



Analysis of Aggregates and Additives in Antibody Drugs

Introduction

Antibody drugs are important potent therapeutic drugs. Since antibody drugs specifically target the cancer cells, they can cause less side effects. However, it is known that they may aggregate to form dimers and other larger aggregates during manufacturing process and/or during storage period. There are concerns that those larger aggregates may produce antibodies or become immunogenic conjugates that have properties of eliciting cell immune responses inside the body, which can lead to side effects.. This may lead to cause side effects. Therefore, monitoring of such aggregates is an important quality control (QC) criteria.

Silica-based aqueous size exclusion chromatography (SEC) columns are generally used for the separation of aggregates. Shodex PROTEIN LW-803 is one of them and it has high separation capability for the purpose. This application demonstrates the effectiveness of LW-803 by comparing it to similar columns from other companies. IgG (molecular weight (MW) about 150,000) and Bovine serum albumin (BSA, MW 66,000) were analyzed for the comparison. The effects of injection volume for the analysis of polyclonal IgG was also tested.

During the production of antibody drugs, small amounts of surfactants may be added to solubilize or stabilize IgG, and thus management of surfactants' concentrations is also important QC criteria. Quantification of surfactants can be difficult, as they exist as complex matrixes and have low UV absorption. In this application, we developed a rapid LC/MS method with a use of polymer-based column, Shodex ODP2 HP-2D, for the quantification of polysorbate (Tween) in the presence of IgG and NaCl as matrixes. The method does not require sample pretreatment prior to the injection.

2. Comparison of SEC columns

2.1 Analysis of IgG

The test samples were prepared by dissolving polyclonal IgG from human serum or monoclonal IgG from recombinant CHO cell in the eluent. Figure 1 shows the chromatograms of polyclonal IgG by injecting 5 μ L of 10-mg/mL sample. The monomer as well as dimer and even larger aggregates were detected. It can be seen that LW-803 demonstrated a superior separation efficiency between monomer and dimer compared to that of KW-803 and a column from another company, which is recommended for the analysis of antibodies.

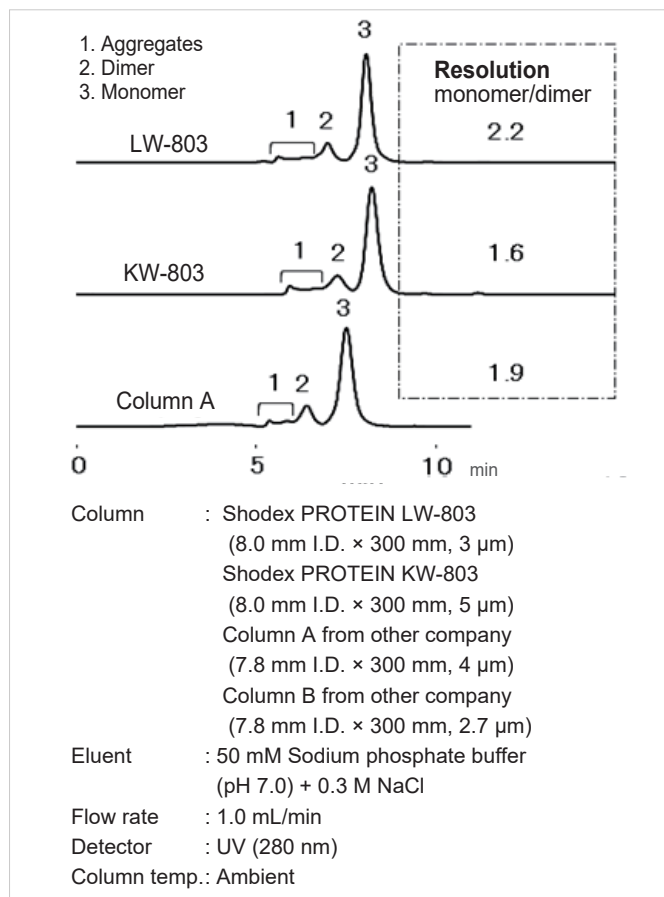


Fig. 1. Chromatograms of polyclonal IgG

Next, monoclonal IgG was analyzed by injecting 20 μ L of 1-mg/mL sample (Fig. 2). Similar to polyclonal IgG analysis, monomer as well as dimer, trimer, and even larger aggregates were detected. The resolutions between monomer and dimer and between dimer and trimer obtained by LW-803 was superior than that of columns from other company.

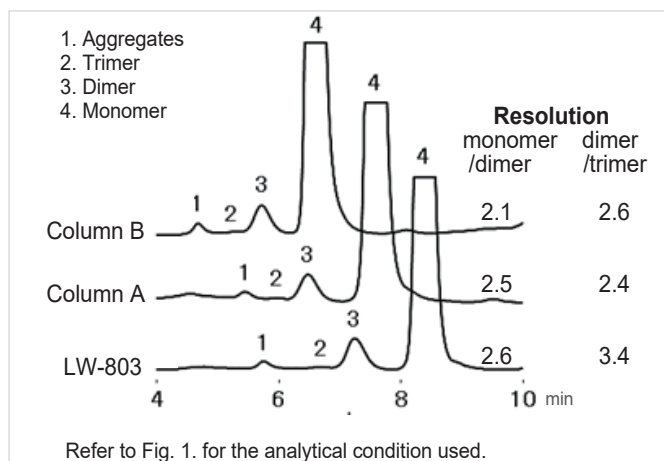


Fig. 2. Chromatograms of monoclonal IgG

2.2 Analysis of BSA

Figure 3 shows the chromatograms of BSA obtained by injecting 5 μL of 2-mg/mL sample. The monomer as well as dimer and trimer were detected.

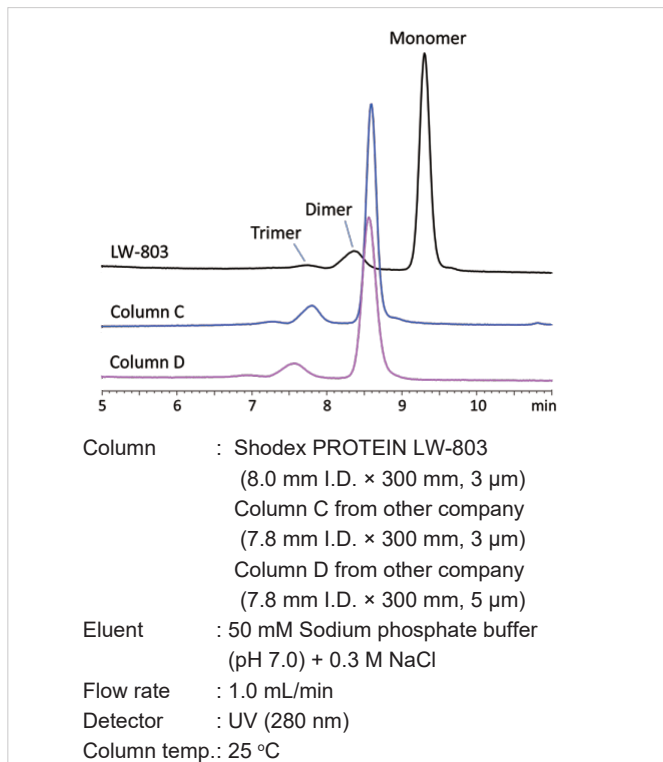


Fig. 3. Chromatograms of BSA

Figure 4 is a superimposed chromatograms of the above three analysis by aligning the BSA monomer peaks.

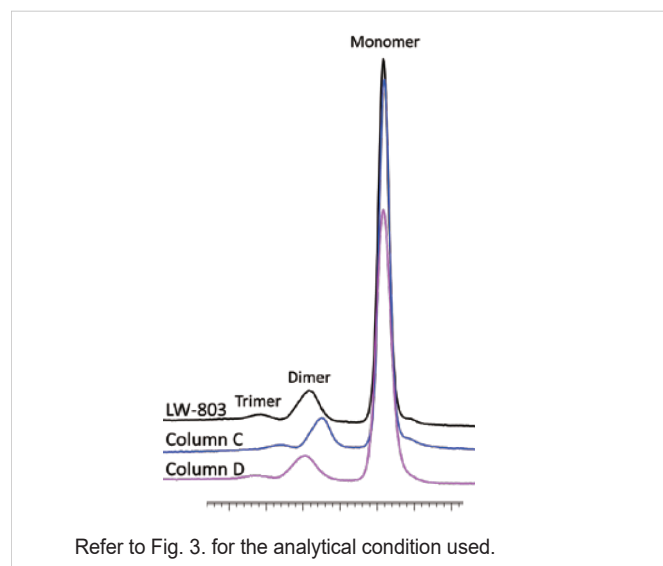


Fig. 4. Superimposed chromatograms of BSA

Theoretical plate number (TPN) and resolution (R_s) between the monomer and dimer for each column are summarized in Table 1. It demonstrated that LW-803 achieved the highest resolution among the three columns studied.

Table 1. TPN and R_s (monomer/dimer) for the analysis of BSA

	RT (min)	TPN	R_s (M/D)
LW-803	9.30	17,478	2.33
Column C	8.59	14,986	2.07
Column D	8.56	8,594	2.07

2.3 Effects of sample injection volume

Effects of sample injection volume for the analysis of IgG was studied. The volume between 1 and 100 μL of 10-mg/mL polyclonal IgG was injected to a LW-803. Figure 5 shows the chromatograms and a graph presenting the relationships between the injection volume and TPN and resolutions between monomer and dimer.

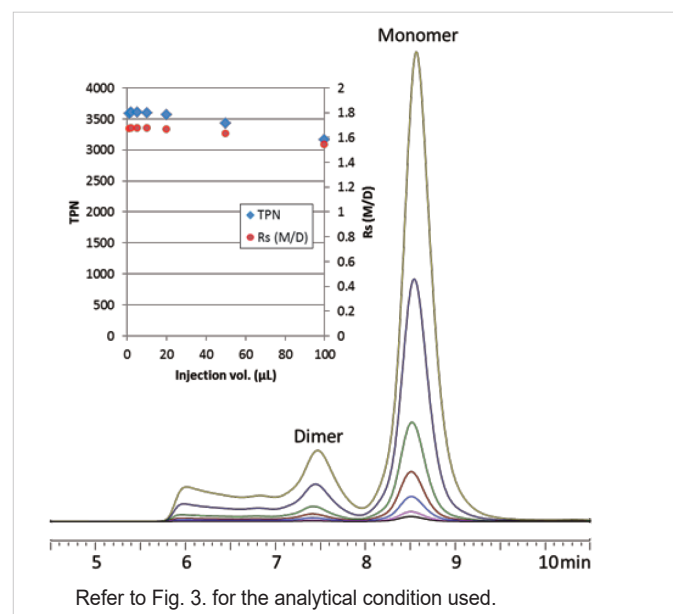


Fig. 5. The effects of sample injection volume

The separation efficiency was consistent among all injection volumes in the 1 - 20 μL range. It also demonstrated the method's capability for handling larger injection volume (100 μL , absolute sample loading of 1 mg) as there was no significant decrease in its separation efficiency (R_s larger than 1.5). The R_s obtained in this experiment was lower than the data obtained in experiment 2.1. This is due to the differences in the analytic systems used. Thus, it is worth noting that the system's dead volume may affect the resolution.

3. Analysis of surfactants in antibody drugs

ODP2 HP-2D is packed with polyhydroxy methacrylate gels. Unlike regular ODS columns, ODP2 HP is highly hydrophilic and this prevents proteins to be retained inside the column. Hydrophobic interaction which is the cause of protein retention can be suppressed almost to none by using alkaline eluent. Also, ODP2 HP's small pore size (40 Å) adds a size exclusion effect that helps proteins to eluting at V_0 . By well-controlling those features, the ODP2 HP column completely separates IgG from surfactants. The surfactants are retained by reverse phase mode. In order to prevent IgG from entering the MS, a flow-switching valve was placed after the UV detector. By using the valve, the column eluate obtained between 0 and 5 minutes is collected in a waste bottle and the eluate obtained after 5 minutes is injected into the MS.

Tween is typically used as an additive in antibody drugs, thus we selected it as a test surfactant in this application. Tween consists of a sorbitan backbone, polymers of ethylene oxide (EO), and long chain fatty acids connected by ester bonding (Fig. 6).

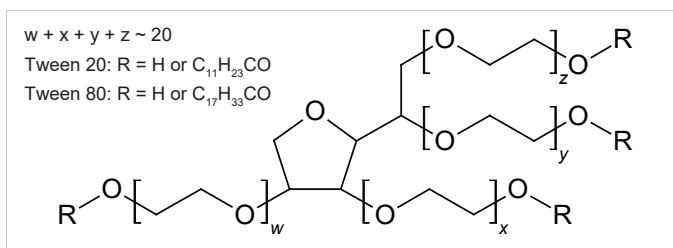


Fig. 6. Structure of Tween

Tween does not exist as a single compound, but exists in several different forms. The most abundant component was selected by pre-scanning the sample. Quantification curve was prepared using the result obtained by an MS (SIM mode) by measuring selected ions. Among the available Tween standards, Tween 20 and Tween 80 were analyzed. As a test sample, a mixture containing 50-mg/L Tween 20 or Tween 80, 1-g/L polyclonal IgG, and 0.3-mol/L NaCl was prepared. Injection volume used was 2 μ L throughout the experiment.

3.1 Analysis of Tween 20

Figure 7 (blue line) shows the SIM chromatogram of Tween 20. The eluent used was ammonim water. Ion at m/z 735.7 was monitored. This ion is assumed to be a divalent ion consisting of a sorbitan backbone, 20 or more EO units, glycerol diesters (MW 1435) of C14 and C12 with either one of them having a double bond, and two ammonium ions. Two chromatograms, one includes IgG and NaCl (Fig. 7(a)) and the other without IgG and NaCl (Fig. 7(b)) demonstrated very similar results, showing that there was minimum ion-suppression effect. Also, a good linearity was obtained for the calibration curve of Tween 20.

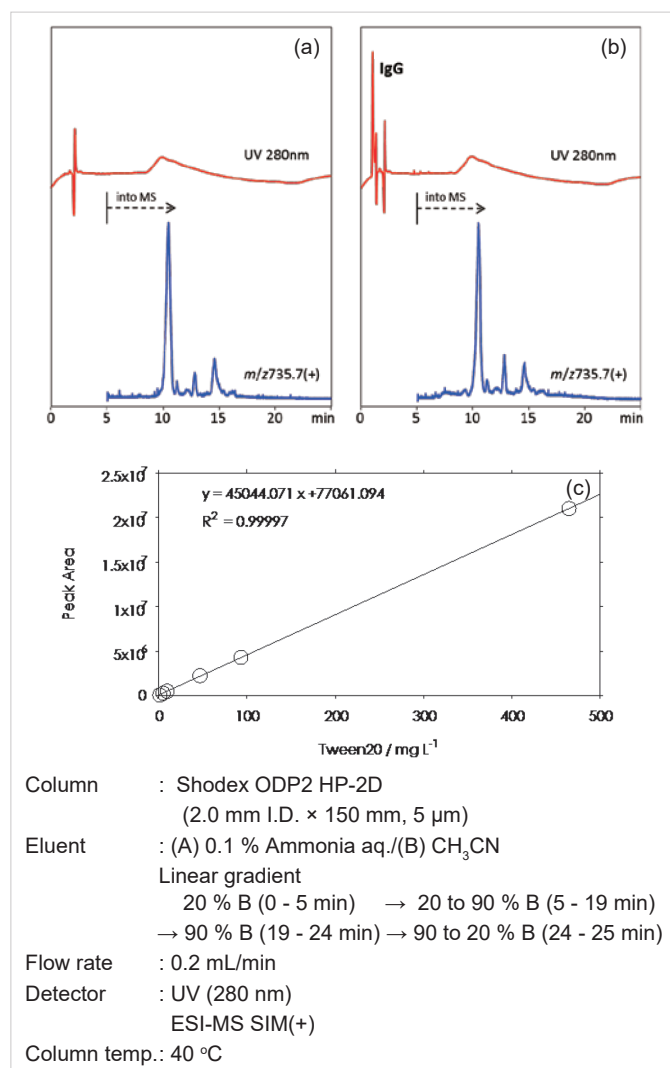


Fig. 7. Analysis of Tween 20. Chromatograms of (a) without and (b) with 1-g/L IgG and 0.3-mol/L NaCl. (c) calibration curve

3.2 Analysis of Tween 80

Tween 80 was analyzed using the same method as for Tween 20. Ion at m/z 848.8 was monitored (Fig. 8). This ion is assumed to be a divalent ion consisting of a sorbitan backbone, 28 or more EO units, glycerol monoester (MW 1661) of C18 with a double bond, and two ammonium ions. Consistent with Tween 20's analysis result, the chromatograms of samples containing with and without IgG and NaCl were very similar, showing the presence of minimum ion suppression. Again, a good linearity was obtained for the calibration curve of Tween 80.

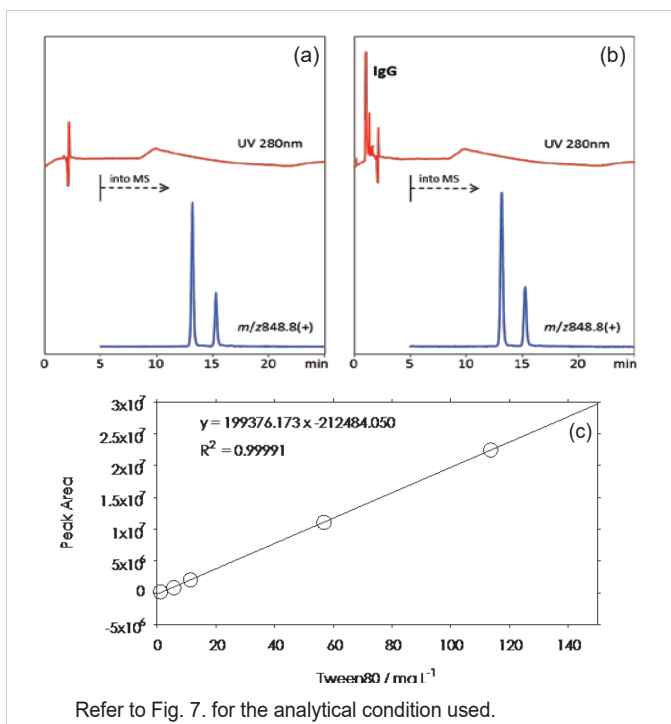


Fig. 8. Analysis of Tween 80. Chromatograms of (a) without and (b) with 1-g/L IgG and 0.3-mol/L NaCl. (c) calibration curve

Conclusions

Shodex PROTEIN LW-803 demonstrated its superior separation capability for IgG monomer and its aggregates. It also has high separation capability in even lower molecular weight ranges, and this allows the analysis of antibody metabolites and other proteins. Moreover, the column is capable of handling high loading volumes. Therefore, LW-803 has a wide usability for the separation of not only antibody aggregates, but for other proteins.

Moreover, Shodex ODP2 HP-2D proved its separation capability of analyzing non-ionic surfactants, Tween 20 and Tween 80, in the presence of IgG and NaCl. The method does not require the use of deproteinization nor desalting sample pretreatment. The method showed the effectiveness of the alkaline eluent for separating IgG at V_0 . The use of the alkaline condition was possible because of the polymer-based packing material used in ODP2 HP. The method developed in this application is fast and high-sensitive for the analysis of surfactants which would be applicable in antibody drug QC analysis. Even faster analysis can be expected by using a shorter column of the ODP2 series, ODP2 HP-2B (2.0 I.D. x 50 mm L), and coupling it with MS.

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