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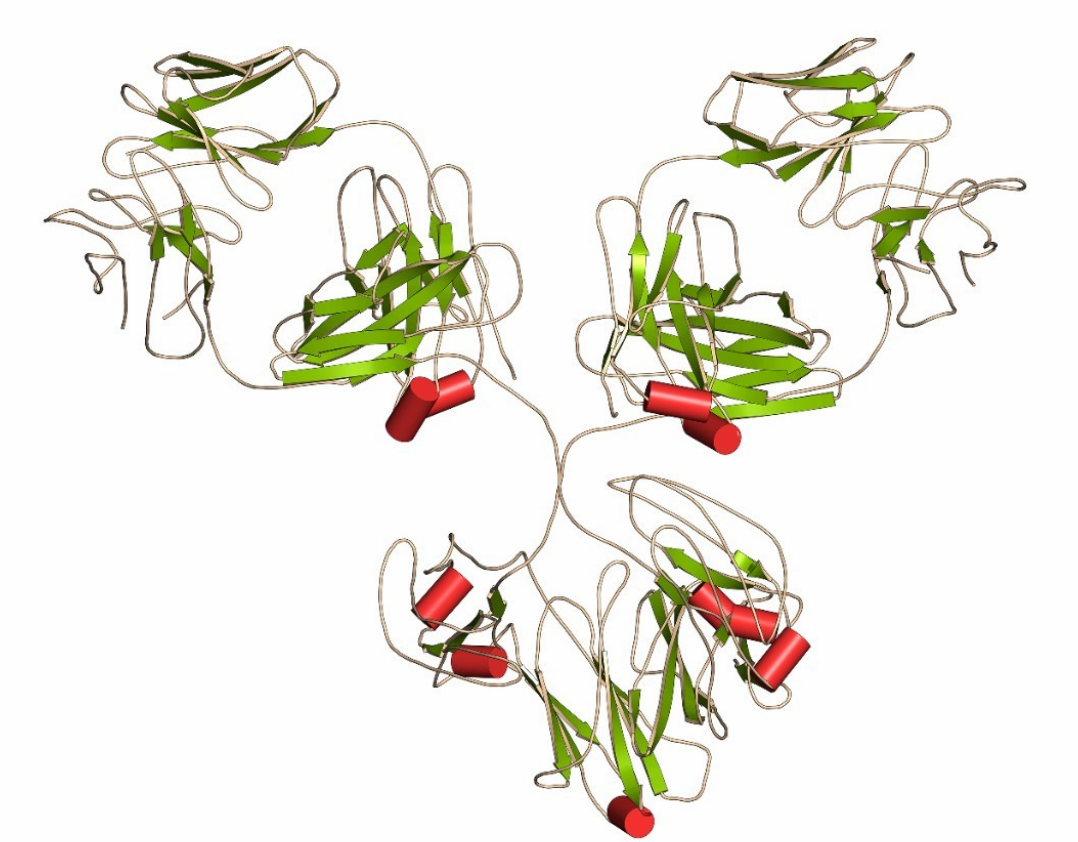
應用手冊

Ion Exchange (IEX) using ACQUITY UPLC H-Class Bio System

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

In this application brief, to simplify ion exchange chromatography (IEX) methods development for the analysis and characterization of proteins using the quaternary-based ACQUITY UPLC H-Class Bio System in combination with Auto•Blend Plus Technology.

Benefits

The ACQUITY UPLC H-Class Bio System and Auto•Blend Plus Technology together help IEX users streamline the development of methods for the analysis of proteins and their charge variants.

Introduction

The complete analysis and characterization of proteins requires orthogonal analytical techniques focusing on different physical and chemical properties. Ion exchange chromatography is often utilized to assess the distribution of proteins or the presence of protein variants formed by post-transitional modifications (***e.g.***, deamidation) that can be recognized by charge differences. For these analyses, adjustments in mobile phase pH are the most useful parameter for method development. Such experiments are, however, time-consuming and cumbersome. The ACQUITY UPLC H-Class Bio System and its quaternary solvent manager takes advantage of UPLC Technology and Auto•Blend Plus Technology to simplify IEX method development.

Auto•Blend Plus Technology allows users to manipulate pH and ionic strength by calculating and delivering the proportions of buffer stocks required for the desired conditions. The introduction of this new system provides users a robust, efficient tool for method development for IEX separations of proteins.

Results and Discussion

Ion exchange chromatography of proteins combines the ACQUITY UPLC H-Class Bio System with Auto•Blend Plus Technology and Protein-Pak Hi Res Columns for simplified method development. The ACQUITY UPLC H-Class Bio System is an inert system that provides stability in the aqueous, high ionic strength buffers used for IEX separations, while also giving the highest recovery of the sample.

Auto•Blend Plus Technology takes advantage of the system's four-solvent blending capabilities to prepare and adjust chromatographic mobile phases using pure solvents and concentrated stocks of acid, base, salt, and water. In the newest implementation, its user interface allows for expressing the chromatographic method in

parameters that are most familiar to the biochemist, specifically pH and ionic strength.

Using Auto•Blend Plus Technology, a series of experiments were performed to demonstrate the effect of buffer composition and pH on IEX separations of proteins. To illustrate the effect of buffer composition, a mixture of proteins was separated using weak cation-exchange chromatography. Two common cation-exchange buffers, Sodium phosphate and MES ((N-Morpholino)ethanesulfonic acid), were compared. At a pH of 6, different selectivity was observed for the most basic proteins, as shown in Figure 1.

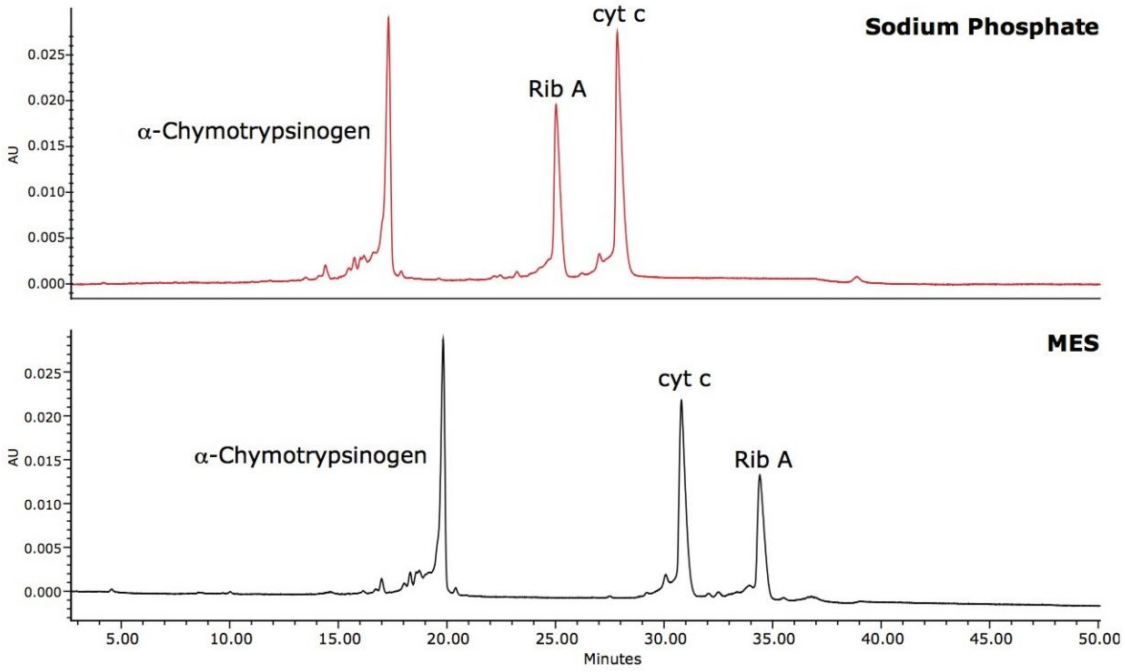


Figure 1. Effect of buffer composition on the IEX separation of proteins.

Sample: Bovine, α-Chymotryspinogen, Bovine Ribonucelase A, Equine cyctochrome c.

Column: Protein-Pak Hi Res CM 7-µm, 4.6 x 100 mm.

Conditions: 20 mM buffer (MES or Sodium Phosphate) pH 6, 1 mL/min, 0 to 0.2 M NaCl in 34 min at 30 °C.

The buffer system also influenced overall retention: MES buffers resulted in longer retention times for the proteins. In a second experiment, a monoclonal antibody containing lysine variants was separated with phosphate buffer at different pHs. The monoclonal antibody separations (Figure 2) demonstrate the influence of pH on retention time and selectivity for a humanized IgG and its variants. In these experiments, the use of Auto•Blend Plus Technology and a four-buffer blending system allowed for simple, fast method development for IEX separation of proteins.

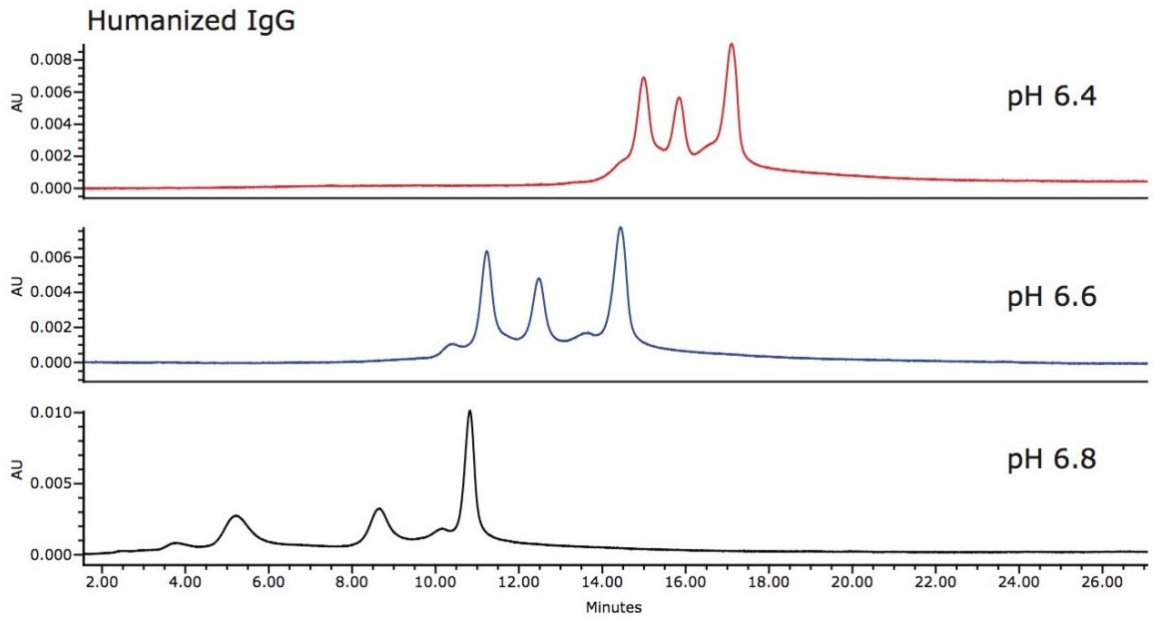


Figure 2. Effect of pH on IEX separation of humanized IgG.

Sample: Humanized IgG, 1.5 mg/mL.

Column: Protein-Pak Hi Res CM 7-µm, 4.6 x 100 mm.

Conditions: 20 mM Sodium Phosphate, 0.5 mL/min, 0 to 0.1 M NaCl in 40 min at 30 °C.

Conclusion

The ACQUITY UPLC H-Class Bio System and Auto▪Blend Plus Technology together provide IEX users with a fast and easy method development system for the analysis of proteins and their charge variants. Adjusting pH is simplified with the use of a four-solvent blending system (acid, base, salt, and water) in combination with Auto▪Blend Plus Technology software. These improved protocols translate to savings in both time and reagent costs, increasing overall productivity.

Featured Products

- ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>
- Auto▪Blend Plus <https://www.waters.com/134623262>

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