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Life-time Stability of Size Exclusion Chromatography Columns for Quality Control of Therapeutic Protein Aggregates

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PURPOSE

Size Exclusion Chromatography (SEC) columns are typically run at conditions that generate relatively low back pressure. In spite of the mild operation condition, the columns show tendency to short lifetime. This is related to the inherent mechanical fragility of wide pore particles and column fouling due to contamination. In this work we evaluated the lifetime of commercially available columns used for mAb aggregate analysis operated with a state of the art biocompatible UHPLC system. The evaluation was performed by large number of injections of a mAb drug product. Peak efficiency, retention time, and resolution between monomer and dimer were evaluated as stability-indicating parameters. The performance of all columns tested was preserved for at least 1000 injections, up to almost 2000 on occasion, without guard columns, contradicting the expectation of fragility for SEC columns.

OBJECTIVE(S)

Aggregation is a common degradation process occurring to therapeutic proteins. Aggregates are regarded as critical quality attributes that require monitoring during the development and production of biotherapeutics. SEC is the most common analytical tool used in biopharmaceutical laboratory to quantify protein aggregates and fragments. Suppliers of SEC columns have illustrated column stability in the order of 500 injections (without a column guard) as satisfactory number. However end-users often report much smaller numbers.
In this work, the lifetime of SEC columns with dimensions of 4 x 300 mm and 7.8 x 300 mm were evaluated with a state of the art biocompatible UHPLC system. Columns were tested without guards.

METHOD(S)

Instrumentation
Thermo Scientific™ Vanquish™ Flex quaternary UHPLC system was utilized to monitor protein aggregation of the commercial drug substance bevacizumab. Detection was performed using the Thermo Scientific™ Vanquish™ Diode array detector with a Thermo Scientific™ LightPipe™ 10 mm standard flow cell.
The default column inlet tubing was replaced with silica nanoViper 0.075x350 mm for the experiments with the 4.0 mm id column, and with MP35N Viper 0.180x350 mm for the experiments with the 7.8 mm id column.
Protein Standards were also used to monitor column performance. The following molecules (with a range of sizes) were used to verify size exclusion: Thyroglobulin, BSA, Ribonuclease A, Myoglobin & (the small molecule) Cytidine.

Chromatographic Conditions

Column:	Thermo Scientific™ MAbPac™ SEC-1, 5µm, 300 Å, **7.8 x 300 mm**	Thermo Scientific™ MAbPac™ SEC-1, 5µm, 300 Å, **4.0 x 300 mm**
Mobile Phase:	**A:** 50 mM sodium phosphate, pH 6.8 in 300 mM NaCl, filtered through 0.2 µm filter membrane before use.	**A:** 100 mM sodium phosphate, pH 6.8 in 300 mM NaCl, filtered through 0.2 µm filter membrane before use.
Gradient:	100% A, Isocratic	100% A, Isocratic
Flow Rate:	0.8 ml/min	0.25 ml/min
Temperature:	Column Oven: 30 °C forced-air Auto Sampler: 4 °C	Column Oven: 30 °C forced-air Auto Sampler: 4 °C
Injection Volume:	**Bevacizumab:** 0.5 µL of 2.5 µg/µL diluted (in mobile phase) sample **Protein Standards:** 1.0 µL	**Bevacizumab:** 2.0 µL of 2.5 µg/µL diluted (in mobile phase) sample
UV Detection:	280 nm, DAD	280 nm & 214 nm, DAD
Data collection & processing:	Thermo Scientific™ Chromeleon™ CDS 7.2 SR4	Thermo Scientific™ Chromeleon™ CDS7.2 SR4

RESULT(S)

As Figure 1 & Table 1 show, relatively consistent retention time was observed for the monomer peak over the course of >1950 injections, with a retention time range of just 0.043 minutes determined between injection numbers 25 and 1953. Similarly, excellent peak symmetry was detected throughout the column lifetime stability study (asymmetry range 0.88 to 0.93) further demonstrating no increase of secondary interactions with the column packing material and hardware across the lifetime of the column. Column efficiency, based on European Pharmacopeia plate count, was found to be >85% of initial efficiency following 1953 injections on the column (equivalent to 1866 injections of mAb). Subsequent to 1866 injections of bevacizumab, a loss in column performance was observed as shown in Figure 2. The loss in column performance (<85% theoretical plate count) did not appear to affect the aggregation profile of bevacizumab (Figure 1).

Figure 1. Aggregate analysis of bevacizumab using a MabPac SEC-1, 4.0 x 300 mm analytical column.

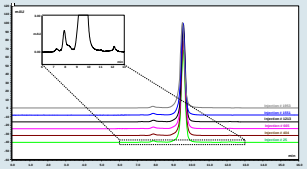
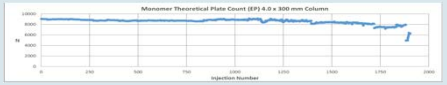


Table 1. Monomer peak information acquired following aggregate analysis of bevacizumab using the MAbPac SEC-1, 4.0 x 300 mm column

On Column Injection #	Retention Time (min)	Monomer Relative Peak Area (%)	Monomer Peak Width @ 50% Height (min)	Asymmetry (EP)	Theoretical Plates (EP)
25	9.554	96.96	0.237	0.92	9032
404	9.558	97.21	0.240	0.92	8797
665	9.558	96.96	0.241	0.93	8736
1213	9.567	97.27	0.243	0.89	8604
1551	9.544	96.99	0.244	0.88	8460
1953	9.524	96.30	0.255	0.89	7737

Figure 2. Graph showing theoretical plate count for the mAb monomer in each injection. Greater than 85% of initial column efficiency was preserved following 1866 injections of bevacizumab (corresponding to 1953 on column injections). A loss of column performance was observed subsequent to 1866 injections of bevacizumab.



During the experiments with the MAbPac SEC-1 7.8 x 300 mm, two injections of a protein check standard were performed every ten mAb injections. This injection cycle was repeated until column degradation was observed. Figure 3 & Table 2 show highlighted chromatography data for bevacizumab analysed using a MAbPac SEC-1, 7.8 x 300 mm column.
The final line of table 2 displays information for the final injection that was above the column efficiency specification for monoclonal antibodies, namely injection number 1292 (>6300 theoretical plates (EP) for the monomer peak). Similar to the MAbPac SEC-1, 4.0 x 300 mm column, the MAbPac SEC-1, 7.8 x 300 mm column also displayed relatively consistent retention time, peak symmetry and excellent column efficiency over the lifetime of the column (Figure 4).

Figure 3. Aggregate analysis of bevacizumab using the MAbPac SEC-1, 7.8 x 300 mm analytical column.

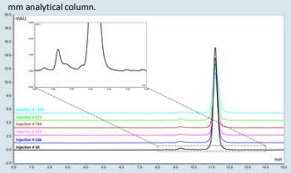
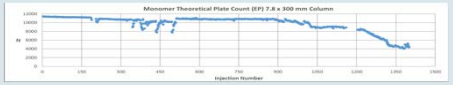


Table 2. Monomer peak information acquired following aggregate analysis of bevacizumab using the MAbPac SEC-1, 7.8 x 300 mm column

On Column Injection #	Retention Time(min)	Monomer Relative Peak Area (%)	Monomer Peak Width @ 50% Height (min)	Asymmetry (EP)	Theoretical Plates (EP)
30	11.192	97.56	0.247	1.08	11349
236	11.183	97.30	0.252	1.11	10870
555	11.192	97.74	0.252	1.11	10918
769	11.200	97.95	0.253	1.06	10876
975	11.225	97.92	0.262	0.93	10158
1292	11.225	97.40	0.333	1.08	6301

Figure 4. Graph showing theoretical plate count for the mAb monomer in each injection. Greater than 55% of initial column efficiency was preserved following 1077 injections of bevacizumab (corresponding to 1292 on column injections). A loss of column performance was observed subsequent to 1077 injections of bevacizumab.



RESULTS (CONT)

The efficiency drops observed between 300 and 500 injections (Figure 3) were not attributed to column degradation. It was observed that the number of injections made from each sample vial negatively affected peak width. When sample and sample vial were replaced, high efficiency values were restored. The root cause for this behaviour could not be found. When the column degraded, the replacement of the sample vial with a fresh one did not have any beneficial effect on peak width. We tested this further with an additional column and changed the column vials after every 180 injections (Figure 5). It is clearly visible that every time the vial was changed the efficiency increased back to initial level. All vials contained the same mAb sample solution and were stored at 4 °C in the same autosampler. Unlike efficiency, retention time was not affected by number of injections (Figure 6), thus it can be concluded that the changes in the sample did not affect either the hydrodynamic radius of mAb or change the secondary interaction between sample and column.

Figure 5. Aggregate analysis of bevacizumab using the MAbPac SEC-1, 7.8 x 300 mm analytical column with modified sample exchange procedure

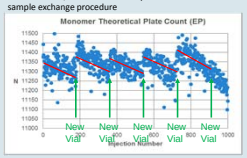
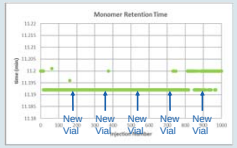


Figure 6. Aggregate analysis of bevacizumab using the MAbPac SEC-1, 7.8 x 300 mm analytical column with modified sample exchange procedure



CONCLUSION(S)

The MAbPac SEC-1 column coupled to the Vanquish Flex Quaternary UHPLC system is a robust platform for aggregate analysis of mAbs.

- Consistent retention time, excellent peak symmetry and exceptional column efficiency were observed over the course of 1953 on-column injections for the MAbPac SEC-1, 4.0 x 300 mm column and for 1292 on-column injections on the MAbPac SEC-1, 7.8 x 300 mm column.
- Data were measured with one specific UHPLC instrument without the use of guard columns. Column lifetime with other instrumentation was not assessed.

TRADEMARKS/LICENSING

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