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PRODUCT SPECIFICATIONS

CX-1 pH 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 MAb 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

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individual charge variants. pH gradient buffer solutions and kits from Thermo Scientific 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

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 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.

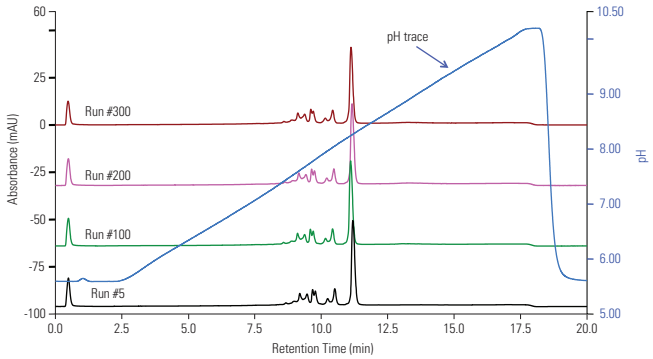


Figure 1. pH gradient platform delivers reproducible gradients. Column. MAbPac SCX-10, 5 µm, 4 × 50 mm; sample: ribonuclease A

Simple Method Optimization

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.

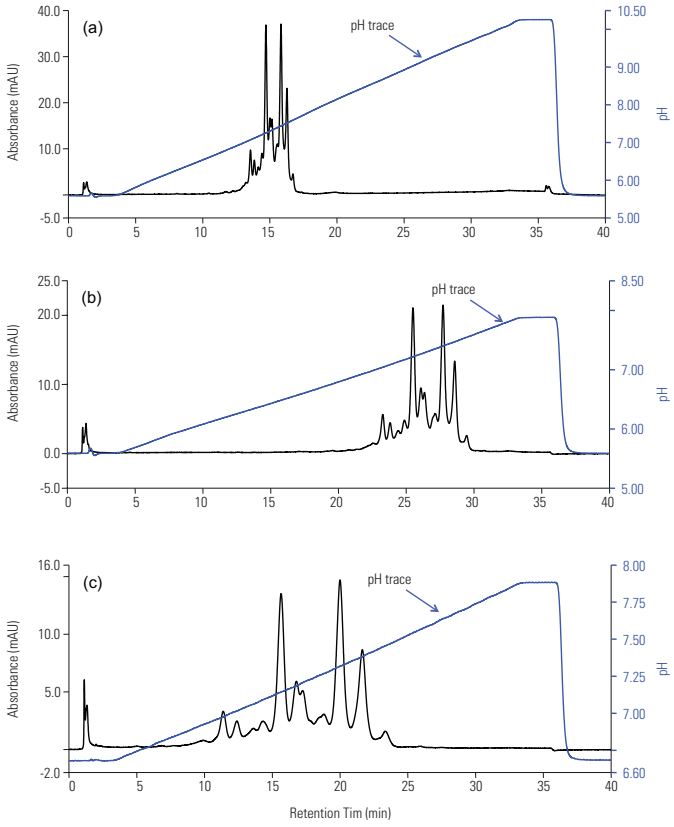


Figure 2. Optimiztion of MAb charge variant separation using a linear pH gradient. Column. MAbPac SCX-10, 10 µm, 4 × 250 mm
(a) Separation by pH gradient, 0% B (pH 5.6) to 100% B (pH 10.2)
(b) Separation by pH gradient, 0% B (pH 5.6) to 50% B (pH 7.9)
(c) Separation by pH gradient, 25% B (pH 6.75) to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

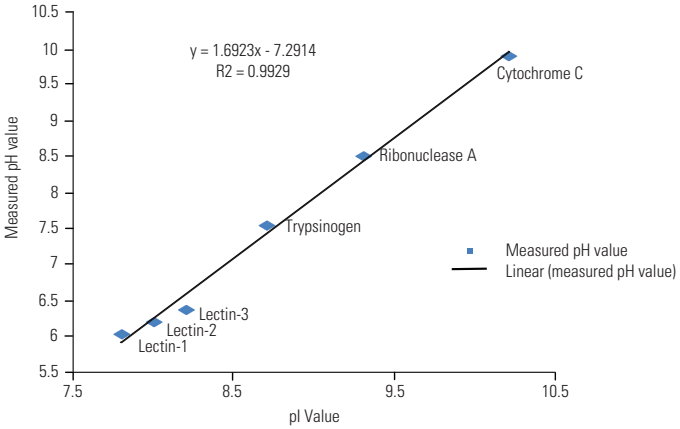


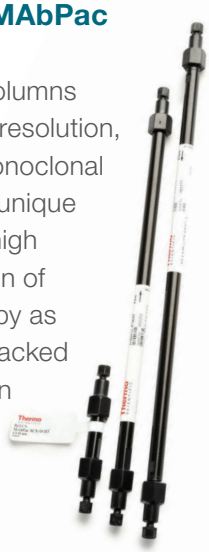
Figure 3. The measured pH values for six protein component peaks correlates with their pI values.

Related Products
pH Designer Software

Thermo Scientific™ pH Designer Software makes it even easier to tailor pH gradients to individual requirements. 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.

Thermo Scientific™ ProPac™ and MAbPac Cation Exchange Columns

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.



Thermo Scientific™ Dionex™ PCM-3000 pH Gradient Separation Module

The PCM-3000 pH gradient separation module can be added to the Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system to serve as a platform for pH gradient ion exchange chromatography. This is for compounds such as monoclonal antibodies and charge variants, conductivity monitoring in gradient separations of biomolecules, or any other application where conductivity or pH monitoring is of interest.

UltiMate 3000 BioRS System

The UltiMate 3000 BioRS system is powered by UltiMate 3000 rapid separation technology to support the high pressures required for the separation of bioanalytes on high resolution bio UHPLC columns. This state-of-the-art technology combined with a biocompatible, low-dispersion flow path, provides the highest peak capacity and sensitivity for complex samples, whether proteins, peptides, or biotherapeutics. The Thermo Scientific™ Dionex™ Viper™ Fingertight fitting technology ensures robust system connections with virtually zero-dead volume for maximum performance. Peptide mapping, monoclonal antibody charge variant analysis, glycan analysis, or nucleic acid analysis, the UltiMate 3000 BioRS system is ready to meet the high chromatographic demands of any of these biomolecule analyses.

Ordering Options

Thermo Scientific pH buffer concentrates used in the pH gradient platform can be purchased individually or as a pair, in quantities of 125 mL or 250 mL. For added convenience, the 125 mL buffers can also be bundled with columns in a number of specifically preconfigured kits.

- The CX-1 pH gradient starter kit contains 125 mL each of buffers A and B, plus a MAbPac SCX-10, 10 µm, 4 × 250 mm column
- The CX-1 pH gradient high throughput kit contains 125 mL each of buffers A and B, plus a MAbPac SCX-10, 5 µm, 4 × 50 mm column
- The CX-1 pH gradient high resolution kit contains 125 mL each of buffers A and B, plus a MAbPac SCX-10, 5 µm, 4 × 250 mm column

For the ultimate flexibility, the preconfigured kits are also available as platforms, including the pH Designer Software. The options are listed in the table below:

Operational Specifications

Description	Part Number
CX-1 pH Gradient Buffer A (pH 5.6), 125 mL	083273
CX-1 pH Gradient Buffer B (pH 10.2), 125 mL	083275
CX-1 pH Gradient Buffer Kit (pH 5.6 to 10.2), 125 mL	083274
CX-1 pH Gradient Buffer A (pH 5.6), 250 mL	085346
CX-1 pH Gradient Buffer B (pH 10.2), 250 mL	085348
CX-1 pH Gradient Buffer Kit (pH 5.6 to 10.2), 250 mL	085349
CX-1 pH Gradient Buffer A, 500 mL	302779
CX-1 pH Gradient Buffer B, 500 mL	302780
CX-1 pH Gradient Buffer A, 1000 mL	303274
CX-1 pH Gradient Buffer B, 1000 mL	303275
CX-1 pH Gradient Starter Kit (pH 5.6 to 10.2), Includes 125mL bottles of both Buffer A & Buffer B + MAbPac SCX-10 column (p/n 074625)	083381
CX-1 pH Gradient High Throughput Kit (pH 5.6 to 10.2), Includes 125mL bottles of both Buffer A & Buffer B + MAbPac SCX-10 column (p/n 078656)	083378
CX-1 pH Gradient High Resolution Kit (pH 5.6 to 10.2), Includes 125mL bottles of both Buffer A & Buffer B + MAbPac SCX-10 column (p/n 078655)	083272

Find out more at **thermofisher.com/pHgradientbuffer**

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