APPLICATION NOTE

Protein Purification -
Sample Preparation

Authors:

Lynn Jordan
Jeremy Lambert
Michael Mayer
Andrew Lindeman
Kevin McGuire
Nico Verlinden

Matteo Costioli
Clément Kaltenbach
Xavier LeSaout

PerkinElmer, Inc.
Hopkinton, MA

Merck KGaA
Darmstadt, Germany

Automated Small Scale Protein Purification and Characterization: Resin Characterization and Elution Profiling

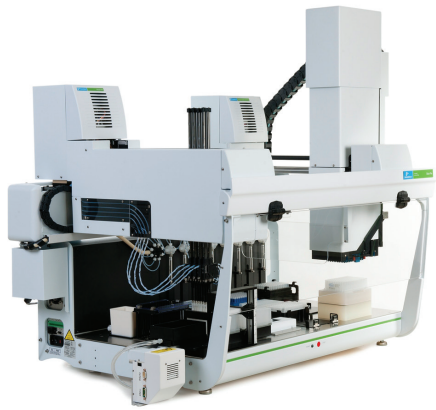

Figure 1. The JANUS BioTx Pro Plus Workstation

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 are developed as intuitive, flexible, automated instruments capable of performing parallel small-scale analytical protein purification. A graphical user interface was developed to accommodate a variety of screening experiments designed to determine the optimal conditions for purification of biotherapeutic proteins using small-scale commercially available ion exchange and affinity chromatography solutions.

PerkinElmer®
For the Better

We investigated the predictive capabilities of the JANUS B ioTx Pro Plus Workstation to provide fundamental control of experimental parameters required for screening studies. A series of experiments was performed to characterize resin types and buffer screening conditions of varying formulations. Results obtained using the JANUS BioTx Pro Plus were compared to those obtained from a larger scale GE ÄKTA Explorer™ FPLC instrument.

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 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 at volume ranges between 50 µL – 2 mL (Figure 2a).

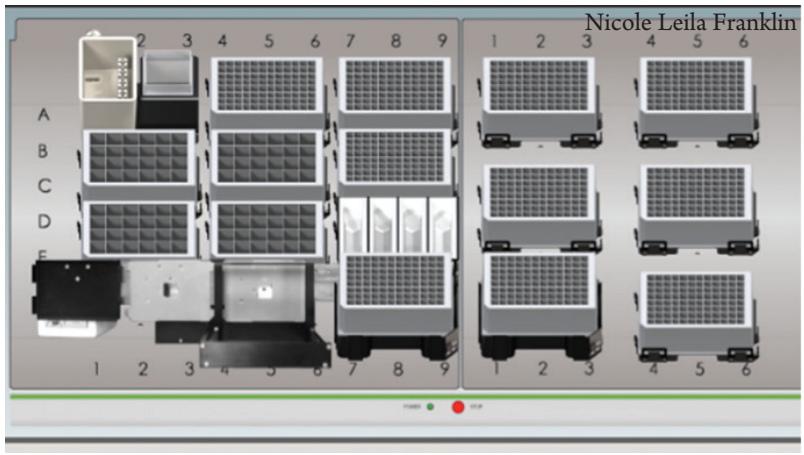

Figure 2a. The JANUS BioTx Pro Plus interfaces directly with Atoll® Robocolumns®, miniaturized chromatography columns that can be filled with a wide variety of commercially available and custom resins. *2b.* The JANUS Application Assistant (JAA) interface simplifies configuration and setup of the automated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

Programming of Experimental Conditions

The JANUS BioTx Pro and Pro Plus workstations utilize the JANUS Application Assistant (JAA) (Figure 2b) to provide a simple intuitive graphical user interface allowing the user easy control to modify parameters central to the goals of the particular study. This simple interface eliminates the need for any programming; yet, it allows the user to customize protocols for the chromatography steps, such as input sample volume, and the parameters for the collected fractions. A report file is generated that couples the entered parameters with fraction collection locations to allow for seamless integration with downstream analytical data associated with each fraction; enabling more efficient analysis and accelerating protein therapeutic development.

Results

Resin Characterization

Chromatographic experiments were conducted across five cation exchange (CEX) resins on the JANUS BioTX as well as on the GE ÄKTA Explorer™ platform to determine process yield and elution purity of recovered fractions (Figure 3). Collected fractions were analyzed for overall yield using UV absorbance at 280 nm and assessment of high and low molecular weight impurities were calculated through electrophoretic separation using the LabChip GXII platform.

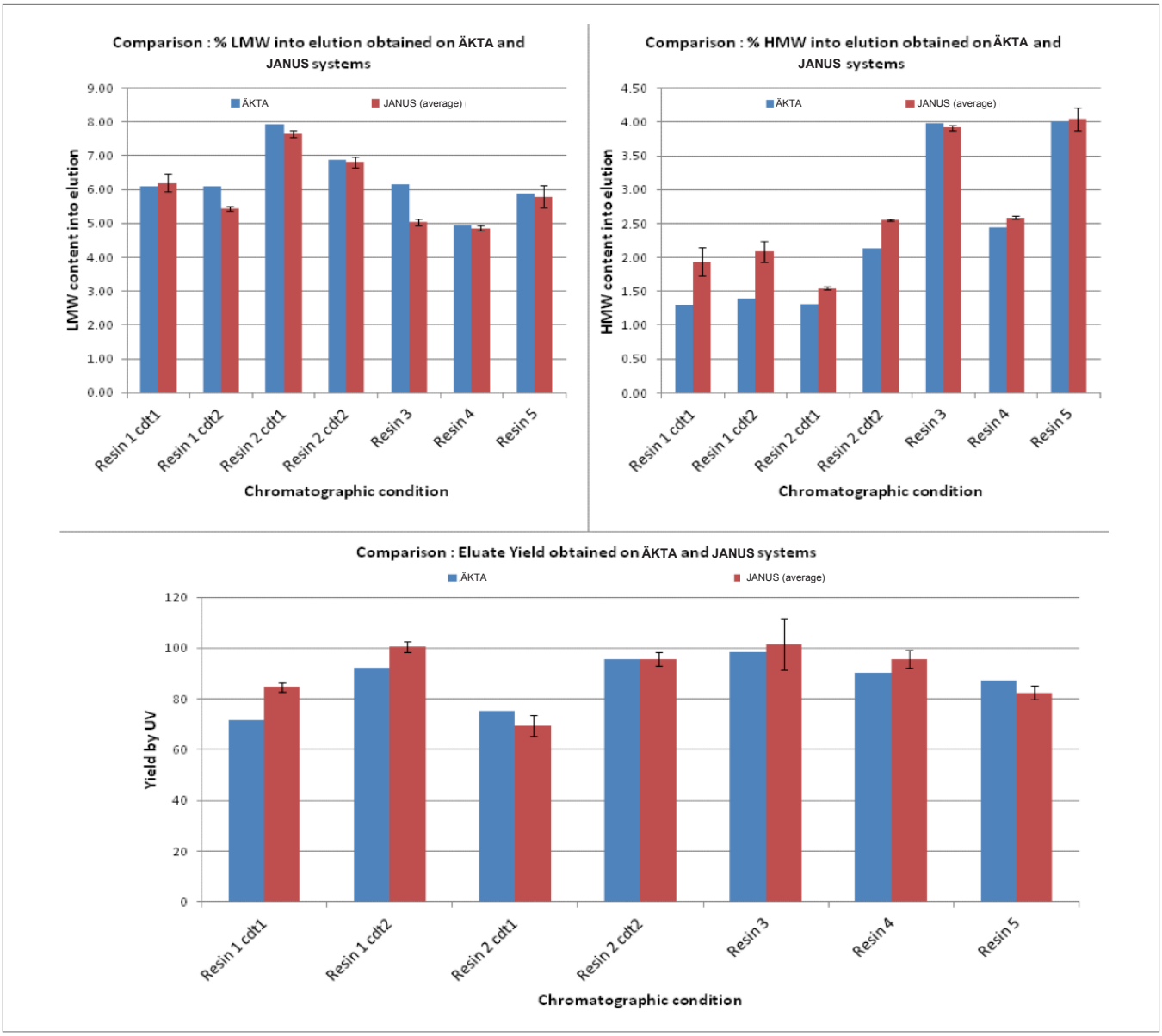

Figure 3 Chromatographic results obtained using the JANUS BioTx Pro Plus were compared to those obtained from a larger scale GE ÄKTA Explorer™ FPLC instrument.

Results show comparable profiles for eluates across conditions tested (e.g. yield, high and low molecular weight eluates) irrespective of platform. However, the seven chromagraphic runs were performed within a 1.5 hour period on the JANUS BioTx Workstation as compared to a 36 hour period on the GE ÄKTA Explorer™ platform. Because of the decreased run times on the JANUS instrument, the results were performed in triplicate, showing statistical similarity across replicates.

Elution Profiling

A stepwise gradient elution study was performed on the JANUS BioTx Pro Plus platform to determine optimal salt concentration for maximum yield of a desired protein (Table 1). A single CEX resin was used to perform the purification. For the elution step, the instrument loaded equal volumes of prepared solutions ranging 250-400 mM NaCl. Collected fractions were analyzed for overall yield using UV absorbance at 280 nm. In order to further understand the composition of the collected fractions, the eluted fractions were run on the Protein Express assay on the LabChip GXII automated capillary electrophoresis system. The LabChip GXII utilized microfluidic CE-SDS to assess the presence of high and low molecular weight impurities as calculated by relative abundance of species of different molecular weights (Figure 4).

Table 1. A stepwise gradient elution study was performed to determine optimal salt concentration for maximum yield of a desired protein.

Step	Volume (BV)	Remark
Equilibration	5 BV	25 mM acetate pH5.5
Load	To reach 20 g/L as capacity	Degraded load at pH5.5 containing HMW and LMW material
Wash	3 BV	25 mm acetate pH5.5
Elution	Increase of 50 mM NaCl per CV	25 mM acetate pH5.5 to 25 mM acetate pH5.5+0.8 M NaCl
Regeneration	3BV	0.5 M NaOH + 1 M NaCl

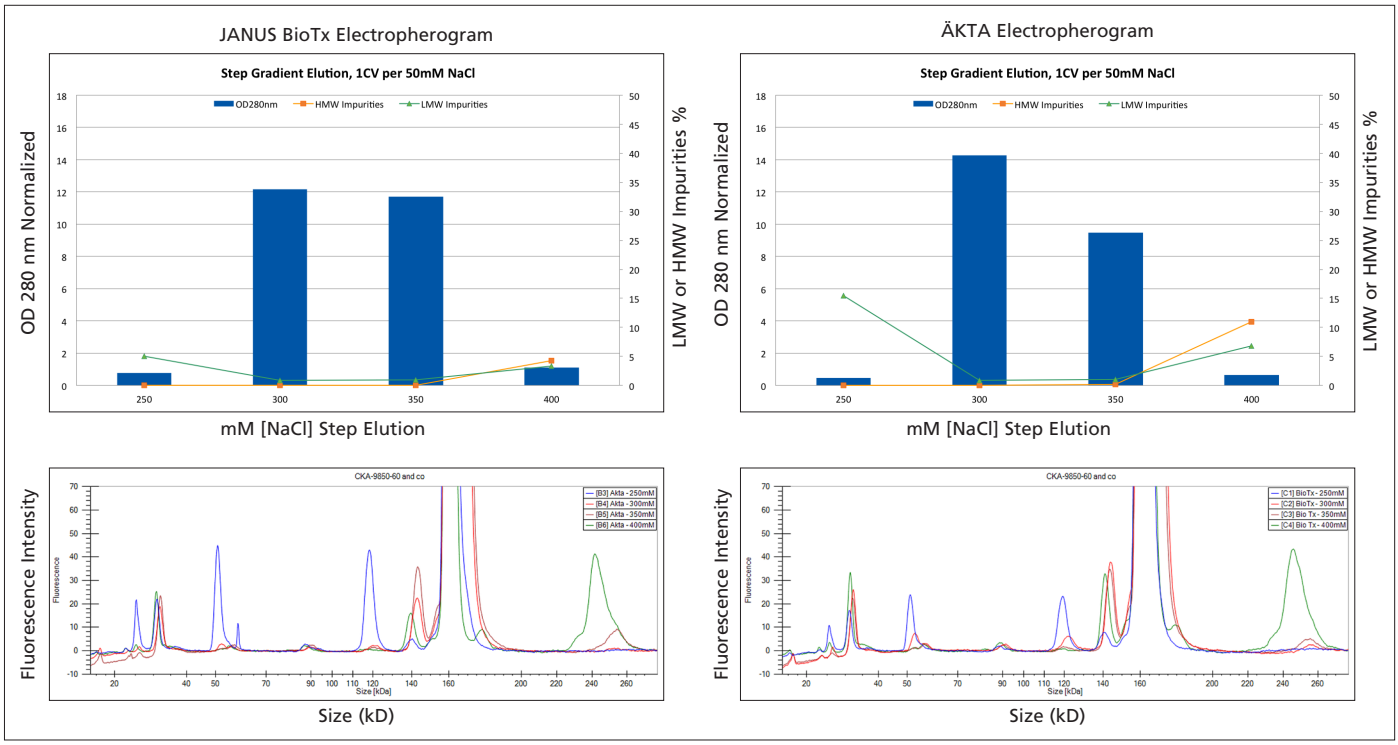

Figure 4. Optimal salt concentration for maximum yield study on a desired protein. Study achieved similar results irrespective of platform. Collected fractions were analyzed for overall yield (UV absorbance at 280nm) and the presence of high and low molecular weight impurities (LabChip GXII).

Discussion

The JANUS BioTx Pro Plus presents a platform with tenfold advantages in throughput, 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.

For research use only. Not intended for diagnostic procedures.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

011868_01 PKI