Introduction

- IgG is one of the most complex biomolecules that require highly selective chromatographic media to separate from its impurities and aggregates.
- Aggregates are generated easily during manufacturing processes and storage. The removal of IgG dimer and aggregates from its monomer before clinical usage is critical.

Objectives

- Evaluation of weak anionic chromatographic media for selectivity and separation performance.
- Develop an efficient flow through process for aggregate removal using a weak anion exchange resin.
- Use of PolyPEI for removal of aggregated IgG from monomer using flow through mode.

Experimental

- All separations were performed in 100 × 7.75 mm ID columns packed with various media using an Äkta Explorer chromatographic system (GE Healthcare).
- Aggregated human IgG was generated by heating a concentrated IgG solution at pH 7.2 solution to 64 °C for 20 minutes.
- Sample 1: IgG and aggregate (about 1:1) in binding buffer; total protein content 1.5 mg/mL.
- Sample 2: 0.08 mg/mL IgG aggregate, 1.0 mg/mL IgG monomer in binding buffer.
- BAKERBOND™ PolyPEI (35 µ, multimode weak anion exchanger) is a product of Avantor Performance Materials Inc. (formerly Mallinckrodt Baker).
- Commercial anion exchangers were purchased from respective manufacturers.
- Human IgG and other proteins were purchased from Sigma.
- Tris (hydroxymethyl)aminomethane, 2-(N-morpholino)ethanesulfonic acid (MES) and other chemicals used are Ultrapure Bioreagents from Avantor Performance Materials, Inc., 1904 J T Baker Way, Phillipsburg NJ 08865.
- All fractions from separations were analyzed by SEC with a Wyatt SEC Tris (Hydroxymethyl)aminomethane, 2-(N-morpholino)ethanesulfonic acid (MES) and other chemicals used are Ultrapure Bioreagents from Avantor Performance Materials, Inc., 1904 J T Baker Way, Phillipsburg NJ 08865.

SEC conditions—

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Conclusions

- BakerBond PolyPEI is shown to be highly selective compared to competitive anion exchange media under similar conditions.
- PolyPEI is effective for the removal of IgG aggregates under wide pH range (5.5 – 7.0) under flow-through mode. IgG monomer was obtained with high yield (>90%) and high purity (>99%).