SHIMADZU
Excellence in Science

Application News

High Performance Liquid Chromatograph Nexera™ XS inert

Analysis of protein drugs aggregation Using Size Exclusion Chromatography

D. Fujimura, E. Ando, R. Suzuki

User Benefits

- Improving the separation of protein drugs and those aggregates and/or decompositions by suppressing secondary interactions such as electrostatic or hydrophobic interaction.
- Highly reproducible data can be acquired even when using a mobile phase containing high concentrations of corrosive salts.

■ Introduction

Therapeutic proteins such as monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) easily aggregate due to changes in temperature, pH, concentration and so on. Aggregation in the production of mAb and ADC negatively affects their efficacy and safety[1]. Therefore, the amount of aggregation should be monitored. Size exclusion chromatography (SEC), which separates molecules by differences in size, is one of the most utilized analytical methods for the detection of aggregation in therapeutic proteins,. It is known that adsorption occurs due to electrostatic interactions between mAb and stationary phases, and adsorption occurs due to hydrophobic interactions between ADC and stationary phases. These secondary interactions cause poor separation of aggregates, monomers, and fragments[2], [3]. Hence, it is necessary to consider these secondary interactions to ensure the reliability of data acquisition.

This article describes an aggregate analysis using a Shimadzu "Shim-pack™ Bio Diol" , size exclusion chromatography column with "Nexera XS inert", an ultra high performance liquid chromatograph. This chromatograph has high salt tolerance and metal-free flow path and allows to use highly salted mobile phases and prevents sample adsorption.

■ Reagents and Analytical Conditions

1mg of mAb (commercially available) and 0.5 mg of ADC (commercially available) were diluted in water to be final concentrations of 1 mg/mL. The analytical conditions for mAb and ADC are shown in Tables 1 and 2 respectively. It is known that electrostatic interaction which causes absorption in mAb analysis can be suppressed by adding salts to the mobile phase[3]. Therefore, 150 mmol/L sodium chloride was added to the 100 mmol/L phosphoric acid buffer solution. In the ADC analysis, in order to suppress hydrophobic interactions with the stationary phase, various concentrations of organic solvent were added to the 100 mmol/L phosphoric acid buffer solution, and then, the effect of organic solvent concentration was evaluated. Alcohol or acetonitrile are commonly used as the organic solvent[2], [3]. On the other hand, the pressure limit of SEC columns is generally lower. Hence, in this study, acetonitrile was used due to its low viscosity.

■ Analysis of mAb Aggregates

Fig. 1 shows the chromatogram of two different analysis on the same mAb. In the first case (Figure 1 , red chromatogram) the mobile phase contained 150 mmol/L sodium chloride, in the second one (Figure 1, black chromatogram) sodium chloride was not added to the mobile phase. In the enlarged figure, aggregates (peak 1) and fragments (peak3) were found before and after the monomer (peak2). When sodium chloride was not added to the mobile phase, the peak of the monomer tailed. However, 150 mmol/L of sodium chloride contained in the mobile phase suppressed peak tailing. It is clear the sodium chloride suppresses the electrostatic interaction between mAbs and stationary phases[3], improves the separation.

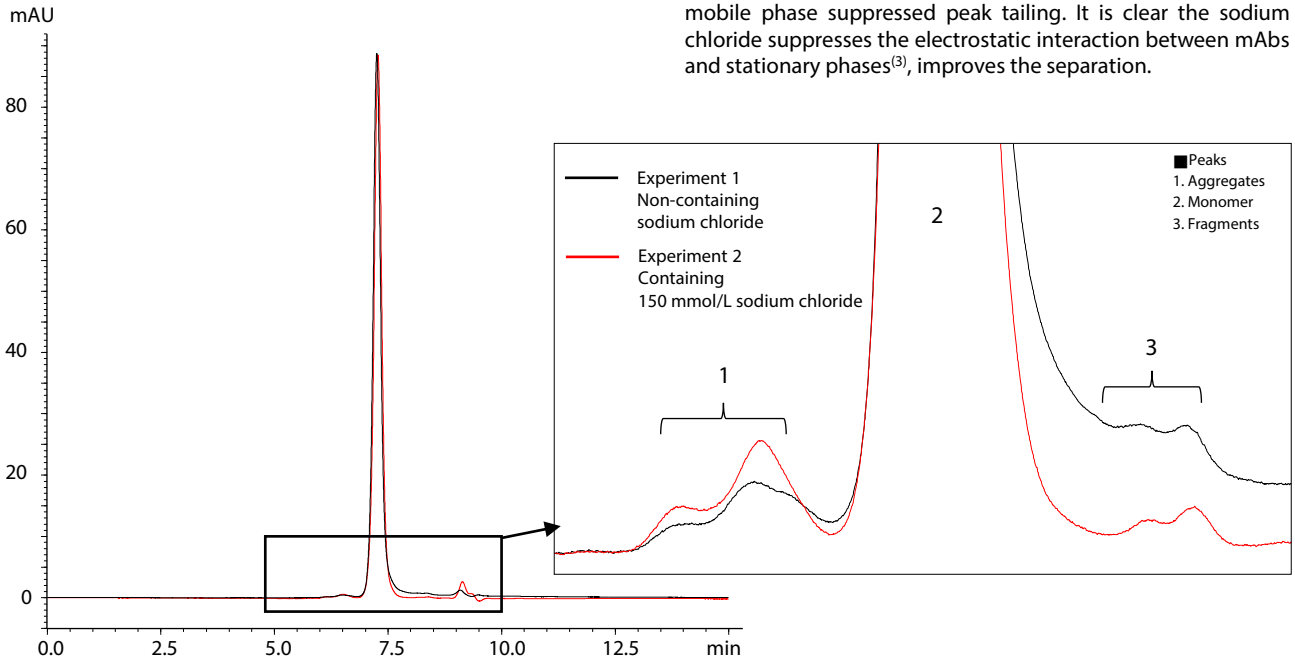

Fig. 1 mAb Chromatograms Comparison of Experiment 1 (mobile phase non-containing sodium chloride) and Experiment 2 (containing 150 mmol/L sodium chloride)

■ Analysis of ADC Aggregates

We evaluated the impact of acetonitrile concentration on the ADC aggregates analysis. Fig. 2 shows overlaid chromatograms of varying concentrations of acetonitrile in the mobile phase.

The peak shape of monomer was broad and showed a strong tailing in the case of a mobile phase without acetonitrile. The peak shape was improved by increasing the acetonitrile concentration. These results show that increasing the acetonitrile concentration suppresses the hydrophobic interaction between ADCs and the stationary phases(2). The degree of hydrophobic interaction between ADCs and stationary phases depends on number of bonds between antibodies and small molecule pharmaceuticals and their hydrophobicities. It is important to highlight that, despite the good results obtained, a further increment in the organic solvent concentration could result in sample denaturation.

■ Conclusion

We introduced examples of mAb and ADC aggregates analysis using the Nexera XS inert and a Shim-pack Bio Diol column. In these analyses, it was necessary to establish the optimal concentration of additives (organic solvents or salts) in the mobile phase. High concentration of salt or organic solvent in mobile phase are strongly improving the chromatographic separation of mAb or ADC aggregates. However, under this extreme conditions, reliable results cannot be obtained on a metal-based chromatograph. The Nexera XS inert, which has metal-free flow path and high corrosion resistance components, is optimal design for those extreme conditions.

Table 1 Analytical Conditions (mAb)

System:	Nexera XS inert
Column:	Shim-pack Bio Diol-300*1 (150 mm × 4.6 mm I.D., 2 µm)
Mobile Phase:	Experiment 1: 100 mmol/L (sodium) phosphate buffer (pH 6.9) (non-containing sodium chloride) Experiment 2: 100 mmol/L (sodium) phosphate buffer (pH 6.9) containing 150 mmol/L sodium chloride
Flowrate:	0.2 mL/min
Column Temp.:	25 ℃
Vial:	TORAST™-H Glass Vial (Shimadzu GLC)*2
Injection Volume:	5 µL (1 mg/mL mAb)
Detection:	280 nm (SPD-M40, UHPLC inert cell)

*1: P/N 227-31010-01, *2: P/N 370-04301-01

Table 2 Analytical Conditions (ADC)

System:	Nexera XS inert
Column:	Shim-pack Bio Diol-300 (150 mm × 4.6 mm I.D., 2 µm)
Mobile Phase:	0, 5, 10, 15 % acetonitrile in 100 mmol/L (sodium) phosphate buffer (pH 6.9)
Flowrate:	0.2 mL/min
Column Temp.:	25 ℃
Vial:	TORAST-H Glass Vial (Shimadzu GLC)
Injection Volume:	5 µL (1 mg/mL ADC)
Detection:	280 nm (SPD-M40, UHPLC inert cell)

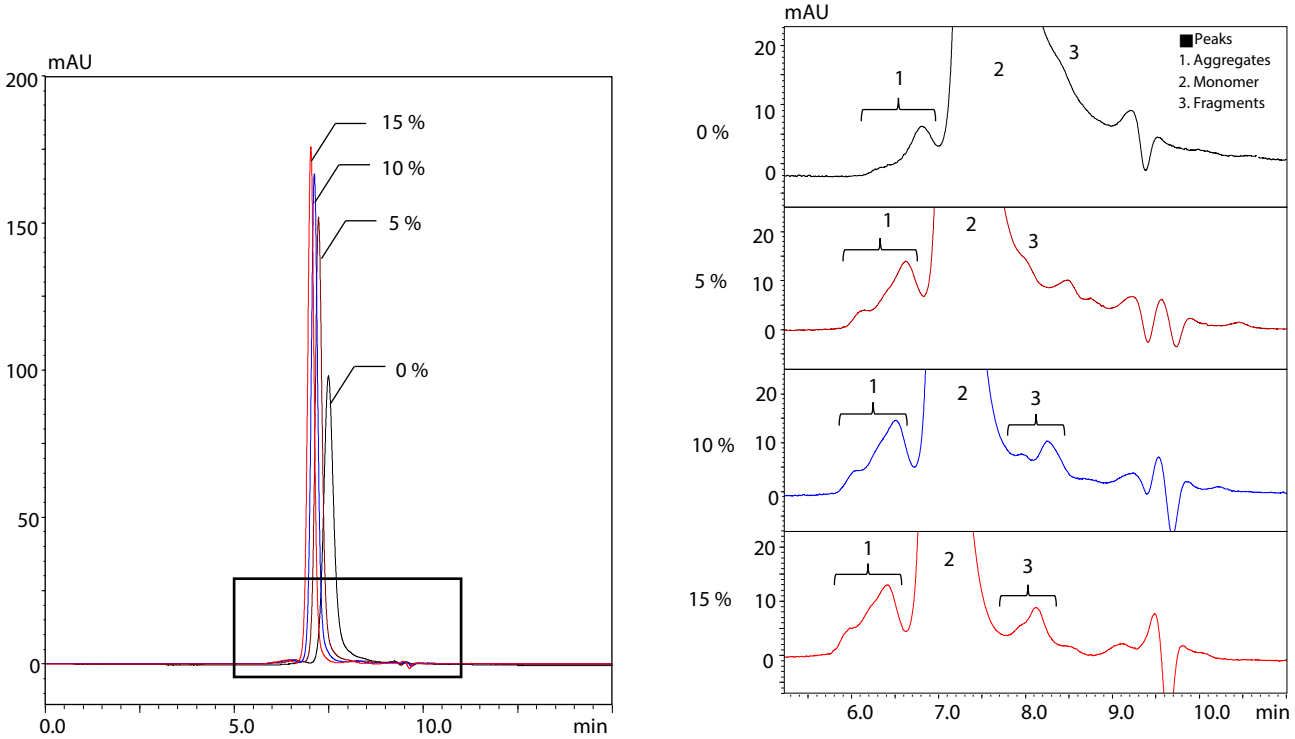

Fig. 2 Effect of Acetonitrile Concentration in the Mobile Phase (right panel is zoomed view of each chromatogram)

References
(1) Rosenberg AS. Effects of protein aggregates: an immunologic perspective. AAPS J., 8, E501-E507 (2006).
(2) Striegel, A. M.; Yau, W. W.; Kirkland, J. J.; Bly, D. D. Modern Size-Exclusion Liquid Chromatography, 2nd ed.; John Wiley & Sons, Inc.: Hoboken, NJ, (2009).
(3) Tsutomu Arakawa, Daisuke Ejima, Kouhei Tsumoto, and Pete Gagnon: Solvent modulation of chromatography, Journal of Japanese Biochemical Society 80 (1) pp 45-51.

Nexera and Shim-pack are trademarks of Shimadzu Corporation in Japan and other countries.
TORAST is a trademark of Shimadzu GLC Ltd.

01-00273-EN First Edition: Feb. 2022

SHIMADZU
Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedure.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.
The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu.
See http://www.shimadzu.com/about/trademarks/index.html for details.
Third party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.
The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2022