TOSOH

Application Note

Fast Analysis of IgG Charge Heterogeneity by Ion Exchange Chromatography

Therapeutic antibodies are enjoying high growth rates in the pharmaceutical market. A majority of the top bestselling global drug brands are monoclonal antibodies (mAbs). The main product characteristics to be analyzed in process monitoring and quality control of these antibodies are aggregation, fragmentation, glycosylation, and charge heterogeneity. Charge isoforms of proteins result from deamidation of asparagine or glutamine residues or from incomplete removal of the C-terminal lysine residue Besides isoelectric focusing, ion exchange chromatography is the method of choice to analyze charge heterogeneity of proteins.

TSKgel® STAT columns are packed with a non-porous polymer based stationary phase. Proprietary surface modification technology ensures a high density of charged groups. Compared to traditional porous ion exchange phases, non-porous particles yield outstanding resolution of charged species at shorter analysis time. TSKgel STAT columns are available with a weak cation exchanger containing a weak acid (carboxymethyl) and as a strong cation exchanger containing a bonded sulfonic acid group (sulfopropyl). Best performance is achieved when the columns are used with low dead volume chromatography systems, such as modern UHPLC systems. This application note presents the fast analysis of mAb charge heterogeneity by cation exchange chromatography using TSKgel STAT columns.

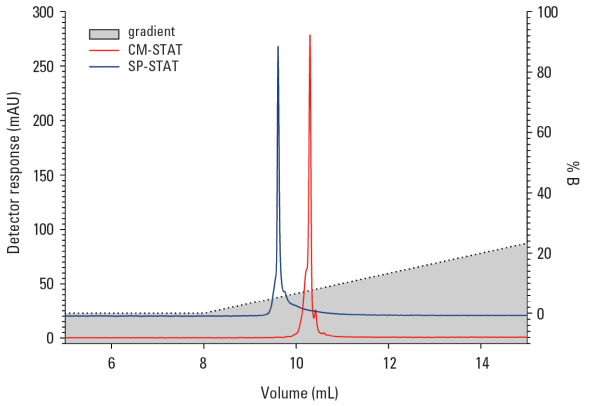
Materials and Methods

Columns:	TSKgel SP-STAT, 7 µm, 4.6 mm ID × 10 cm TSKgel CM-STAT, 7 µm, 4.6 mm ID × 10 cm
Mobile phase:	A: 10 mmol/L sodium phosphate buffer, pH 7.0 *(Figures 1, 2, 3)* 10 mmol/L sodium phosphate buffer, pH 6.0 *(Figure 3)* 10 mmol/L sodium acetate buffer, pH 5.0 *(Figure 3)* B: 100 mmol/L phosphate, pH 7.0 + 500 mmol/L NaCl *(Figures 1, 2, 3)* 100 mmol/L phosphate, pH 6.0 + 500 mmol/L NaCl *(Figure 3)* 100 mmol/L acetate, pH 5.0 + 500 mmol/L NaCl *(Figure 3)*
Gradient:	0 - 100% B in 30 min
Flow rate:	1 mL/min
Detection:	UV @ 280 nm
Injection vol.:	10 µL
Samples:	mAb A (2 g/L), mAb B (2 g/L)

Results

The charge isoforms of two monoclonal antibodies were separated on a TSKgel CM-STAT weak cation exchange column and a TSKgel SP-STAT strong cation exchange column. *Figure 1* shows the analysis of mAb A on both columns at pH 7. For this IgG, the weak cation exchange column delivers a better separation of the basic variant from the main peak.

Figure 1. Analysis of mAb A on Weak and Strong Ion Exchange Columns at pH 7



As shown in *Figure 2*, this is not the case for mAb B, where the strong cation exchange column shows a better separation. Retention and resolution of the charged isoforms are dependent on the buffer pH, as can be seen in *Figure 3* for the analysis of mAb B on TSKgel SP-STAT.

Figure 2. Analysis of mAb B on Weak and Strong Ion Exchange Columns at pH 7

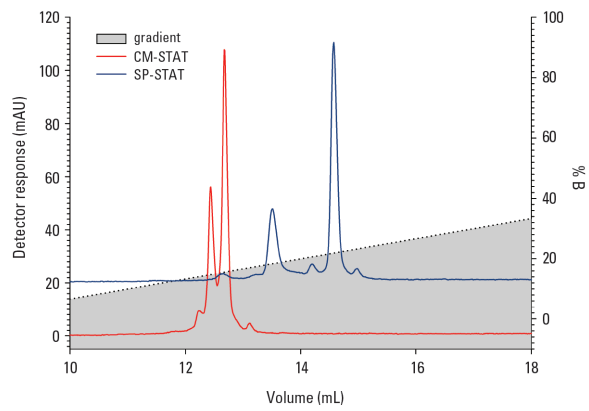
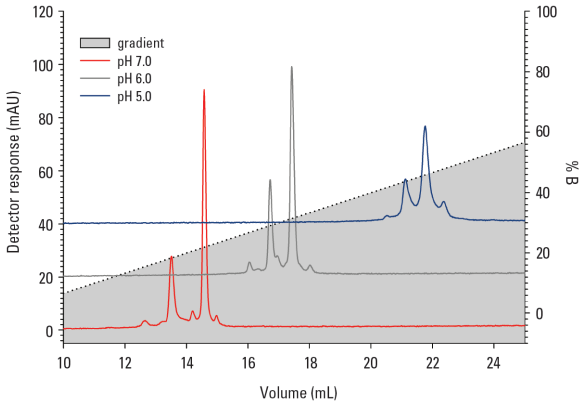


Figure 3. Dependence of Cation Exchange Separation on Mobile Phase pH



Conclusions

Weak and strong cation exchange columns provide different selectivities for the analysis of charge heterogeneity of proteins. In order to reach the best separation of acidic and basic isoforms from the main peak, both types should be evaluated at various pH values of the mobile phase during method development. TSKgel STAT series columns provide a high resolution of isoforms in a short analysis time and are ideally suited for the QC of biotherapeutics by UHPLC or HPLC.

Featured Products

Part #	Description
0021964	TSKgel SP-STAT, 7 µm, 4.6 mm ID × 10 cm
0021966	TSKgel CM-STAT, 7 µm, 4.6 mm ID × 10 cm

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