

# Technical Report

## Improvement of Data Reliability by Real-Time Measurement of Eluent pH – Application of pH Monitor to the Analysis of Aggregates and Charge Variants in Monoclonal Antibodies –

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### Abstract:

Biopharmaceuticals require appropriate quality control of each process, from development to shipment, increasing the importance of improved analytical reliability and data traceability. Because proteins and peptides, the main components of biopharmaceuticals, have many dissociable groups in their structures, the pH of the eluent affects peak separation in high-performance liquid chromatography analyses. For that reason, pH plays a critical role when optimizing analytical conditions. However, the pH of the eluent is usually checked only during the preparation process, even though variations in pH during long periods of continuous analysis could negatively affect analytical performances (such as resolution and reproducibility). The pH monitor pHM-40 can measure pH in real time. The LabSolutions software helps improve data reliability and traceability by managing chromatograms together with pH within a single data file. This article introduces an example of the use of pH monitor in the automatic preparation and confirmation of eluent pH using the mobile phase blending function for the analysis of aggregates and fragments in monoclonal antibodies, and in the pH gradient analysis of charge variants.

**Keywords:** pH monitor pHM-40, monoclonal antibody, biopharmaceutical

### 1. Background

Quality control of biopharmaceuticals during all stages from development to shipment is extremely important, and the quality, efficacy, and safety of biopharmaceuticals must be assessed in accordance with specified guideline<sup>[1]</sup>.

Because proteins and peptides, which are the main components of biopharmaceuticals, have many dissociable groups in their structures, the pH of the eluent may change the charge state, affecting resolution and reproducibility<sup>[2]</sup>. Therefore, it is desirable to monitor eluent pH not only during preparation but also during analysis. Data traceability is also important in pharmaceutical manufacturing, so the ability to readily verify analytical conditions and results is also desirable.

### 2. pH Monitor pHM-40

The pH monitor pHM-40 is installed after the detector (Fig. 1), so it can record changes in pH in real-time. That information is automatically stored in a data file together with all other analytical information (such as, analytical conditions, chromatograms, and quantitation parameters), which can be crucial when investigating the causes of poor results and during method development. It is also very useful for ensuring the data traceability and reliability. The wide measurable pH range (1 to 14) offers the possibility to benefit of pHM-40 features in many different applications. This article introduces the use of pHM-40 to examine eluent pH conditions in the analysis of aggregates of monoclonal antibodies using size exclusion chromatography and pH gradient conditions during the analysis of charge variants of monoclonal antibodies using ion exchange chromatography.

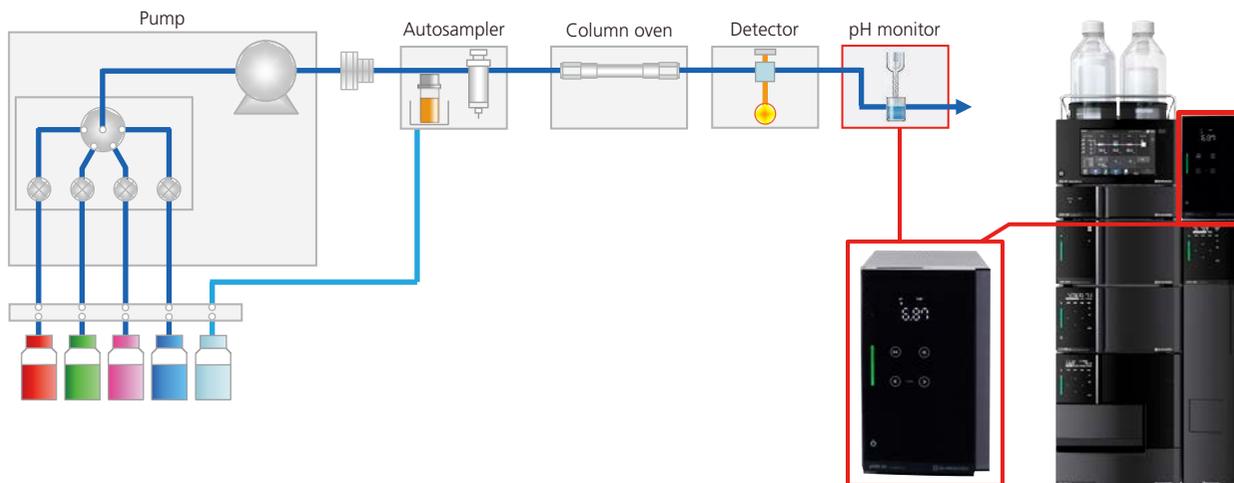


Fig. 1 Flow Diagram and Instrument Configuration Example of Low-Pressure Gradient System with pH Monitor

### 3. Optimization of Eluent pH

One method of analyzing impurities such as aggregates and fragments in antibody drugs is size exclusion chromatography. In size exclusion chromatography of monoclonal antibodies, eluent pH can alter the charge state of monomer, aggregates, and fragments, modifying chromatographic separation. In this example, eluent pH was varied from pH 5.7 to pH 7.2, and the pH during analysis is confirmed by pHM-40 to determine the optimum pH for separation. The eluent was automatically prepared using the mobile phase blending function<sup>[2]</sup> (Table 1).

Fig. 2 shows the chromatogram and pH data at pH 7.2 and Fig. 3 shows the enlarged chromatograms and pH data for the monomer peak. From the change in pH, it was confirmed that the eluent pH prepared automatically was the desired value and that the pH was stable during analysis. By overlaying the chromatogram and pH data, the separation results can be correlated with the pH.

Table 1 Analytical Conditions

System	: Nexera™ XS inert
Column	: Shim-pack™ Bio Diol-300 *1 (150 mm × 4.6 mm I.D., 2 μm)
Mobile phase	: A: 200 mmol/L (Monosodium) phosphate buffer B: 200 mmol/L (Disodium) phosphate buffer C: 1 mol/L NaCl D: Water
Flow rate	: 0.25 mL/min
Column temp.	: 25 °C
Sample	: Monoclonal Antibody Standard (Conc. 500 μg/mL)
Vial	: TORAST™-H Glass Vial *2 (Manufactured by Shimadzu GLC)
Injection vol.	: 5 μL
Detection	: 280 nm (SPD-M40, inert cell)

\*1 P/N: 227-31010-01 \*2 P/N: 370-04300-01

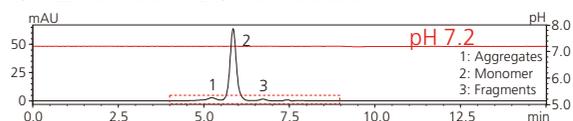


Fig. 2 Chromatogram at pH 7.2

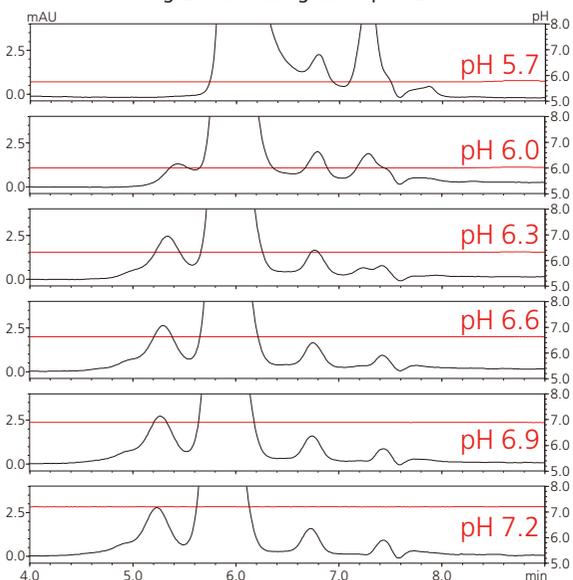


Fig. 3 Chromatograms and pH

### 4. Examples of Use in pH Gradient Analysis

Charge variants in antibody drugs are analyzed by evaluating antibody charge heterogeneity. An example of pH gradient analysis of a cetuximab biosimilar by ion exchange chromatography is shown. An equimolar mixture of HEPES, MES and sodium acetate solutions with different buffering capacities was adjusted to pH 5.0 and pH 8.2 with NaOH solution for use as eluents. The analytical conditions are shown in Table 2.

From Fig. 4, it was confirmed that the pH changed linearly from pH 5.5 to around pH 8.0 with increasing mobile phase B concentration. After the eluent concentration was returned to the initial concentration, the chromatogram decreased to the baseline level (approximately 18 min) and the pH returned to the initial value. (The time difference is the time it took for the eluent to reach the pH monitor from the detector after the column reached equilibrium in response to changes in the eluent pH.). The measured pH can be useful for accurately estimating the column equilibration time.

Table 2 Analytical Conditions

System	: Nexera XS inert
Column	: Shim-pack Bio IEX SP-NP*3 (100 mm × 4.6 mm I.D., 3 μm)
Mobile phase	: A: 20 mmol/L HEPES-MES-Sodium acetate (pH 5.0) B: 20 mmol/L HEPES-MES-Sodium acetate (pH 8.2)
Gradient program	: B Conc. 0% (0-0.5 min) – 100% (0.5-10 min) – 0% (15.1-25 min)
Flow rate	: 0.6 mL/min
Column temp.	: 25 °C
Sample	: Cetuximab Biosimilar (Conc. 1 mg/mL)
Vial	: TORAST-H Glass Vial (Manufactured by Shimadzu GLC)
Injection vol.	: 5 μL
Detection	: 280 nm (SPD-M40, inert cell)

\*3 P/N: 227-31005-03

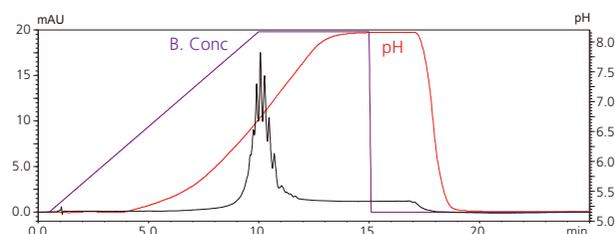


Fig. 4 Chromatogram of Cetuximab and pH

### 5. Summary

Since proteins and peptides, which are the main components of biopharmaceuticals, are easily affected by eluent pH, it is desirable to monitor eluent pH during analysis. In this study, a pH monitor pHM-40, was used to measure the pH during analysis of impurities in monoclonal antibodies. The eluent pH was stable at the intended value and the pH gradient was linear. Moreover, the pH profile can be easily overlaid on chromatograms to make the resulting datafile more complete and to improve data traceability and reliability.

### Reference

- [1] Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (PMSB/ELD Notification No. 571 dated May 1, 2001)
- [2] Emiko Ando, Daiki Fujimura, Keiko Matsumoto, 2022. Method Optimization for the Analysis of Monoclonal Antibodies by Size-Exclusion Chromatography

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