APPLICATION NOTE

Protein Purification -
Sample Preparation

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Automated Small Scale Protein Purification and Characterization for Accelerated Development of Protein Therapeutics

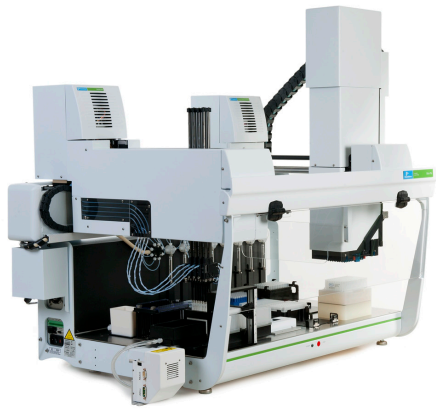

Figure 1. The JANUS BioTx Pro Plus Workstation

Introduction

Small scale protein purification presents opportunities for accelerated process development of biotherapeutic molecules. Miniaturization of purification conditions reduces time and allows for parallel processing of samples offering increased statistical significance and greater breadth of variables. Critical to the utility of this approach is the ability of the miniaturized platform to be predictive of larger scale purification schemes.

The JANUS® BioTx Pro and Pro Plus Workstations (Figure 1) were developed as intuitive, flexible, automated devices capable of performing parallel small-scale analytical protein purification. Pre-programmed methods automate a variety of commercially available ion exchange and affinity chromatography solutions, including miniaturized chromatography columns, resin-packed pipette tips, and resin filled microtiter vacuum filtration plates.

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A series of experiments was performed comparing the JANUS BioTx Pro Plus with GE ÄKTA Explorer™ chromatography to demonstrate the capabilities of a robotic platform to miniaturize chromatographic purification of proteins that is predictive of higher scale purification platforms. Key attributes tested were the ability to match elution profiles, determine column dynamic binding capacity and characterize separation resins.

Materials and Methods

A monoclonal antibody was used as source protein material for the experiments performed. Experiments were performed on the JANUS BioTx Pro Plus Workstation and GE ÄKTA Explorer™ 3.5 mL (0.66 x 10 cm) chromatography platform. All purifications were performed using 0.6 mL Atoll® Robocolumns® packed with proprietary resins. Recovered fractions were analyzed using UV absorbance and the LabChip® GX II microfluidic CE-SDS instrument electrophoresis platform.

Robotic Sample Processing

Monoclonal antibodies were purified using cation exchange chromatography. Automated loading of sample buffers and protein is achieved through the unique 4-way valve, allowing sample loops with continuous flow of >5 mL of loading material. Protein sample and buffers were preconfigured for elution profiling from microtitre plates and reservoirs, spanning a volume range of 0.2-50 mL. Precise control of flow rates allow direct control for resin screening and capacity determination studies, with minimum flow rates capable of achieving residence times of 11.83 minutes for 0.6 mL columns. Analytical fractions were collected in 96-well UV plates for direct analysis, or into standard 2 mL 96 deepwell plates using the integrated plate::shuttle (Figure 2).

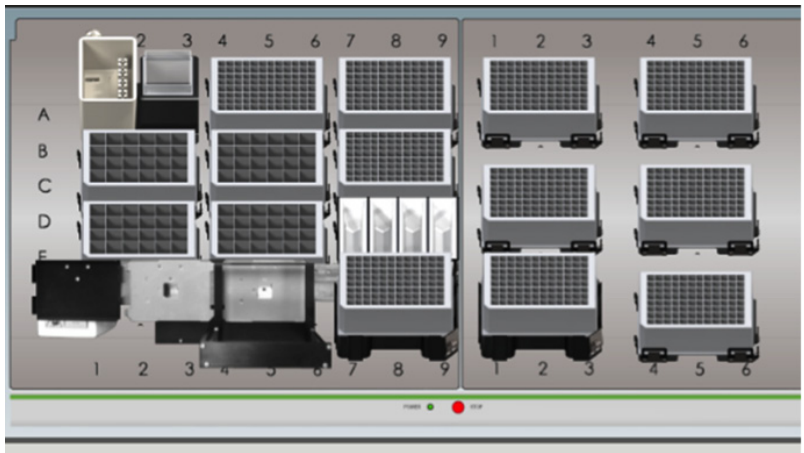


Figure 2a. The JANUS BioTx Pro Plus interfaces directly with Atoll® Robocolumns®, miniaturized chromatography columns that can be filled with a wide variety of commercially available and custom resins. *2b.* The JANUS Application Assistant (JAA) interface simplifies configuration and setup of the automated purification protocol.

Results

Column Reproducibility

A step elution of increasing NaCl in elution buffer from 50 to 600 mM was performed in parallel on four Atoll® Robocolumns® using the same resin. UV monitoring of each collected fraction was used to evaluate system reproducibility. A similar experiment was performed on ÄKTA™ (3.5 mL scale) for comparison. Results demonstrate a high level of reproducibility across replicates while obtaining similar results to those obtained on the ÄKTA™ platform (Figure 3).

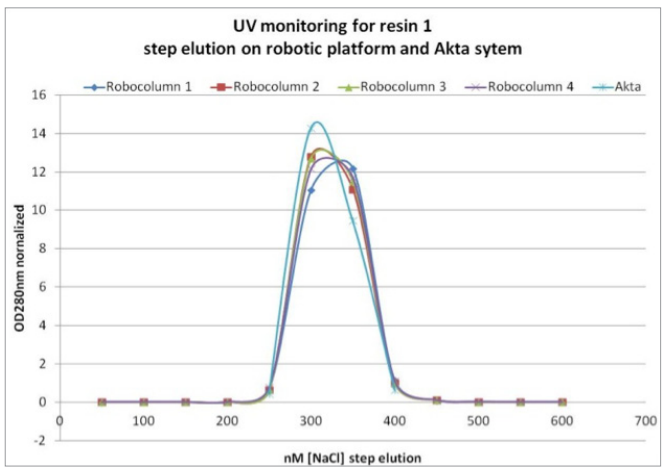


Figure 3. Reproducibility of the JANUS BioTx Pro Plus is demonstrated looking at four replicates as compared with traditional FPLC platforms.

Capacity Determination

To evaluate the ability of the JANUS BioTX Pro Plus to determine and compare residence time in a similar manner to the GE ÄKTA™ 3.5 mL scale column, a monoclonal antibody was purified using four strong and one weak cation exchange (CEX) resins packed in 0.6 mL Atoll® Robocolumns®. Flow rates for the JANUS BioTx Pro Plus were varied between 2 and 20 µL/s, resulting in varying residence times in order to obtain profiles deterministic of dynamic binding capacity for the various resins analyzed (Figure 4).

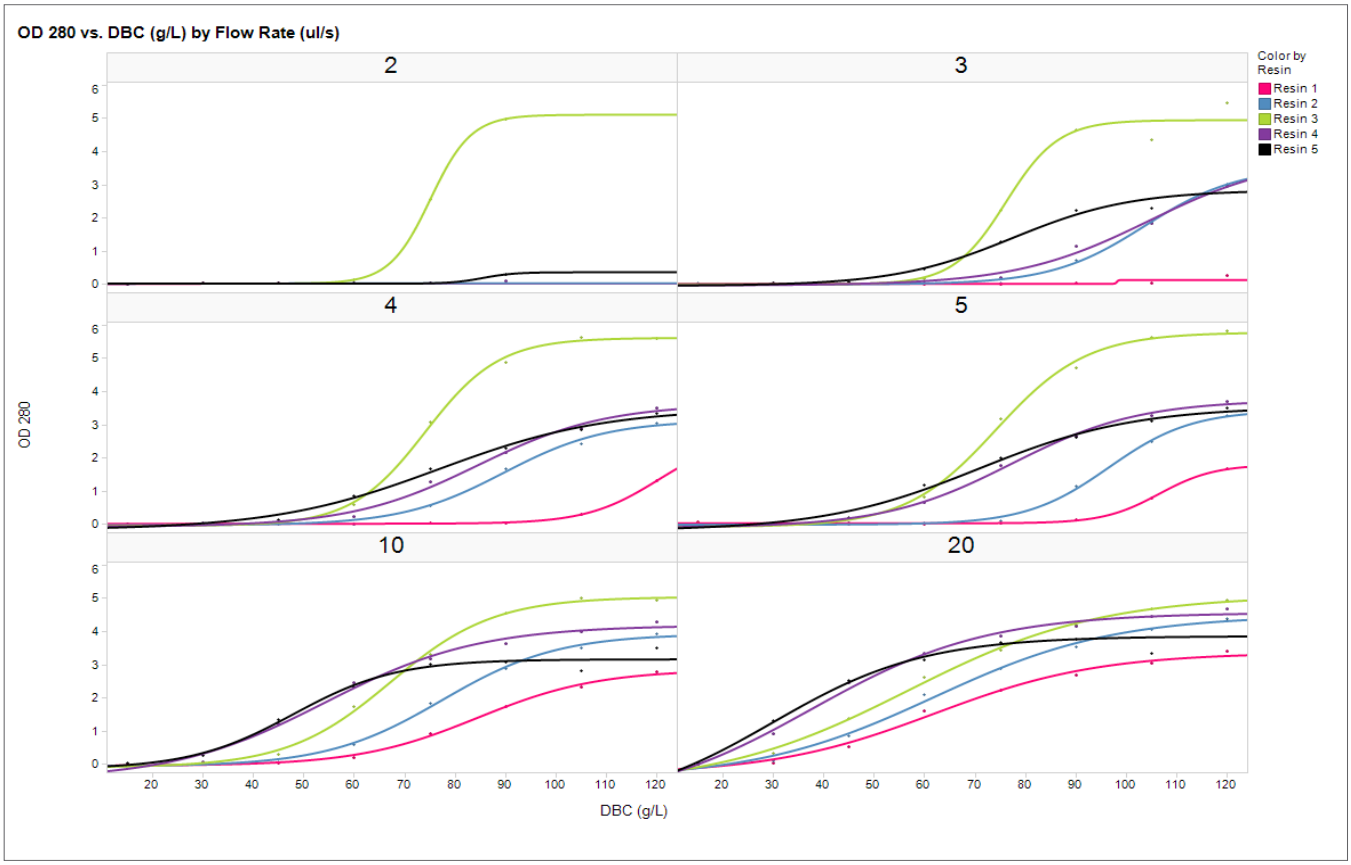
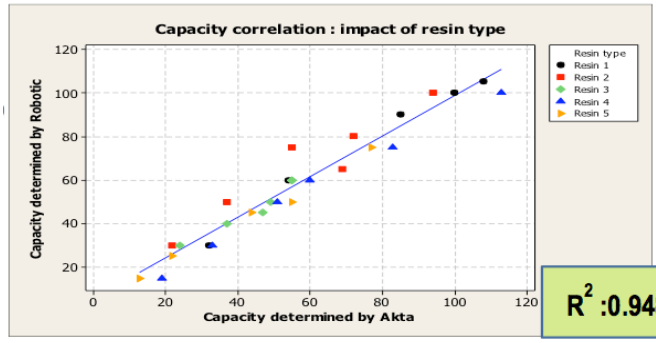


Figure 4. Capacity determination using robotic platform across 5 CEX resins using flow rates of 2, 3, 4, 5, 10 & 20µL/s.

Comparisons between these profiles and those obtained on a 3.5 mL ÄKTA™ Explorer platform were drawn to understand the predictability of these profiles to higher scale purification schemes. The required runs were accomplished in 1.5 days on the JANUS BioTx system, compared with a five day run time on the ÄKTA™ platform (Figure 5).

Akta explorer	Resin				
Residence time (min)	Resin 1	Resin 2	Resin 3	Resin 4	Resin 5
0.5	32	22	24	19	13
1	54	37	37	33	22
2	85	55	47	51	
2.5	100	69	49	60	44
3.3	108	72	55	83	55
5	124	94	55	113	77

Robotic platform	Resin				
Residence time (min)	Resin 1	Resin 2	Resin 3	Resin 4	Resin 5
0.5	30	30	30	15	15
1	60	50	40	30	25
2	90	75	45	50	45
2.5	100	65	50	60	45
3.3	105	80	60	75	50
5		100	60	100	75



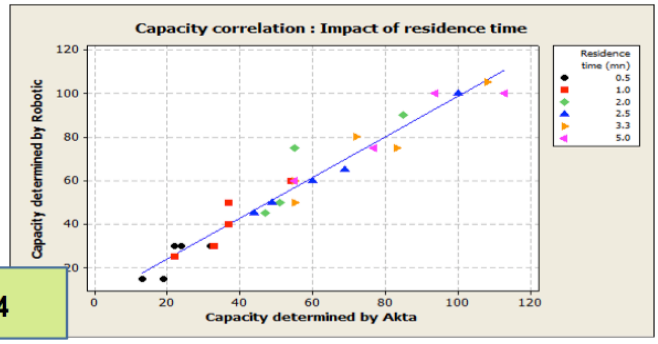


Figure 5. Comparison of the JANUS BioTx Pro Plus to AKTA Explorer FPLC platform across (a) resin type and (b) residence time.

Discussion

The JANUS BioTx Pro Plus presents in a platform with 10x advantages in throughput, and 5x reduction in protein mass requirement over higher scale Fast Protein Liquid Chromatography platforms. The capability of generating predictive data for a variety of process development experiments presents a tractable platform for high throughput acceleration of biotherapeutic protein development.

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