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Application News

No. AD-0226

Biopharma / Nexera Bio UHPLC / Shim-pack Bio Diol

Optimizing Aggregates Analysis – Mobile Phase

❑ Introduction

Protein-based therapeutic products such as monoclonal antibodies (mAbs) have shown to be effective against a variety of diseases. However, a slight difference or modification in the protein can alter its activity. Protein aggregation is one of the factors that causes the product unfit for use. Thus, accurate analysis of protein aggregates is required to ensure the applicability of therapeutic proteins.

Size exclusion chromatography (SEC) is one of the chromatographic separation modes used for large molecules such as proteins and polymers by their sizes. It has been the main technique for analysis of fragments, monomers, dimers, and higher aggregates of therapeutic proteins. Despite being a simple assay, mobile phase condition is often needed to be optimized to improve peak shape and resolution of proteins. In this study, we describe analysis of trastuzumab, an anti-HER2 mAb using Shim-pack Bio Diol column on Nexera Bio UHPLC. The effect of mobile phase composition (ionic strength and pH) on chromatographic separation of trastuzumab is presented in this application news.

❑ Experimental

Trastuzumab sample was diluted to 1 mg/mL with water. The sample was analyzed by a Shimadzu Nexera Bio UHPLC with a UV detector. Sodium phosphate buffer was prepared by mixing 50 mmol/L NaH_2PO_4 + x mmol/L NaCl and 50 mmol/L Na_2HPO_4 + x mmol/L NaCl stock solutions to the desired pH. Analytical conditions are shown in Table 1.

Table 1: SEC analysis conditions of trastuzumab

LC system	:	Nexera Bio UHPLC
Column	:	Shim-pack Bio Diol-300, 300 mm x 4.6 mm I.D., 3 µm (P/N: 227-31010-03)
Column temp.	:	25 ℃
Mobile phase	:	50 mmol/L (sodium) phosphate buffer + x mmol/L NaCl (pH 6 – 7.5)
Flow rate	:	0.4 mL/min
Elution mode	:	Isocratic flow
Injection vol.	:	5 µL
Detector	:	UV, 280 nm

Table 2: Other consumables

Item description and P/N*
1.5ml Screw-thread amber vial with write on spot, caps with PTFE/white silicone septa (P/N: 226-54111-41)
1L Solvent bottle (P/N: 226-88583-02)
Solvent safety caps kit (P/N: 226-50319-01)
LC solvent waste kit (P/N: 226-50330-00)
PEEK fitting (P/N: 226-50106-02)

*Please contact your local Shimadzu representative for more information on these consumables.

❑ Results & Discussions

Proteins are prone to interact with stationary phase in analytical column via two main interactions, electrostatic interactions and hydrophobic interactions.[1] These non-ideal interactions can lead to protein adsorption, peak tailing, and shift in retention time. The resulting poor peak resolution and poor peak performance would impact on accuracy in quantitative and qualitative analytical results. Thus, optimization of chromatographic conditions such as mobile phase composition, is necessary to mitigate the non-ideal interactions. The SEC analysis of trastuzumab was evaluated over 4 NaCl concentrations (100 mmol/L, 150 mmol/L, 200 mmol/L, 250 mmol/L) at different pH conditions (6.0, 6.5, 7.0, 7.5).

Ionic strength (salt concentration)

An overview of the results indicates that mobile phase composition had an effect on peak shape of trastuzumab (Figure 1). It is known that electrostatic interactions in SEC can be suppressed by increasing ionic strength or salt concentration of mobile phase.[2] A decrease in peak tailing was observed with mobile phase containing higher NaCl concentration (higher ionic strength) across all tested pH (Figure 1, Table 3). The increase in salt concentration suppressed electrostatic interactions, resulting in improved aggregate recovery and peak symmetry. However, peak tailing was observed at salt concentration > 200 mmol/L (Figure 1, Table 3). Hydrophobic interactions between mAbs and stationary phase might be attributed to the increased retention time that resulted in peak tailing.[2]

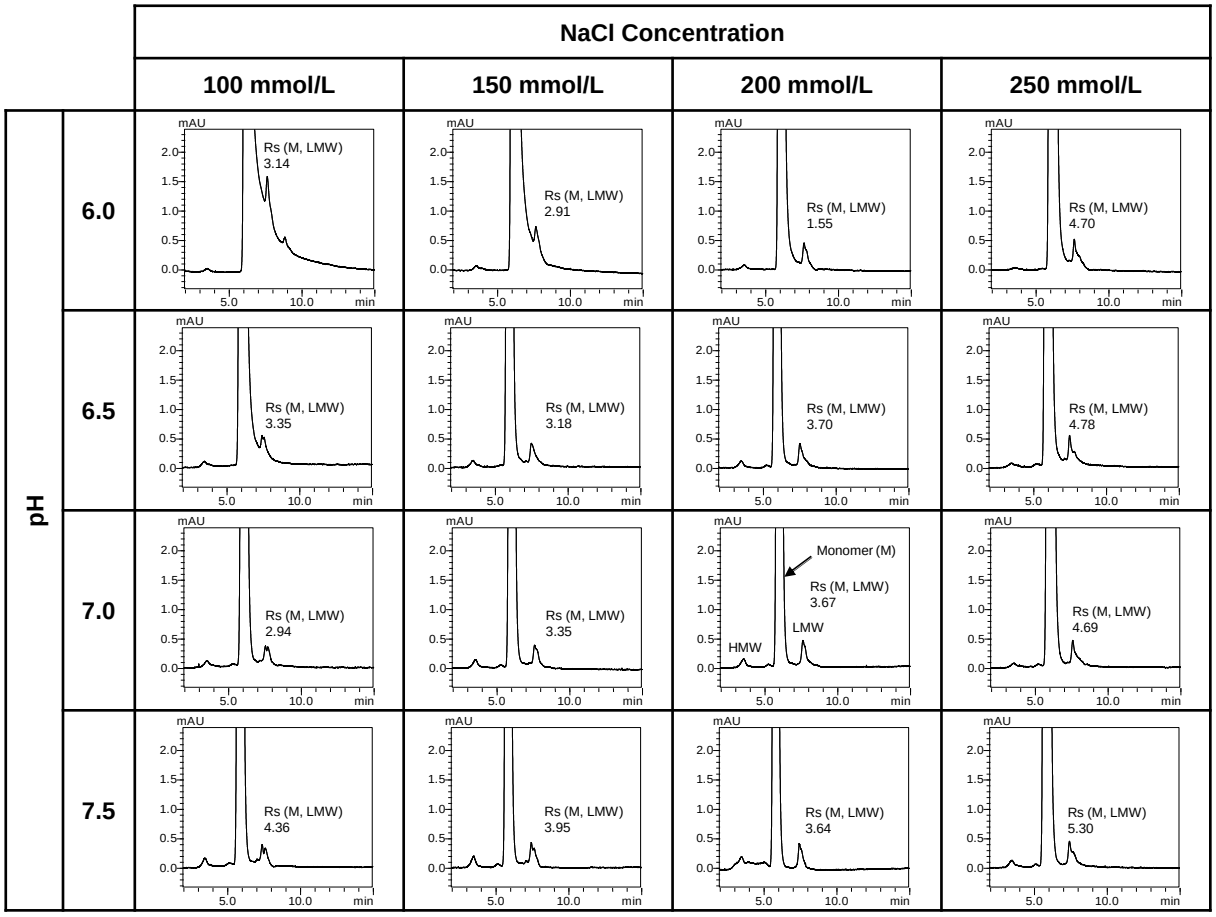

Figure 1. Tabulated chromatograms on SEC of trastuzumab.

pH

Varying mobile phase pH had an effect on peak shape (Figure 1). Protein conformation changes at different pH. This may lead to a different elution profile due to changes in hydrodynamic sizes and/or non-ideal interactions with stationary phase.[3] Optimal separation profile of trastuzumab was observed at 50 mmol/L (sodium) phosphate buffer with 200 mmol/L NaCl at pH 7.0 where good monomer peak shape was observed and aggregates were well separated from monomer peak.

Table 3: USP peak tailing of trastuzumab monomer.

[NaCl] / pH	100 mmol/L	150 mmol/L	200 mmol/L	250 mmol/L
6.0	3.72	2.14	1.48	1.81
6.5	1.92	1.41	1.35	1.70
7.0	1.47	1.37	1.30	1.76
7.5	1.45	1.34	1.27	1.73

❑ Conclusion

SEC is important for characterization of protein-based therapeutic products, and a robust method for SEC analysis is desired. In this application, mobile phase optimization was performed on a Shim-pack Bio Diol-300 column. Results demonstrate that mobile phase compositions (ionic strength and pH) are important factors and needed to be evaluated for effective SEC analysis.

❑ References

1. Kopaciewicz W., Regnier F. E., *Anal. Biochem.*, **1982**, 126 (1), pp 8-16.
2. Tsumoto K., Ejima D., Senczuk A. M., Kita Y., Arakawa T., *J. Pharm. Sci.*, **2007**, 96 (7), pp 1677-1690.
3. Golovchenko N. P., Kataeva I. A., Akimenko V. K., *J. Chromatogr.*, **1992**, 591 (1-2), pp 121-128.

SHIMADZU (Asia Pacific) Pte. Ltd
79 Science Park Drive, #02-01/08 Cintech IV, Singapore 118264,
www.shimadzu.com.sg; Tel: +65-6778 6280 Fax: +65-6778 2050