Protein A Affinity IgG Capture Followed by AdvanceBio SEC Aggregation Analysis

Using the Agilent 1290 Infinity 2D-LC Solution

Application Note

Biotherapeutics and Biologics

Authors

Patrick Cronan, Timothy Rice, and Michael Kruger
Agilent Technologies, Inc.

Abstract

This Application Note describes the quantification of aggregates in biotherapeutic samples using the Agilent 1290 Infinity 2D-LC solution. Protein A affinity capture was performed in the first dimension, and size exclusion chromatography in the second dimension. Multiple heart-cutting was used to transfer the samples from the first to the second dimension. This technique enabled the entire characterization to be done with a single analysis, eliminating the need for fraction collection and multiple injections.

Agilent Technologies

Introduction

Protein therapeutics have a complexity far exceeding that of small molecule drugs, therefore, unraveling this complexity represents an analytical challenge. During the development and lifetime of these molecules, an in-depth characterization is required[1].

Protein A is an immunoglobulin-Fc (IgG) receptor found in the cell wall of *Staphylococcus aureus*. This protein has a strong affinity for polyclonal and monoclonal IgGs such as human IgG 1, IgG 2, IgG 4, and IgGs from other species such as rabbit and mouse. Immobilized Protein A is commonly used for preparative and process scale purifications of IgG. At the analytical scale, the Agilent Bio-Monolith Protein A HPLC column can be used for fast quantification and small-scale purification of IgGs in complex mixtures or pure samples[2,3].

Size exclusion chromatography (SEC) is the method of choice for the quantification of aggregates in biotherapeutic samples. Protein A IgG capture, followed by SEC for aggregation analysis, yields more accurate characterization, as purer sample is analyzed. With the Agilent 1290 Infinity 2D-LC solution, this analysis can be done with one injection. Protein A capture of IgG is performed in the first dimension. Then, eluted IgG is trapped in the multiple heart-cutting loops, and eluted in the second dimension onto an Agilent AdvanceBio SEC sizing column for accurate aggregate analysis.

Experimental

Instrumentation

The 2D-LC solution used in this study comprised an Agilent 1260 Infinity Bio-Inert LC as the first-dimension system. The 1260 Infinity Bio-Inert LC was coupled through an Agilent 2D-LC Quick Change Valve, a multiple heart-cutting valve (with 12 factory installed 40 µL sample loops), and an Agilent 1290 Infinity high-speed pump for second-dimension chromatography. Diode array detection (DAD) was deployed in the second dimension. Figure 1 shows the instrument modules used.

Software

Agilent OpenLAB CDS ChemStation Edition software with Agilent 1290 Infinity 2D-LC software was used to configure the system, set up the 2D-LC methods, and control the 2D-LC data acquisition (Figure 2).

Sample

The sample was an IgG standard purchased from Sigma (p/n I9640).

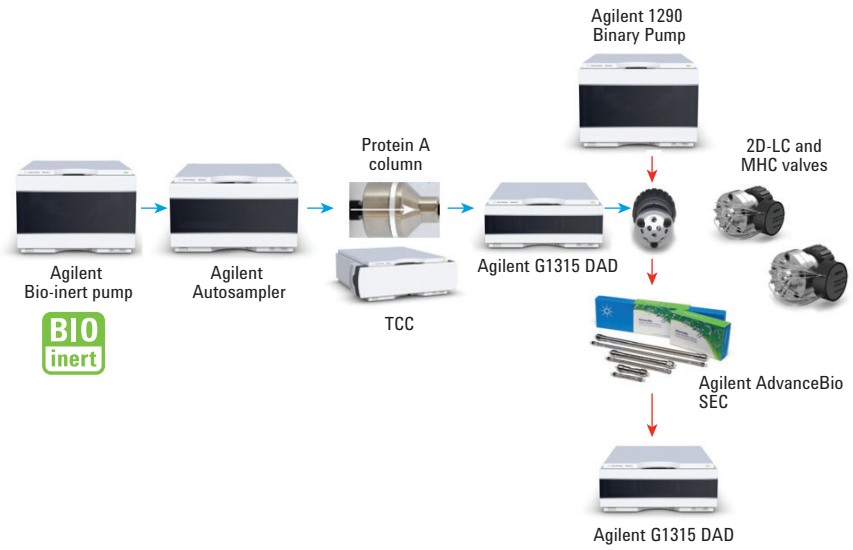
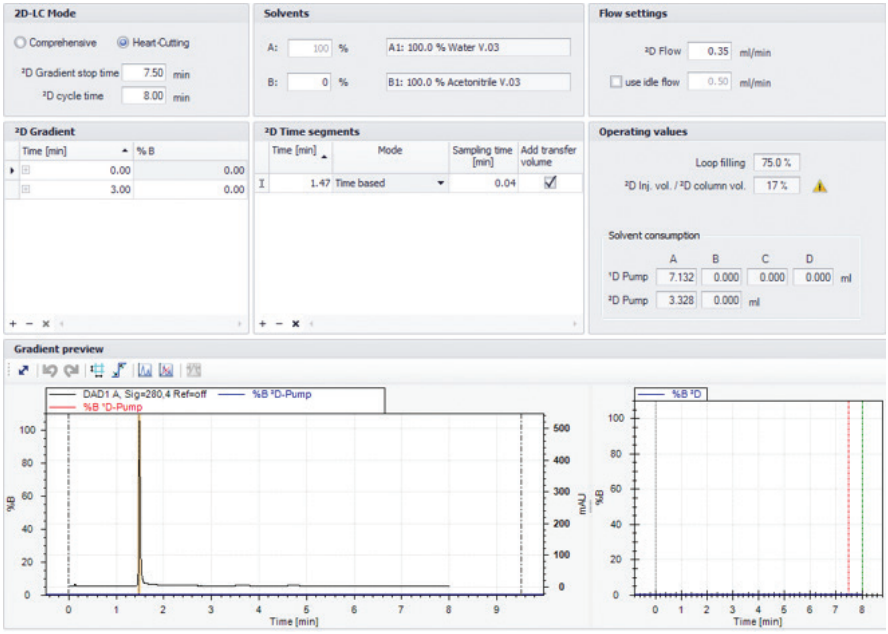


Figure 1. 2D-LC solution configuration with Agilent Bio-Monolith Protein A column in the first dimension and Agilent AdvanceBio SEC in the second dimension.

A



B

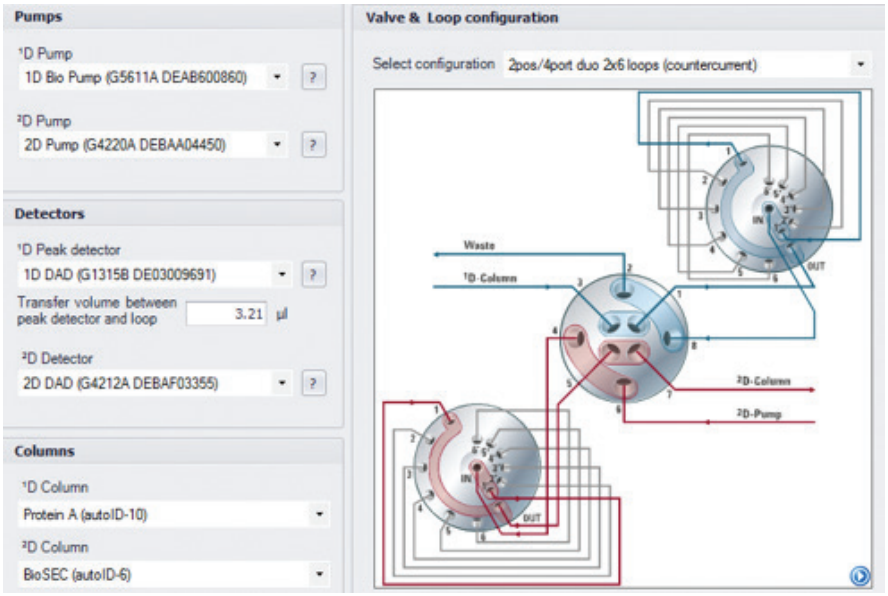


Figure 2. Configuring the 2D-LC system with Agilent OpenLab CDS ChemStation 2D-LC software, and setting up the second gradient and multiple time cuts with a reference first chromatogram along with an overlaid first-dimension gradient. The method setup dialog enables easy setup of multiple times and timing events.

Results and Discussion

This 2D separation served to speed up the analysis of monoclonal antibody (mAb) aggregation. By combining two steps in the aggregation characterization, it was possible to conduct the entire analysis with one injection, thus removing the fraction collection step all together. Figure 3 shows a typical Protein A affinity capture separation. The sample was captured in phosphate-buffered saline, and eluted with 500 mM acetic acid (aqueous).

Figure 4 shows that, when used as a first dimension in a 2D analysis, the SEC step can follow immediately after release from the Protein A.

Methods

First-dimension separation – Protein A affinity capture with UV detection	
Column	Agilent Bio-Monolith Protein A (p/n 5069–3639) at room temperature
Flow rate	0.75 mL/min
Mobile phase A	100 mM PBS
Mobile phase B	500 mM acetic acid
Gradient	0 minutes – 0 %B 0.5 minutes – 0 %B 0.51 minutes – 100 %B 2.5 minutes – 100 %B 2.6 minutes – 0 %B
Second-dimension separation – SEC	
Column	Agilent AdvanceBio SEC 300 Å, 4.6 × 150 mm, 2.7 µm (p/n PL1580-3301), at 25 °C
Flow rate	0.35 mL/min
Mobile phase	10 mM PBS pH 7.4, isocratic
2D-LC mode	Multiple heart-cutting

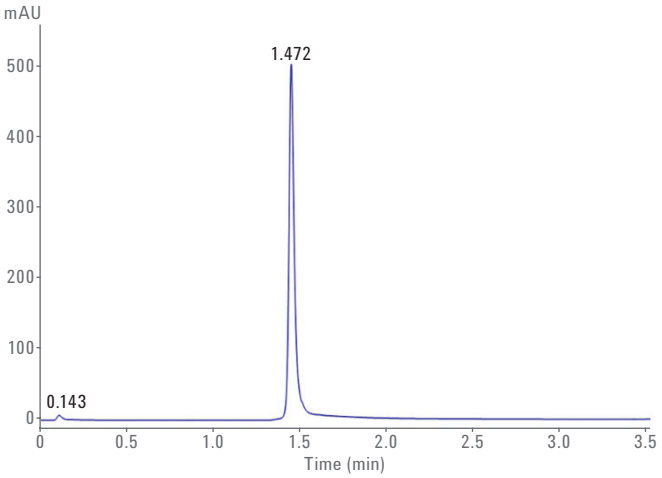


Figure 3. Protein A affinity capture separation of IgG, using an Agilent Bio-Monolith Protein A column.

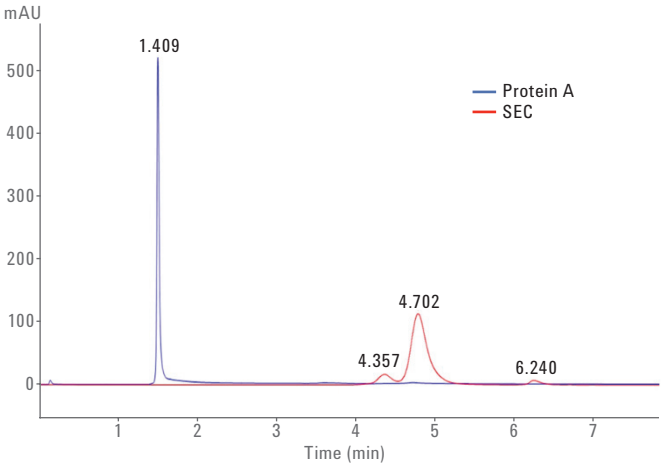


Figure 4. Protein A affinity capture using an Agilent Bio-Monolith Protein A column and size exclusion separation of IgG using an Agilent AdvanceBio SEC column.

This process demonstrates the feasibility of coupling Protein A and SEC techniques together in one analysis using the Agilent 1290 Infinity 2D-LC solution. Following this demonstration, SEC detection of different aggregation concentrations using the 2D method was tested. The IgG standard was heat stressed in a hot water bath at 65 °C for 24 hours. This heating resulted in a larger concentration of aggregates, as shown in Figure 5 and Figure 6. Figure 6 shows clear resolution of aggregates from monomer and dimer species.

Conclusions

The Agilent 1290 Infinity 2D-LC solution offers a versatile and efficient way to determine mAb titer and aggregation in a single method, eliminating the need for fraction collection and multiple injections. Agilent Bio-Monolith Protein A columns used as the first dimension of a 2D-LC workflow are convenient for the analytical-scale purification of mAbs for titer determination, and as an initial purification step before aggregation analysis. The Agilent AdvanceBio SEC 300 Å column demonstrated excellent resolution of aggregate species from the monomer to enable accurate evaluation as the second dimension in a 2D-LC workflow.

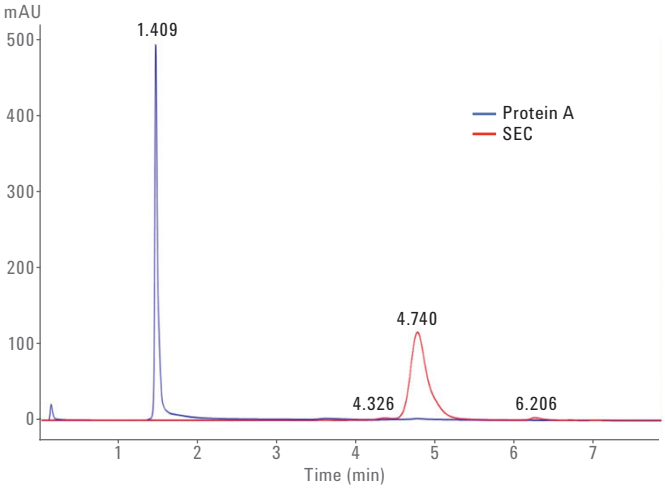


Figure 5. Protein A affinity capture with an Agilent Bio-Monolith Protein A column, and size exclusion separation of heat-stressed IgG using an Agilent AdvanceBio SEC column.

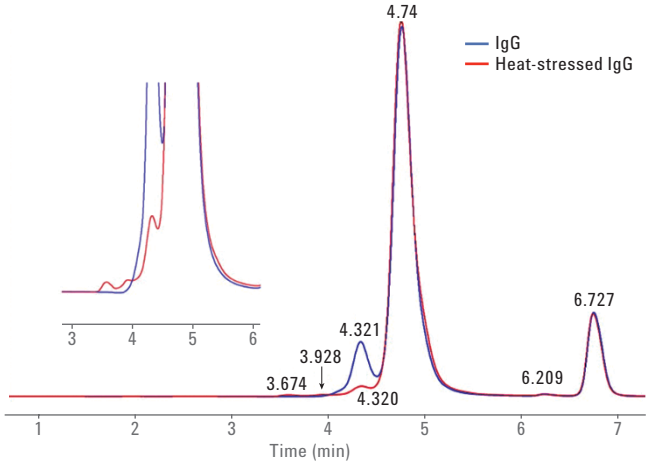


Figure 6. Agilent AdvanceBio SEC 2D size exclusion separation overlay of IgG and heat-stressed IgG.

References

1. Vanhoenacker, G.; *et al.* Analysis of Monoclonal Antibody Digests with the Agilent 1290 Infinity 2D-LC Solution *Agilent Technologies Application Note*, publication number 5991-2880EN, **2013**.

2. Lidija, U.; *et al.* Rapid Human Polyclonal IgG Quantification Using the Agilent Bio-Monolith Protein A HPLC Column. *Agilent Technologies Application Note*, publication number 5989-9733EN, **2008**.

3. Duong, P. T. Agilent Bio-Monolith Protein A Monitors Monoclonal Antibody Titer from Cell Cultures. *Agilent Technologies Application Note,* publication number 5991-2990EN, **2014**.

www.agilent.com/chem

Published in the USA, September 1, 2016
5991-7223EN

Agilent Technologies