Separation of mAb Isoforms Using Controlled pH Gradients and Ion Exchange Chromatography Columns

TSKgel
APPLICATION NOTE

Introduction

Monoclonal antibody isoforms differ in modifications of individual amino acid side chains, or the N- or C-terminus. Typical modifications are deamidation, phosphorylation, acetylation, methylation, oxidation, or glycosylation. Fast separation of monoclonal antibody isoforms is important for profiling and mass spectrometric determination. Isoforms may differ in biological activity and stability, making a thorough characterization and quantification of the isoforms necessary to ensure consistent product quality.

Though Ion Exchange Chromatography (IEC) is a useful separation technique for profiling the charge heterogeneity of monoclonal antibodies, these separations are product specific and time consuming to develop. The utilization of controlled pH gradients, however, can provide significant advantages, including improved separation resolution, lower salt concentration in collected fractions, and the ability to correlate the protein isoelectric point (pI) data with elution profiles. Monoclonal antibody isoforms are separated based on differences in their charge states.

This application note discusses the separation of isoforms of monoclonal antibodies using highly controlled pH gradients on TSKgel STAT columns packed with 7 and 10 µm hydrophilic non-porous resin particles. The innovative bonding chemistry and relatively large particle size of TSKgel STAT columns result in a respectable loading capacity and a low operating pressure, making these columns suitable for all HPLC and FPLC systems in biomolecule separations.

Experimental Conditions

To create controlled pH gradients a pISep© kit (CryoBioPhysica, Silver Spring, MD), consisting of a software package and two nearly identical buffer compositions, acidic and basic, composed of small zwitterions with overlapping pKas, was used. pISep buffer composition possesses strong, relatively uniform buffering capacity throughout the pH range 2-12. The pISep software was used to compute column volume or time-based protocols for the development of single or multistep, linear or nonlinear, pH gradients on ion exchange (IEX) columns over any segment of the pH range 2.4-10.8.

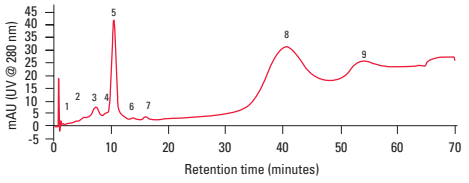
Analyses were carried out using an Agilent-1100 HPLC system running Chemstation (ver B.04.02).

Columns:	Strong cation, polymer: TSKgel SP-STAT, 7 µm, 4.6 mm ID × 10 cm (S0004-501N) Weak cation, polymer: TSKgel CM-STAT, 10 µm, 3.0 mm ID × 3.5 cm (N0018-507N) Strong anion, polymer: TSKgel Q-STAT, 7 µm, 4.6 mm ID × 10 cm (R0087-501N)
Flow rate:	TSKgel SP-STAT (1.0 mL/min or 1.66 min/CV); 1CV = 1.66 mL TSKgel CM-STAT (1.0 mL/min or 2.075 min/CV); 1CV = 0.247 mL TSKgel Q-STAT (0.8 ml/min or 0.247 min/CV); 1CV = 1.66 mL
Detection:	UV @ 280 nm
Temperature:	ambient
Injection vol.:	10 µL
Samples:	monoclonal antibody: BI-mAb-2 from Boehringer-Ingelheim (gift from Tosoh Bioscience GmbH) concentration: 4.5 g/L in glycine/Na phosphate, pH 6.0

Results and Discussion

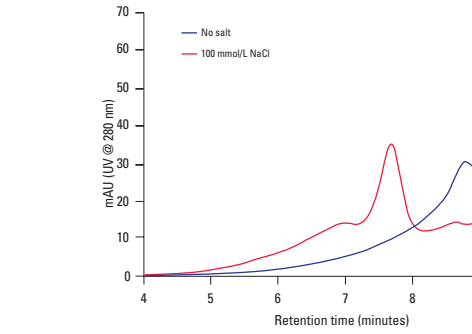
A size exclusion chromatographic separation using a TSKgel G3000SW$_{XL}$, 5 µm, 7.8 mm ID × 30 cm column (data not shown here) was used for a quality control study in the purification of a monoclonal antibody and yielded a predominantly pure monomer peak at ambient temperature with a retention time of 7.9 minutes during isocratic separation using 0.1 mmol/L phosphate buffer at the flow rate of 1 mL/min. Dimer and aggregate peak impurities were resolved from the monomer peak. Linear salt gradient-based separation (not shown here) of a monoclonal antibody using 10 mmol/L phosphate buffer containing 10 mmol/L Na_2SO_4 as a neutral salt (buffer A) and the same with 1 mol/L NaCl (buffer B) on a strong cation exchange TSKgel SP-STAT column was also used to separate the different mAb isoforms and yielded only a few peaks. Controlled pH gradient-based ion exchange separation of a monoclonal antibody using a strong cation exchange TSKgel SP-STAT column under the chromatographic conditions as mentioned above separated 9 different isoforms *(see Figure 1)*. The peaks were further resolved by the precise modification of the pH gradient. Large changes in resolution were achieved in controlled pH gradient elution simply by changing the range of elution (not shown here). *Figure 2* shows the effect of the addition of 100 mmol/L NaCl salt in improving the peak resolution of the two isoform peaks in the analysis of a mAb using a TSKgel CM-STAT column, a weak cation exchange chromatography column. Similarly an anion exchange chromatography column, TSKgel Q-STAT, was also successfully used for the separation of isoforms (data not shown here).

Figure 1. pH Gradient-based Ion Exchange Separation of a Monoclonal Antibody Separation using a Strong Cation Exchange TSKgel SP-STAT Column



Time (min)	%B	pH
0	0	7.6
15.1	21.4	8.6
23.9	36.9	8.7
44.1	77.3	9.7
50.4	87.6	10.1
57.0	94.9	10.4
63.0	100.0	10.8
70.0	100.0	10.8
70.1	0	7.6

Figure 2. Effect of Salt in pH Gradient-based Weak Cation Exchange Separation of a Monoclonal Antibody using a TSKgel CM-STAT Column



Conclusions

Controlled pH gradient-based ion exchange chromatography can be an effective method for the separation of protein isoforms. Good resolution was found for pH gradient-based separations using a broad range universal buffer system such as the pISep kit used in this study. TSKgel STAT columns can be used effectively to separate monoclonal antibody isoforms using a controlled pH gradient. Further studies to monitor the effect of different types of salt and organic solvents in improving peak resolution during a controlled pH gradient separation of mAb isoforms is in progress.

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