doi:10.1017/S1431927616006309
Microsc. Microanal. 22 (Suppl 3), 2016

Using Microscopy to Qualitatively Assess Protein A Resin and Guide Cleaning In Place (CIP) Strategy

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Commercial scale monoclonal antibody (mAb) purification usually employs rProtein A for capture because of its high selectivity for mAb, followed by one or two other chromatography steps [1]. rProtein A resin is very expensive leading to a higher overall cost of antibody based biotherapeutic protein production [2]. Since the cost of the resin is one of the most significant expenses associated with the mAb purification, it is reused after it is cleaned and sanitized. Various solutions and protocols are used on resins for cleaning in place (CIP) to remove strongly bound impurities and sanitizing in place (SIP) to kill microbial contamination [3, 4]. Effective cleaning and sanitizing protocols are essential early in development not only to reduce costs associated with reuse of the resin but also to reduce deviations associated with bioburden during scale up activities at commercial manufacturing.

PAB (120mM Phosphoric acid, 167mM Acetic Acid, 2% Benzyl Alcohol) has historically been used as an effective CIP solution during glass bead based Protein A chromatography. However, when implemented over agarose based rProtein A chromatography, cleaning with PAB resulted in pressure increase across the column over the course of multiple runs. The goal of this work was to determine if Solution A, an alternative solution identified for CIP, could replace PAB. To test whether this increase in pressure was due to resin fouling or damage when using PAB, scanning electron microscopy (SEM) was used to characterize the resin surface.

MabSelect SuRe™ rProtein A agarose based resin (GE Healthcare, Piscataway, NJ, USA) was studied with mAbs A and B. rProtein A resin packed in a chromatography column was loaded with clarified cell culture feed streams from CHO cell lines. The stream was run up to 75 and 48 cycles respectively for mAb A and B. For each mAb, two sets of studies were conducted: one using PAB and the other using Solution A for CIP. The pressure across the columns was monitored during the course of all these runs and product eluting from the column was collected intermittently every 8 cycles and subjected for impurity analysis. Following all chromatography studies, the resin was unpacked from the column and subjected to SEM imaging to assess resin bead and pore structure.

CIP with Solution A had consistent performance over multiple cycles in contrast to the pressure spikes produced by CIP with PAB. The SEM imaging studies allowed for direct visualization of the pore structure of the resin samples collected at the end of the cycling. Samples were prepared for SEM analysis similar to a procedure reported by Close *et al* [5]. Resin slurry was pipetted onto a pre-coated (Pt/Pd) glass coverslip and excess liquid was wicked away. Samples were sputter coated (Cressington 208 HR Auto Sputter Coater, Ted Pella, Inc., Redding, CA, USA) and secondary electron images were acquired at 2keV accelerating voltage (XL30 ESEM FEG, FEI Company, Hillsboro, Oregon, USA).

SEM documented the fouling, with smooth, featureless debris; and cracking of the agarose beads

exposed to PAB. This effect was more pronounced at the top of the column which is consistent with what one would expect as the initial contact takes place at the top of the column. Product quality (% monomer, host cell protein levels, DNA levels) for both PAB and Solution A of the purified mAb showed comparable analytics. Based on the SEM data and product quality, Solution A was recommended as the CIP buffer of choice for scale up.

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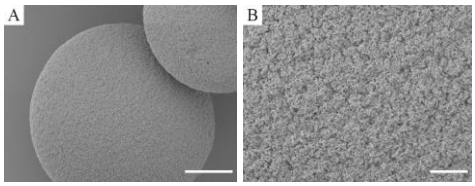

Figure 1. New MabSelect SuRe™ Resin. A) Scale bar is 10 µm. B) Scale bar is 2 µm.

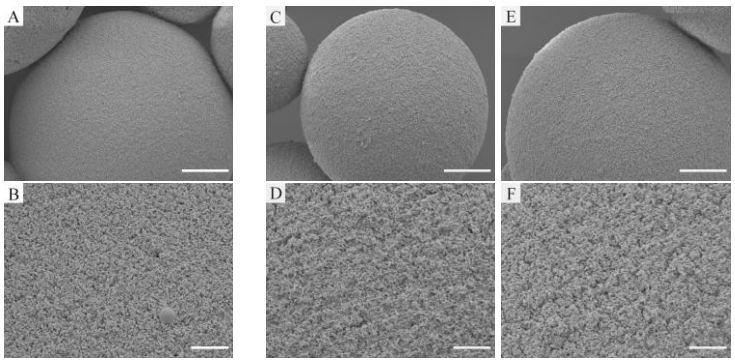

Figure 2. MabSelect SuRe™ Resin after CIP with Solution A shows a clean surface. A - B) Resin cycled with mAb A. C-D) Resin cycled with mAb B, removed from top of column. E-F) Resin cycled with mAb B, removed from bottom of column. Scale bar is 10 µm for A, C, E and 2 µm for B, D, F.

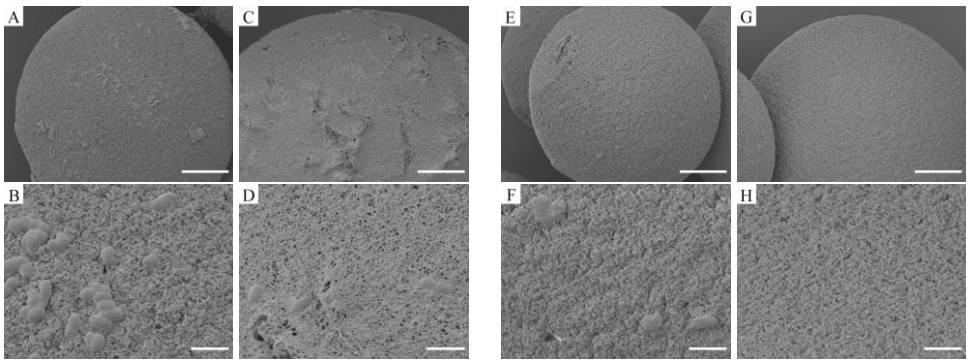

Figure 3. MabSelect SuRe™ Resin after CIP with PAB shows fouling and cracking of the surface. A - D) Resin cycled with mAb B, removed from top of column. E - H) Resin cycled with mAb B, removed from bottom of column. Scale bar is 10 µm for A, C, E, G and 2 µm for B, D, F, H.

https://doi.org/10.1017/S1431927616006309 Published online by Cambridge University Press