A single step procedure for IgG purification from serum was developed using cation exchange chromatography. Three different types of strong cation exchangers were investigated and compared for selectivity. The developed method yields pure protein (>90%) with good recoveries (>95%) and can be easily scaled to a larger column.

Objective

- Develop a single step chromatographic method for the purification of IgG from rabbit serum.
- Investigate and compare performance of commonly available cation exchangers for this application.
- Optimize the conditions for the selected media.
- Scale-up the process from lab to process scale under optimized conditions.

Experimental

- BAKERBOND™ PolyCSX (15µm, strong cation exchanger) is a product of Mallinckrodt Baker, Inc.
- Strong cation exchanger — 90µm (Media-1)
- Strong cation exchanger — 75µm (Media-2)
- Rabbit serum was purchased from Sigma (PN R4505) and Rockland (Product Code 0119-00).
- All laboratory separations were performed in 4.7 ml columns (100 × 200 mm ID) using an ÄKTA Pilot chromatographic system (GE Healthcare).
- The scale-up experiment was carried out in a 3.4 L BPG column (110 × 350 cm; Gradient 20 CV; Flow rate: 306 cm/hr). The ratio of scale-up volume is a factor of 721.

Comparison of lab scale and large scale chromatograms of PolyCSX with 0.5M NaOH has been shown.

Further Investigation of PolyCSX under Various Conditions

Results for IgG separation by PolyCSX at different conditions

Throughput Comparison of Cation Exchange Chromatographic Media

Separation of rabbit Serum on PolyCSX

Analysis of Fractions by Electrophoresis—PolyCSX

Analysis of Fractions by Size Exclusion Chromatography (SEC)

Separation (inset) and Analysis of Fractions by SXC—Media 1

Separation (inset) and Analysis of Fractions by SXC—Media 2

Comparison of lab scale and large scale chromatograms of PolyCSX

Conclusions

- High selectivity of PolyCSX enables a single step purification of IgG from a complex matrix.
- Removes common impurities such as high molecular weight proteins, albumin and hemoglobin.
- Reversible binding-elution process along with cleaning/sanitization shows the media’s use in multiple cycles.
- Facilitates scale-up due to robustness of the media and operating conditions.
- 700 fold scale-up to 20 cm id column was achieved from 0.7 cm id lab scale column.
- Due to the media’s high selectivity, high sample loading was possible.
- IgG purity of more than 90% was achieved in the presence of high concentration of contaminants and other proteins.
- PolyCSX has shown to provide better selectivity in this application compared to two other available strong cation exchangers.

One Step Purification of IgG from Serum: Optimization and Scale-up


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