SHIMADZU TOSOH CORPORATION THE UNIVERSITY OF TOKYO

Automated Preparation and Quality Test for Antibody

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Best Poster Award

1. Introduction

In biopharmaceutical manufacturing, the differences in glycosylation and aggregate formation occur under various conditions, resulting in different activities. Therefore, it is necessary to optimize culture conditions and cell lines based on glycosylation and aggregate evaluation.

These screening assessments are necessary to evaluate several analysis conditions and sample types, which require reduced effort and automation.

Therefore, we attempted to establish an automated preparation and analysis system that simultaneously assesses both glycosylation and aggregation of antibodies directly from the cell culture supernatant using a liquid handler that integrates an auto-sampler and a fraction collector function in the same device.

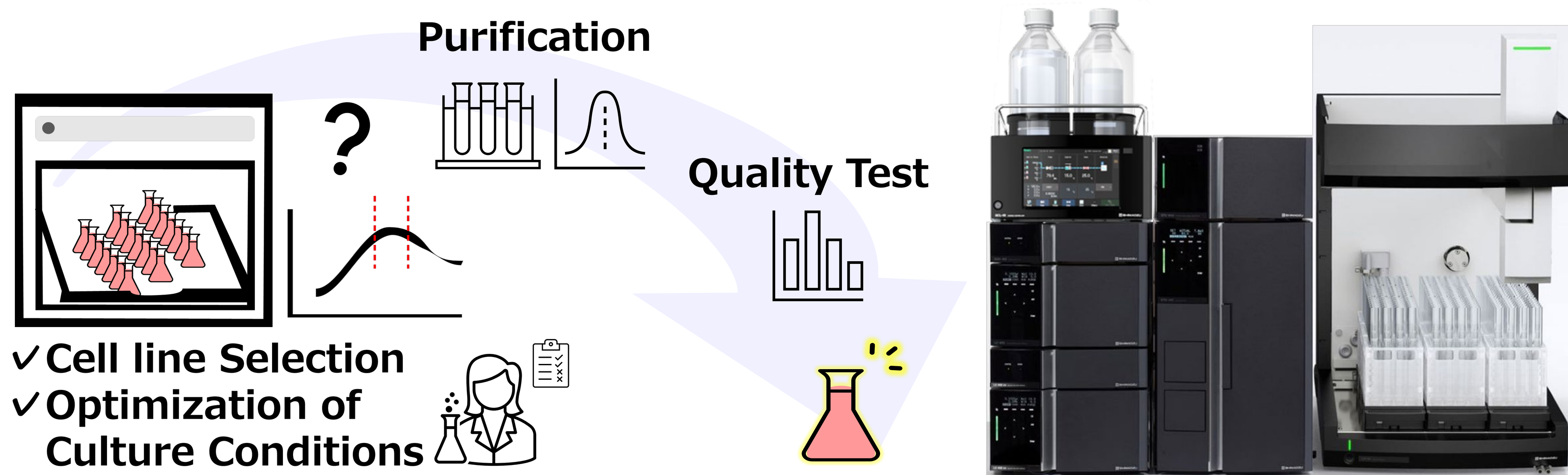

Figure 1. Experimental Strategy

Figure 2. Nexera™ XS inert & Liquid Handler

2. Materials & Methods

2-1. Seamless Analysis with Liquid Handler

The liquid handler serves as an autosampler as well as a fraction collector for the LC system. That means samples fractionated during the first run can be injected directly for the second run without transferring them from a fraction collector to an autosampler. For example, with this system (Figure 2), the target protein is purified by an affinity column and fractionated as the first step, and then the fractionated protein elution is re-injected for glycoform analysis and aggregate analysis as the second step (Flow chart: Figure 3). These two steps can be performed simply by specifying the method and fraction.

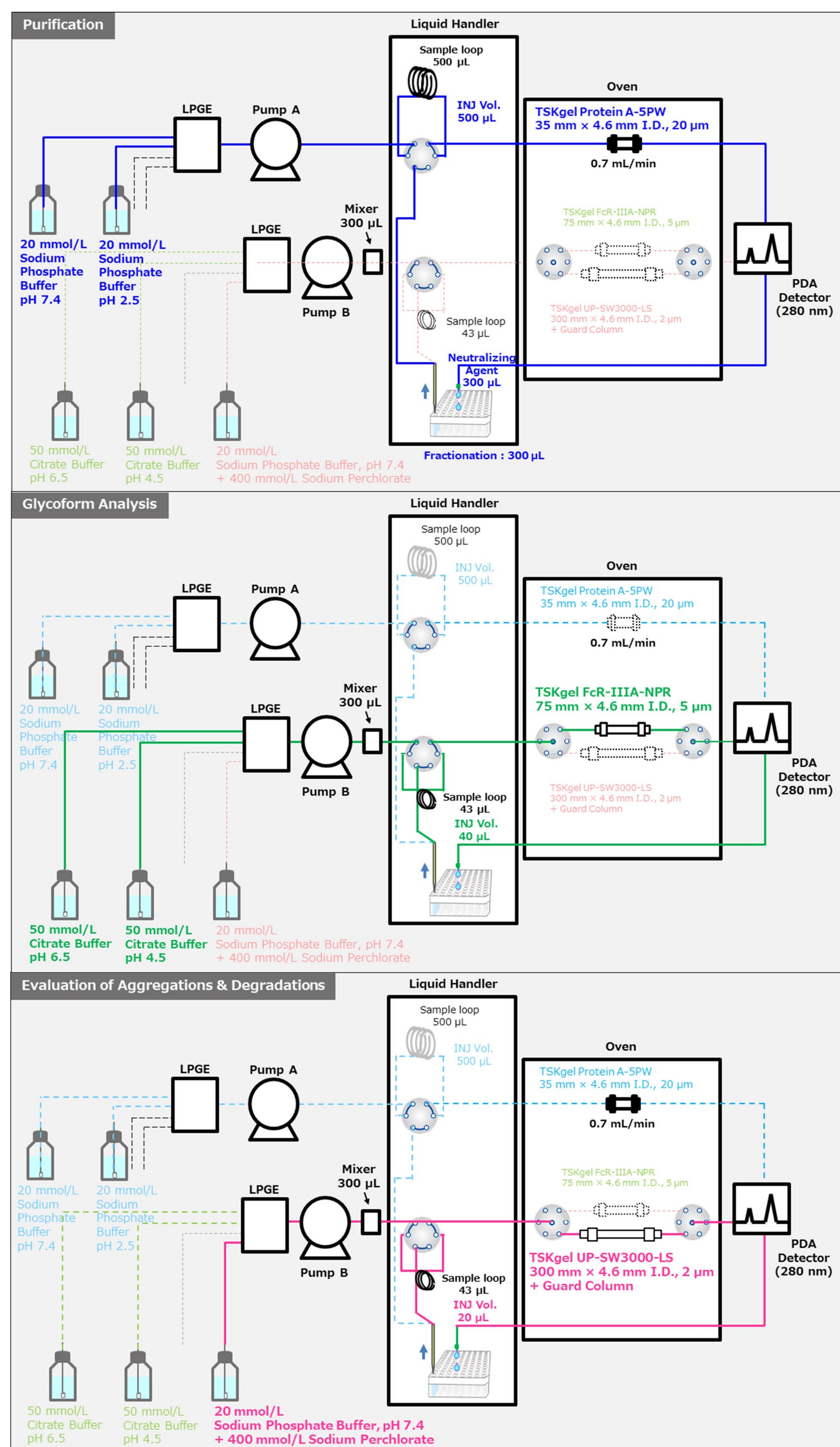

Figure 3. Flow Chart for each Step

3. Results & Discussion

3-1. Results of Affinity Purification

Cell culture supernatants of antibodies with different days of culture (harvested on day 4, day 7, and day 12) were used as samples.

The culture supernatant of the antibody was first set in a liquid handler, then purified with a Protein A column (TSKgel® ProteinA-5PW, Tosoh, Japan) and fractionated.

The samples were injected in the same volume, indicating that the antibodies were capable of higher expression in longer cultures (Figure 4).

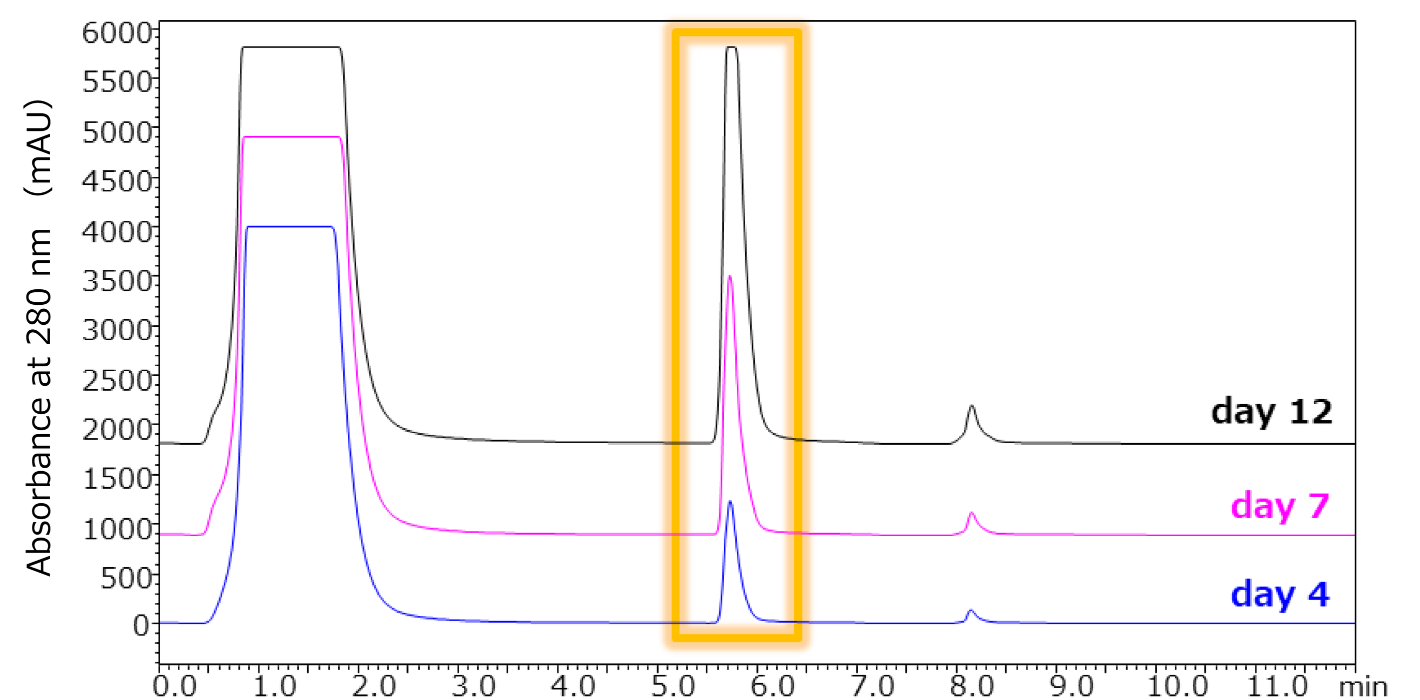

Figure 4. Chromatogram of Purification Step

3-2. Glycoform and Aggregate Analysis

Next, that fraction containing the target purified antibody was subjected to glycoform analysis using an affinity-mode column that recognizes N-linked glycan structural changes in the Fc region (TSKgel® FcR-IIIA-NPR, Tosoh, Japan).

It is known that the higher the antibody-dependent cellular cytotoxic activity (ADCC activity) of cultured antibodies, the more effective they are as antibody drugs.

This column allows the glycosylation of antibodies to be separated into multiple peaks, where each of the three major peaks represents the same antibody with variation in the glycan structures. The later the point of harvest, the lower the ADCC activity (Figure 5).

Furthermore, the fraction containing the purified antibody was also evaluated for aggregates using an SEC column (TSKgel® UP-SW3000-LS, Tosoh, Japan).

As shown in Figure 6, The later the point of harvest, the more agglomerates appeared.

These results suggest that harvested on day 4 is optimal in this culture condition.

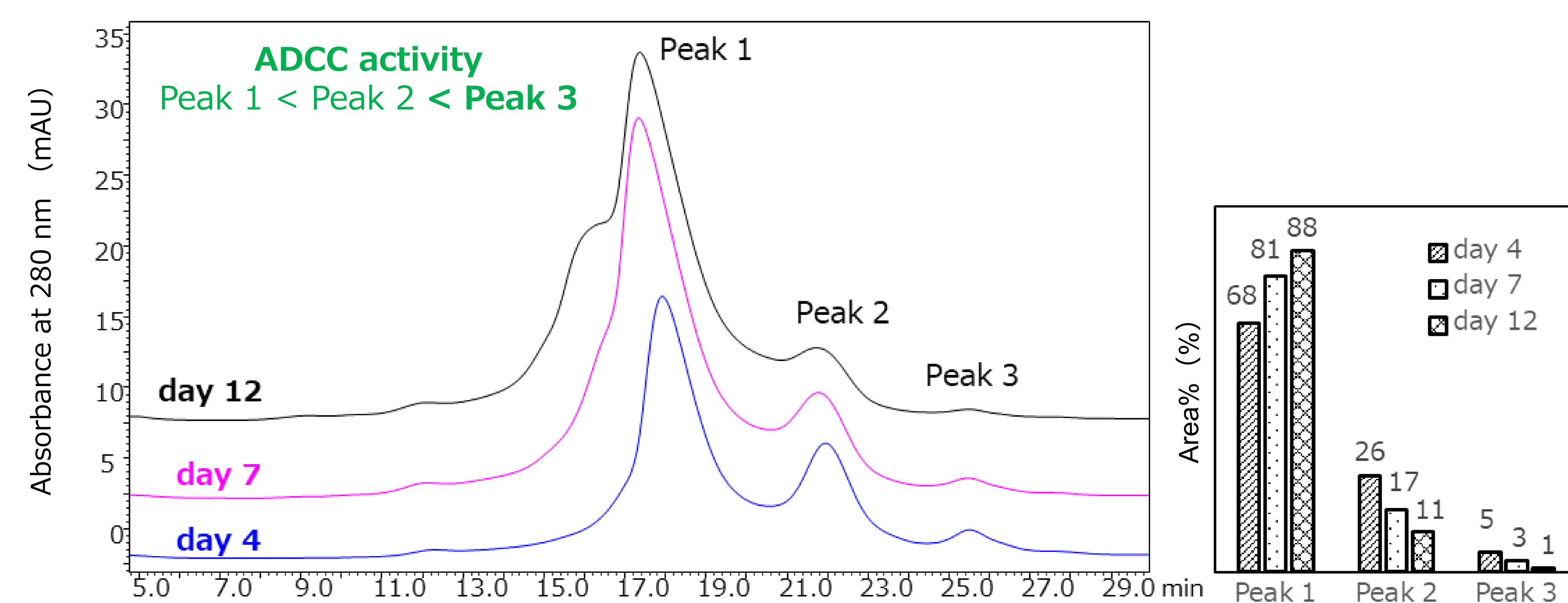

Figure 5. Results of Glycoform Analysis

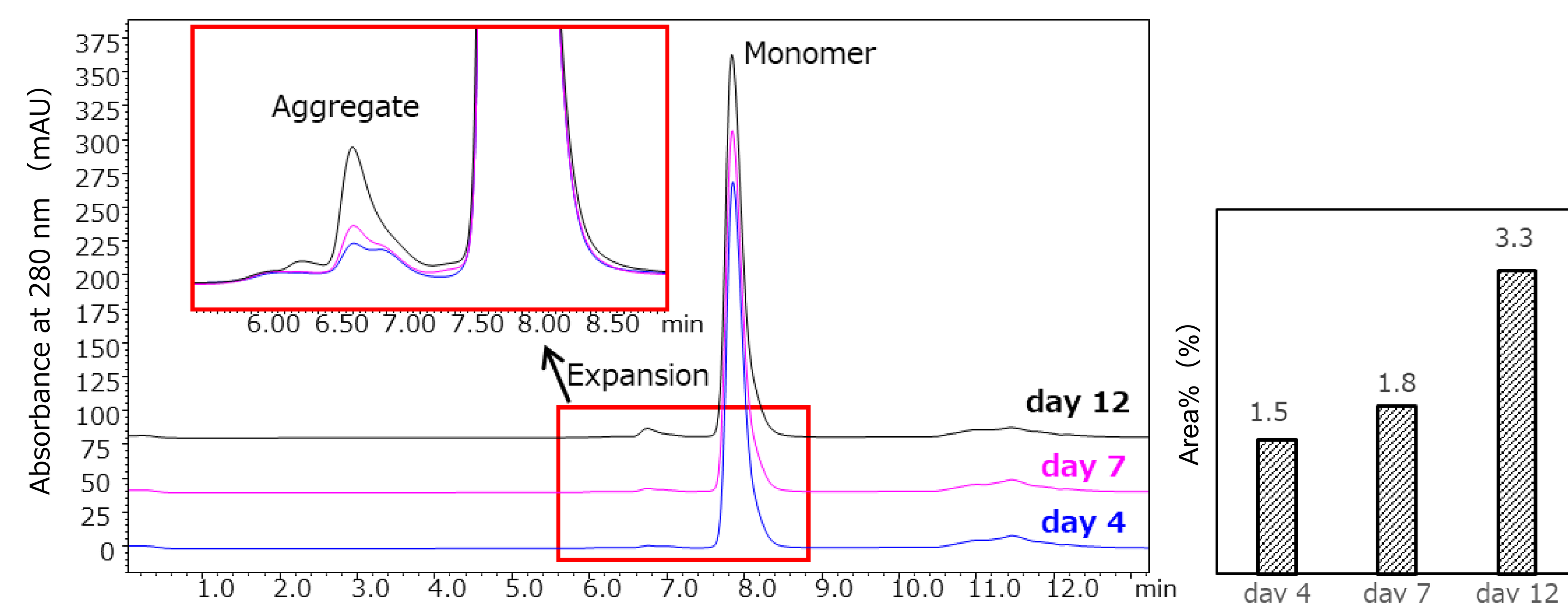

Figure 6. Results of Aggregate Analysis

4. Conclusion

This analysis system made it possible to automatically perform activity evaluation and aggregate evaluation of antibodies simultaneously from the cell culture supernatant. The system reduces the workload and enables highly reproducible data collection independent of the experimenter's skill. In addition, rapid evaluation results allow for smooth feedback to optimize culture conditions.

Moreover, since various columns can be attached, charge variant evaluation can also be performed. Therefore, it is also helpful as quality control profiling in the lot control of manufactured antibody production.

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