Downstream Processing: Purification of anti IL-8 mAb by Protein A Chromatography on the AKTA PURE System

Conditioned media was harvested from the bioreactor by centrifugation followed by microfiltration. The media was then concentrated using tangential flow filtration and a 9.5 ml sample of the concentrate was used for protein A chromatography on the AKTA pure system.

Purpose:

To purify anti IL-8 mAb from concentrated conditioned media and analyze the purity of the samples using SDS PAGE

Materials:

- Concentrated conditioned media from bioreactor harvest
- Materials are listed in the corresponding SOPs

Documents used:

- SOP: Isolation of Anti IL-8 mAb from Conditioned Medium by Protein A Chromatography on the AKTA Pure
- Batch Record: Downstream Processing of Anti IL-8 mAb
- SOP**:** AKTA Pure Operation
- SOP: SDS PAGE

The SOPs above and other related resources can be found on the NBC2 website at the link below:
http://biomanufacturing.org/curriculum-resources/program-units/downstream-processing

Anti IL-8 mAb, Protein A Sepharose Chromatography

<u>Sample chromatogram 1</u>

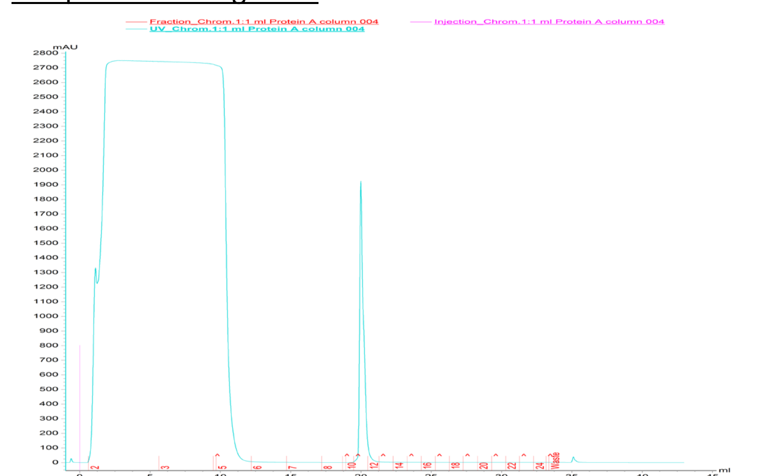


Figure 1: A typical protein A chromatograph showing flow through and a single purified mAb peak

Protein A sepharose chromatogram 2 –sample experimental data

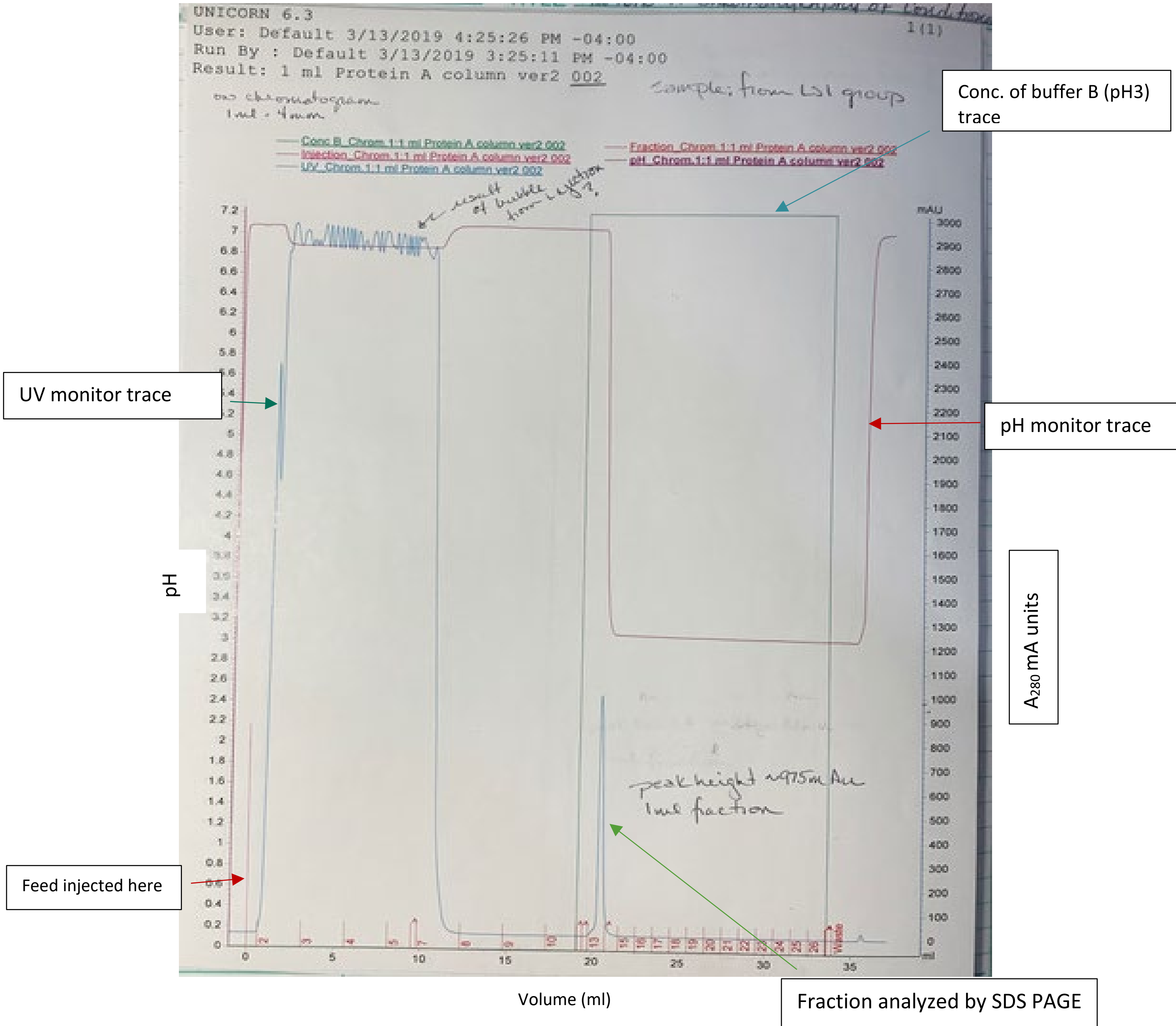


Figure 2: A typical protein A chromatogram

- Large peak from fraction 2 to fraction 7 representing the flow through material which consists of in-process impurities like host cell proteins, DNA, and media components which do not bind to the protein A column. The anti IL-8 mAb was eluted in fraction 13 by the introduction of 100% buffer B (pH 3).

A representative flow through fraction (3), peak fraction (13) and peak tail fraction (14) were stored at 4°C then analyzed by SDS PAGE.

Nanodrop Data:

The total protein concentration of fraction 3 (flow through), fraction 13 (peak) and fraction 14 (peak tail) was measured using the Nanodrop total protein setting or IgG setting to measure the mAb fractions.

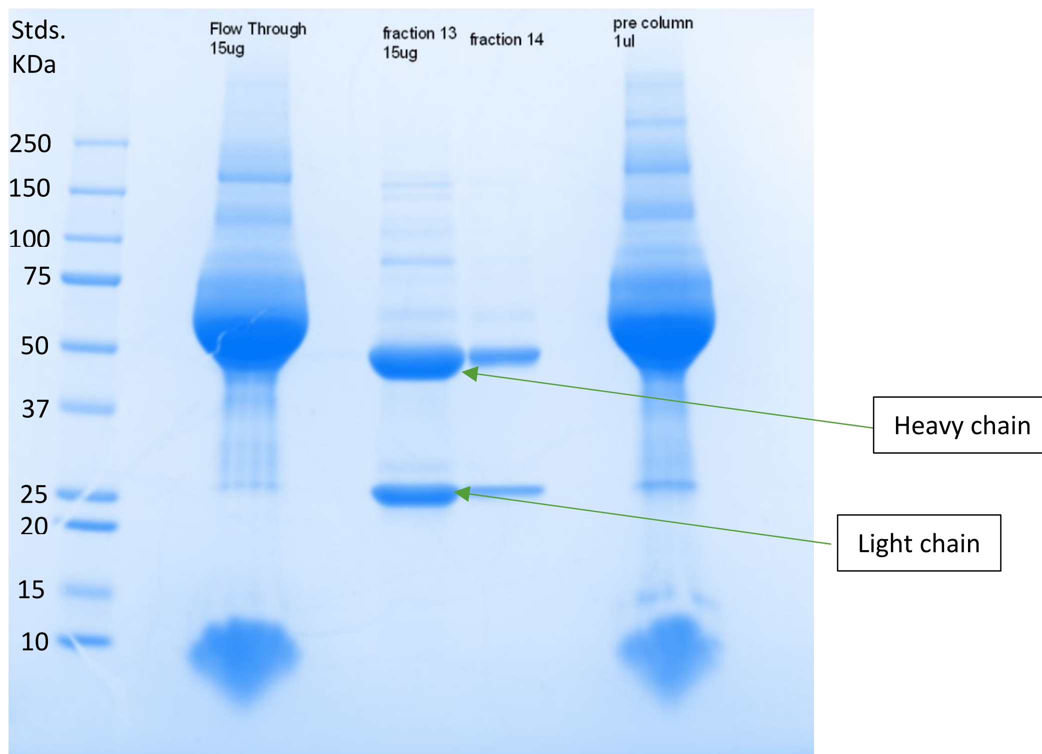
Protein Concentration from Nanodrop of Protein A chromatography Fractions

Fraction #	Sample ID	A280	Sample Type	Protein Conc.	Unit
3	Flow through	13.935	total protein	13.935	mg/ml
13	Peak	1.381	IgG	1.008	mg/ml
14	Peak tail	0.212	IgG	0.155	mg/ml

15ug of flow through (fraction 3) and peak (fraction 13) was loaded onto the gel and 6.36ug of post peak (fraction 14) was loaded. The pre column material could not be measured using the nanodrop due to the presence of detergent so approximately 15ug or 1ul was loaded.

SDS PAGE analysis of fractions:

Figure 3. 4-20% SDS-page gel



SDS-page gel analysis:

- Lane 1: 5 µl of Bio-Rad Kaleidoscope protein standards
- Lane 3: The large band at approximately 60 kDa is most likely of bovine serum albumin from the low IgG FBS added to the media. Several other fainter bands are observed.

- Lane 5: Intense bands at about 50KDa and 25KDa representing the heavy and light chain of mAb are seen. The denaturing conditions used for the gel denatures the 150KDa mAb into 2 heavy and 2 light chains. Other faint bands are seen at about 27KDa, 75KDa, 80KDa, 100KDa and 150KDa. The peak fraction is overloaded to analyze for the presence of host cell proteins. The visual analysis indicates that the peak mAb fraction approximately 95% pure.
- Lane 6: Two intense bands at 50 and 25 KDa representing the heavy and light chain of the mAb are visible along with very faint bands at 57, 75 and 80 KDa
- Lane 8: Similar to FT lane, strong band at approximately 60kDa representing BSA and several bands representing host cell proteins. A band at 25KDa is observed representing the mAb light chain, the 50Kda heavy chain band is obscured by the BSA band.

The anti IL-8 monoclonal antibody is approximately 95% pure after this chromatography step. Protein A chromatography is highly efficient in purifying mAbs and is used in the capture step of a typical commercial scale downstream process. This step is followed by intermediate and polishing chromatography step which involve ion exchange, hydrophobic interaction or mixed mode chromatography.

Experiments performed and recorded by Dr. Maggie Bryans, Hetal Doshi and Robin Zuck at Montgomery County Community College. Questions regarding data can be sent to mbryans@mc3.edu. This work was funded by NSF ATE DUE 1501631, the Northeast Biomanufacturing Center and Collaborative.