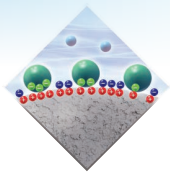
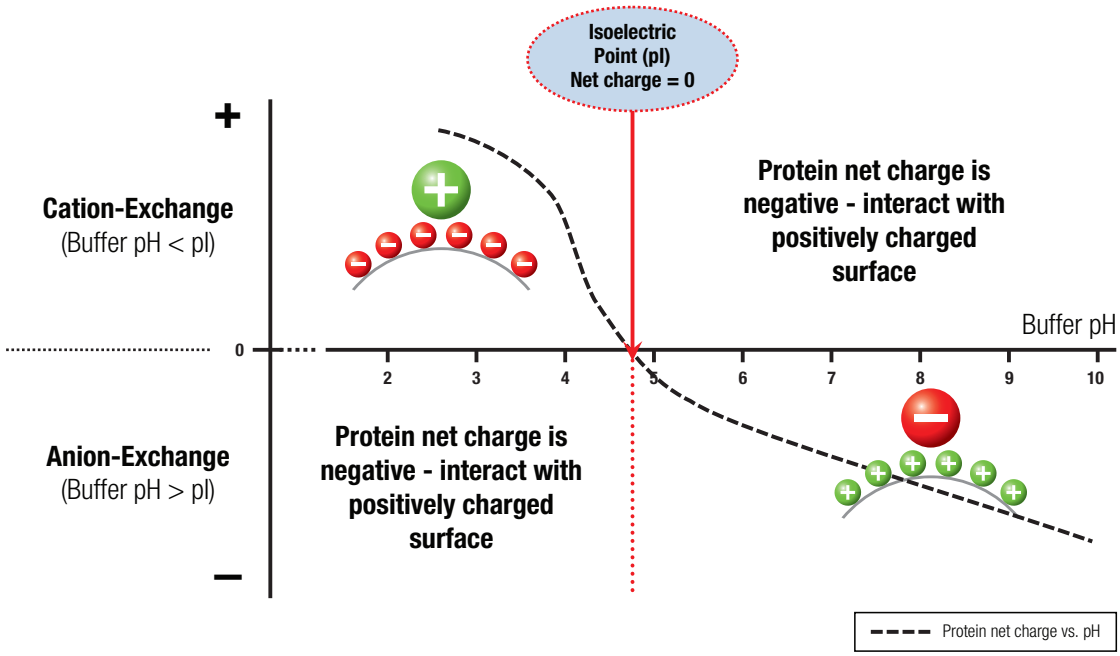


Charge Variant Analysis Using Ion Exchange Chromatography

Ion Exchange Chromatography (IEC) utilises a stationary phase with a charged ligand on the surface interacting with analytes of the opposite charge. The technique is divided between anion exchange and cation exchange chromatography. In anion exchange a positively charged surface ligands interacts with negatively charged analytes, whilst in cation exchange negatively charged surface ligands interact with positively charged analytes. In IEC, elution of the mAb charge variants can be performed by employing either a salt gradient or a pH gradient

In salt-gradient-based IEC, the pH of the buffer is fixed. In addition to choosing the appropriate pH of the starting buffer, its ionic strength is kept low since the affinity of proteins for IEC resins decreases as ionic strength increases. The proteins are then eluted by increasing the ionic strength (salt concentration) of the buffer to increase the competition between the buffer ions and proteins for charged groups on the IEC resin. As a result, the interaction between the IEC resin and proteins is reduced, causing the proteins to elute.

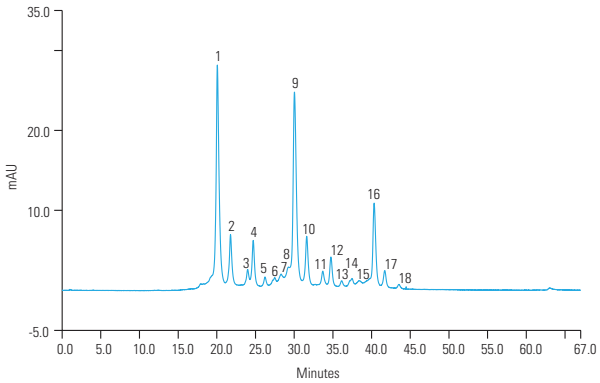
In pH-gradient-based IEC, the pH of the starting buffer is maintained at a constant level to ensure the proteins obtain the opposite charge of the stationary phase and bind to it. The proteins are eluted by changing the buffer pH so the proteins transition to a net zero charge (and ultimately the same charge as the resin) and elute from the column.



The mAbs undergo several post-translational modifications including oxidations, deamidations, glycosylation, incomplete C-terminal processing, and others. These modifications cause antibody variants in the cloning process, which in turn can impact the mAb activity and stability as a biotherapeutic agent. Monitoring stability of therapeutic mAb is essential for demonstrating safety and efficacy of the antibody as a drug.

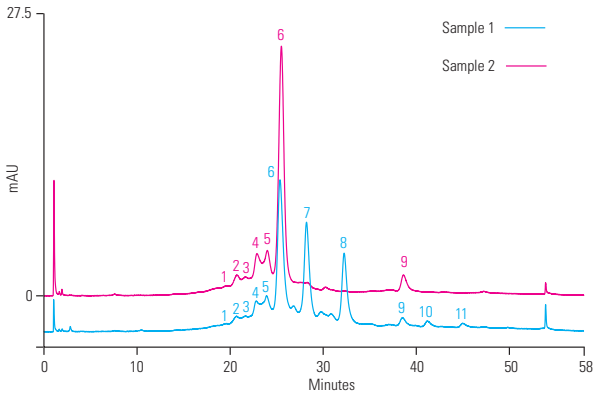
The pI of the majority of antibodies tends to be in the pH 6–10 region, which is the reason why cation exchange is usually the chosen separation mode for mAb charged variants.

Now available is a range of cation exchange MAbPac columns designed specifically for the separation of mAb variants and other variations affected by charge.



Column:	**MAbPac SCX-10**, 10 µm 4.0 × 250 mm
Mobile Phase A:	20 mM MES (pH 5.6) + 60 mM sodium chloride
Mobile Phase B:	20 mM MES (pH 5.6) + 300 mM sodium chloride
Gradient:	5–55% B in 60 min
Flow Rate:	1 mL/min
Inj. Volume:	10 µL
Temp:	30 °C
Detection:	280 nm

One of the most important and common analyses of monoclonal antibody heterogeneity is the monitoring and determination of acidic and basic variants. Thermo Scientific™ MabPac™ SCX-10 column separates lysine truncation variants before and after incubation with carboxypeptidase B.

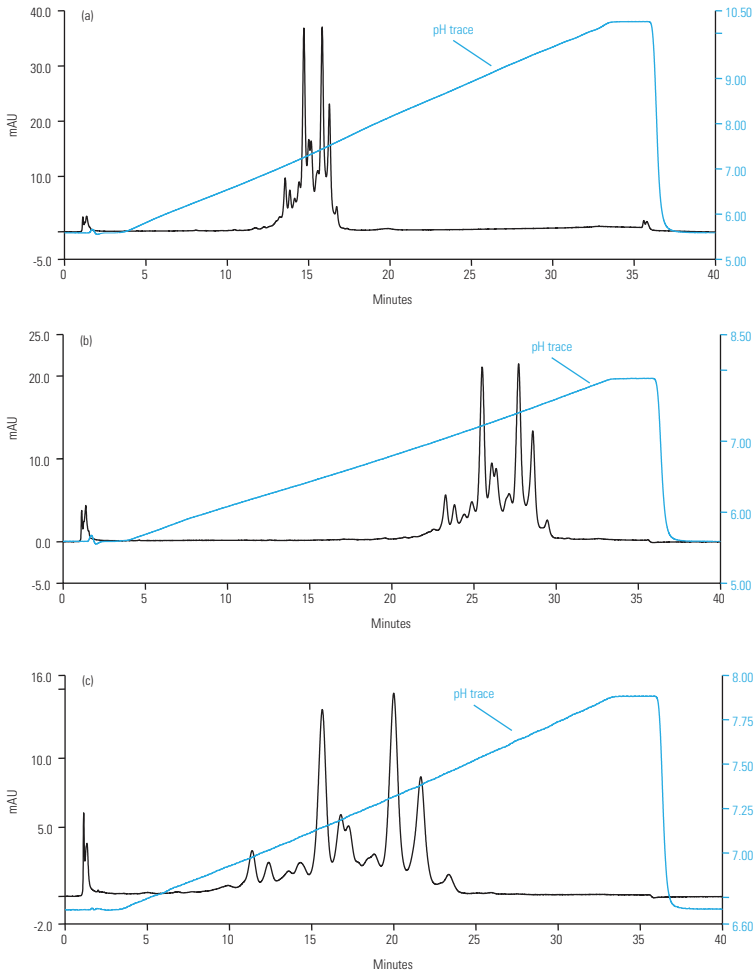


Column:	**MAbPac SCX-10**, 10 µm 4.0 × 250 mm
Mobile Phase A:	20 mM MES (pH 5.6) + 60 mM sodium chloride
Mobile Phase B:	20 mM MES (pH 5.6) + 300 mM sodium chloride
Gradient:	15–36% B in 50 min
Flow Rate:	1 mL/min
Inj. Volume:	5 µL
Temp:	30 °C
Detection:	280 nm
Samples:	1. mAb B, 900 µg in 100 µL (no carboxypeptidase) 2. mAb B, 900 µg in 100 µL + carboxypeptidase, 50 µg, incubation at 37 °C for 3 h
Both Chromatograms:	Peaks 1–5: Acidic variants
Sample 1:	Peaks 6–8: C-Terminal lysine truncation variants of main peak Peaks 9–11: C-Terminal lysine truncation variants of minor variant peak
Sample 2:	Peak 6 results from peaks 6, 7, and 8 after CBP treatment Peak 9 results from peaks 9, 10, and 11 after CBP treatment

Platform Method Using Ion Exchange pH Gradient

In the fast-paced drug development environment, a platform method to accommodate the majority of mAb analyses is desired. Considerable effort is often required to tailor the salt gradient method for a cation exchange separation of an individual mAb. Ion exchange separations by pH gradient provide the advantage of a generic platform approach, thus removing the need for individual sample method development.

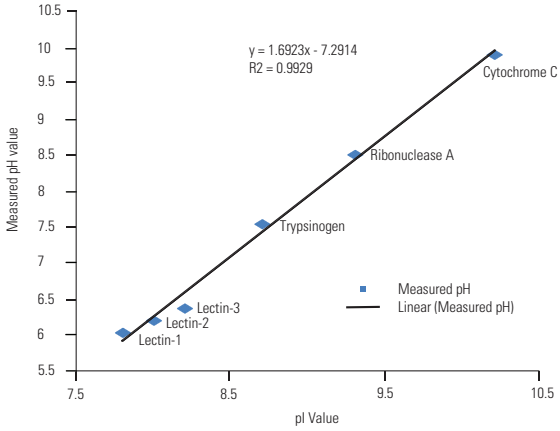
This approach features a multi-component zwitterionic buffer system in which the linear gradient can be run from 100% eluent A (low pH buffer) to 100% eluent B (high pH buffer). Once the approximate pH elution range of the target mAb has been established in the initial run, further optimization of separation can simply be achieved by running a shallower pH gradient in a narrower pH range. The optimization of a mAb variant separation is achieved by addressing the pH gradient range. When a broad pH gradient is applied from 0% to 100% B (pH 5.6 to 10.2 a, top), all analytes are eluted in the pH range 7.0 to 8.0. By progressively adjusting the gradient to 0–50% B (5.6–7.9 pH range, b middle) and 25–50% B (6.75–7.9 pH range, c bottom), resolution is further improved with minimal optimization steps.



Column:	**MAbPac SCX-10**, 10 µm 4.0 × 250 mm
Eluent A:	pH gradient buffer A
Eluent B:	pH gradient buffer B
pH gradient:	(a) 0% B (pH 5.6) to 100% B (pH 10.2)
	(b) 0% B (pH 5.6) to 50% B (pH 7.9)
	(c) 25% B (pH 6.75) to 50% B (pH 7.9)

Using a pH gradient to predict charge variant pI

Monitoring the eluent pH during a pH gradient makes charge variant characterization simpler and more predictable because proteins and mAbs will only elute once the eluent pH is above the biomolecules pI. The measured pH values for six proteins exhibit a strong linear correlation to the literature based pI values. This shows that the pI of a protein component can be estimated based on the peak retention time and measured pH.



Platform Method Using Ion Exchange pH Gradient–pH designer buffer capabilties

Tailoring pH gradients for individual needs is easily achieved using the Thermo Scientific™ pH Designer Software. The package describes how to create unique buffer formulations from a multitude of components and even predicts ionic strength, buffering capacity as well as pH profiles through a separation gradient.

The linearity of the pH gradient can be further verified using the Thermo Scientific™ Dionex™ UltiMate™ 3000 pH and conductivity online monitor, which can be added to UltiMate 3000 BioRS system to serve as a platform for pH gradient ion exchange chromatography.

The UltiMate 3000 pH and conductivity monitor is a valuable tool for HPLC method development, particularly for protein and nucleic acid separations. It enables linking the mobile phase pH and/or conductivity with the elution time of the components. The pH and conductivity monitor allows for the monitoring of gradient formation, column equilibration, and understanding column buffering effects in pH gradient ion-exchange chromatography.