

APPLICATION NOTE

Impact of Tip-based Systems and Jetting Technology on High Throughput Process Development

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Introduction

High throughput process development (HTPD) is crucial for screening resins and optimizing bioprocessing parameters. By mitigating risk and aligning process development with quality by design (QbD) principles, HTPD drives cost efficiency and reduces time to market for biopharmaceuticals.

Study Overview

One method for achieving HTPD is through a tip-based system, utilizing technology from Integrated Micro-Chromatography Systems (IMCS). IMCStips® utilizes dispersive solid-phase extraction to rapidly mix loose resin and sample in a pipette tip format. In conjunction with a liquid handling robot, this format has the capability to operate 96 tips at once. For best results operating this system, chromatography resins must demonstrate high binding capacity and fast mass transfer. Similar to batch binding in which resin is incubated with sample and mixed, IMCStips contain loosely packed resin inside pipette tips retained by two porous filters. Resin parameters such as ligand, ligand density, porosity, and composition can affect performance in tip-based systems. This study examines Purolite Resins and the impact of Jetting manufacturing on mass transfer, dynamic binding capacity, and yield in a high throughput process development (HTPD) format.

Methods

Praesto Jetted A50 and two leading protein A resins were compared for binding and elution efficiencies across a range of protein concentrations. In this series of experiments, loose resin is rapidly mixed with sample inside the tip, allowing a rate of binding similar to an agitated batch system. Nine experiments were conducted with different concentrations of human IgG (0.50–7.0 mg) to measure dynamic binding capacity (DBC) across the three resins. Binding experiments we performed in 1.5 ml PBS plus a set concentration of human IgG, this process was repeated for a total of 40 cycles at each set concentration level. Purolite™ resins demonstrated a high degree of rectangularity. This is caused by a steep initial gradient enabling maximum binding to be reached in fewer binding cycles, especially noticeable for samples with a higher initial concentration (figure 2). Binding to resin is governed by fast (k_f) and slow (k_s) kinetics. Fast kinetics represents easily accessible surface binding, while slow kinetics represents binding to the pores in the resin beads. At low concentrations of IgG, reactions are primarily governed by fast kinetics. As easily accessible sites decrease, slow kinetics become increasingly important as IgG must penetrate deeper into the resin pores to bind to the resin bead. With higher protein loads, a 50:50 balance of fast and slow kinetics (displayed by Jetted beads, Figure 2) proved to be the most effective strategy for maximizing binding while minimizing the number of cycles required. In this study, Praesto Jetted A50 shows the highest percentage of binding to the surface binding sites, likely due to the consistent resin bead size and even surface distribution of ligands.

Figure 1

Binding of Human IgG to Protein A Resins

Human IgG bound over 40 cycles for nine different concentrations of IgG across three Affinity Resins, Competitor A, Competitor B, and Praesto Jetted A50.

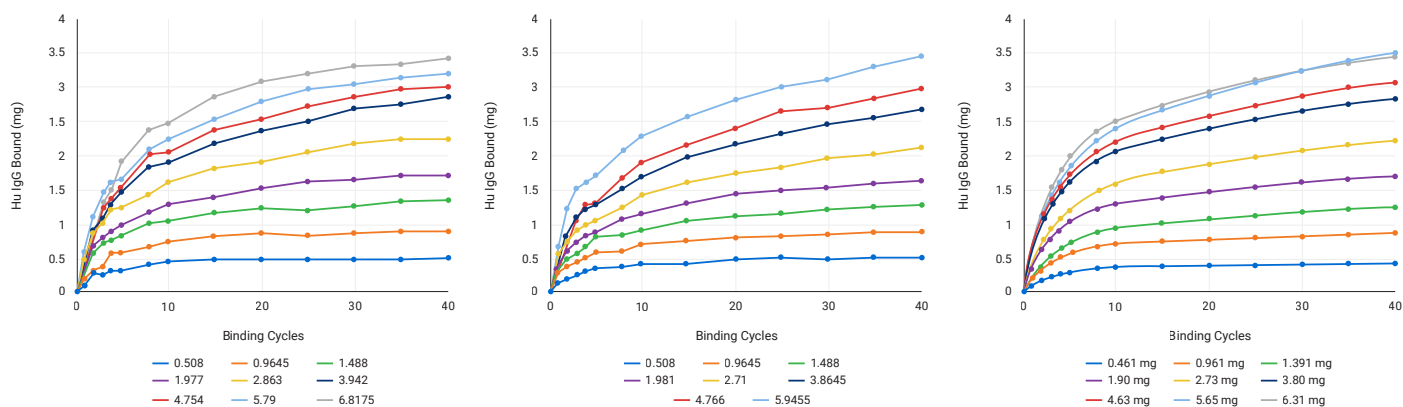
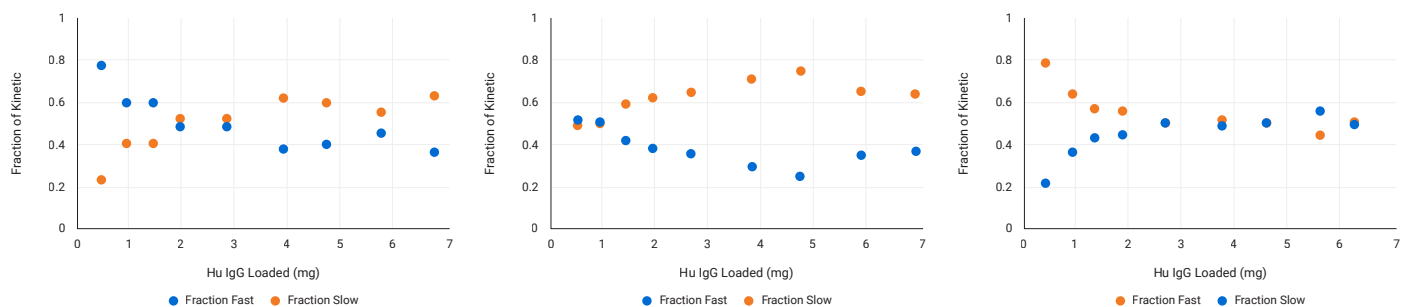


Figure 2

Fraction of Fast and Slow Kinetics of Protein A Resins



The Langmuir isotherm is a simple model used to describe the adsorption of molecules onto a surface. It provides a mathematical equation for the relationship between concentration and adsorption. It is commonly used to understand and model adsorption processes in chromatography. Datasets generated during this study were plotted according to a Langmuir isotherm model.

The function becomes more rectangular as the EBC (equilibrium binding capacity) is approached. As demonstrated in this study for Praesto Jetted A50, the apparent affinity (K_d) decreases as the cycle number increases, showing the resin’s efficiency in binding the target even as the concentration of human IgG remaining in the solution decreases. The efficiency of Praesto Jetted A50 is also demonstrated by the increase in the apparent binding capacity (Q_{max}) as the cycle number increases. In approximately 30 binding cycles, the majority of IgG has been bound. Fewer cycles are needed when using Praesto Jetted A50.

Equation 1

Langmuir Isotherm Model

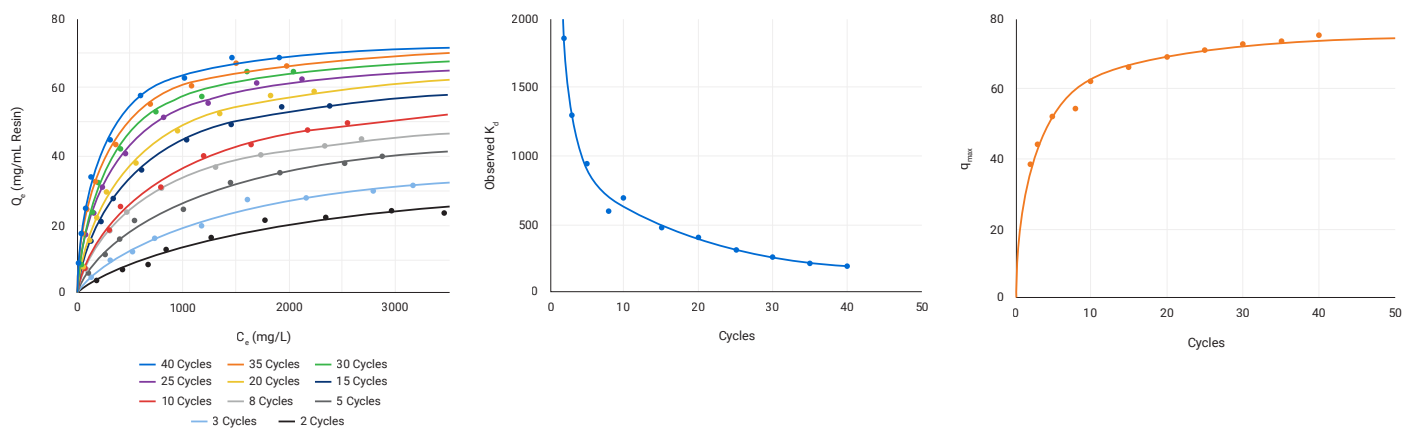
In a typical Langmuir isotherm model, a lower K_d value indicates a higher binding affinity and stronger molecule attraction. Conversely, a higher K_d value represents a weaker binding affinity.

$$\frac{q_{max}c_e}{K_d + c_e} = Q_e$$

Figure 3

Langmuir Isotherm Model for Praesto Jetted A50

Langmuir isotherms for Praesto A50 (left), Affinity (K_d) (center) and binding capacity (q_{max}) values for Praesto Jetted A50 vs cycles.



The dynamic binding capacity of the three resins was tested in a cycle-based system versus that of resin mixing system, with the sample in an Eppendorf tube for 24 hours on a tube rotator. While all three resins demonstrated positive aspects of performance in a tip, resin B and Praesto Jetted A50 showed the highest binding capacity, implying their superior use for high titer samples (Table 1). In addition, the low K_d values for resin A and Praesto Jetted A50 suggest their superior use for a low titer system. While each resin has a similar particle size, Praesto Jetted A50 resin beads are more uniform due to the Jetting manufacturing procedure, promoting faster mass transfer and facilitating target binding.

TABLE 1 Buffers for anion exchange chromatography. Comparison of data from all 3 resins.

Resin	DBC ₄₀ (mg/mL)	K_{d40} (mg/L)	k_f (cycle ⁻¹)	k_s (cycle ⁻¹)	k_f/k_s	Fraction Fast Sites	Median Particle Diameter* (µm)
A	69.2	168	0.322	0.044	7.28	0.45	50
B	79.4	329	0.617	0.050	12.3	0.37	60
Praesto Jetted A50	76.1	201	0.363	0.029	12.7	0.50	50

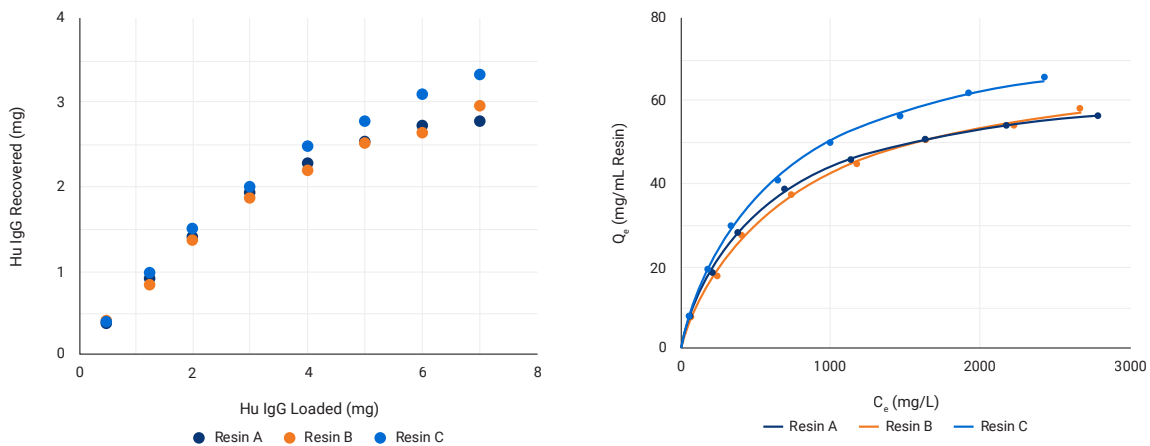
Elution efficiency was investigated (Table 2). Praesto Jetted A50 shows the highest protein eluted in the first two elutions, resulting in a more concentrated elution pool.

TABLE 2 Dynamic Binding Capacity Comparison of Protein A Resins

Resin	Sample Load (mg)	Bound (mg)	Eluted (E1+E2) (mg)	Overall Yield (%)
A	1.66 ± 0.02	1.51 ± 0.02	1.35 ± 0.03	81.7 ± 2.2
B	1.89 ± 0.03	1.50 ± 0.01	1.39 ± 0.07	73.5 ± 3.2
Praesto Jetted A50	1.86 ± 0.01	1.54 ± 0.02	1.49 ± 0.02	80.2 ± 1.7

Figure 4**Recovered Protein A Resins**

Recovered versus loaded protein (left). Protein remaining in solution (flow-through, C_e) and protein bound to resin (eluent, Q_e) (right). Praesto Jetted A50 outperformed both resins across the protein range.

**Results**

The study determined that the method of resin production will affect the properties of the chromatography column. The traditional method of agarose bead production, batch emulsification, requires extensive sieving of the resultant beads to narrow the size range distribution. Jetting manufacturing of resin beads requires no solvents to dissolve agarose nor extensive sieving to narrow the bead size, resulting in greater yield and narrow size range distribution. A narrower bead distribution gives a resin with higher mass transfer, resulting in greater efficiency in binding and elution. It also means rapid buffer transitions, optimizing washes, and reducing buffer consumption and cycle times.

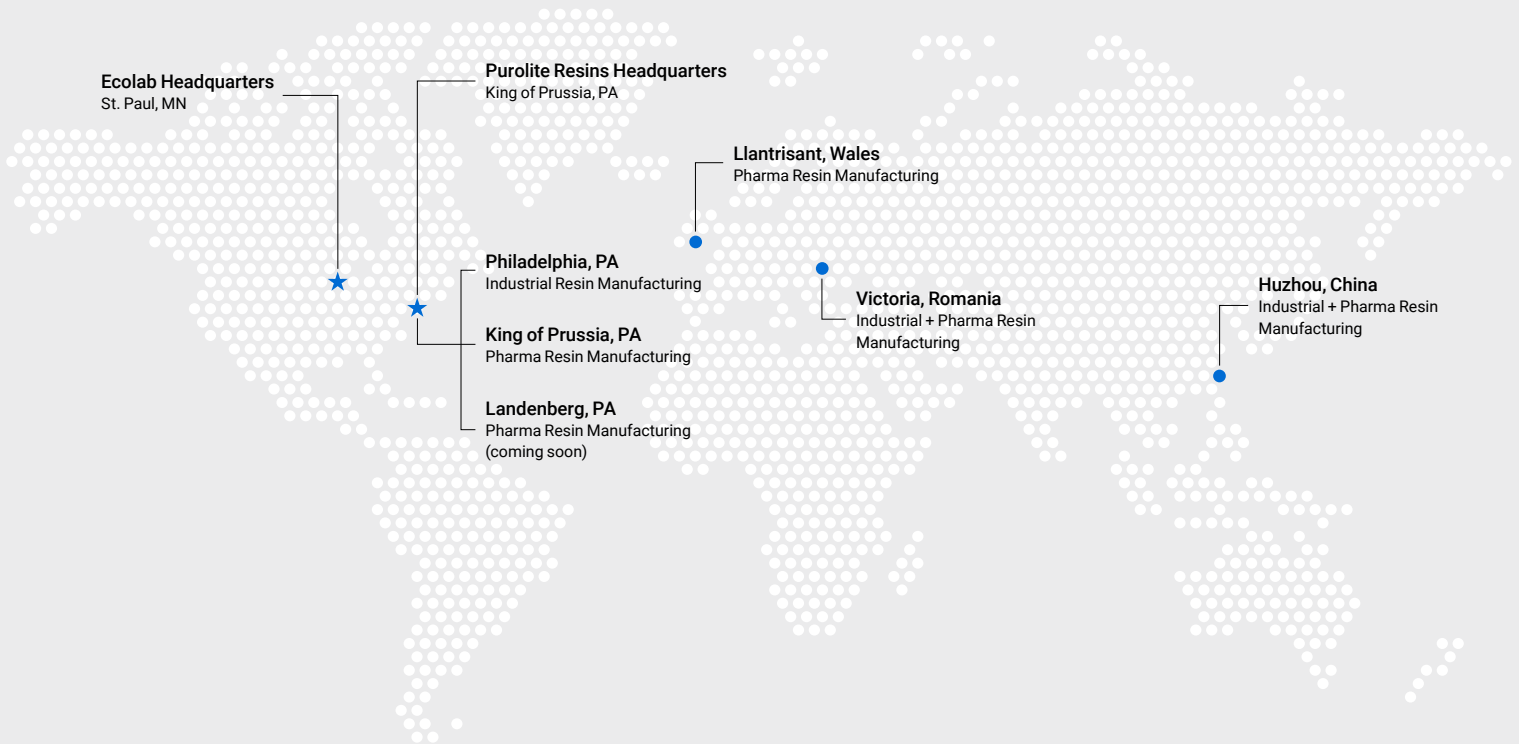
These properties support Praesto Jetted A50 as the most efficient resin for purification within the IMCS tip-based system. In this study, Praesto Jetted A50 demonstrated:

- High DBC and $I_{0.5}$ indicating it is ideal for samples with high titers
- Low K_d indicating it is ideal for samples with low titers
- Higher elution efficiency, compared to competitor resins evaluated

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