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Performance comparison of protein A affinity resins for the purification of monoclonal antibodies☆

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Abstract

During the selection of protein A affinity resin for the purification of monoclonal antibodies, dynamic binding capacity ($Q_{dyn10\%}$), volumetric production rate (Pr_{vol}) and 'process robustness' are essential parameters to be evaluated. In this article, empirical mathematical models describe these parameters as a function of antibody concentration in load (C_0), load flow rate (u_{load}) and bed height (L). These models allow us to select optimal process conditions for each of the evaluated protein A affinity resins. C_0, u_{load} and L largely affect dynamic binding capacity ($Q_{dyn10\%}$) and volumetric production rate (Pr_{vol}). Maximum $Q_{dyn10\%}$ is generally obtained at high C_0 and at low u_{load}. Maximum Pr_{vol} is obtained at high C_0 and at lowest L, run at high u_{load}. All evaluated resins have a relatively high robustness against variations in C_0. $|\delta Q_{dyn10\%}/\delta C_0|$ ranges from 0.0 to 7.8. It is clear that $Q_{dyn10\%}$, Pr_{vol} and 'process robustness' cannot be maximized all at the same time. Furthermore, some other aspects like IgG recovery, protein A leaching, easiness to pack, easiness to clean, number of re-uses and cost of production might be important to be taken into the equation. Certain evaluation parameters may be more important than others, depending on the specific situation. Therefore, a case-by-case evaluation is recommended.

Keywords: Protein A; Affinity chromatography; Monoclonal antibody; Purification; Dynamic binding capacity; Process robustness; Breakthrough curve; Production rate; Design of experiments; DOE; Packed bed absorption; PBA

1. Introduction

To date, 17 monoclonal antibodies (mAbs) have received approval by US FDA. In 2003, market demand for individual mAbs ranged from few grams per year to even as high as 480 kg per year. The current global antibody pipeline, which comprises more than a hundred antibodies in development, is ready to deliver 16 new products by 2008; total mAb market demand is expected to triple by 2008. Hence, cost-effective mAb manufacturing is gaining importance and will become a pre-requisite of biotech industry's success [1–4].

At first, cell culture contributed the most to cost of production. In addition to this, cell culture was the most common process bottleneck; in this situation the number of bioreactors, bioreactor volume, cell culture cycle time and expression level determine overall process throughput. In response to increasing market demands and pressure for cost reduction, the biotech industry has successfully increased bioreactor volumes up to 20,000 L. Furthermore, cell line expression levels are currently up to 5.0 g/L, thanks to improvements in cell line selection, growth medium, production medium, feed strategy, process control and process design [5]. Consequently, process bottlenecks have moved downstream and mAb manufacturing cost structure has changed; purification costs are now outbalancing cell culture costs [6].

Protein A affinity chromatography is the predominant capture step for purification of mAbs. This is mainly because of its high selectivity, which leads to high purity. Protein A affinity chromatography has to meet the demands of high dynamic binding capacity and high throughput to keep pace with increasing bioreactor volumes and cell culture expression levels and thereby prevent it from becoming a process bottleneck. Moreover, an optimal usage of expensive protein A affinity resins (6000–9000 €/L resin) significantly reduces cost of production.

Several published studies have dealt with the determination of adsorption isotherms, dynamic binding capacity and produc-

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tion rate for commercially available protein A affinity resins [7–10]. This article in particular evaluates first order, interaction and quadratic effects of load flow rate and hIgG concentration in load on dynamic binding capacity at 10% breakthrough for recently developed protein A affinity resins; Prosep vA Ultra and MabSelect Xtra together with Prosep A High Capacity, Rmp Protein A Sepharose FF and MabSelect. Furthermore, obtained mathematical models are used to evaluate 'process robustness' and calculate volumetric production rate.

2. Experimental

2.1. Materials

2.1.1. Protein A affinity resins

Prosep A High Capacity and Prosep vA Ultra were obtained from Millipore (Bedford, MA, USA). Rmp Protein A Sepharose 4FF, MabSelect and MabSelect Xtra were obtained from Amersham Biosciences (NJ, USA). Table 1 shows characteristics of above protein A affinity resins.

2.1.2. Clarified cell culture harvest

Murine hybridoma cells (NS0) were used to produce a humanized monoclonal IgG_4 (hIgG) in a fed-batch process at laboratory scale (5–40 L). Cell culture harvests were clarified by consecutive filtration through a depth lenticular filter (150 L/m^2/h, B1HC, Millipore, Bedford, MA, USA) and an absolute 0.22 μm filter (Opticap 4″, Millipore, Bedford, MA, USA) and stored aseptically at 4 °C.

2.1.3. Purified hIgG solutions

Purified hIgG solutions were obtained by purification of clarified cell culture harvest by protein A affinity chromatography with Prosep A High Capacity resin, packed in a Vantage L 22 mm/250 mm column (Millipore, Bedford, MA, USA) at a bed height of 161 mm.

The column was pre-rinsed with 3 CV of 0.03 M HCl pH 1.5 and equilibrated with 3 CV of 20 mM sodium succinate pH 5.8. Clarified harvest was loaded at 20 g hIgG/L resin. After loading, the column was washed with 5 CV of 20 mM sodium succinate pH 5.8, eluted with 5 CV of 20 mM sodium succinate pH 3.65, regenerated with 5 CV of 0.03 M HCl pH 1.5, neutralized with 5 CV of 20 mM sodium succinate pH 5.8 and stored with 3 CV of 20% EtOH. All steps were run at a flow rate of 31.1 CV/h.

The eluate fraction was adjusted to pH 6.0 with 500 mM sodium succinate pH 8.4, filtered through an absolute 0.22 μm cellulose acetate filter (Corning Inc., NY, USA) and stored aseptically at 4 °C. The pH adjusted and filtered eluate was diluted with phosphate buffered saline pH 7.5 into hIgG solutions with predefined hIgG concentration (0.5–2.0 g hIgG/L).

All buffers and solvents used in this study were filtered through an absolute 0.22 μm cellulose acetate filter (Corning Inc., NY, USA).

2.2. Analytical methods

hIgG concentration was determined by UV-spectrophotometry at 280 nm (Agilent 8453, Agilent Technologies, CA, USA) or by Protein A HPLC. Protein A HPLC was performed with a liquid chromatography system (LC-10AD VP, Shimadzu, Duisburg, Germany) equipped with a protein A affinity column (PA ID, Applied Biosystems, CA, USA) and UV–vis detection (SPD-10A VP, Shimadzu, Duisburg, Germany). The sample is loaded with 10 mM phosphate + 150 mM NaCl pH 7.2 and eluted with 12 mM HCl + 150 mM NaCl pH 2.0.

2.3. Procedures and equipment

Dynamic binding capacity and breakthrough curves were determined on all above-mentioned protein A affinity resins (Section 2.1.1), packed in Vantage L 11 mm/250 mm columns (Millipore, Bedford, MA, USA) at a bed height of 194–200 mm and installed on an Äkta Explorer 100 chromatography system (Amersham Biosciences, NJ, USA). All packed columns were in accordance with suppliers' instructions for HETP and asymmetry.

2.3.1. Dynamic binding capacity

A design of experiments (Table 2), to evaluate effects (first order, interaction and quadratic effects) of hIgG concentration in load (C_0) and load flow rate (u_{load}) on dynamic binding capacity at 10% breakthrough ($Q_{dyn10\%}$), was set up with a statistical software package (JMP V5.1, SAS Institute Inc., Cary, NC, USA). The tested range for C_0 (0.5–2.0 g hIgG/L) was based on projections of hIgG expression levels in cell culture. The tested ranges for u_{load} were determined from typical operating ranges as indicated by suppliers. For each resin, a symmetric design was set up. Centre point and occasionally other design points were replicated to estimate mathematical model's pure error. Order of experiments was randomized.

Experiments were performed with purified hIgG solutions (Section 2.1.3) and hIgG breakthrough was monitored with

Table 1
Characteristics of protein A affinity resins

	Matrix beads	Protein A origin	d_p d_{50v}[a] (μm)
Prosep A high capacity	Rigid porous glass	Mammalian free, native	100
Prosep vA Ultra	Rigid porous glass	Mammalian free, native	100
Rmp Protein A Sepharose 4FF	Cross-linked agarose	Mammalian free, recombinant	90
MabSelect	Cross-linked agarose	Mammalian free, recombinant	85
MabSelect Xtra	Cross-linked agarose	Mammalian free, recombinant	77

[a] d_{50v} is the median particle size of the cumulative volume distribution.

Table 2
Design of experiments for dynamic binding capacity determination by loading purified hIgG solutions

	C_0 (g hIgG/L)	u_{load}[a] (cm/h)	u_{load} (CV/h)	Number of experiments	Pure error d.f.[b]
Prosep A high capacity	0.5–2.0	250–750	12.5–37.5	13	4
Prosep vA Ultra	0.5–2.0	250–750	12.5–37.5	12	3
Rmp Protein A Sepharose 4FF	0.5–2.0	100–300	5.15–15.5	14	2
MabSelect	0.5–2.0	100–500	5.15–25.0	10	5
MabSelect Xtra	0.5–2.0	100–300	5.15–15.5	8	3

[a] u_{load} ranges are based on typical operating ranges as indicated by suppliers.
[b] Pure error d.f.: degrees of freedom to estimate the mathematical model's pure error.

an online UV-detector, because no online assay (e.g. protein A HPLC) was at our disposal. These results are compared to $Q_{dyn10\%}$ as determined with clarified harvest and offline protein A HPLC (Section 2.3.2).

The protein A affinity column was pre-rinsed with 3 CV of 12 mM HCl + 150 mM NaCl pH 2.0 in order to clear the column from potentially present free impurities (protein A, proteins, . . .). Subsequently, the columns are equilibrated with 3 CV of 20 mM sodium succinate pH 5.8. Purified hIgG solution (2.1.3) was loaded until the flow through reached 10% of the UV-280 nm absorption of the loaded purified hIgG solution. Subsequently, the column was washed with 3 CV of 20 mM sodium succinate pH 5.8 and eluted (5.0 CV/h) with 5 CV of 20 mM sodium succinate pH 3.65, regenerated (12.5 CV/h) with 5 CV of 12 mM HCl + 150 mM NaCl pH 2.0, neutralized (12.5 CV/h) with 5 CV of 20 mM sodium succinate pH 5.8 and stored (12.5 CV/h) with 3 CV of 20% EtOH. Pre-rinse, equilibration and wash steps were run at the same flow rate as for loading.

$Q_{dyn10\%}$, mass balance and recovery are respectively calculated with Eqs. (1)–(3).

$$Q_{\text{dyn }10\%}\ (\text{g hIgG/L resin}) = \frac{[V_{FT}(C_0 - C_{FT}) - V_{WA}C_{WA}]}{V_{column}} \quad (1)$$

$$\text{Mass balance } (\%) = \frac{100\,[V_{FT}C_{FT} + V_{WA}C_{WA} + V_{EL}C_{EL}]}{V_{FT}C_0} \quad (2)$$

$$\text{Recovery } (\%) = \frac{100V_{EL}C_{EL}}{V_{FT}C_0} \quad (3)$$

C_0, C_{FT}, C_{WA} and C_{EL} (respectively hIgG concentration of load fraction, flow through fraction, wash fraction and elution fraction) were determined by protein A HPLC. V_{FT}, V_{WA}, V_{EL} and V_{column} are respectively volume of load fraction (=volume of flow through fraction), wash fraction, eluate fraction and column volume.

2.3.2. Breakthrough curves

Breakthrough curves were determined for each resin by loading clarified cell culture harvest (Section 2.1.2, $C_0 = 0.68 \pm 0.05$ hIgG/L) until $C_{FT}/C_0 = 100\%$ at upper and lower flow rates of the resin's typical operating conditions. C_0 and C_{FT} were determined by protein A HPLC. Based on obtained breakthrough curves, $Q_{dyn10\%}$ was determined for $C_{FT}/C_0 = 10\%$.

2.3.3. Production rate calculations

Volumetric production rate (Pr_{vol}; g hIgG/h/L resin) is the amount of material purified per hour per liter resin. Pr_{vol} is calculated by dividing dynamic binding capacity at 10% breakthrough ($Q_{dyn10\%}$; g hIgG/L resin) by cycle time (h). Cycle time is calculated with Eq. (4). The first term indicates the time needed for loading. The second term indicates the time needed for 22 CV of pre-conditioning, equilibration, wash, elution and regeneration. Pr_{vol} is maximized by assuming that these 22 CV are run at theoretical maximum flow rate (u_{max}; cm/h).

$$\text{Cycle time (h)} = \frac{Q_{\text{dyn }10\%}}{C_0 u_{load}} + \frac{22L}{u_{max}} \quad (4)$$

U_{max} was calculated as a function of bed height (L) taking into account bed compression limitations and a maximum column inlet pressure ($P_{column\ inlet}$) of 2 bar. $P_{column\ inlet}$ is the sum of pressure drop over a packed bed ($\Delta P_{packed\ bed}$) and system pressure drop (ΔP_{system}). ΔP_{system} is estimated to be equal to 0.03 bar and considered independent of flow rate, under the assumption that chromatography skids can be dimensioned accordingly[11]. $\Delta P_{packed\ bed}$ is directly proportional to linear flow rate (u), bed height (L) and an apparent friction constant (γ), as shown by the Blake–Kozeny equation (Eq. (5)). γ is dependent on liquid viscosity (μ), interstitial bed porosity (ε), resin bead diameter (d_p) and an empirical constant K_0 [12].

$$\Delta P_{packedbed} = \gamma u L = \frac{\mu K_0 (1-\varepsilon)^2}{d_p^2 \varepsilon^3} u L \quad (5)$$

For incompressible resins (rigid porous glass), such as Prosep A High Capacity and Prosep vA Ultra, γ is assumed to be equal to 4.00×10^{-5} bar h/cm^2 as reported in literature [7]. The maximum u_{max} is set to 900 cm/h. For these resins, there are no bed compression limitations.

For compressible resins (cross-linked agarose), such as Rmp Protein A Sepharose 4FF, MabSelect and MabSelect Xtra, bed compression (λ) limitations are more restrictive for u_{max} than $P_{column\ inlet}$ limitations. Based on supplier's information, we set u_{max} for Rmp Protein A Sepharose 4FF, MabSelect and MabSelect Xtra at respectively 300 cm/h, 500 cm/h and 300 cm/h for $L \leq 20$ cm. For $L > 20$ cm, u_{max} is adjusted according to Eq. (5) in order to maintain the same $\Delta P_{packed\ bed}$ as at $L = 20$ cm. For compressible resins, the required packing flow rate to reach a specific λ decreases for increasing column diameter, due to loss in wall support. For this reason u_{max} – always lower than the packing flow rate – decreases as a function of column diameter. Data pub-

lished in literature [12] shows that Sepharose 4FF resin, packed (L = 20 cm, λ = 17%) in columns of 1.1 cm and 100 cm in diameter, has a u_{max} of 485 cm/h ($\Delta P_{packed\ bed}$ = 1.0 bar) and 140 cm/h ($\Delta P_{packed\ bed}$ = 0.3 bar), respectively. In this study however, we did not take column diameter and its effect on u_{max} and Pr_{vol} into account.

3. Results and discussions

3.1. Dynamic binding capacity

Table 3 shows that mass balance (84–106%) and recovery (84–106%) are acceptable for all 57 experiments. MabSelect Xtra (56–69 g hIgG/L resin) has a significantly higher dynamic binding capacity ($Q_{dyn10\%}$) compared to Prosep vA Ultra (37–53 g hIgG/L resin), MabSelect (35–48 g hIgG/L resin) and Rmp Protein A Sepharose 4FF (31–48 g hIgG/L resin). Prosep A High Capacity (26–33 g hIgG/L resin) has a significantly lower $Q_{dyn10\%}$. Above $Q_{dyn10\%}$ ranges were obtained for varying hIgG concentration in load (C_0) and load flow rate (u_{load}) as shown in Table 2.

C_0 and u_{load} effects on $Q_{dyn10\%}$ are adequately modeled, as indicated by R^2_{adj} values (87–97%). To broaden the applicability of the contour plots to columns packed at different bed height, u_{load} is expressed in CV/h, i.e. u_{load} = 1/residence time. This is done under the assumption that $Q_{dyn10\%}$ is constant for constant residence time, as supported by several publications [7,8]. Contour plots (Fig. 1) show that maximum $Q_{dyn10\%}$ is generally obtained at high C_0 and at low u_{load}. With these mathematical models, we can identify operating conditions that maximize $Q_{dyn10\%}$, i.e. we can purify a maximum amount of antibody for a given quantity of resin. In this way, the number of cycles needed to purify a specific amount of antibody are minimized.

Above described effects of C_0 and u_{load} on $Q_{dyn10\%}$ are in accordance with what is described in literature. Transport of hIgG molecules to binding sites (protein A) is said to be dominated by slow diffusive mass transport. For this reason, u_{load} negatively affects hIgG breakthrough. Langmuir type adsorption isotherms (Eq. (6)) – describing monolayer adsorption of hIgG to protein A – show that stationary phase hIgG concentration (Q^* = equilibrium capacity) asymptotically approaches maximum capacity (Q_{MAX}) of the resin as a function of mobile phase hIgG concentration (C^*). Maximum capacity will be reached at lower C^* for hIgG-protein A combinations of lower dissociation constants (k_D) [13].

$$Q^* = Q_{MAX}\frac{C^*}{C^* + k_D} \tag{6}$$

Another interesting evaluation parameter is process robustness. Process parameters, that potentially affect product yield, are typically evaluated as part of a robustness study. For these parameters a proven acceptable range of operation is defined. C_0 and u_{load} largely affect $Q_{dyn10\%}$; the effect of u_{load} (−33% to −6%) is generally larger compared to the effect of C_0 (0 to +20%). From that perspective, C_0 and u_{load} appear both to be critical and need to be well controlled. Chromatography skids usually have a good control on u_{load}. For C_0 however, one needs to assess the natural

Table 3
Data evaluation of dynamic binding capacity ($Q_{dyn10\%}$) as determined with purified hIgG solutions

	Comparison for all pairs[a]		$Q_{dyn10\%}$ (g hIgG/L resin)			$\delta Q_{dyn10\%}/\delta C_0$[b]		Mass balance (%)	Recovery (%)	R^2_{adj}[c] (%)
			Mean	Minimum	Maximum	Minimum	Maximum	Minimum–maximum range	Minimum–maximum range	
MabSelect Xtra	A		62	56	69		+2.5	95–106	95–106	88
Prosep vA Ultra		B	43	37	53	+0.1	+5.8	94–105	93–104	94
MabSelect	B	C	41	35	48		0.0	84–103	84–103	91
Rmp Protein A Sepharose 4FF		C	38	31	48	–4.2	+7.8	92–103	90–102	87
Prosep A High Capacity		D	30	26	33	0.0	+3.2	92–102	92–99	97

[a] Comparison for all pairs is done by using Tukey–Kramer honestly significant difference test: protein A affinity resins not connected by same letter are significantly different.
[b] $\delta Q_{dyn10\%}/\delta C_0$ is the first derivative of the mathematical model for $Q_{dyn10\%}$.
[c] R^2_{adj} is the adjusted R^2 value of the mathematical models as represented by contour plots in Fig. 1.

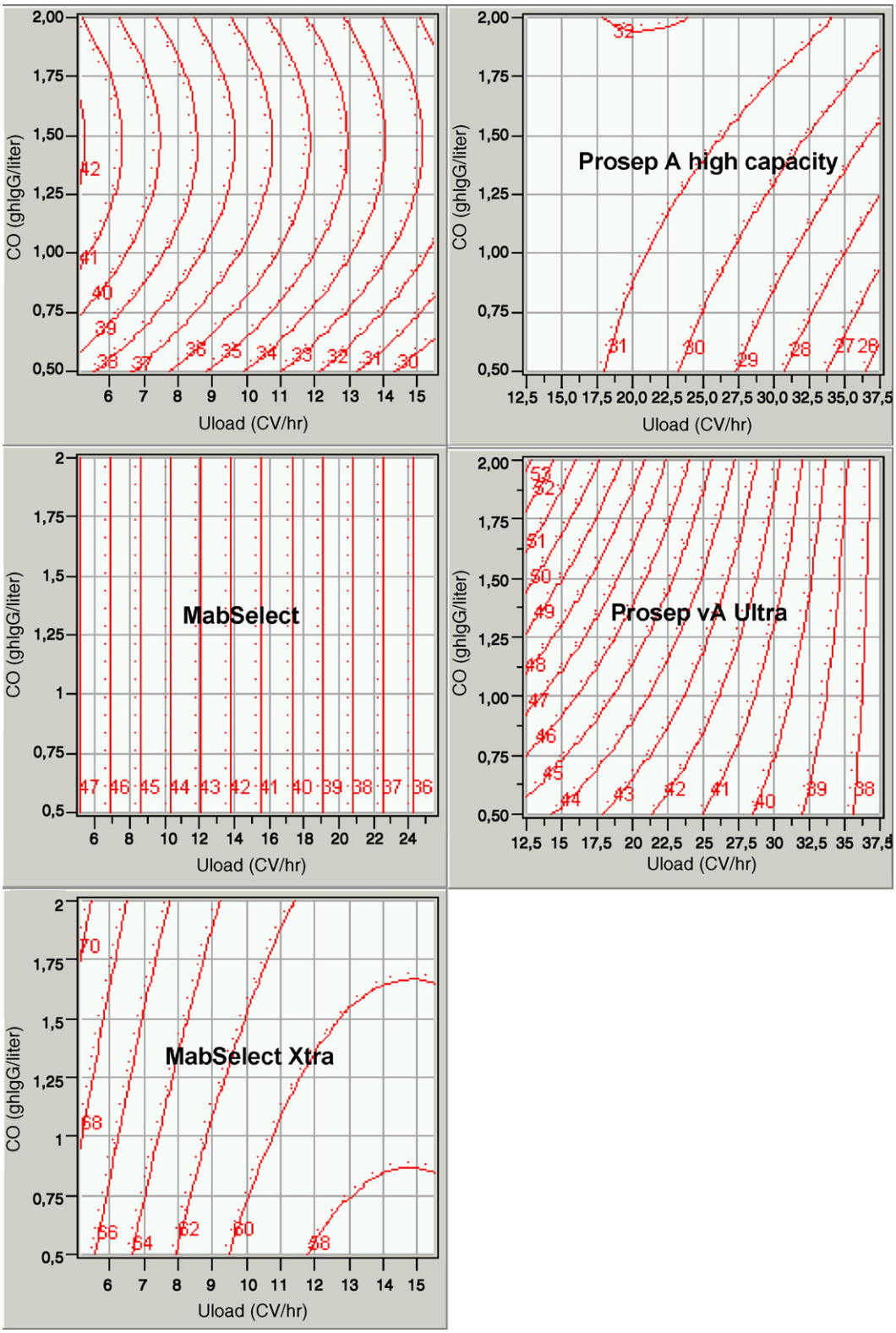

Fig. 1. Contour plots visualize the effect of hIgG concentration in load (C_0; g hIgG/L) and flow rate in load (u_{load}; CV/h) on dynamic binding capacity ($Q_{dyn10\%}$; g hIgG/L resin).

process variation and foresee enough safety margin as an excursion of C_0 on the lower side might otherwise cause early IgG breakthrough and cause yield loss. For this reason, we now will focus on how sensitive $Q_{dyn10\%}$ is to variations in C_0. This sensitivity is quantified by the first derivative in C_0 ($\delta Q_{dyn10\%}/\delta C_0$) of the mathematical model for $Q_{dyn10\%}$, as shown in Fig. 2.

MabSelect has the highest 'process robustness' of all evaluated resins, i.e. $Q_{dyn10\%}$ changes the least as a function of

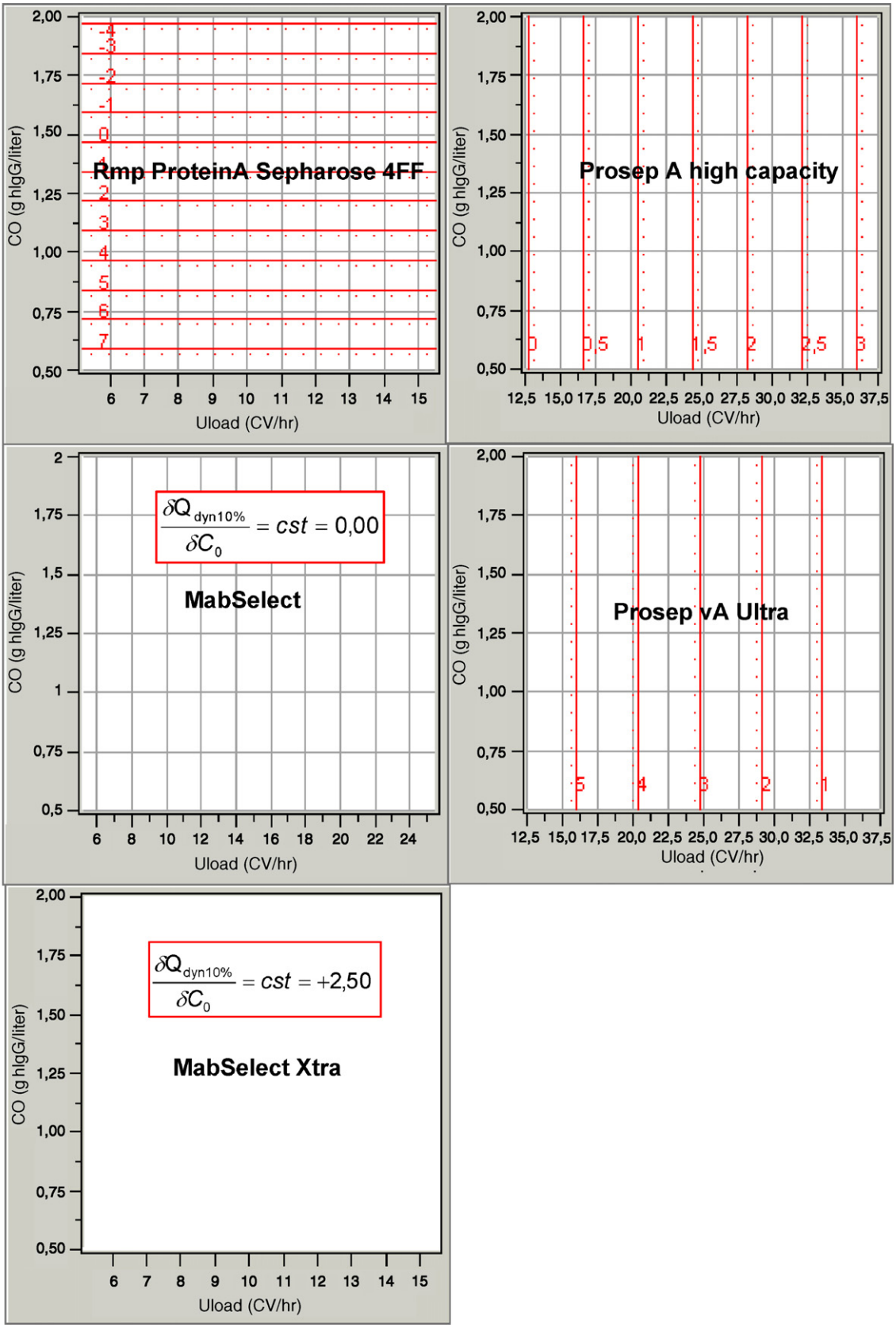

Fig. 2. Contour plots visualize the first derivative of dynamic binding capacity in C_0 ($\delta Q_{dyn10\%}/\delta C_0$) as a function of hIgG concentration in load (C_0; g hIgG/L) and flow rate in load (u_{load}; CV/h).

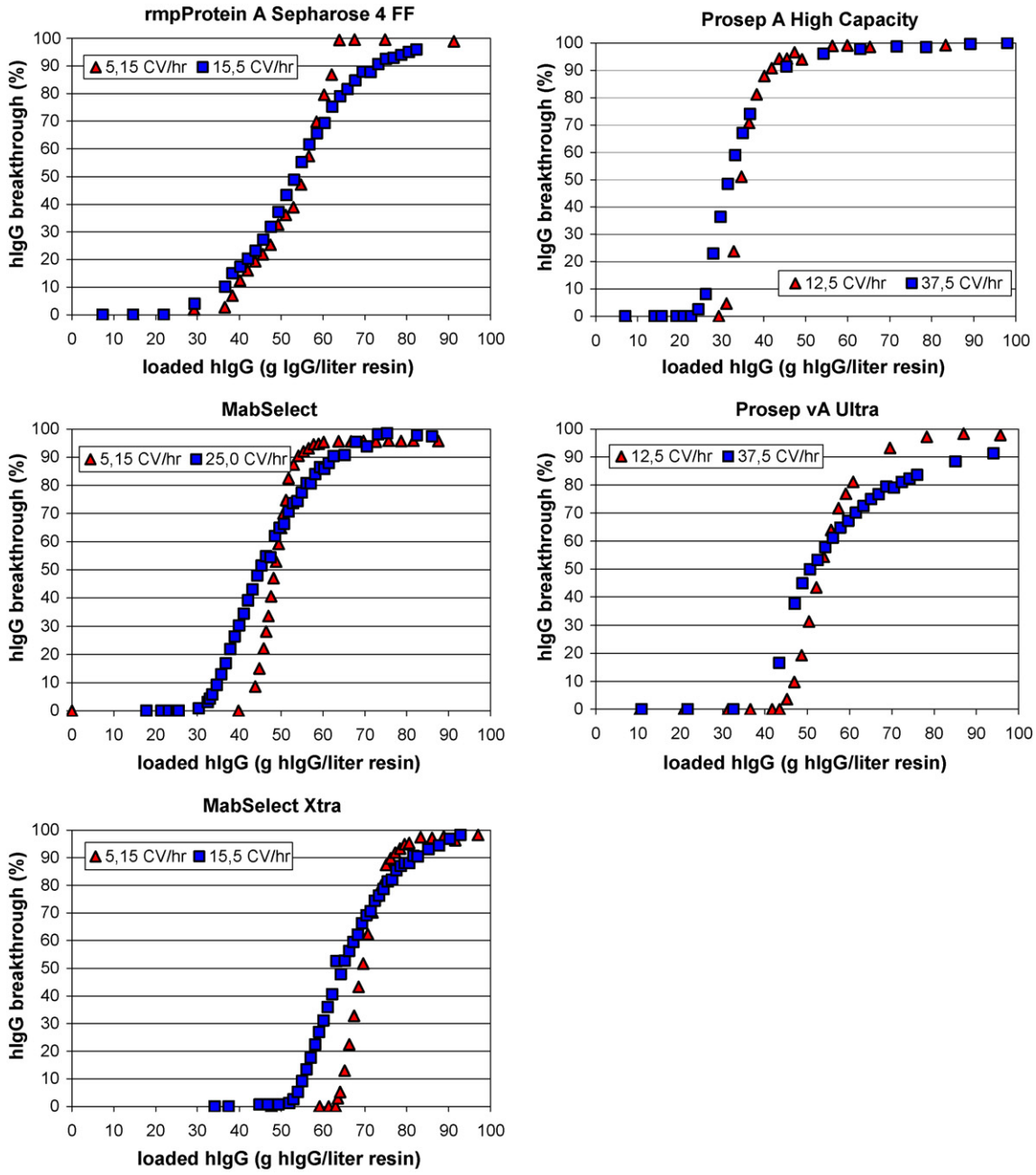

Fig. 3. Breakthrough curves of clarified cell culture harvest ($C_0 = 0.68 \pm 0.05$ hIgG/L) loaded on Prosep A High Capacity, Prosep vA Ultra, Rmp Protein A Sepharose 4FF, MabSelect and MabSelect Xtra, packed in Vantage L 11 mm/250 mm columns at a bed height of 194–200 mm, run at upper and lower flow rates of the resin's evaluated operating range.

C_0. $\delta Q_{dyn10\%}/\delta C_0$ of MabSelect equals 0.0 over the whole tested range of u_{load} and C_0. This means that, for MabSelect, $Q_{dyn10\%}$ is totally independent of C_0. For MabSelect Xtra ($\delta Q_{dyn10\%}/\delta C_0 = +2.5$) $Q_{dyn10\%}$ does change as a function of C_0. Prosep A high capacity (0.0 to +3.2) and Prosep vA Ultra (+0.1 to +5.8) have a broader range of $\delta Q_{dyn10\%}/\delta C_0$. 'Process robustness' can be maximized for each individual resin by operating at u_{load} and/or C_0 for which $\delta Q_{dyn10\%}/\delta C_0$ is closest to zero. For Prosep A high capacity and Prosep vA Ultra, $Q_{dyn10\%}$ is minimally affected by C_0 variations at $u_{load} = 12.9$ CV/h and $u_{load} = 37.5$ CV/h, respectively. Rmp Protein A Sepharose 4FF (−4.2 to +7.8) has the broadest range of $\delta Q_{dyn10\%}/\delta C_0$. However, $\delta Q_{dyn10\%}/\delta C_0$ is not affected by u_{load}. For Rmp Protein A Sepharose 4FF the sensitivity of $Q_{dyn10\%}$ to C_0 variations can be minimized by operating at $C_0 = 1.47$ g hIgG/L. If we take into account all evaluated resins, $|\delta Q_{dyn10\%}/\delta C_0|$ ranges from 0.0 to 7.8, this means that $Q_{dyn10\%}$ maximally changes ± 1.95 g hIgG/L resin for a C_0 variation of ± 0.25 g hIgG/L. We can conclude that all evaluated resins have a relatively high robustness against variations in C_0.

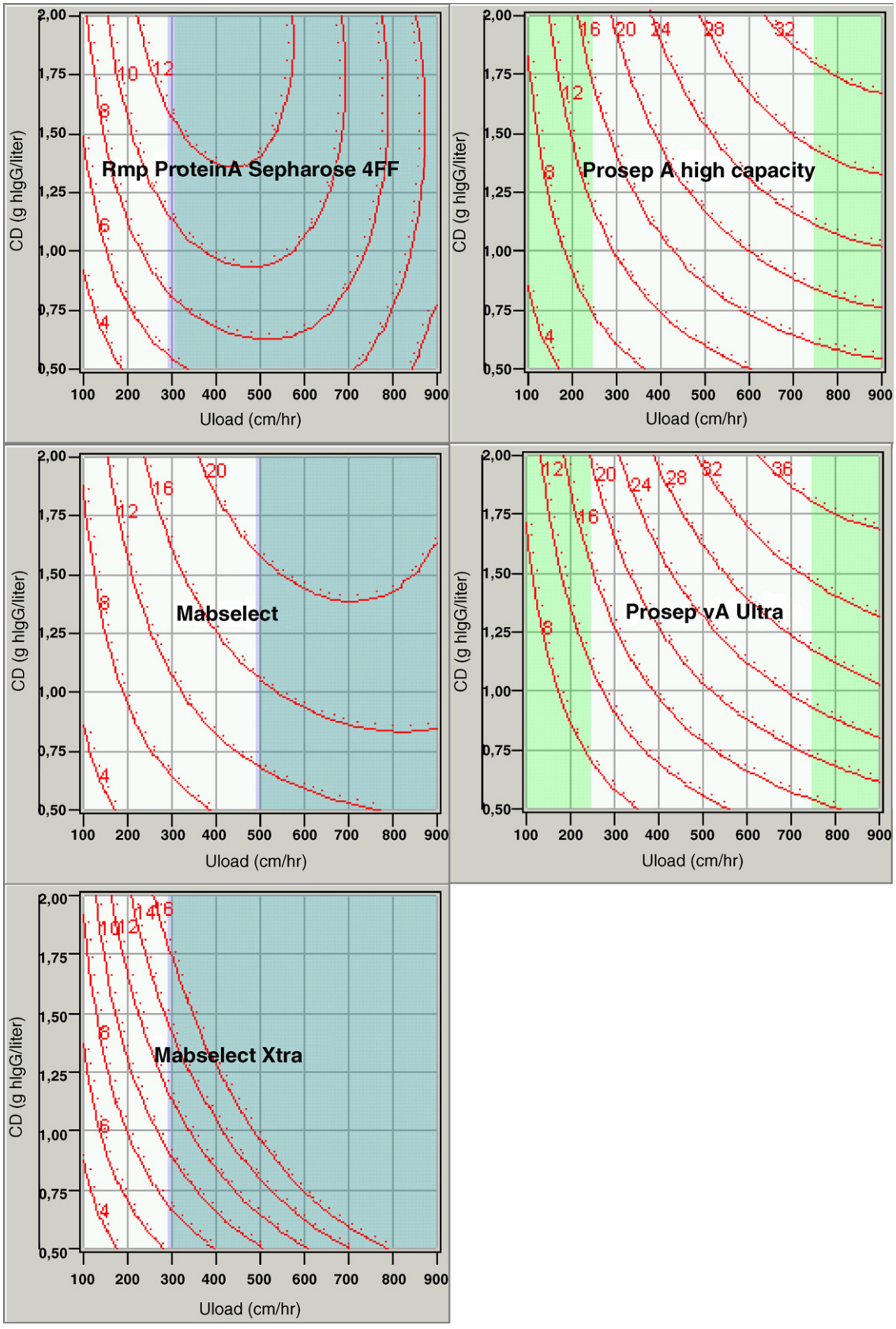

Fig. 4. Volumetric production rate (g hIgG/L resin/h) as a function of hIgG concentration in load (C_0; g hIgG/L) and linear flow rate in load (u_{load}; cm/h) at a bed height of 20 cm. White area represents the evaluated operating range. Green area is outside this range and blue area indicates bed compression limitations or pressure limitations.

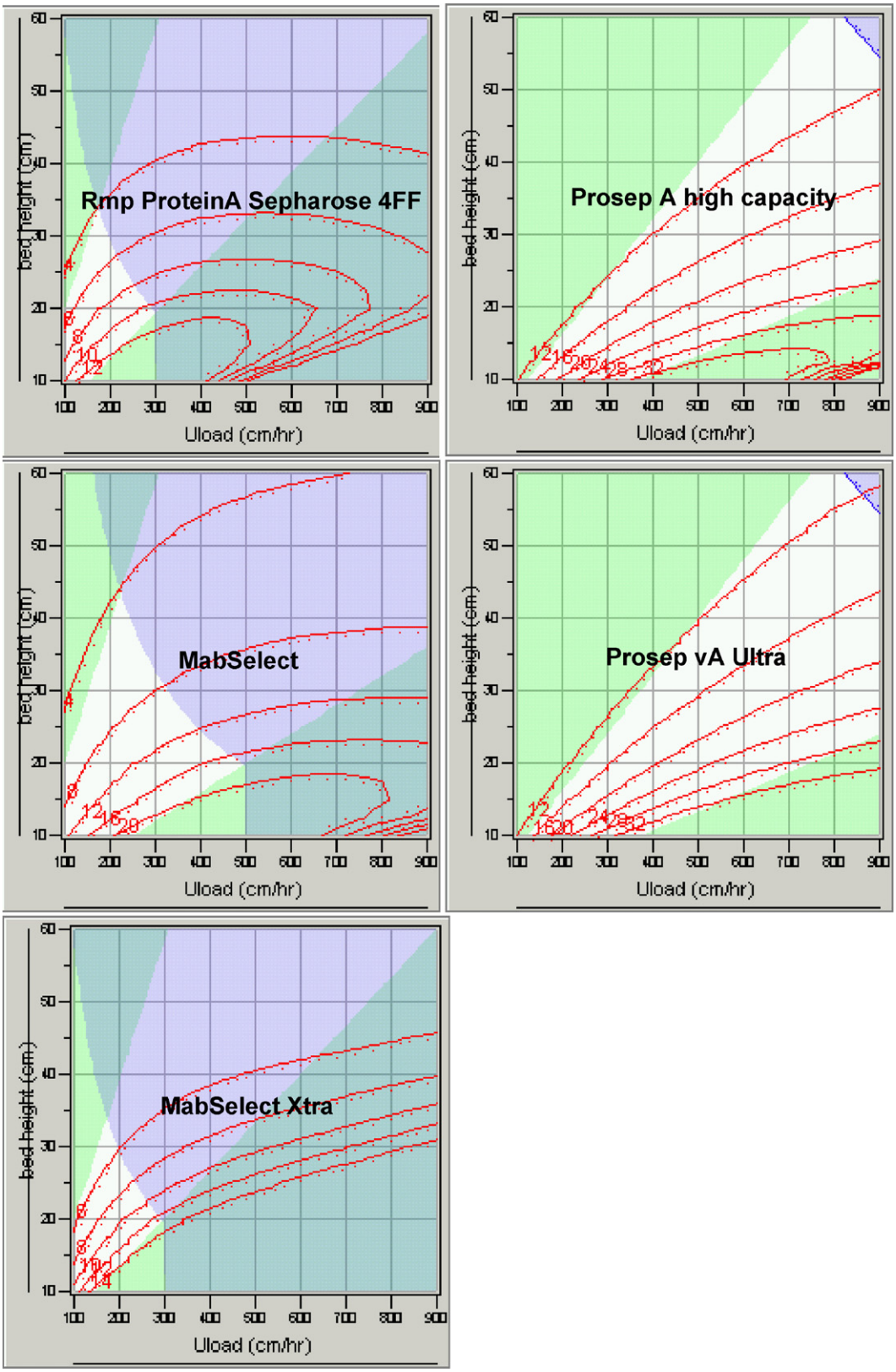

Fig. 5. Volumetric production rate (g hIgG/L resin/h) as a function of bed height (cm) and linear flow rate in load (u_{load}; cm/h) at hIgG concentration in load of 1.25 g hIgG/L. White area represents the evaluated operating range. Green area is outside this residence time range and blue area indicates bed compression limitations or pressure limitations. Volumetric production rate is calculated for varying bed heights, under the assumption that $Q_{dyn10\%}$ is constant for constant residence time, as supported by several publications [7,8].

Table 4
Comparison of dynamic binding capacity ($Q_{dyn10\%}$) as determined with purified hIgG solutions and clarified harvest

	u_{load}		$Q_{dyn10\%}$	
	cm/h	CV/h	Purified hIgG solution 2.1.3 (g hIgG/L resin)	Clarified harvest 2.1.2 (g hIgG/L resin)
Prosep vA Ultra	250	12.5	45.5	46.3
Prosep vA Ultra	750	37.5	37.5	40.7
Prosep A High Capacity	250	12.5	31.1	31.2
Prosep A High Capacity	750	37.5	27.2	26.0
Rmp Protein A Sepharose 4FF	100	5.15	43.1	39.5
Rmp Protein A Sepharose 4FF	300	15.5	36.5	35.9
MabSelect	100	5.15	47.7	45.2
MabSelect	500	25.8	35.7	35.0
MabSelect Xtra	100	5.15	67.4	64.8
MabSelect Xtra	300	15.5	57.6	55.4

A pair-wise comparison shows that $Q_{dyn10\%}$ as determined with purified hIgG solutions and clarified harvest is not significantly ($P(T \leq t) = 0.17$) different.

3.2. Breakthrough curves

Fig. 3 shows breakthrough curves for Prosep A High Capacity, Prosep vA Ultra, Rmp Protein A Sepharose 4FF, MabSelect and MabSelect Xtra loaded with clarified harvest. Breakthrough curves confirm the negative effect of u_{load} on $Q_{dyn10\%}$. Table 4 shows that above mathematical models for $Q_{dyn10\%}$ – as determined with purified IgG solutions – can accurately predict $Q_{dyn10\%}$ of clarified harvest based on C_0 and u_{load}. The differences between dynamic binding capacity as determined with purified IgG solutions and clarified harvest are not significant and range from −9.1% to +7.9%.

3.3. Production rate

Figs. 4 and 5 show that hIgG concentration in load (C_0), load flow rate (u_{load}) and bed height (L) largely affect volumetric production rate (Pr_{vol}). For all tested resins, highest Pr_{vol} is obtained at high C_0, low L and high u_{load}. A closer look at how each of these variables affect the main contributors to Pr_{vol} (Eq. (4)) explains why. $Q_{dyn10\%}$ generally increases for increasing C_0, whereas cycle time decreases for increasing C_0. For decreasing L or increasing u_{load} – i.e. decreasing residence time – the decrease in cycle time is higher than the decrease in $Q_{dyn10\%}$. Analogous effects are present at different L and C_0 levels.

In the section below we compare all evaluated protein A affinity resins at maximum Pr_{vol}, i.e. at $C_0 = 2.0$ hIgG g/L, $L = 10$ cm and high u_{load}. Prosep vA Ultra resin (50 g hIgG/h/L resin) and Prosep A High Capacity (47 g hIgG/h/L resin) have a higher maximum Pr_{vol} compared to MabSelect (31 g hIgG/h/L resin), MabSelect Xtra (22 g hIgG/h/L resin) and Rmp Protein A Sepharose 4FF (18 g hIgG/h/L resin). This is so because the operational window (white area on contour plots) is bigger for incompressible resins (porous glass) compared to compressible resins (cross-linked agarose). As such, we can apply much higher flow rates on Prosep resins not only during loading but also during other chromatography steps (pre-conditioning, equilibration, wash, elution and regeneration). Consequently, cycle time is much shorter for porous glass resins compared to cross-linked agarose resins. In spite of the fact that the evaluated compressible resins have equal or higher $Q_{dyn10\%}$ compared to the evaluated incompressible resins, their Pr_{vol} is lower because of the significant role that flow rate plays in the productivity calculation. This corresponds to earlier findings in other publications [8,14]. However, we have to be conscious of the fact that applying theoretical maximum flow rate for all other chromatography steps besides loading might have a negative impact on protein A eluate recovery and impurity profile. Moreover, it might lead to insufficient contact time for regeneration or might be practically infeasible for a particular chromatography skid.

The implications of maximizing Pr_{vol} are numerous. Purification cycles are run as fast as possible. This can be important when short hold and processing times are required because of limited product stability of clarified cell culture fluid. Furthermore, shorter process times are especially beneficial when protein A affinity chromatography becomes a process bottleneck. This is exactly what happens when expression levels are up to 5.0 g/L in 20,000-L scale bioreactors as reported in literature [5]. Another consequence of maximizing Pr_{vol} is that less antibody can be purified in one cycle and therefore relatively more cycles might be needed to purify a specific amount of antibody. In this situation, the amount of antibody purified per quantity of resin is not maximized. However, the total amount of time needed to run these multiple cycles is shorter.

4. Conclusions

During a selection of protein A affinity resin, $Q_{dyn10\%}$, Pr_{vol} and 'process robustness' are essential parameters to be evaluated. Above mathematical models allow us to determine process conditions (u_{load}, C_0 and L) for each specific resin to maximize $Q_{dyn10\%}$, 'process robustness' or volumetric production rate (Pr_{vol}). However, as shown in Table 5, these parameters cannot be maximized all at the same time. Moreover, some other aspects like IgG recovery, protein A leaching, easiness to pack, easiness to clean, number of re-uses and cost of production might also be important to be taken into the equation. Depending on the specific situation, certain evaluation parameters may be more important than others. Therefore, a case-by-case evaluation is recommended.

Table 5
Overview of scores

	Dynamic binding capacity	Volumetric production rate	'Process robustness'
Prosep A High Capacity	+	+++	++
Prosep vA Ultra	++	+++	++
Rmp Protein A Sepharose 4FF	++	+	++
MabSelect	++	++	+++
MabSelect Xtra	+++	+	++

(+) moderate; (++) high; (+++) highest.

5. Nomenclature

C_0	load hIgG concentration
C_{EL}	elution fraction hIgG concentration
C_{FT}	flow through fraction hIgG concentration
C_{WA}	wash fraction hIgG concentration
CV/hr	column volumes per hour
d_p	resin bead diameter
hIgG	humanized monoclonal IgG_4
HETP	Height Equivalent to a Theoretical Plate
L	bed/column height
mAbs	monoclonal antibodies
Pr_{vol}	volumetric production rate
$Q_{dyn10\%}$	dynamic binding capacity at 10% breakthrough
u_{load}	load flow rate
V_{column}	column volume
V_{EL}	eluate fraction volume
V_{FT}	flow through fraction volume
V_{WA}	wash fraction volume

Greek letters

ε	interstitial bed porosity
γ	apparent friction constant
λ	bed compression
μ	liquid viscosity

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