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Comparison of protein A affinity sorbents II. Mass transfer properties

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Abstract

Protein A affinity chromatography is the standard purification method for isolation of therapeutic antibodies. Due to improvements in expression technology and optimization of fermentation, culture supernatants with high antibody content must be processed. Recently protein A affinity media with improved adsorption characteristics have been developed. The agarose media MabSelect Xtra and MabSelect SuRe are recent developments of the existing protein A affinity medium MabSelect. MabSelect Xtra is designed to exhibit a higher binding capacity for IgG, and MabSelect SuRe is functionalized with an alkaline stabilized protein A. ProSep-vA Ultra is a porous glass medium with a pore size of 70 nm, also developed to improve the binding capacity. Adsorption was measured in a finite and infinite bath. Mass transfer in these systems could be well described by a model including film and pore diffusion. Mass transfer parameters were used to accurately predict IgG breakthrough in packed bed mode. The dynamic binding capacity of all three media did not change when residence time was at least 4 min. All three media are suited for capture of feed stocks with high antibody content.

Keywords: Staphylococcus protein A; Antibody; Immunoglobulin; Pore diffusion; Film diffusion

1. Introduction

Affinity chromatography with staphylococcal protein A as ligand is a standard unit operation for antibody purification at both laboratory and industrial scales [1,2]. The method has become popular due to high purification efficiency, stability and the possibility to develop generic purification platforms for purification of human antibodies with the subclasses 1, 2 and 4 [3]. In the past, the protein A ligand has been attached to a large variety of different base matrices such as cross-linked agarose, surface modified porous glass, coated polystyrene, hydrogel filled into a ceramic shell and other materials based on organic polymers [4–9]. Many of these matrices suffer from low binding capacity and are thus not suited for industrial applications. As previously reported, eight different sorbents were found well suited for large scale operation due to their high binding capacities, good mass transfer properties and other operational characteristics required for production of biopharmaceuticals [5]. Due to increasing economic pressure on monoclonal antibody production, feedstock expression levels are continuously increasing, already reaching up to 3 g/l [10]. Such a development requires protein A media with higher binding capacity and better mass transfer properties than the existing commercially available affinity chromatography media. Another demand from industry has been the caustic stability to allow efficient CIP procedures. Thus protein A ligands have been developed by selective substitution of asparagine residues in the protein A molecule in order to improve stability in an alkaline environment [11]. To facilitate a high dynamic binding capacity (DBC), a careful balance between particle size, pore size and ligand density is required. As a rule-of-thumb, large pores enable fast mass transfer but also reduced surface area, which may lead to lower equilibrium capacities. High ligand densities result in high capacities but reduced mass transfer, since the immobilized protein A is

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a large molecule and reduces the pore diameter when immobilized in excess.

We compared three different media with improved binding capacity: MabSelect Xtra, and MabSelect SuRe (GE Healthcare, Uppsala, Sweden), and ProSep-vA Ultra (Millipore, Consett, UK). MabSelect Xtra is based on highly cross-linked agarose and has a higher ligand density and a larger pore size than MabSelect, but with decreased particle size. MabSelect SuRe is also based on a highly cross-linked agarose, functionalized with a modified protein A produced by genetic engineering and having increased stability in alkaline solutions compared to the wild-type protein. ProSep-vA Ultra is based on specifically surface modified porous glass. In this development the surface area was increased by reduction of pore size compared to ProSep vA-high capacity.

Principally, mass transfer properties of chromatography media can be studied in two different modes. Uptake kinetics can be measured in a finite [12] or infinite bath [13]. The first is typically a batch adsorption in an agitated vessel or in an rotated tube. The latter can be simulated in so-called shallow bed reactor, a micro-column with only approximately 5–20 μl stationary phase. Parameters characterizing the morphology of the stationary phase are required to fit the uptake curves by an appropriate model assuming various mass transfer and/or kinetic resistances. These parameters include particle diameter, intraparticle porosity and optionally the pore size. The particle diameter can be calculated from pressure drop measurements, coulter counter measurements, or visual inspection from scanning electron microscopy. The intraparticle porosity is determined by pulse experiments in packed beds by injecting inert tracer substances, one that is totally excluded from the pores and one that can completely penetrate the pores [14]. Especially in case of affinity chromatography, the ligand density is also an important parameter to help study the adsorption process [4,15].

We determined intraparticle transport rates of the new protein A affinity chromatography media. Based on these results prediction of breakthrough curves were carried out and then compared to experimental runs. The improvements will be discussed in context of previously reported data on existing versions of protein A affinity media.

2. Materials and methods

2.1. Chromatography media

MabSelect Xtra, MabSelect SuRe and MabSelect media were from GE Healthcare and Prosep-vA Ultra, ProSep-vA High Capacity ProSep media from Millipore.

2.2. Chemicals

Ingredients for buffer preparation were purchased from Sigma–Aldrich (Vienna, Austria). Human polyclonal IgG, which was used for all adsorption experiments, was a kind gift from Octapharma Pharmazeutische Produkte (Vienna, Austria).

2.3. Batch adsorption

A 50% gel slurry (v/v) of the respective sorbents was prepared in equilibration buffer (PBS). Gel samples were drawn by quickly pipetting the corresponding volume of suspension of the desired gel amount from a homogenized gel slurry into an IgG solution. The sorbents were gently rotated for 3 h. Samples were drawn from the experimental stock solution at different time intervals and analyzed by measuring the UV absorbance on a spectrophotometer at 280 nm to determine the IgG concentration. Additional stocks were analyzed after 3 h to determine the equilibrium capacity.

2.4. Shallow bed adsorption

Shallow bed experiments were performed as described by Lewus et al. [13]. Approximately 10 μl of individual gel samples were transferred into HR 5 columns (GE Healthcare) and connected to an ÄKTA explorer 100 equipped with a P 960 sample pump (GE Healthcare). The system was configured in a way that buffer streams were recycled. The IgG solution was passed through the bed at a flow rate of 4 ml/min which corresponds to a linear velocity of 1224 cm/h. After a specified time interval, desorption of IgG was perfomed with a 0.1 M glycine buffer at pH 2.5. The elution peak was integrated and the amount of IgG was calculated from a calibration curve. By varying the contact times the kinetic of adsorption can be constructed. The exact volume of the bed was determined by comparing the equilibrium uptake capacity of the shallow bed with known equilibrium values determined by other methods (batch adsorption, column experiments).

2.5. Column chromatography

All chromatography runs were performed on an ÄKTAexplorer 100 system at room temperature. The capacity studies were performed in XK 16 columns (GE Healthcare) with a bed height of 11–12 cm, the gel volumes varied between 22 and 24 ml. Columns were slurry-packed at a velocity of 800 cm/h. For breakthrough curves, a 20 mM $Na–PO_4$, 150 mM NaCl, pH 7.3 buffer (PBS incomplete, buffer A) was prepared as equilibration buffer. Elution buffer was 0.1 M glycine pH 3.0 (buffer B) and regeneration buffer was 0.1 M glycine pH 2.5 (buffer C). Polyclonal IgG, a gift from Octapharma, Vienna, Austria was prepared as feed stock. The stock solution (50 mg/ml) was diluted with PBS to a concentration of 0.4 and 1.0 mg/ml. For longer runs all buffers and the feedstock were supplemented with 0.05% sodium azide in order to avoid bacterial growth.

The columns were first regenerated with two column volumes (CV) buffer C and then equilibrated with 2CV buffer B. After sample loading bound IgG was desorbed with 3CV buffer B and then regenerated again with 2CV buffer C. Flow

rates were adjusted in a way to cover residence times from 1 to 12 min. The data evaluation accounted for the fact polyclonal IgG contains approximately 5–6% IgG 3 which does not bind to Protein A. The immediate breakthrough was subtracted from the total absorbance and the concentration was corrected individually for each run.

Pulse response experiments were performed by injecting 100 μl pulses of 1 M sodium nitrate in Na–PO_4 buffer containing 0.5 M sodium chloride at flow velocities from 50–600 cm/h. 100 μl pulses of 2 mg/ml blue dextran were performed in PBS buffer containing 20% ethanol at 50 cm/h. Pulses with IgG under non-binding conditions were performed with 0.1 M glycine buffer at pH 2.5. Peaks were fitted with an exponential modified Gaussian function to determine HETP. Blank runs were performed with empty columns to determine extra column dead volumes and band spreading. These values were subtracted from the data obtained with the packed columns.

3. Theory

3.1. Batch and shallow bed uptake

The transport of a solute within a spherical particle is described by a pore diffusion model and is given by the following equations and boundary conditions [16–19]:

$$\varepsilon_p \frac{\partial c}{\partial t} + (1-\varepsilon_p)\frac{\partial q}{\partial t} = \frac{D_e}{r^2}\frac{\partial}{\partial r}\left(r^2 \frac{\partial c}{\partial r}\right) \tag{1}$$

$$t = 0 \quad c = 0 \quad q = 0 \tag{2}$$

$$r = 0 \quad \frac{\partial c}{\partial r} = 0 \tag{3}$$

$$r = R_p \quad D_e \frac{\partial c}{\partial r} = k_f(C - c) \tag{4}$$

with c as the concentration in the mobile phase of the particle and q as the concentration in the stationary phase of the particle. The particle porosity is given as ε_p and the effective pore diffusion coefficient as D_e.

The conservation equation and the initial condition for the bulk fluid are given as

$$\frac{dC}{dt} = -\frac{3k_f}{R_p}\frac{V_m}{V}(C - c|_{r=R_p}) \tag{5}$$

$$t = 0 \quad C = C_0 \tag{6}$$

with C as the concentration in the bulk fluid, k_f as the external film mass transfer coefficient, R_p as the particle radius, V_m as the volume of particles, V as the volume of the bulk fluid and $c|_{r=R_p}$ as the concentration of the mobile phase at the particles surface. C_0 is the initial concentration of the bulk fluid.

The relationship between adsorbed phase and pore fluid concentration is defined by the Langmuir adsorption isotherm:

$$q = \frac{q_m c}{c + K_d} \tag{7}$$

with the maximum binding capacity q_m and the equilibrium dissociation constant K_d. Local equilibrium for the adsorption of protein is assumed.

For external film mass transfer control an analytical solution of Eq. (5) is given by Weaver and Carta [18]:

$$\frac{C}{C_0} = \exp\left(-\frac{3k_f}{R_p}\frac{V_m}{V}t\right) \tag{8}$$

Data points for batch adsorption at very low protein concentration were fitted to Eq. (8) to calculate to film mass transfer coefficient k_f.

For shallow-bed adsorption the same model (Eqs. (1)–(7)) can be applied as for finite bath experiments though for shallow-bed the volume of the media V_m is infinitely small compared to the volume of the protein solution V, simplifying the conservation equation for the bulk fluid (Eq. (5)) to

$$\frac{dC}{dt} = 0 \tag{9}$$

For the calculations using the data from the shallow-bed the following correlation was used for calculation of the Sherwood number

$$Sh = 1.15\left(\frac{Re}{\varepsilon}\right)^{1/2} Sc^{0.33} \tag{10}$$

using the Reynolds number Re, the void fraction ε and the Schmidt number Sc.

For nonlinear isotherms the above model needs a numerical solution. This was obtained using orthogonal collocation on finite elements. All simulations were carried out using MatLab 6.5 Release 13 (MathWorks, Aachen, Germany).

3.2. Column operation

Eqs. (1)–(4) are also used to describe the transport of a solute in a particle in column operation mode. The bulk fluid equation for batch adsorption (Eqs. (5) and (6)) are replaced by the conservation equation for a column with plug flow and can be written as

$$D_L \frac{\partial^2 C}{\partial z^2} = v\frac{\partial C}{\partial z} + \phi\frac{3k_f}{R_p}(C - C_{p,R=R_p}) + \frac{\partial c}{\partial t} \tag{11}$$

with initial and boundary conditions

$$C(t=0) = 0 \tag{12}$$

$$\left.\frac{\partial C_{b,A}}{\partial t}\right|_{z=0} = \frac{u}{D_{L,A}}(C_{b,A} - C_{feed}) \quad \text{with}$$

$$C_{feed} \neq 0 \quad \text{for} \quad 0 \le t \le t_{inject} \tag{13}$$

and

$$\frac{\partial C}{\partial z} = 0 \quad \text{for} \quad z = L \tag{14}$$

D_L is the axial dispersion, v the interstitial velocity, C the bulk fluid concentration, $C_{p,R=R_p}$ the concentration of the mobile phase at the border of the particle, k_f the film mass transfer coefficient, R_p the radius of the particle and c_{feed} the concentration of the feed during the injection time t_{inject}. Neglecting axial dispersion an analytical solution could be derived for a rectangular isotherm [20,21].

4. Results and discussion

4.1. Equilibrium uptake

Adsorption isotherms of the three novel protein A affinity media (MabSelect Xtra, MabSelect SuRe and ProSep-vA Ultra) are shown in Fig. 1. To facilitate a comparison under identical experimental conditions, Fig. 1 also includes the isotherms of MabSelect and ProSep-vA High Capacity. The Langmuir isotherm was fitted to the equilibrium capacities determined by batch uptake, shallow bed uptake and fixed bed uptake. The latter were determined by integration of the breakthrough curves obtained on small scale columns (~1 ml) fed with polyclonal IgG at a velocity of 15 cm/h. All isotherms are very favorable and can be regarded as almost rectangular. MabSelect Xtra has the highest equilibrium capacity of all media. The capacities of MabSelect SuRe, ProSep-vA Ultra and MabSelect are about 10% lower whereas that of ProSep-vA High Capacity lies about 25% below. The equilibrium dissociation constants are in the range of $\sim 5 \times 10^{-7}$ M, a typical value for strong, high selective affinity systems [22]. Table 1 provides a summary of the isotherm data.

4.2. Morphology of stationary phases

Parameters characterizing the particle morphology are required to determine mass transfer rates. Particle diameters were obtained from the manufacturers. Porosities were calculated from retention times of small samples of blue dextran, IgG and sodium nitrate, according to Eq. (15)

$$\varepsilon_b = \varepsilon + (1 - \varepsilon)\varepsilon_p \tag{15}$$

where ε_b is the total porosity, ε the void volume and ε_p the intraparticle porosity. For Prosep-vA Ultra the void fraction could not be experimentally determined because blue dextran could not be eluted from the column. Data were taken from the report by McCue et al. [6]. The value of ε_b could be confirmed with our experiments. All parameters are summarized in Table 2.

HETP was calculated from a moment analysis of the peak profiles obtained from the nitrate pulses. HETP showed the expected dependence on the velocity (Fig. 2). The packing

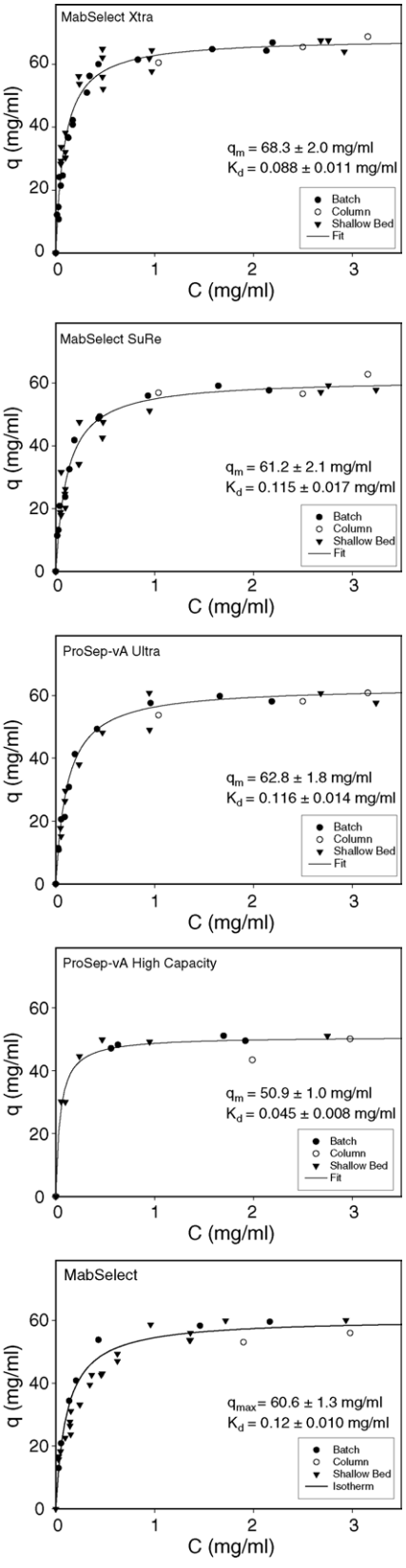

Fig. 1. Adsorption equilibrium data of polyclonal IgG adsorbed per ml protein A affinity medium. Data were derived from batch uptake, shallow bed uptake and breakthrough curves. Lines represent the Langmuir isotherm model.

Table 1
Isotherm parameters of protein A affinity media

		Protein A affinity medium				
		MabSelect Xtra	MabSelect SuRe	ProSep-vA Ultra	ProSep-vA High Capacity	MabSelect
q_m	(mg/ml)	68.3 ± 2.0	61.2 ± 2.1	62.8 ± 1.8	50.9 ± 1.0	60.6 ± 1.3
	(M)	$4.6 \times 10^{-4} \pm 1.3 \times 10^{-5}$	$4.1 \times 10^{-4} \pm 1.4 \times 10^{-5}$	$4.2 \times 10^{-4} \pm 1.2 \times 10^{-5}$	$3.4 \times 10^{-4} \pm 6.7 \times 10^{-6}$	$4.0 \times 10^{-4} \pm 8.7 \times 10^{-6}$
K_d	(mg/ml)	0.088 ± 0.011	0.115 ± 0.017	0.116 ± 0.014	0.045 ± 0.008	0.120 ± 0.010
	(M)	$5.9 \times 10^{-7} \pm 7.3 \times 10^{-8}$	$7.7 \times 10^{-7} \pm 1.1 \times 10^{-7}$	$7.7 \times 10^{-7} \pm 9.3 \times 10^{-8}$	$3.0 \times 10^{-7} \pm 5.3 \times 10^{-8}$	$8.0 \times 10^{-7} \pm 6.6 \times 10^{-8}$

Table 2
Morphology of protein A affinity media

Protein A medium	d_p (μm)	ε_b	ε_p	ε	ε_b[a]	ε_p[a]
MabSelect Xtra	75	0.92	0.87	0.38	0.70	0.52
MabSelect SuRe	85	0.92	0.88	0.36	0.69	0.52
ProSep-vA Ultra	100	0.82[b] 0.81[c]	0.68[b]	0.43[b]	0.69	0.46

[a] Porosities determined with IgG pulses under non-binding conditions.
[b] Data from McCue et al. [6].
[c] Own determinations with nitrate pulses.

quality for MabSelect Xtra and SuRe was almost identical. HETP data for ProSep-vA Ultra were about two times higher, which is due to the fact that the material is irregular shaped. Increased eddy diffusion and lower packing quality due to higher particle density is made responsible for this effect.

4.3. Kinetics of adsorption

Batch adsorption was performed in rotated tubes. In Fig. 3 the IgG uptake at different initial concentration is shown in terms of dimensionless solution concentration (C/C_0) and of the amount of protein adsorbed per ml of settled gel particles. The latter was calculated on the base of transferring 100 μl of a 1:2 slurry of gel into the reaction tube and assuming that the amount of gel corresponds to 50 μl of a packed bed. The initial stage of adsorption (200 s) at the lower concentration was fitted with Eq. (5). In this regime film mass transfer is the determent resistance [18]. We calculated a film mass transfer coefficient k_f of $3 \pm 0.5 \times 10^{-4}$ cm/s for all media. We compared this value with a correlation obtained from stirred tank experiments [19]. Our calculated values would correspond to an agitation with an 2 cm impeller at a stirrer speed of ∼30 rotation per minute, which can be regarded as very gentle stirring similar to the agitation in an rotating tube.

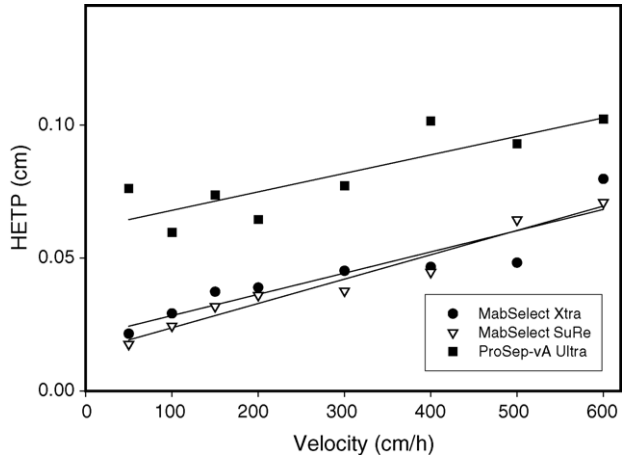

Fig. 2. HETP determined with pulse response experiments as a function of velocity. The column dimensions were 16 mm i.d. and the bed length was 11 cm.

Shallow bed uptake curves are shown in Fig. 4. The IgG solutions were passed through the columns at a velocity of 1250 cm/h. Based on Eq. (10) a k_f of 0.0021–0.0025 cm/s was calculated which is one order of magnitude higher compared to the batch systems. In the shallow bed system, since the protein concentration in the bulk fluid remains constant, and the pore liquid concentration is zero, integration of Eq. (5) assuming film mass transfer control yields

$$q = \frac{3k_f}{R_p} C_0 t \tag{16}$$

We fitted the very initial shape of the uptake curve at 0.05 and 0.1 mg/ml with Eq. (16). The obtained values of 0.003–0.004 cm/s are in reasonably good agreement with the packed bed correlation and confirm that film resistance is not important in the experimental shallow bed set-up. In fact, equilibrium is obtained somewhat faster in the shallow bed system.

The fractional saturation with time was plotted for three different feed concentrations (Fig. 5). At low feed concentrations all investigated media showed identical mass transfer rates. Although the effective pore diffusivity is highest for ProSep-vA High Capacity fractional saturation was reached at same time as the other media. ProSep-vA High Capacity has a steeper adsorption isotherm, thus at low feed concentrations more IgG can be bound. Here we observe a interdependence between affinity and diffusivity. At a feed concentration of 0.5 mg/ml the initial slope of the isotherm became of lesser influence, corresponding to a stationary phase concentration close to q_m. Then the diffusional resistance is controlling the rate of adsorption. At a feed concentration of 3 mg/ml it can be clearly seen that these novel media have been particularly designed for capture of high-concentration feed-stocks. Saturation is achieved as fast as the medium with the largest pores (ProSep-vA High Capacity) but the total flux of antibody into the beads is much higher. Uptake at low feed concentration also benefits from these improved diffusion properties. Effective diffusivities D_e were obtained by fitting the uptake curve from shallow bed experiments with a pore

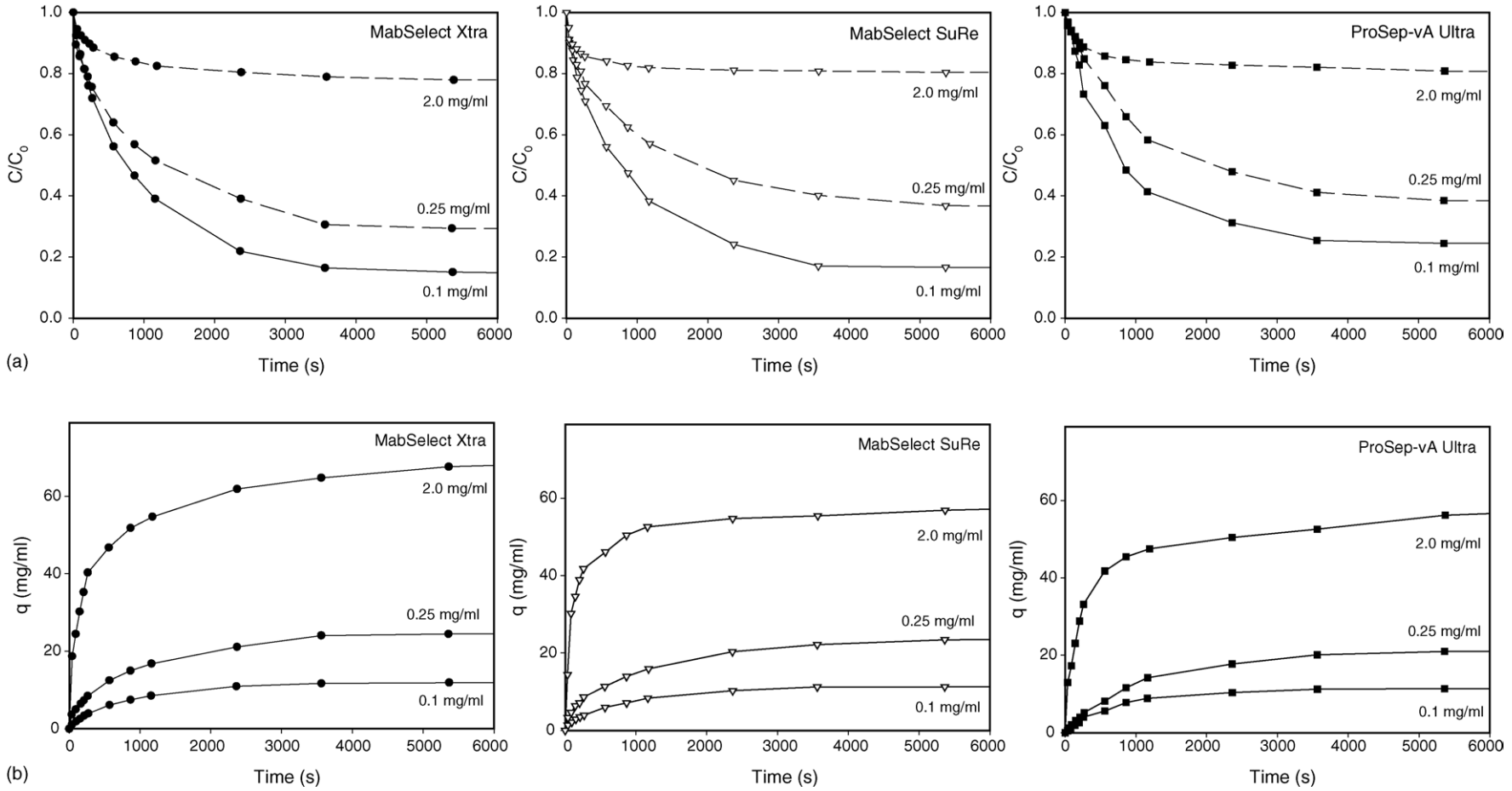

Fig. 3. Batch adsorption kinetics of IgG on protein A affinity sorbents at 2.0, 0.25 and 0.1 mg/ml. The amount of sorbent was 50 μl suspended in a reaction volume of 7 ml.

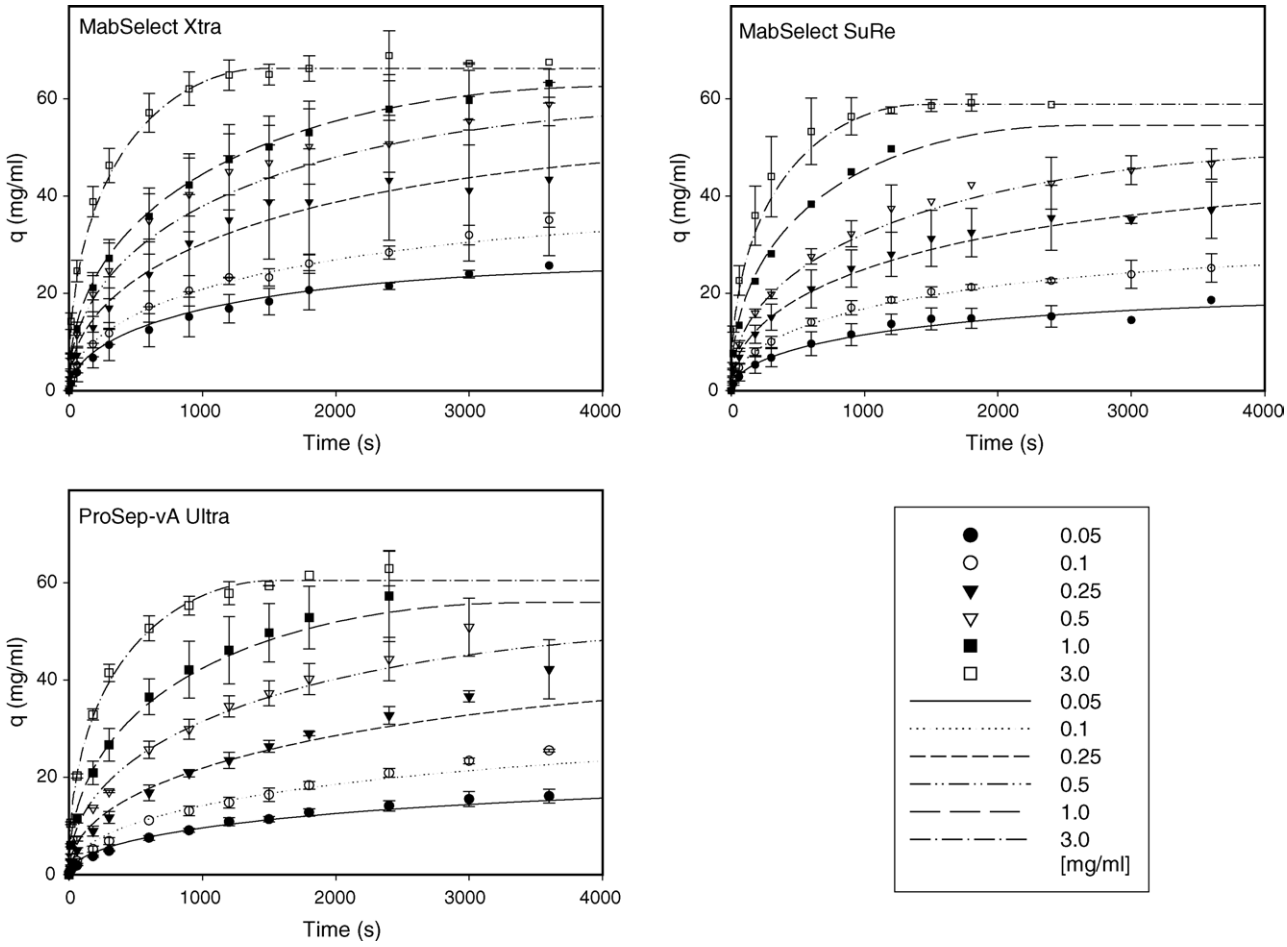

Fig. 4. Shallow bed uptake kinetics of IgG on protein A affinity media.

diffusion model as described in the theory section. Data are summarized in Table 3. We observed a slight difference of effective pore diffusion coefficients measured in our laboratory and the data provided by McCue et al. [6]. We assume that a prototype material was used which was different than the currently commercially available media. A similar observation was made with the static binding capacities.

D_e values are inversely proportional to the feed concentration. The same was observed by Mc Cue et al. [6] and Horstmann and Chase [4]. Two different effects may contribute to this observation; (a) at low feed concentration we might still not have reached equilibrium conditions and that the actual isotherm is steeper than shown in Fig. 1, which would give rise to a smaller D_e-value. This is experimentally difficult to obtain, since long incubation times were required and IgG tended to aggregate with time. (b) the mass transport mechanism might have changed with feed concentration. The applied methodology did not allow a further discrimination of transport mechanisms. Horstmann and Chase have characterized protein A affinity prototype media which were based

Table 3
Effective pore diffusion coefficients D_e ($\times 10^7$ cm^2/s) calculated from shallow bed and batch uptake curves

Conc. (mg/ml)	MabSelect Xtra		MabSelect SuRe		MabSelect		ProSep-vA Ultra			ProSep-vA HC		
	Shallow bed	Batch	Shallow bed	Batch	Shallow bed	Batch	Shallow bed	Batch	Batch[a]	Shallow bed	Batch	Batch[a]
0.05	3.5	–	3.2	–	4.0	–	2.7	–	–	20	–	–
0.1	2.2	–	2.3	–	1.5	–	1.9	–	5.1	4.5	–	81
0.25	1.2	–	1.2	–	–	–	1.1	–	–	4.5	–	–
0.5	0.8	–	0.8	–	0.5	–	1.0	–	1.8	3.5	–	2.0
1.0	0.7	–	0.8	–	–	–	0.9	–	1.6	1.5	–	2.3
2.0	–	0.4	–	0.8	–		–	0.6	1.2	–	–	1.0
3.0	0.5	–	0.5	–	0.3	–	0.6	–	1.0	1.0	–	1.2

[a] Data from McCue et al. [6].

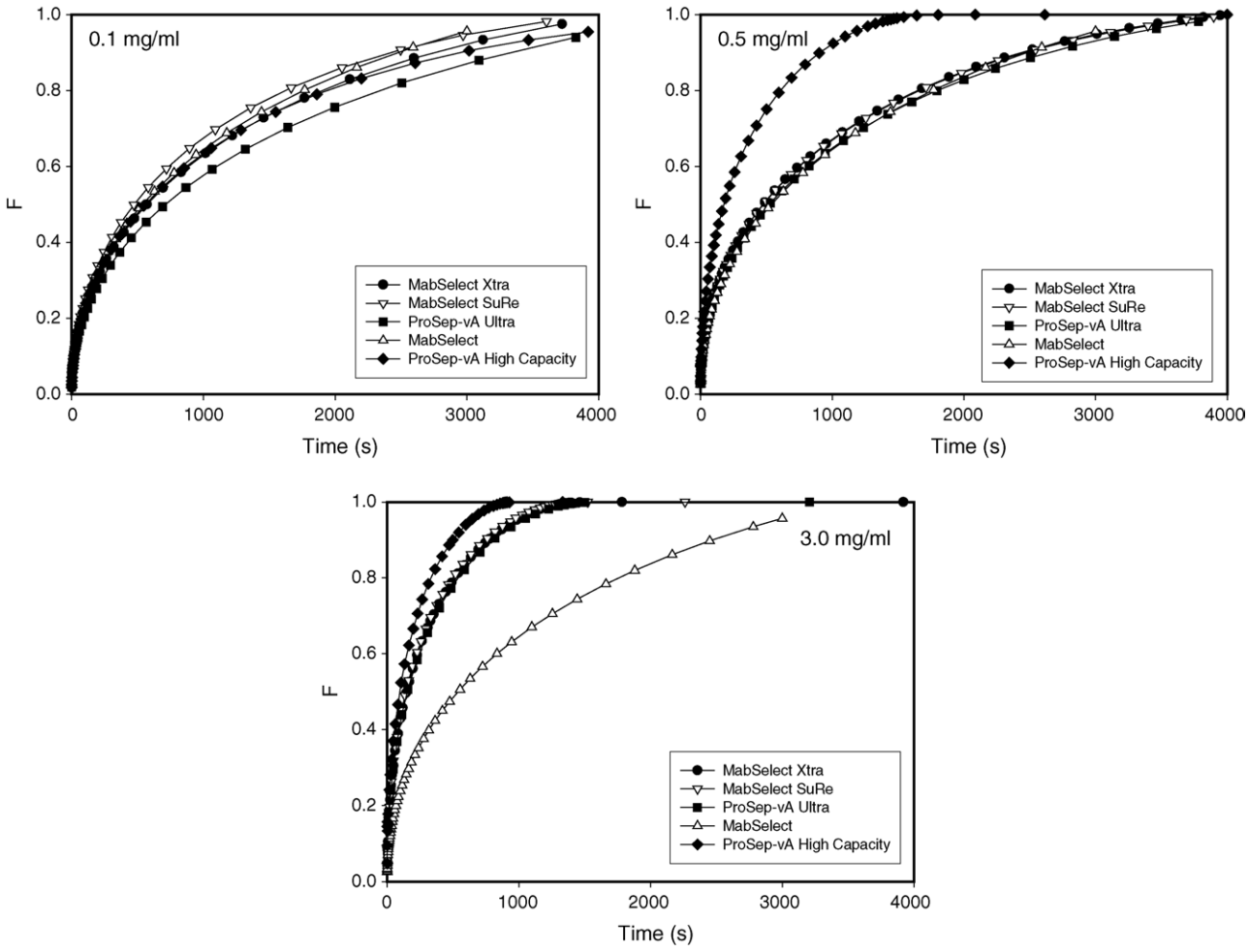

Fig. 5. Shallow bed uptake kinetics of IgG on protein A affinity sorbents plotted as fractional saturation *F* as a function of time. The data represent the numerical solution obtained from a fit to 2–4 individual shallow bed experiments.

on conventional Sepharose or Superose type. They used the same model to approximated batch uptake. Diffusivities in their case were lower indicating the advancements of the latest versions of Protein A affinity chromatography media.

4.4. Column operation

Breakthrough curves with human polyclonal IgG as feed stock were carried out at different scales. In Fig. 6 a comparison of earlier with new generation protein A media is shown. The chromatography runs were performed in HR 5 columns with identical bed height of 10 cm. Comparing the breakthrough profiles it can be seen that for MabSelect media mass transfer rates remains almost constant while increasing the capacity. For ProSep the capacity increased at the expense of a much reduced mass transfer rate indicated by a wider mass transfer zone.

The dependence of the dynamic binding capacity on flow velocity and residence time, respectively, was studied using XK 16 columns with a bed height of approximately 11 cm and a total bed volume of 22 ml. This scale was a compromise between sufficient accuracy whilst still allowing performance of the experiments within a reasonable time period. All media exhibit a comparable dependence of DBC on flow velocity (Fig. 7). At residence times higher than 4 min DBC approaches equilibrium conditions (Fig. 8) which are excellent properties for process chromatography media. With such media highly productive processes can be designed. Under static conditions ProSep-vA Ultra exerts a slightly higher capacity than MabSelect SuRe as can be seen from the adsorption isotherms. However, in a dynamic situation the capacities were reversed, and MabSelect SuRe has a higher DBC than ProSep-vA Ultra at both feed concentrations 0.4 and 1.0 mg/ml. This behavior can be explained by the larger particle diameter of ProSep-vA. Both media exhibit almost identical pore diffusion coefficients but mass transfer resistance increases with the square of the particle diameter.

A long time was required to reach the full saturation of the column (Fig. 9). This was observed for all three media in this study. According to current understanding

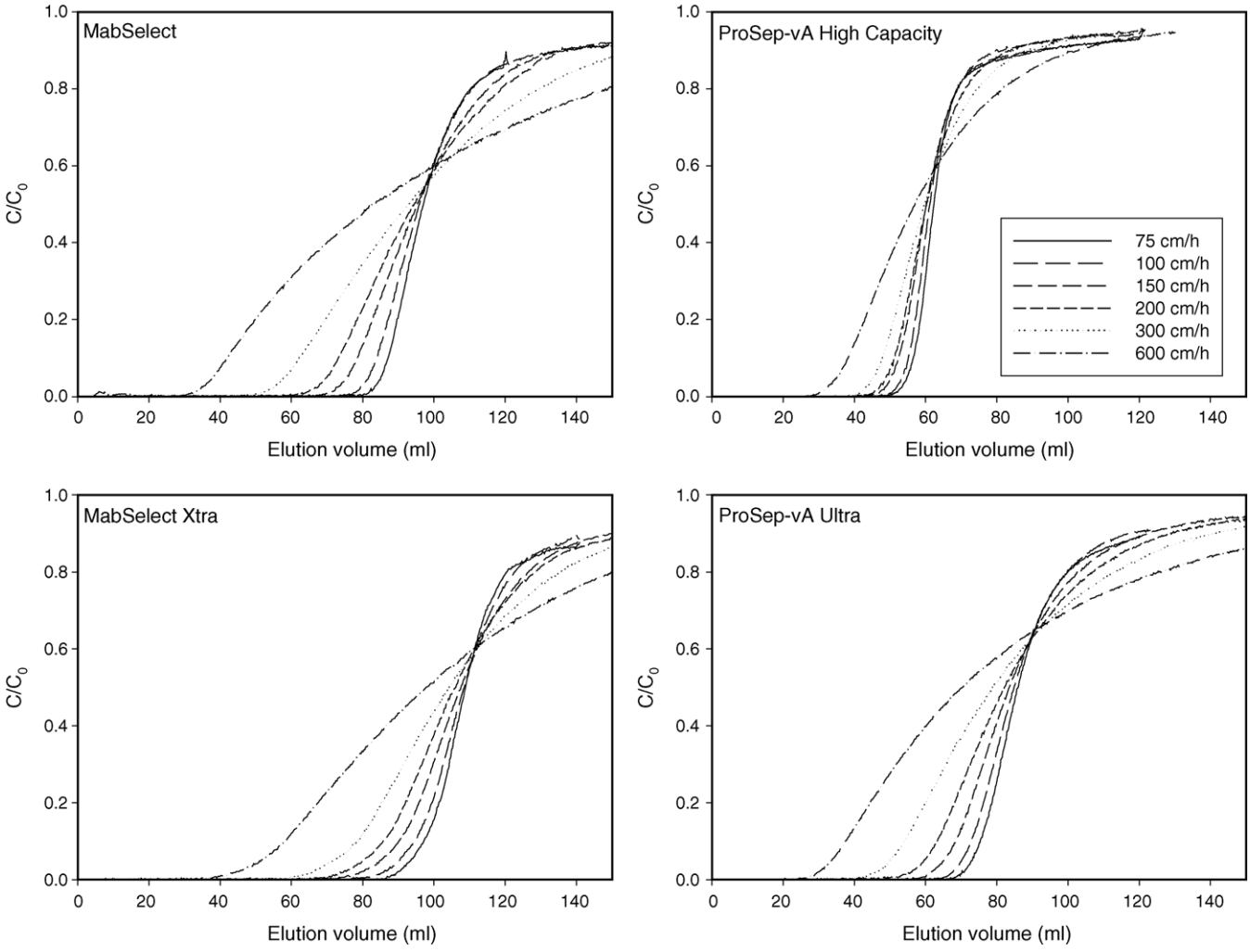

Fig. 6. Breakthrough curves on protein A affinity media in HR 5 columns (column height 10 cm, column volume ~2 ml) with 1.0 mg/ml polyclonal IgG as feed solution at different velocities.

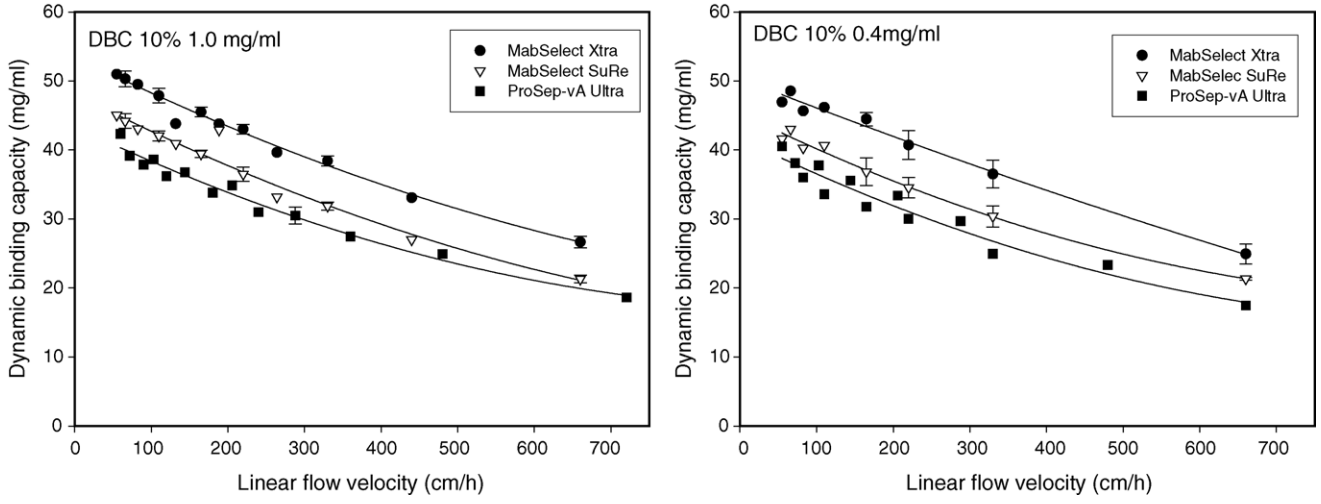

Fig. 7. Dynamic binding capacity at 10%breakthrough dependent on velocity at 1.0 and 0.4 mg/ml polyclonal IgG.

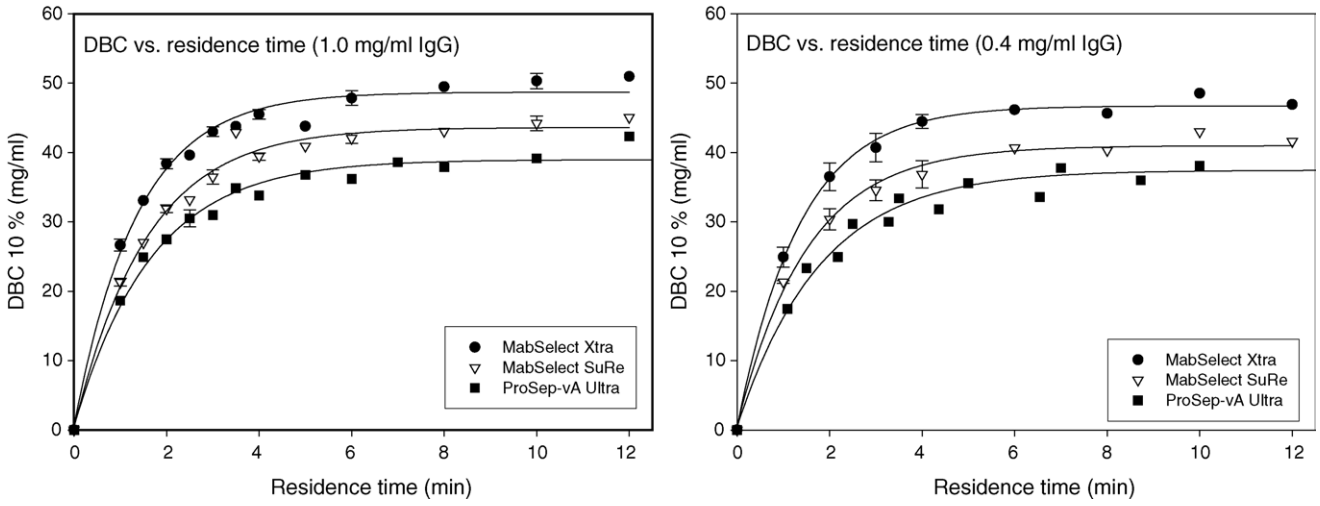

Fig. 8. Dynamic binding capacity at 10%breakthrough dependent on residence time at 1.0 and 0.4 polyclonal mg/ml IgG.

this phenomenon could be caused by aggregates competing with the monomers for binding sites [23], stretching of the molecules [24], or changing of transport mechanism upon changing of pore radius with adsorption [25]. In our case the physical reason for this observation is not yet known.

According to theory we could also deduce a q_0 for sufficient long residence times for loading. The model here applied is a rearranged form of the analytical model for pore diffusion control and rectangular isotherm from Cooper and Liberman [26] and Carta [27]. At first glance the underlying assumptions of this model are valid for our case. The dimensionless time τ_1 represents the ratio of the dynamic to the static binding capacity. At $C/C_0 = 0.1$(10% DBC)

$$\tau_1 = 1 - \frac{1.03}{N} \tag{17}$$

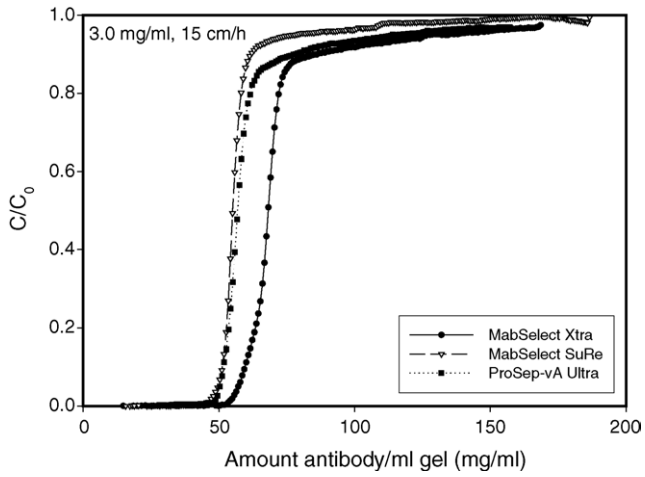

Fig. 9. Breakthrough curves at high feed concentration (3 mg/ml) and low feed velocity (15 cm/h).

is obtained from the Cooper solution, with N the number of transfer units, which is given by

$$N = \frac{15(1-\varepsilon)D_e L}{u r_p^2} \tag{18}$$

Eq. (17) is valid for $N\tau_1 \geq 2.5$

For short residence times the following numerical estimation was used as described by Carta et al. [27]:

$$\tau_1 = 0.364N - 0.0612N^2 + 0.00423N^3 \tag{19}$$

At 50% breakthrough ($C/C_0 = 0.5$) τ_1 is calculated as

$$\tau_1 = 1 - \frac{0.179}{N} \tag{20}$$

From the fit using Eqs. (17) and (20) we could estimate D_e and an apparent maximum binding capacity (q_0). q_0 was around 15–20% lower than the capacity estimated from the Langmuir isotherm (Fig. 10). D_e obtained with this method was identical as determined by uptake experiments with finite and infinite baths. Using these estimated parameters an accurate prediction of DBC could be obtained (Fig. 11) for the initial part of the curve. It is obvious that the phase to reach equilibrium is not well described. Other physical principles, which do not underlie the applied model, are responsible for the slow equilibrium. Although there is a discrepancy between the binding capacities measured by batch and shallow bed uptake and fitting from column $DBC_{10\%}$ data, an accurate prediction of breakthrough was possible. Maximum binding capacities extracted form uptake curves overpredicted breakthrough. This phenomenon was observed with all three media. Further experiments are required to explain this discrepancy. Ghose et al. [28] and McCue et al. [6] also tried to fit the breakthrough curve using a homogenous diffusion model. The uptake curves could be predicted, but also in these cases a further insight into the transport mechanism was not gained.

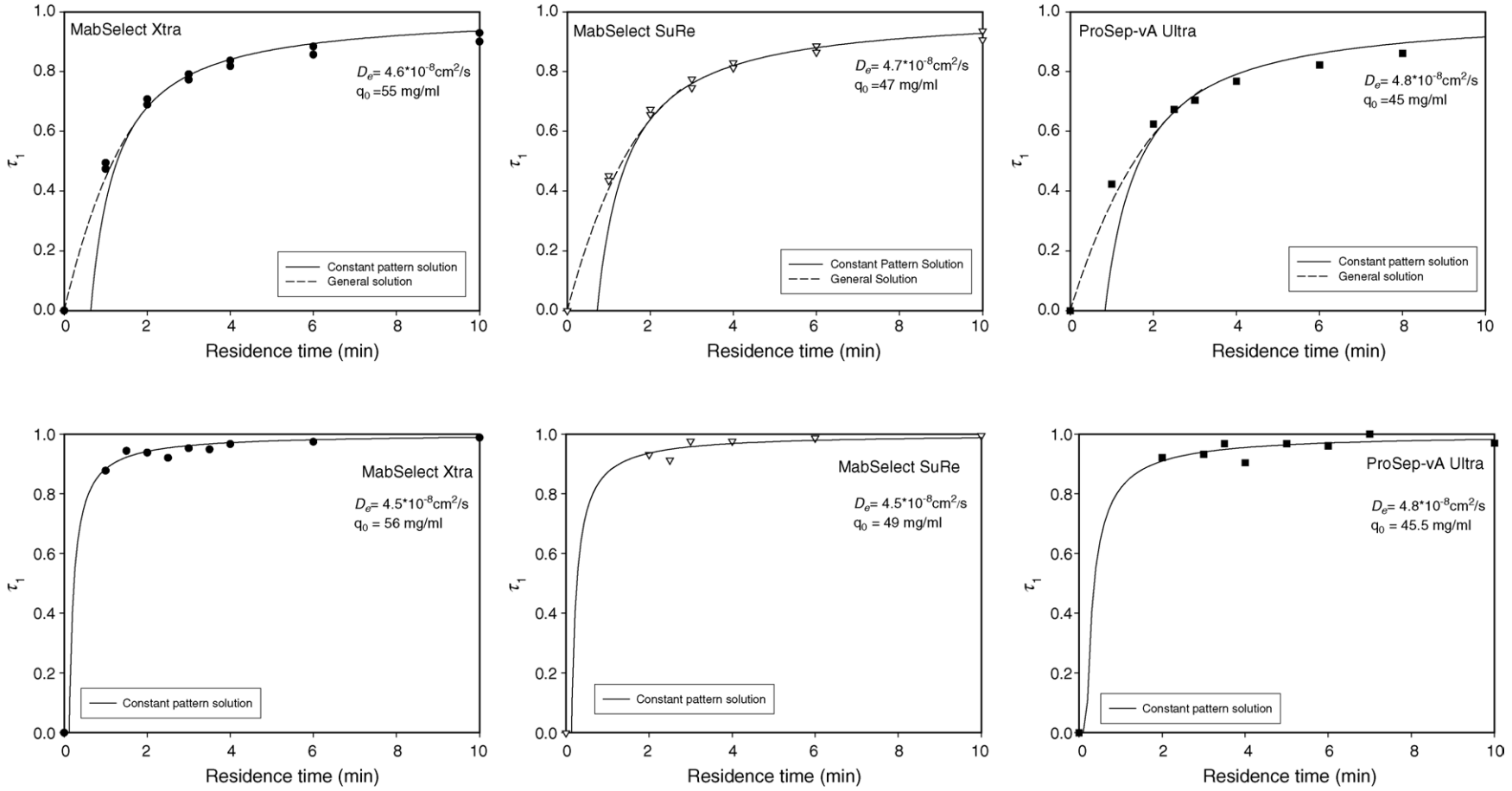

Fig. 10. Ratio of DBC at 10% breakthrough to static capacity as a function of residence time.

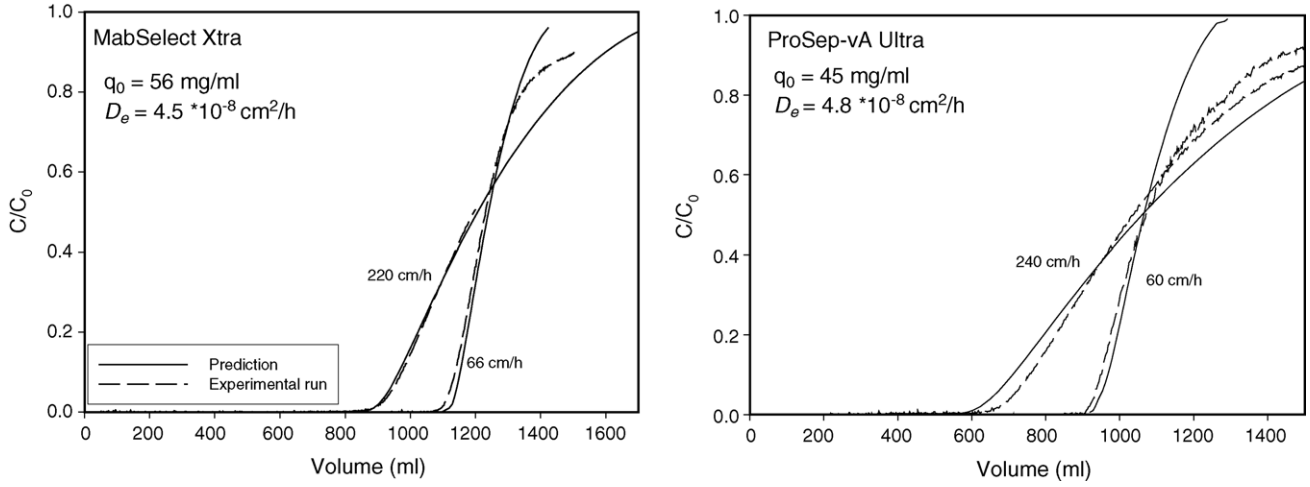

Fig. 11. Breakthrough curves of IgG at 1.0 mg/ml on MabSelect Xtra and ProSep-vA Ultra packed in XK 16 columns. Solid lines represent predictions with a film and pore diffusion model assuming a rectangular isotherm. Dashed lines represent experimental runs.

Table 4
Apparent friction factors of protein A affinity chromatography media

Protein A medium	d_p (μm)	Experiment (bar h cm^{-2})	Theoretical[a] (bar h cm^{-2})	McCue et al [6] (bar h cm^{-2})	Fahrner et al. [7] (bar h cm^{-2})
MabSelect Xtra	75	12.7	5.2	–	–
MabSelect SuRe	85	11.0	5.1	–	–
ProSep-vA Ultra	100	7.4	1.7	4.0	–
Prosep-A	100	–	–	–	2.1
rPA-Sepharose FF	90	–	–	–	7.6

[a] Calculated using Kozeny-Karman equation.

To be able to design optimal column geometry, information on pressure drop versus velocity is necessary. Pressure drop was measured in 10 cm columns at different velocities. Apparent friction factors were estimated and compared to theoretical values using the Kozeny Karman equation (Table 4)

$$\frac{\Delta P}{L} = 150\frac{u\eta}{d_p^2}\frac{(1-\varepsilon)^2}{\varepsilon^3} \quad (21)$$

using a form factor of 150. MabSelect Xtra and MabSelect Sure exhibited as expected a higher pressure drop due to smaller particles. A linear pressure-flow relationship was observed up to 600 cm/h.

4.5. Conclusions

This generation of Protein A affinity chromatography media is characterized by high static and dynamic capacities, and almost rectangular adsorption isotherms. MabSelect Xtra, MabSelect SuRe and ProSep-vA Ultra have been engineered to achieve higher binding capacity and faster mass transfer compared to previous generations. ProSep-vA Ultra has a reduced pore size compared to the previous generation and thus a higher capacity and much lower mass transfer was obtained. A pore diffusion model allowed successful prediction of breakthrough. A discrepancy was found between binding capacities determined by batch adsorption and column methods. Further experimental research and simulation will be required to find out how close this generation of media is to the theoretical possible mass transfer and binding properties. In a forthcoming study, operational characteristics such as life-time, recovery, ligand leakage and host cell removal will be presented using a real feed stock.

5. Nomenclature

C	concentration in solution (mg/ml)
C_0	initial concentration (mg/ml)
c	pore liquid concentration in fluid phase of particle (mg/ml)
D	molecular diffusion coefficient (cm^2/s)
D_e	effective pore diffusion coefficient (cm^2/s)
D_L	axial dispersion coefficient (cm^2/s)
d_p	particle diameter (cm)
K_d	equilibrium dissociation constant (ml/mg)
k_f	external film mass transfer coefficient (cm/s)
P	power input per unit mass of fluid (cm^2/s^3)
q	concentration in solid phase of particle (mg/ml)
q_m	Langmuir isotherm equilibrium parameter (mg/ml)

q_0 solid phase concentration in equilibrium with the feed concentration C_0 (mg/ml)
r particle radial coordination (cm)
Re Reynolds number ($d_p \varepsilon v/\nu$)
R_p particle radius (cm)
Sc Schmidt number (ν/D)
Sh Sherwood number ($k_f d_p/D$)
t time (s)
u superficial velocity (cm/s)
v interstitial velocity (cm/s)
V volume of solution (ml)
V_m volume of particles (ml)

Greek symbols
ε void fraction of packing
ε_p intraparticle void fraction
η dynamic viscosity (Pa s)
ν kinematic viscosity (cm^2/s)
τ_1 dimensionless time

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MabSelect, MabSelect Xtra, MabSelect SuRe and ÄKTA are trademarks of GE Healthcare Ltd, a General Electric Company. ProSep-vA Ultra and ProSep-vA High Capacity are trademarks of Millipore Corporation. We would like to thank Octapharma Pharmazeutika, Vienna, Austria for donation of polyclonal IgG. The work has been supported by GE Healthcare, Uppsala, Sweden.

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