GE Healthcare
Life Sciences

Application note 29-0642-95 AA

Chromatography systems

Depletion of albumin from serum samples using ÄKTA™ start

Albumin comprises 55% to 65% of the total protein in serum. The abundance of this protein makes it difficult to study other serum proteins, hence removal of albumin in a single step will speed up the process and aid in easy identification and characterization of low-abundance serum proteins, such as disease-specific biomarkers. Yang used UNICORN™ start 1.0 control software to develop a method for quick and easy removal of albumin from human serum using ÄKTA start chromatography system, Frac30 fraction collector and a C 10/10 column packed with Blue Sepharose™ 6 Fast Flow (FF) medium (resin).

Introduction

Serum is comprised of thousands of proteins and the composition varies with different physiological or pathological conditions. Some serum proteins may be potential biomarkers that are used for monitoring either disease progression or treatment. Effective depletion of albumin is a key step in the isolation and analysis of these biomarkers, which are typically present in low amounts.

Yang is a research scholar in a research laboratory focusing on identifying and characterizing disease-specific biomarkers in human serum. Yang felt too much of his time was being spent on manual purification tasks such as continuously monitoring the run at each step, collecting fraction manually and identifying protein fractions in the absence of an on-line UV detection facility. To save time and to make the process simple and effective, Yang switched to ÄKTA start with a Frac30 fraction collector. He used UNICORN start software from a neighboring lab to develop a method to remove albumin from human serum.

Blue Sepharose is a chromatography medium commonly used to selectively remove albumin from serum samples or culture media. Blue Sepharose 6 FF is Cibacron™ Blue 3G coupled to Sepharose 6 FF. Albumin binds to Cibacron Blue 3G, a synthetic polycyclic dye that acts as an aromatic anionic ligand binding the albumin via electrostatic and/or hydrophobic interactions. Albumin depletion in turn enriches the other serum proteins, which are usually present only in very low concentrations.

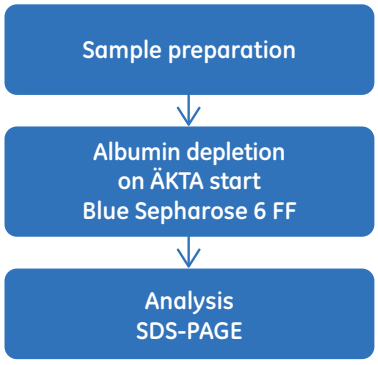


Fig 1. Workflow illustrating the steps involved in albumin depletion with a Blue Sepharose 6 FF column.

Methods

Yang followed the workflow illustrated in Figure 1 to perform albumin depletion using ÄKTA start. The depletion method was developed using the UNICORN start Method Editor module. The optimized run parameters Yang used are described in Table 1.

Table 1. UNICORN start method overview

Method flow	Method settings
Method settings	User defined column, Column volume: 3 ml Pressure: 0.1 MPa[1] Flow rate: 3 ml/min
Prime and equilibration	3 CV[2]
Sample application	Apply sample using loop, Sample volume: 1 ml Flow rate: 0.5 ml/min Fixed volume fractionation, Fraction volume: 1 ml
Wash out unbound	3 CV[2] Flow rate: 1 ml/min Fixed volume fractionation, Fraction volume: 1 ml
Elution and fractionation	Isocratic elution: % B concentration 100%, Elution volume: 5 CV[2] Fractionation; Peak fractionation, level based (start/end: 10 mAU)
Prime and equilibration	3 CV[2]

[1] 0.3 MPa = 3 bar (43.5 psi)
[2] CV = Column volumes

Yang exported the method from UNICORN start to a USB memory stick and imported the method into ÄKTA start instrument placed in a cold cabinet. Yang manually packed a C 10/10 column (i.d. 10 mm) with Blue Sepharose 6 FF medium (3 ml). The column was mounted onto ÄKTA start instrument and equilibrated with binding buffer (20 mM sodium phosphate buffer, pH 7.0). A sample loop was used to load 1 ml of sample (human serum) onto the column. The sample was loaded at a flow rate of 0.5 ml/min to ensure efficient binding of albumin to the media. Flowthrough was collected as 1 ml fixed volume fractions using Frac30 fraction collector. To remove unbound proteins, the column was washed with 3 column volumes (CV) of binding buffer at 1 ml/min flow rate.

Prepare ÄKTA start system by priming with desired buffers

Bound albumin was eluted from the column using 5 CV of 100% elution buffer (20 mM sodium phosphate buffer, 2 M NaCl, pH 7.0) in a single step. The protein peak was fractionated using UV level-based fractionation and 1.5 ml fractions were collected using Frac30 fraction collector. The run was monitored in real time using the UV trace from the instrument display. Using the bitmap (.bmp) result file, Yang identified the protein fractions collected during the sample application, wash unbound and elution steps. The pooled fractions were analyzed by 12.5% SDS polyacrylamide gel electrophoresis (SDS-PAGE).

Results

In this study Yang depleted albumin from serum samples in a single depletion step, using a C 10/10 column manually packed with Blue Sepharose 6 FF media. Yang took advantage of the additional options available in UNICORN start to develop a method that included multiphase fractionation. Fixed volume fractionation was used to collect flowthrough and peak fractionation (UV level-based) was used to collect bound albumin. The compact size of ÄKTA start, and the ability to run the instrument in a standalone mode (i.e. without being connected to UNICORN start software) with the Frac30 fraction collector allowed Yang to perform the depletion run in a cold cabinet. The Frac30 fraction collector allowed him to collect fractions at multiple phases without manual intervention.

Customize your method using UNICORN start Method Editor

To quickly view the results and to identify the peak fractions for pooling Yang printed the bitmap (.bmp) image file that had been generated during the purification run and saved onto a USB memory stick (Fig 2). He also imported the result file from the USB memory stick into UNICORN start (Fig 3A) and used Evaluation module functions such as peak integration for further analysis of the results and PDF report generation for project archival.

The SDS-PAGE profile of serum fractions showed > 95% removal of albumin from the human serum sample (Fig 3B). The flowthrough was free from albumin and suitable for further characterization.

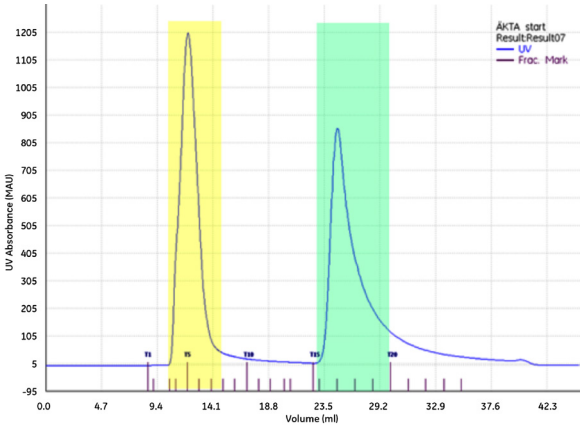
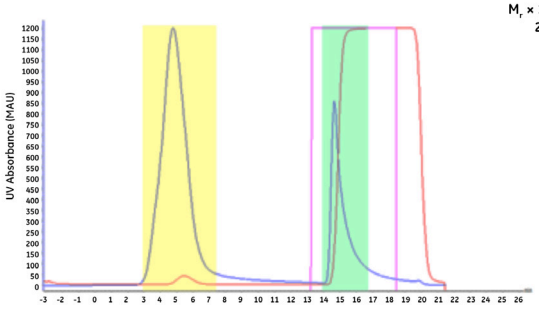


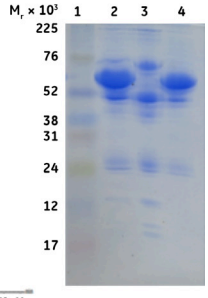
Fig 2. Chromatographic profile (.bmp) of albumin depletion from human serum sample on ÄKTA start. The highlighted areas (yellow: Flowthrough; green: Eluate) represent the pooled fractions.

Column:	C 10/10 Column, packed with 3 ml Blue Sepharose 6 FF
Sample:	Human serum
Sample load:	1 ml
Binding buffer:	20 mM sodium phosphate buffer, pH 7.0
Elution buffer:	20 mM sodium phosphate buffer, 2 M NaCl, pH 7.0
Flow rate:	3 ml/min (equilibration and elution) 0.5 ml/min (sample application) 1 ml/min (wash out unbound)
Gradient:	100% step
System:	ÄKTA start, Frac30 fraction collector and UNICORN start
Detection:	UV (280 nm)

(A)



(B)



Lanes

1. High-range Rainbow™ molecular weight markers
2. Load
3. Flowthrough
4. Eluate

Fig3. (A) Chromatogram showing albumin depletion from human serum sample on ÄKTA start (data imported from USB memory stick to UNICORN start software). The area highlighted in yellow represents the flowthrough fractions, and the green-highlighted area is the albumin-containing eluate). The red lines represents the conductivity curve and the pink line shows the gradient concentration. (B) SDS-12.5% PAGE profile of the sample following albumin depletion.

Summary

Yang used the intuitive UNICORN start software to quickly develop a method for effective depletion of albumin from human serum using ÄKTA start, Frac30 fraction collector, and a manually-packed column with Blue Sepharose 6 FF media. By using an automated process, Yang no longer needed to spend so much time monitoring the run. The bitmap result file generated by ÄKTA start enabled quick and easy identification of relevant fractions, and automatic fraction collection with Frac30 saved Yang time and ensured that the relevant fractions could be easily pooled.

Depletion of albumin helped Yang enrich the serum sample, facilitating biomarker isolation and characterization. Yang is now able to purify multiple batches to obtain low-abundance serum proteins for further characterization.

Ordering information

Product	Quantity	Code number
ÄKTA start	1	29-0220-94
UNICORN start 1.0 software	1	29-0187-51
Frac30 fraction collector	1	29-0230-51
Blue Sepharose 6 Fast Flow	50 ml	17-0948-01
C 10/10 column	1	19-5001-01
High-range Rainbow molecular weight markers	1 × 250 µl	RPN756E

Related literature

Product	Code number
Purification of N-terminal histidine-tagged protein using ÄKTA start, Application note	29-0642-77
Purification of GST-tagged protein using ÄKTA start, Application note	29-0642-98
Purification of antibodies using ÄKTA start and HiTrap Protein G HP column, Application note	29-0643-02

For local office contact information, visit
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