ThermoFisher SCIENTIFIC

CX-1 gradient buffers

Simple method development for charge variant characterization

Benefits

- Proprietary buffer formulations enable fast, robust and reproducible pH gradients that are simple to optimize and easily automated
- Ready to use with existing LC columns and systems, without the need for time-consuming mobile phase adjustments
- Applicable to the majority of mAbs

Keywords
CX-1 pH buffer, pH gradient, charge variant analysis, MAbPac, monoclonal antibody, mAb, biopharmaceutical, protein, biomolecules

The Thermo Scientific™ CX-1 pH gradient platform accelerates method development and facilitates method transfer to QA/QC for a wide range of protein and mAbs charge variants, through a generic LC-based approach to charge variant characterization.

Introduction

As biotherapeutic drugs are becoming more popular, chemists in biopharmaceutical laboratories are under increasing pressure to adopt a fast, generic, and robust approach to clone screening, method development, and method transfer to QA/QC. Recombinant monoclonal antibodies (mAbs) can be highly heterogeneous due to modifications such as sialylation, deamidation and C-terminal lysine truncation. During their development and production, it is essential to detect, characterize, and quantify impurities as well as structural variants and modifications, and to monitor product stability.

This is key to demonstrating their safety and efficacy as biotherapeutics and is required by the U.S. FDA and other regulatory agencies.

Traditionally, cation exchange chromatography (IEC) using salt gradients has been successfully used to characterize mAb charge variants. However, additional effort is often required to tailor the salt gradient method for individual charge variants. Thermo Scientific™ pH gradient buffer solutions and kits can be used to generate highly reproducible, linear pH gradients using cation exchange chromatography. This generic LC-based platform approach saves time in method development and facilitates method transfer to QA/QC for a wide range of mAb charge variants. Unlike traditional salt gradients, it is possible to predict the pI and the expected retention of the charge variants and use a narrow pH range.

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pH gradient buffer concentrates

The building blocks of the pH gradient platform are two multicomponent zwitterionic buffer concentrates, prepared using a patent pending formulation. Buffer A is titrated to pH 5.6 and buffer B is titrated to pH 10.2. In this pH range, each buffer species is either neutral or negatively charged. Therefore, they will not be retained by the cation exchange column stationary phase and serve as good buffers for the mobile phase and the stationary phase.

Simple to use

All that is required to generate a pH gradient is a 1:10 dilution of the pH buffer concentrates–then you're ready to go. A linear pH gradient from pH 5.6 to 10.2 can simply be delivered by running a pump gradient from 100% eluent A to 100% eluent B.

Rugged reproducible pH gradients

Figure 1 shows the elution of ribonuclease A using the pH gradient run on a Thermo Scientific™ MAbPac™ SCX-10, 5 µm, 4 × 50 mm column. The gradient time was 15 min with a total run time of 20 minutes, as shown in Figure 1. The retention time RSD of the ribonuclease A peak was less than 0.8% over 300 runs. This demonstrates the high level of reproducibility with which the pH gradient can be applied to charge variant separations.

Simple method optimization

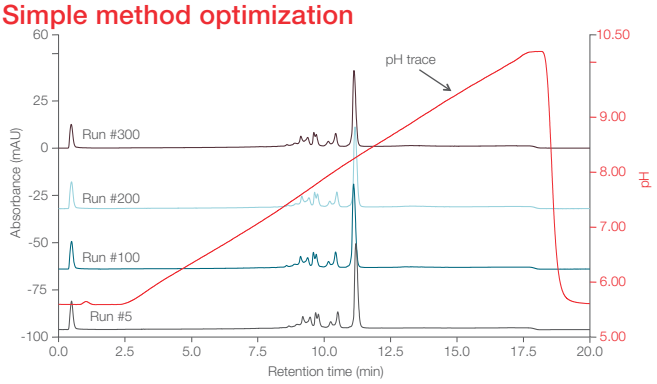

Figure 1. pH gradient platform delivers reproducible gradients.
Column: MAbPac SCX-10, 5 µm, 4 × 50 mm (PN 078656)
Sample: Ribonuclease A

Optimization of the separation may be necessary in order to improve the resolution of the charge variants. This can simply be achieved by running a shallower pH gradient over a narrower pH range. The chromato-graphic profile and therefore the elution order of the variants remains predictable when running a shallower pH gradient. This predictability means that it is simple to automate the optimization process by selecting a narrower eluent range (e.g. 25 to 50% B instead of 0 to 100%), as shown in Figure 2.

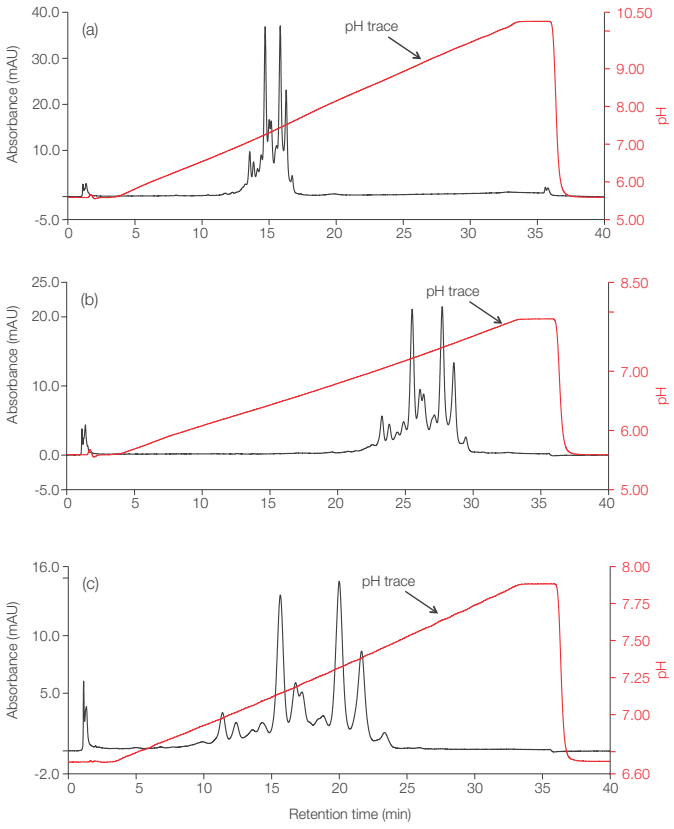

Figure 2. Optimiztion of MAb charge variant separation using a linear pH gradient.
Column: MAbPac SCX-10, 10 µm, 4 × 250 mm (PN 074625)
(a) Separation by pH gradient, 0% B (pH 5.6) to 100% B (pH 10.2)
(b) Separation by pH gradient, 0% B (pH 5.6) to 50% B (pH 7.9)
(c) Separation by pH gradient, 25% B (pH 6.75) to 50% B (pH 7.9)

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.

As shown in Figure 3, the measured pH values for six protein peaks exhibited a strong linear correlation to the literature based pI values. This supports the fact that linear regression coupled with the pH gradient method described here can be used to estimate the pI of a protein component based on the peak retention time
and measured pH.

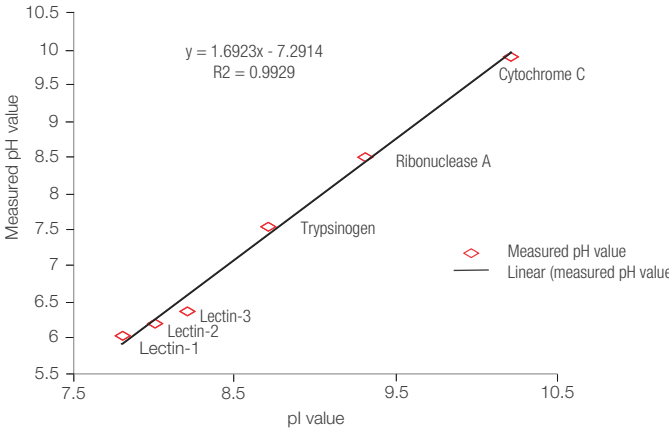

Figure 3. The measured pH values for six protein component peaks correlates with their pI values.

ProPac and MAbPac Cation Exchange Columns

Thermo Scientific™ ProPac™ and MAbPac cation exchange columns have been designed specifically for high resolution, high efficiency separation of proteins, monoclonal antibodies and associated variants. The unique nonporous resin provides exceptionally high resolving power, permitting the separation of monoclonal antibody variants that differ by as little as one charged residue. Columns packed with 3 µm and 5 µm particles deliver even more speed and resolution for mAb charge variant characterization.

Vanquish Flex UHPLC systemm

The Thermo Scientific™ Vanquish™ Flex™ UHPLC system is designed for the needs of UHPLC and LC-MS scientists to provide the high pressures required for the separation of bioanalytes on high resolution bio UHPLC columns. Whether you are analyzing innovative biotherapeutics or novel chemical medicines, method development has never been easier. The Vanquish Flex UHPLC system brings exceptional analytical precision, detector sensitivity and operational simplicity to enable unprecedented robustness and flexibility while providing full biocompatibility to address the most challenging biotherapeutic applications. The Thermo Scientific™ Dionex™ Viper™ Fingertight fitting technology ensures robust system connections with virtually zero-dead volume for maximum performance.

Ordering options

Thermo Scientific pH buffer concentrates used in the pH gradient platform can be purchased individually in quantities of 125 mL, 250 mL, 500 mL or 1000 mL.

Ordering information

Description	Size	pH	Cat. no.
pH gradient buffer A	125 mL	pH 5.6	083273
	250 mL		085346
	500 mL		302779
	1000 mL		303274
pH gradient buffer B	125 mL	pH 10.2	083275
	250 mL		085348
	500 mL		302780
	1000 mL		303275

Learn more at **thermofisher.com/pHgradientbuffer**