Does Tween 20 Affect Monoclonal Antibody Separation?

Application Note

Biotherapeutics & Biosimilars

Authors

Keeley Mapp and Phu T Duong

Agilent Technologies, Inc

Introduction

Tween 20 is commonly used during the preparation of biological samples. Typically, its concentration ranges from 0.01 to 0.1%. Tween 20 has various beneficial properties, such as stabilizing proteins, solubilizing protein membranes, and preventing nonspecific binding of primary and secondary antibodies. Therefore, Tween 20 is often present in samples of monoclonal antibodies that require analysis using techniques such as size exclusion chromatography (SEC). There is concern that the presence of Tween 20 can interact with the column matrix and change the column's performance. This application note investigates whether the presence of Tween 20 detergent directly in the sample and in the eluent, during multiple injections of an IgG sample onto an Agilent Bio SEC column, affects the analysis or the column.

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Materials and Methods

Conditions, Figures 1 and 2

Column:	Agilent Bio SEC–3, 300Å, 7.8 x 300 mm, 3 µm (p/n 5190–2511)
Samples:	Bio-Rad Gel Filtration Standard (thyroglobulin, γ-globulin, ovalbumin, myoglobin, and vitamin B12) for column performance testing
	PEG/PEO mix (PEG 106, PEG 12, 140, PEO 77, 350, 1 mg/mL in eluent) for system performance test
	IgG (Sigma-Aldrich Corp. I5506), 1 mg/mL in eluent and in 0.1% Tween 20 solution
	Tween 20 (Sigma-Aldrich Corp. P9416), 1 mg/mL in eluent
Eluent:	150 mM Sodium phosphate buffer, pH 7
Flow rate:	1.0 mL/min
Detectors:	UV at 220 nm for proteins and IgG samples
	RI detector for PEG/PEO mix and Tween 20 samples
System:	Agilent 1260 Infinity Bio-inert Quaternary LC System

Conditions, Figures 3 and 4

Column:	Agilent Bio SEC-3, 300Å, 7.8 × 300 mm, 3 µm (p/n 5190–2511)
Samples:	IgG (Sigma I5506), 1 mg/mL in eluent
Eluent:	150 mM sodium phosphate buffer, pH 7 containing 0.1% Tween 20
Flow rate:	1.0 mL/min
Detectors:	UV at 220 nm
System:	Agilent 1260 Infinity Bio-inert Quaternary LC System

Results and Discussion

No co-elution between Tween 20 and IgG

The column and LC system were first evaluated for performance using the protein standard test mix (with UV detector) and PEG/PEO mix (with RI detector as Tween 20 does not have a UV chromophore); data not shown. Injections of a Tween 20 solution and the IgG sample, prepared with and without Tween 20, were then injected onto the column.

Data obtained (Figure 1) show that the Tween 20 retention time was 11.26 minutes, the retention time of IgG dimer was 6.69 minutes, and that of the monomer was 7.58 minutes. The IgG and Tween 20 did not coelute and the chromatographic profile data of the IgG sample remained relatively unchanged whether it contained Tween 20 or not. The analysis of ratios and resolution factors of aggregates, dimers, and monomer (Table 1) indicates little difference.

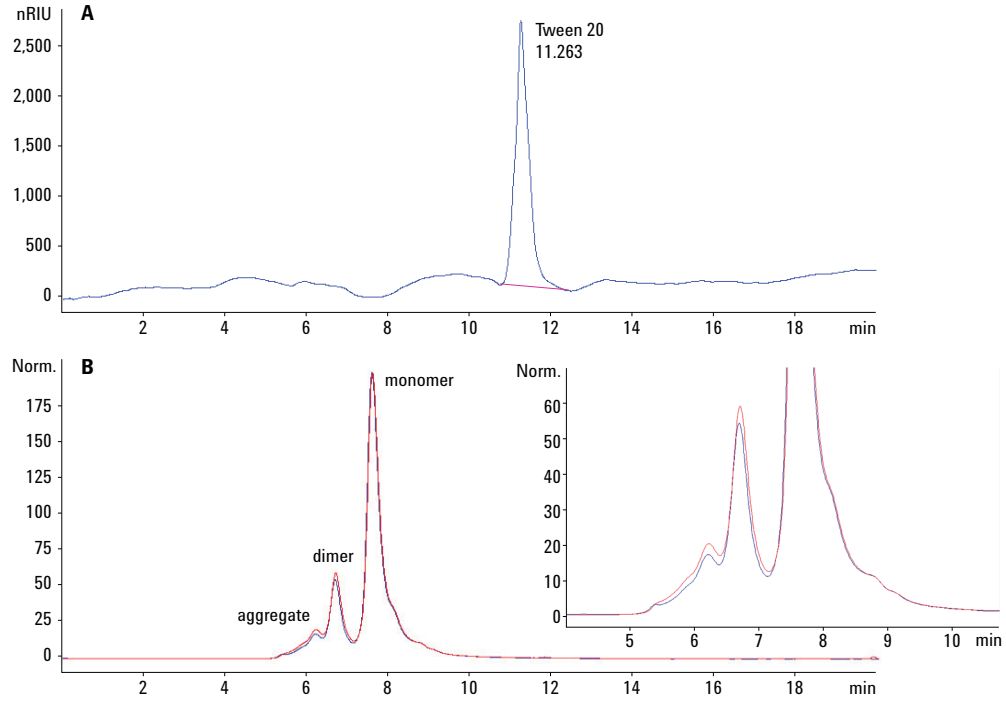

Figure 1. The elution of Tween 20 detected by the refractive index detector (A), and the elution profiles for IgG, with Tween 20 (red chromatogram) and without Tween 20 (blue chromatogram) (B).

Table 1. Area percentage ratios and resolution factors for the analysis of IgG.

Injection of 5 µL IgG without Tween 20			
	Aggregations	**Dimer**	**Monomer**
% Ratio	7.32	16.83	75.85
RS factor	–	0.72	1.63
Injection of 5 µL IgG with Tween 20			
	Aggregations	**Dimer**	**Monomer**
% Ratio	8.74	17.92	73.34
RS factor	–	0.67	1.61

Analysis of multiple injections of IgG containing Tween 20

To study the column's performance and stability when exposed to Tween 20 solution, multiple injections were conducted, as follows:

1) Two injections of IgG in buffer, containing no Tween 20, were made as reference injections.
2) Ten injections of Tween 20 (20 µL)
3) Two injections of IgG in buffer, containing no Tween 20
4) Ten injections of Tween 20 (20 µL)
5) Two injections of IgG in buffer

The elution profiles were calculated for their percentage ratio and resolution factors to determine if there were any changes in elution profiles after multiple injections with and without Tween 20.

Data in Figure 2 showed that after multiple injections of Tween 20, the column could still generate the same profile of IgG with very little change.

Very little change was observed in the column's ability to resolve the IgG components after multiple injections of Tween 20.

Table 2. Percentage ratio and resolution factors of aggregates, dimers and monomers for IgG after multiple injections of Tween 20 using the Agilent Bio SEC column.

	Aggregates	**Dimer**	**Monomer**
% Ratio	6.09	16.07	77.84
RS factor	–	0.76	1.63
Ten injections of Tween 20			
% Ratio	6.05	15.87	78.08
RS factor	–	0.75	1.63
Ten injections of Tween 20			
% Ratio	6.06	15.87	78.08
RS factor	–	0.75	1.62

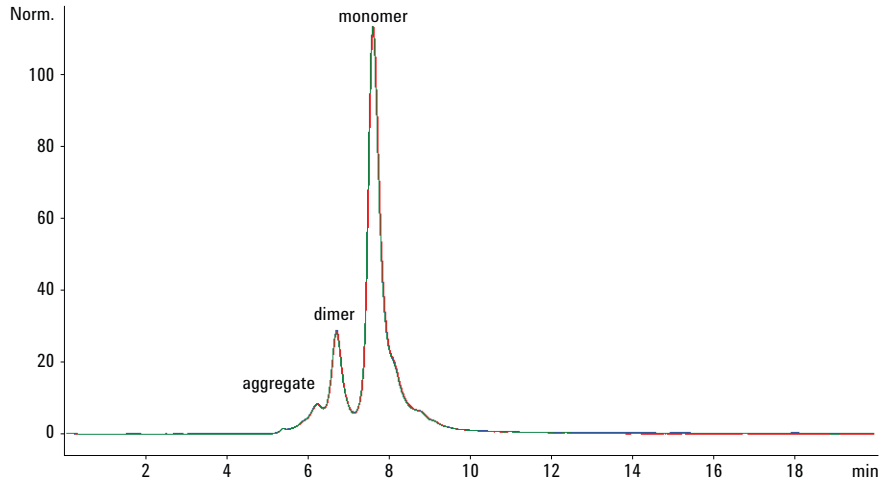

Figure 2. Elution of IgG after zero (in blue), 10 (in red), and 30 (in green) injections of Tween 20.

Multiple injections of IgG with 0.1% Tween 20 in the eluent

This experiment studied the effect of Tween 20 on the Bio SEC column when it was present throughout the analysis. The column was fully conditioned in the buffer containing 0.1% Tween 20, and 50 injections of IgG in this eluent were made onto the column (Figure 3).

The plots in Figure 4 compare the integration data of the resolution factors and the percentage areas. The resolution factors between the monomer and dimer components remain consistent after multiple injections.

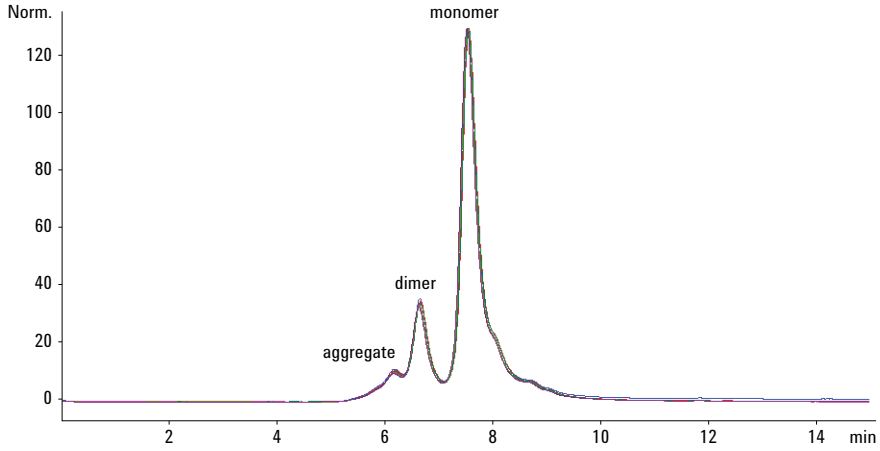

Figure 3. The IgG profile remains very similar throughout 50 injections; resolution factors between the monomer and dimer components remain consistent after multiple injections.

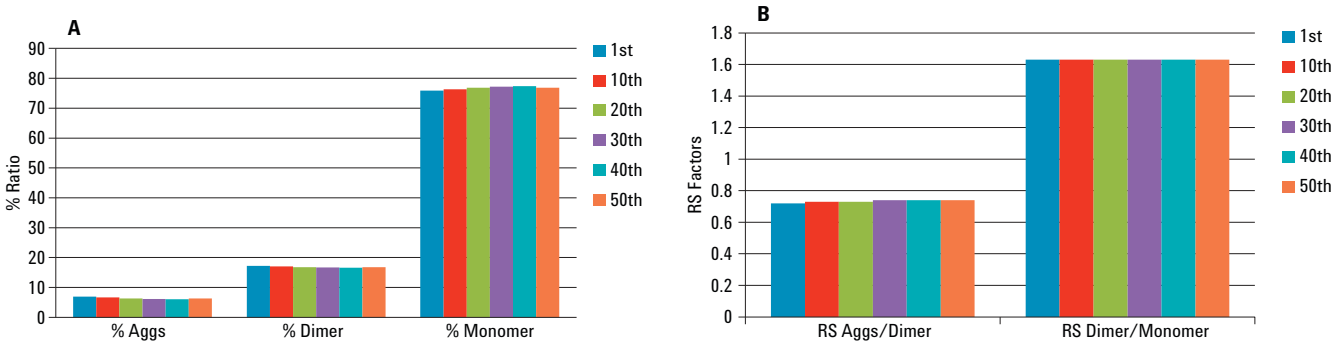

Figure 4. Integration of the IgG elution profile for 50 injections with 0.1% Tween 20 present throughout within the eluent and sample preparation. (A) % ratio and (B) resolution factor.

Evalution of column before and after exposure to Tween 20

A comparison of the column's performance at the start of the study (before the column was exposed to Tween 20) and at the end of the study after being subjected to Tween 20 was then made.

A chromatogram of the column's separation of the Bio-Rad protein standard was recorded (Figure 5A). The column was then used for multiple separations of IgG in the presence of Tween 20. After this, the column underwent a cleanup procedure to remove any possible contamination, by flushing at 1 mL/min with 20% ethanol for five column volumes, reconditioning thoroughly with testing eluent, and then injecting with protein standard to evaluate the separation after cleaning (Figure 5B).

There was no significant change in the performance of the Agilent Bio SEC-3 column in the presence of Tween 20 (Table 3).

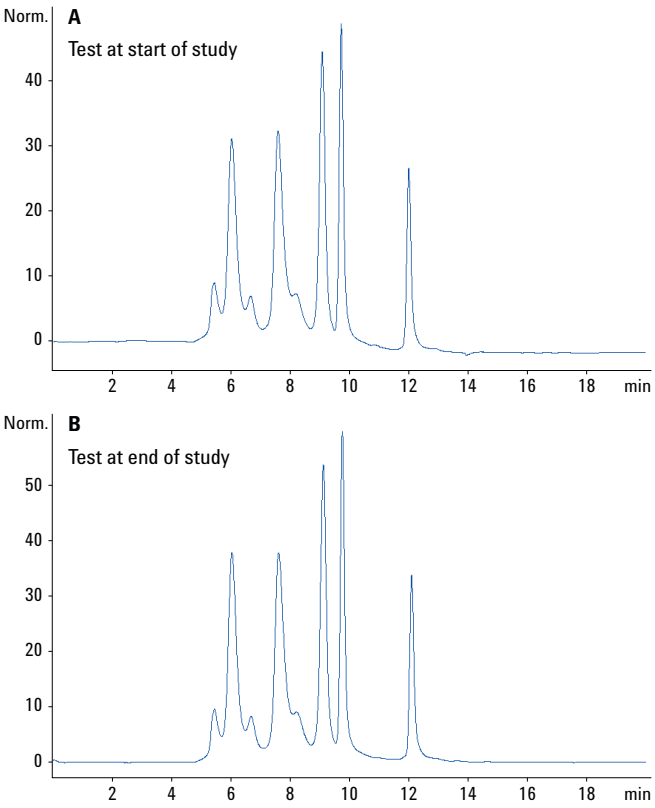

Figure 5. Bio-Rad protein standard test to assess the performance of the Agilent Bio SEC-3 column throughout the study. A) separation at the start of the study; B) separation at the end of the study after the ethanol cleanup procedure.

Table 3. Comparison of retention times, resolution factors, and plate counts before and after column cleanup.

Protein	RT (min) before	RT (min) after	RS factor before	RS factor after	Plates/m before	Plates/m after
1. Thyroglobulin	6.034	6.058	–	–	6,549	6,902
2. γ-Globulin	7.594	7.628	2.81	2.81	9,509	9,106
3. Ovalbumin	9.080	9.125	3.17	3.13	32,413	32,250
4. Myoglobin	9.718	9.764	2.00	1.98	70,050	68,856
5. Vitamin B12	11.991	12.089	8.53	8.60	109,726	108,439

Conclusions

The analyses of IgG in the presence of Tween 20, either in the eluent buffer or sample, showed that Tween 20 had no effect on the performance of Agilent Bio SEC columns or the integration data achieved. The columns maintained their performance throughout the study whether they were exposed to 0.1% Tween 20 in the sample or in eluent buffer after multiple injections. The columns also kept their performance after they were cleaned with 20% ethanol to remove any possible contaminants, and their separation properties were not affected.

For More Information

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