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Protein Aggregation Case Study

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Job Number: J14292

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March 21, 2019

The following test was performed:

1. Standardized Gel Permeation Chromatography (GPC)
2. Tetradetection Gel Permeation Chromatography (GPC-T)

Objective

Albumin (Thermo Scientific, Lot#SD242052, Exp 07/25/2020) and IgG from rabbit serum (Sigma-Aldrich, Lot# SLBX4521, Exp 10/01/2020) and a Protein Standard Mix 15-600 kDa were analyzed on an Agilent Bio SEC column (300Å, 2 µm, 7.8X300 mm). ***The goals of this analysis were to determine the aggregation state for the proteins and to determine the differences in the calculated molecular weights via standardized GPC as compared to GPC with light scattering.***

Introduction

Protein aggregation is a common phenomenon in nature and often occurs during protein manufacturing processes, formulation and storage. For biopharmaceutical manufacturers, protein aggregation poses a key challenge in the development and storage of biologics as it has a great impact on drug efficacy and immunogenicity. Technology to monitor and detect protein aggregation is therefore very important to ensure the quality of protein-based pharmaceuticals. Here, we described a reliable SEC method to achieve this goal quantitatively.

Sample Preparation

Albumin standard was received in a 0.9% aqueous NaCl solution containing sodium azide at 2 mg/mL. The standard was used as received.

IgG was received as a solid. 1.058 mg of IgG was dissolved in 2 mL of PBS buffer (pH=7.4). The solution was then injected without further treatment.

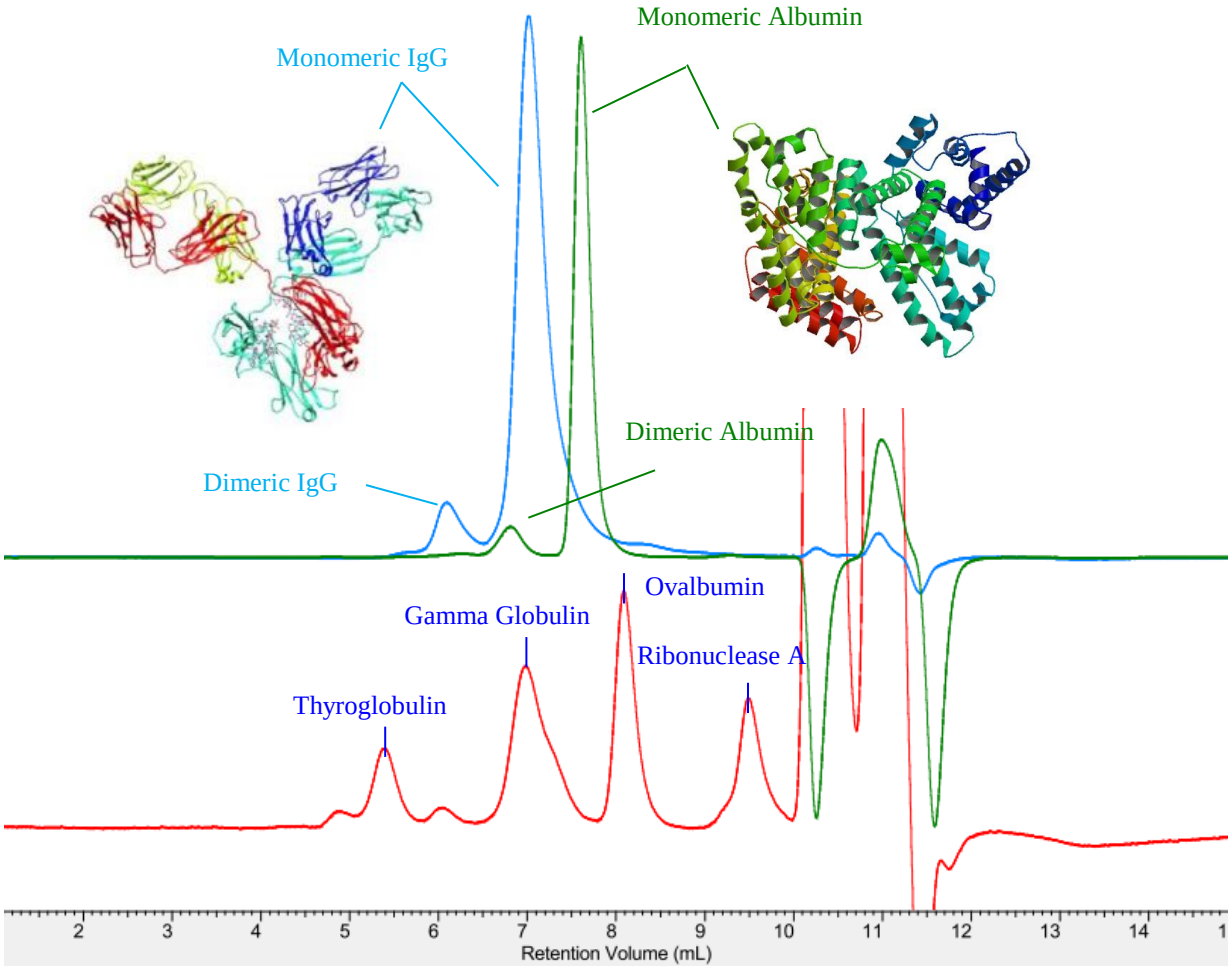
Protein Standard Mix was received as a powder. 1.3078 mg of the protein standard mix was dissolved in 2 mL of PBS buffer (pH=7.4). The solution was then injected without further treatment.

Standardized GPC Results

A set of protein standards with molecular weights ranging from 15 kDa to 600 kDa was successfully separated. This method achieved almost baseline separation of the different sized protein standards. The monomeric and dimeric forms of both *IgG* and *Albumin* were also very well resolved. Therefore, the amount of monomeric, dimeric and oligomeric aggregate species can be quantitatively evaluated.

The molecular weights of each protein species were also determined.

IgG was found to exhibit smaller molecular weight than its actual value, while *Albumin* exhibited significantly higher experimental molecular weights. This is because *IgG* assumes a rigid Y shape and is more compact, while proteins like *Albumin* is more stretched and assumes a larger hydrodynamic volume compared to globularly packed proteins.



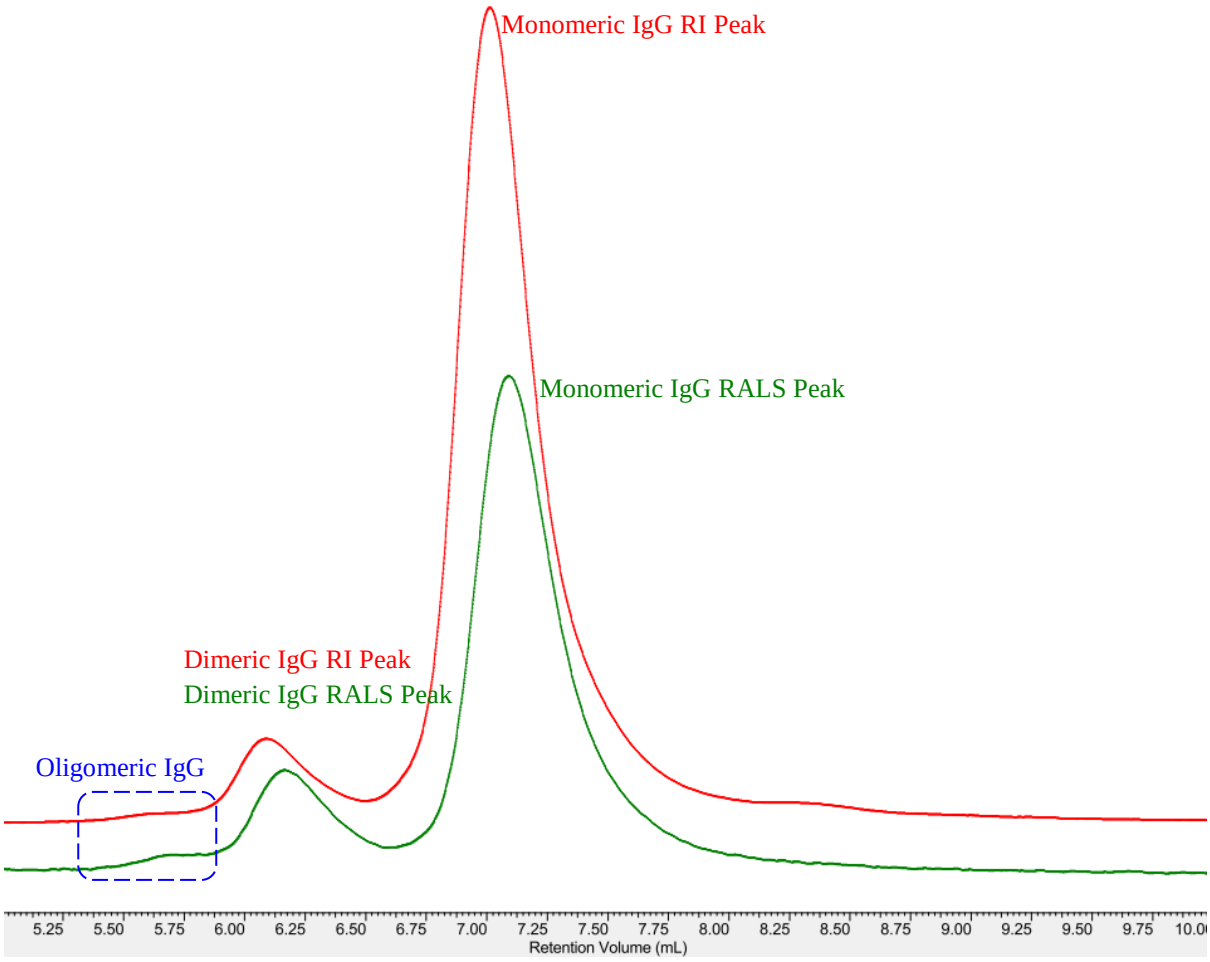
IgG [I]	M_n (Da)	M_w(Da)	M_z (Da)	Dimer/ Monmer	True M_w	Deviation %
Monomer	120,329±472	128,133±324	134,680±679	0.09	150 kDa	**-14.58%**
Dimer	300,117±1,820	301,325±2,014	302,679±2,347		300 kDa	**<1.0%**

Albumin	M_n (Da)	M_w(Da)	M_z (Da)	Dimer/ Monmer	True M_w	Deviation %
Monomer	75,701±252	76,666±252	77,504±257	0.09	66.5 kDa	**15.29%**
Dimer	165,609±948	168,782±1,142	171,868±1,392		130 kDa	**26.90%**

[I] Averages of four injections

GPC-T Results

Standardized GPC determination of protein molecular weight relies on the comparison of the hydrodynamic volume of the protein of interest to the protein standards. As proteins are packed in different shapes, it is very difficult to obtain a commercially available protein standard set suitable for the analysis of a particular protein. GPC-T doesn't require the comparison of hydrodynamic volume to a specific standard set. Instead, it measures the absolute molecular weight of any species that scatters a sufficient amount of light (usually >2,000 Da). Therefore, the accuracy of the method will not be affected by differences in protein shapes. Meanwhile, GPC-T system calibration only requires a single protein standard. Therefore, the results obtained via GPC-T are more accurate and cost-effective. Here, we present an example that accurately measured protein molecular weight with less than 1% error.



IgG [II]	M_n	M_w	M_z	PDI	True M_w	Deviation %
Monomer	148,898±414	150,442±479	152,364±620	1.01	150 kDa	<1.0%
Dimer	300,117±1,820	301,325±2,014	302,679±2,347	1.00	300 kDa	<1.0%

[II] Averages of four injections

Analysis Conditions

GPC-T

This section of a Jordi report provides information on the methods used including instrument type, temperatures, solvents, sample preparation, etc. The specific conditions have been removed for this case study.

Closing Comments

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Sincerely,

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