, elution behavior and regeneration behavior can be seen.

The chromatograms of the various experiments are shown in Fig. 8. The conductivity profile is only shown for rPrA Sepharose. At the time of the start of the study, rPrA Sepharose FF was used as a reference material. For rPrA Sepharose FF, PrA Sepharose 4 FF, Poros 50 A, Prosep-rA and Prosep-A an almost identical profile could be observed. The onset of elution of IgG with PrA HyperD was similar to the previously mentioned sorbents, but the peak was broader and showed a significant shoulder. Such behavior suggests a need to use a lower pH for the elution buffer. Poros 50 A elutes in a smaller volume

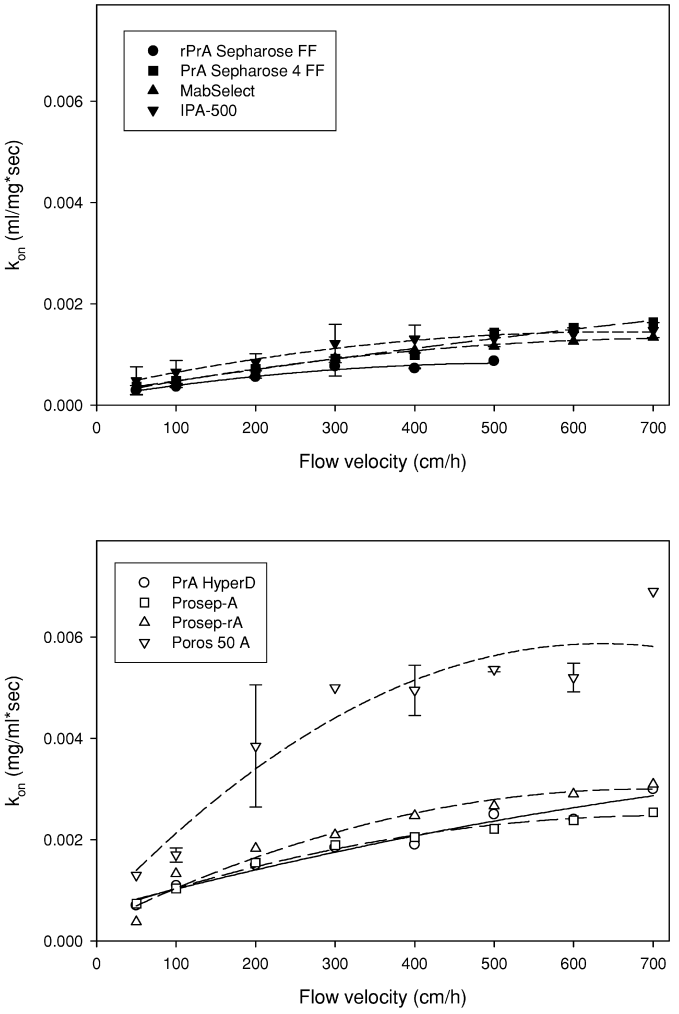


Fig. 7. Apparent rate constants (k_{on}) dependent on the flow velocity. The data were derived by fitting the breakthrough curves at 10-cm column height using the Thomas solution.

compared to rPrA Sepharose which can again be explained by the smaller particle diameter. IPA-500 elutes with the same volume but slightly retarded. Regeneration of most of the tested sorbents did not yield a large peak, but differences can be seen by SDS–PAGE (Fig. 9). After regeneration an aliquot of the medium was further investigated by SDS–PAGE.

The boiled Poros 50 A sorbent gel shows residual IgG and BSA or fragmented IgG. The 60-kDa band cannot be seen with the other media. UltraLink Protein A, UltraLink Protein A Plus and Protein A Toyopearl bind IgG very tightly. The boiled sorbent of Protein A Toyopearl exhibits an extreme smear on the gel indicating aggregated and fragmented IgG sticking on the gel after regeneration. Affi-Prep Protein A and Affi-Gel Protein A elute in a very broad peak which is retarded compared to rPrA Sepharose. These sorbents could not be completely regenerated. Here we also want to emphasize that silver staining is not a quantitative method. The method is very sensitive, however, and small traces of impurities may appear as intensely stained bands. Nevertheless, these experiments provide useful in-

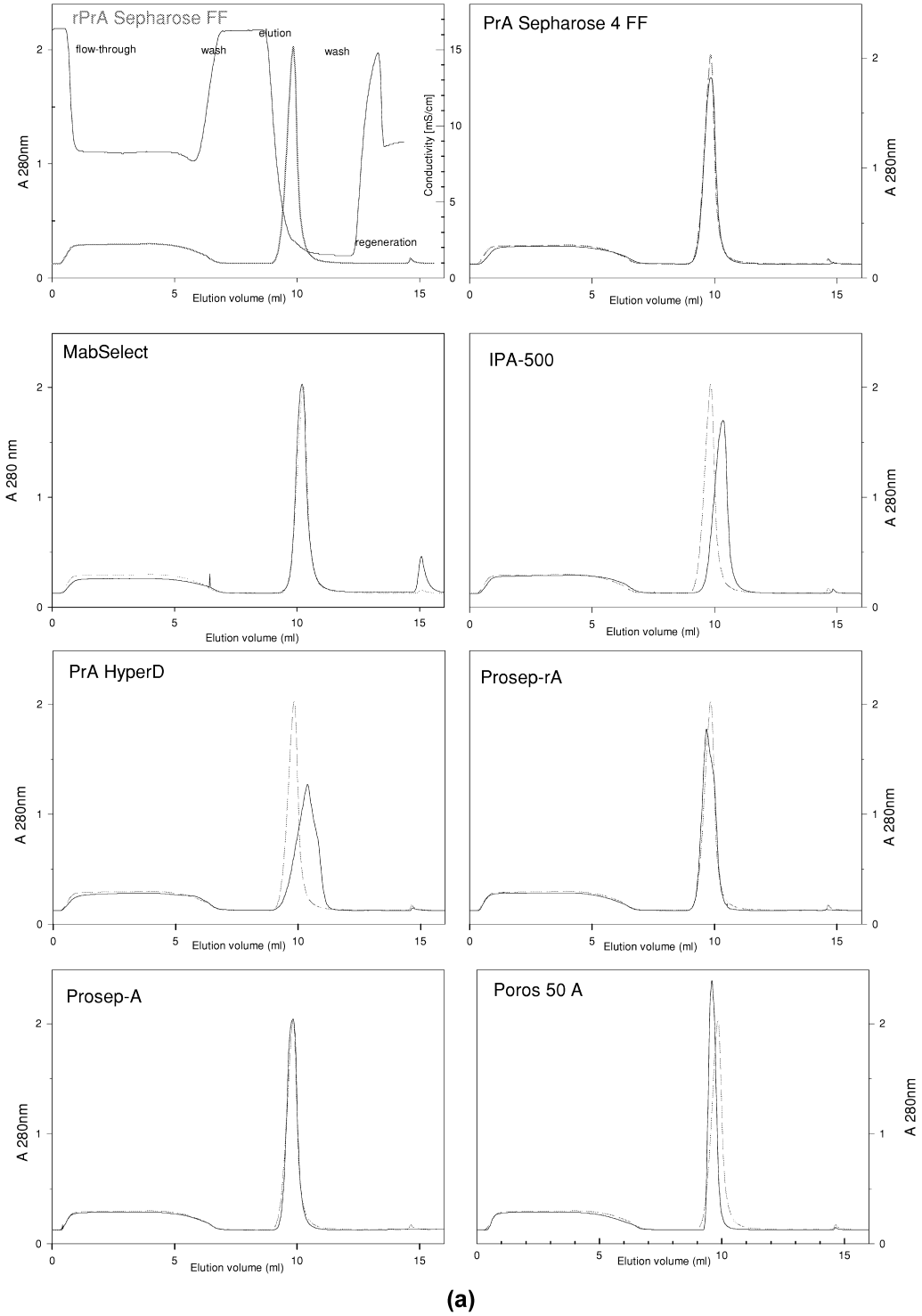
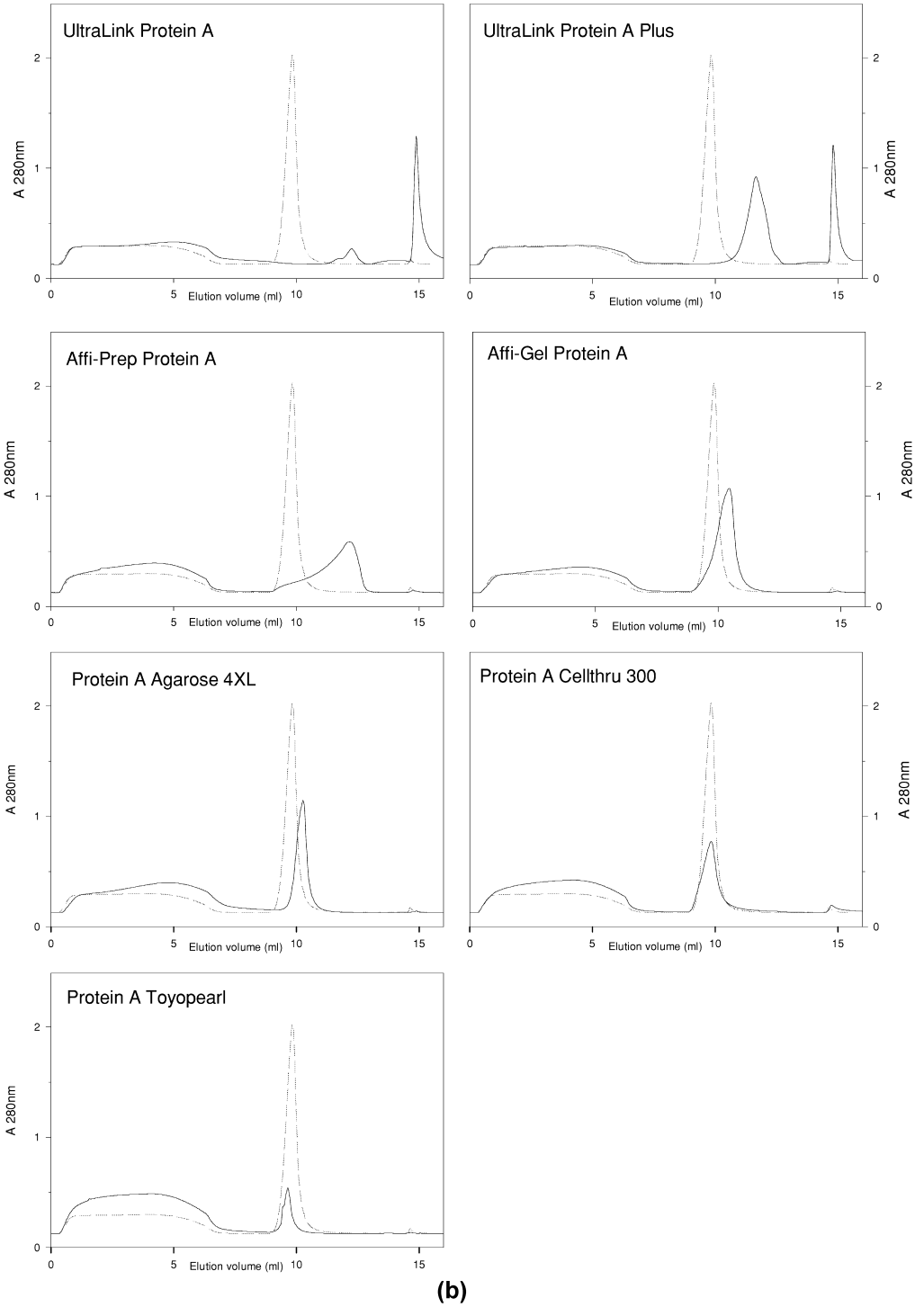


Fig. 8. Purification of human polyclonal IgG from cell culture supernatant containing 2.5% fetal calf serum. Elution was carried out at pH 3.5, and regeneration was carried out at pH 2.5. The chromatogram of rPrA Sepharose FF was superimposed as a reference.



(b)

Fig. 8. (*continued*)

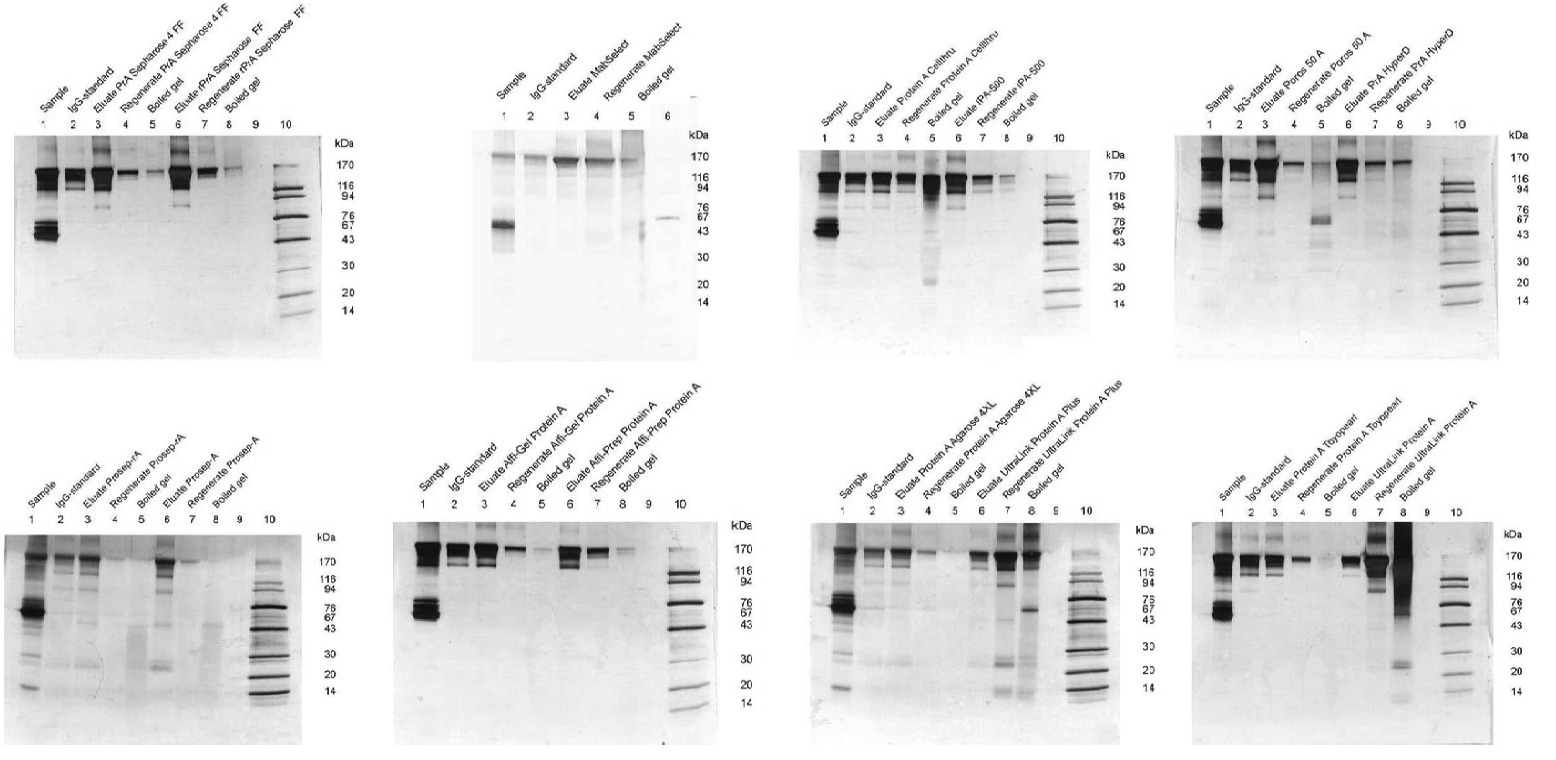


Fig. 9. SDS–PAGE of samples collected from the purification of IgG from cell culture supernatant. The gels were run under non-reducing conditions. The sample is cell culture supernatant spiked with 1 mg/ml human polyclonal IgG. kDa, molecular mass $(M_r)\cdot 10^{-3}$.

formation to optimize elution and regeneration conditions for individual sorbents and feed-stocks.

In conclusion, a large selection of protein A affinity sorbents is available for separation of IgG. Several media were found which were not suited for preparative application. Initial screening of sorbents for the binding capacity must be carefully interpreted. The experiments only provide a crude estimate of the performance. For scale up and engineering, the DBC as a function of the residence time must be considered. In an industrial process, a residence time of 3 min and higher is very relevant. In this range two agarose based media showed higher DBCs compared to the other media. We have also shown that calculations are useful with parameters from the Langmuir isotherm and the Thomas model. These parameters could easily be used for the prediction of scale-up scenarios. The overall performance of an IgG process depends also on the composition of the feed-stock, which impacts the regeneration and the life length of the column. We have presented an operational approach which can help to optimize the elution and the regeneration conditions. For industrial application pressure drop is an additional important issue not addressed in this work. Nevertheless, the methods presented here have allowed a reasonable comparison of protein A affinity media which can be used as a base for optimization and scale up of methods for the capture of human monoclonal antibodies.

5. Nomenclature

A_c	Cross-sectional area of column
C	Concentration of adsorbate in solution
C_0	Initial concentration of adsorbate
f	Volumetric flow rate
h	Column height
J	J-function in the Thomas solution
K_d	Equilibrium dissociation constant
k_{on}	Rate constant for binding between adsorbent and adsorbate
q	Binding capacity of sorbent
q_{max}	Maximum binding capacity of sorbent
ϵ	Void fraction

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