

Capillary Isoelectric Focusing Analysis of Therapeutic Proteins During Process Development

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Abstract

Isoelectric focusing (IEF) is a high resolution technique for the separation of proteins by their isoelectric point (pI). It is routinely used for protein pI determination, identification, characterization, and stability monitoring. Compared to conventional slab gel IEF, capillary IEF (cIEF) offers higher resolution, speed, and quantitation and automation capabilities. An imaged cIEF system has been commercialized by Convergent Bioscience which uses a short separation column and whole-column imaging detector. Imaged cIEF enables cIEF analysis without the mobilization step required in conventional cIEF. As a consequence, the resolution and sensitivity are further improved and the assay runtime is shorter compared to conventional cIEF. In this work, specific applications of imaged cIEF to the development of therapeutic proteins will be presented.

Principle of IEF

- In capillary isoelectric focusing (cIEF), the substances to be separated are applied to the entire capillary together with carrier ampholytes, solutions of amphoteric compounds which have closely spaced pI values encompassing a given pI range.
- The pH gradient is created, and maintained, by the passage of an electric current through the ampholyte solution. Under the influence of an electric field, the charged species will start to migrate through the electrophoresis medium and carrier ampholytes stack according to their pI values.
 - The most acidic carrier ampholyte moves toward the anode, where it concentrates in a zone whose pH equals its pI, while the more basic ampholytes are driven toward the cathode.
 - Less acidic ampholytes will migrate adjacent to and just cathodal to the previous one and so on, until all components of the system reach a steady state.
 - A pH gradient, increasing from anode to cathode is established
- Finally, as time progresses, the sample protein molecules also reach their isoelectric points.
- The surface charge of an amphoteric compound in IEF keeps changing, and decreasing, according to its titration curve, as it moves along a pH gradient until it reaches its equilibrium position – the region where the pH matches its pI. Here, its mobility equals zero and the molecule comes to a stop.
- Protein molecules diffusing out of a focused zone will acquire a charge and are pulled into the center of the zone where the net charge is zero.

cIEF in Bioprocess Development

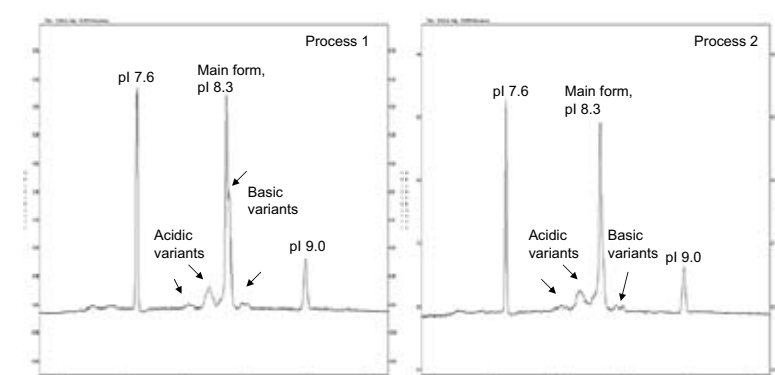
- In bioprocess development for therapeutic proteins, various production cell clones, process conditions and formulations are evaluated for product quality attributes to aid in the selection of the most desirable clone, process and formulation. Imaged cIEF is one technique that is routinely used for the examination of charge-based isoforms of therapeutic proteins.
- Specific applications of imaged cIEF in bioprocess development include:
 - Process Development
 - Clone Selection
 - Stability Monitoring
 - Formulation Screening
 - Product Characterization

Methodology

- cIEF is carried out using a Convergent Bioscience iCE280 Analyzer
- The separation column is a 5cm long, 100um ID, 200um OD silica capillary
 - The outside polyimide coating is removed to facilitate whole column detection
 - The inner wall is coated with fluorocarbon to reduce electroosmotic flow
- Detection
 - Absorbance of the focused proteins is detected at 280nm
 - Focusing is monitored by taking the whole capillary absorption image by a charge-coupled device camera every 30 sec.
- Optimization of Peak Resolution
 - cIEF for each protein is optimized to give the maximum resolution for the sample protein of interest. This is accomplished by selecting the optimal carrier ampholyte mixture and focusing time.
 - Ampholytes with different pH ranges from several different vendors are evaluated. Various combinations of wide and narrow range ampholytes, as well as the total volume of ampholytes are also tested.

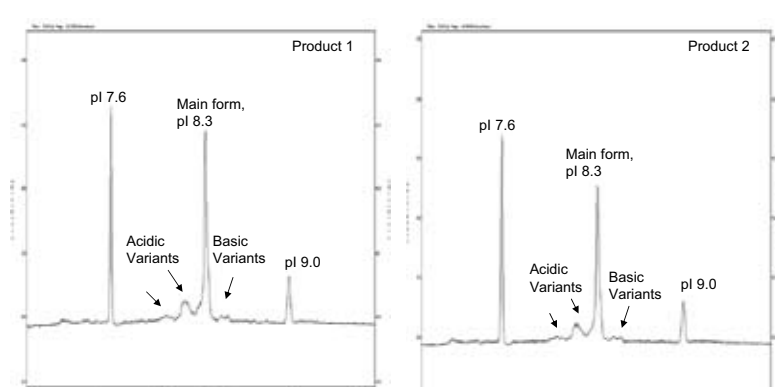
Purification Development

Therapeutic protein A is evaluated for charge heterogeneity during purification process development



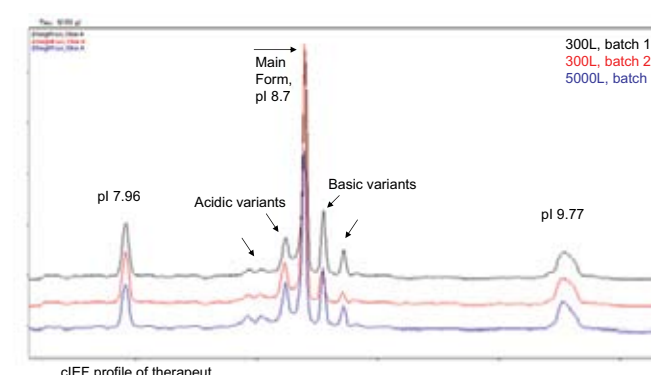
cIEF profile of therapeutic protein A. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10.5 (Pharmalyte), 0.35 mg/ml protein, 1mcl marker, pI 7.6, 1mcl marker, pI 9.0, 117 water, focusing time 1 min at 1500V, 15 min at 3000V

Therapeutic protein A is evaluated for charge heterogeneity during optimization of the ultrafiltration step



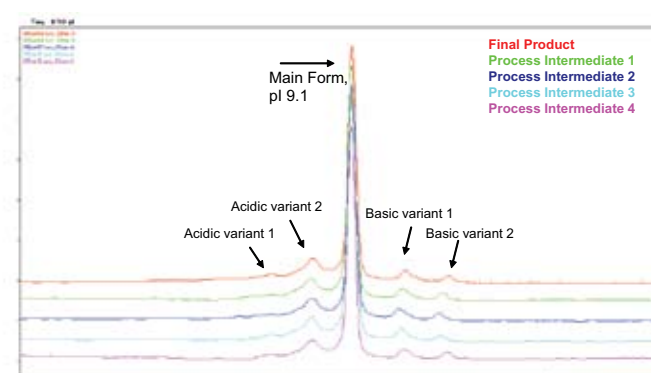
cIEF profile of therapeutic protein A. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10.5 (Pharmalyte), 0.35 mg/ml protein, 1mcl marker, pI 7.6, 1mcl marker, pI 9.0, 117 water, focusing time 1 min at 1500V, 15 min at 3000V

A consistent cIEF profile is observed for therapeutic protein B during scale-up



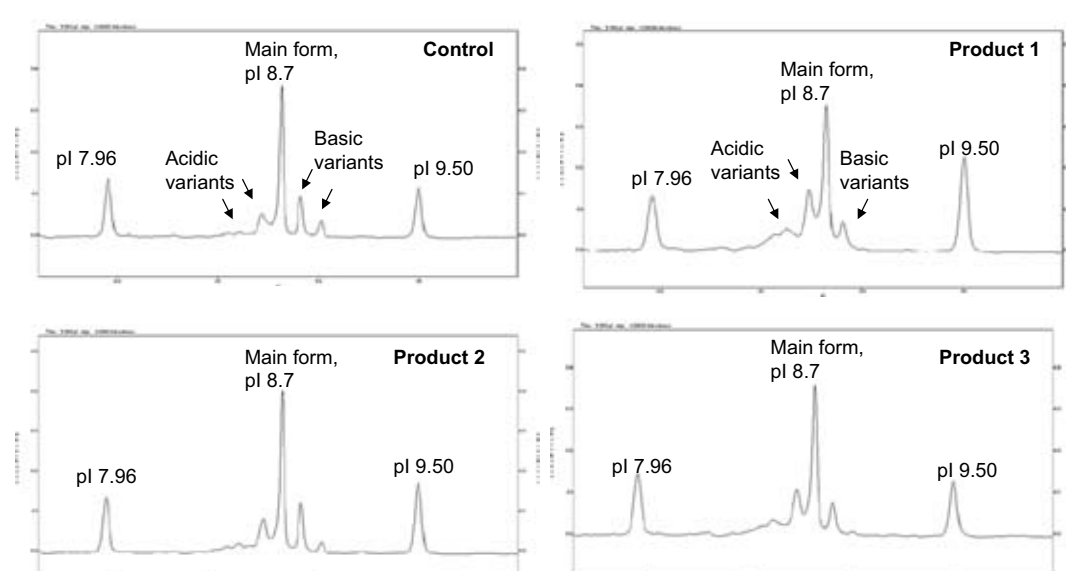
cIEF profile of therapeutic protein B. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10 (Bio-Lyte), 0.25mg/ml protein, 2mcl marker, pI 7.96, 2mcl marker, pI 9.77, 112 water, focusing time 1 min at 1500V, 8 min at 3000V

A consistent cIEF profile is observed for therapeutic protein C throughout the final purification process



cIEF profile of therapeutic protein C. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10.5 (Pharmalyte), 0.25 mg/ml protein, 2mcl marker, pI 8.1, 2mcl marker, pI 9.5, 107 water, focusing time 1 min at 1500V, 8 min at 3000V

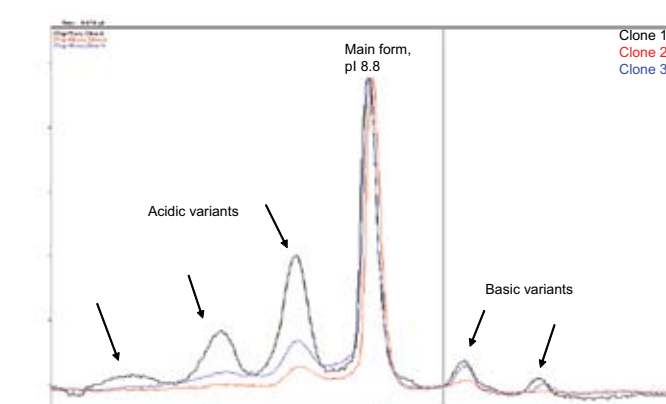
Variations in column volume, injection volume and pH affect the cIEF profile of therapeutic protein B



cIEF profile of therapeutic protein B. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10 (Bio-Lyte), 0.25 mg/ml protein, 2mcl marker, pI 7.96, 2mcl marker, pI 9.50, 112 water, focusing time 1 min at 1500V, 8 min at 3000V

Clone Selection

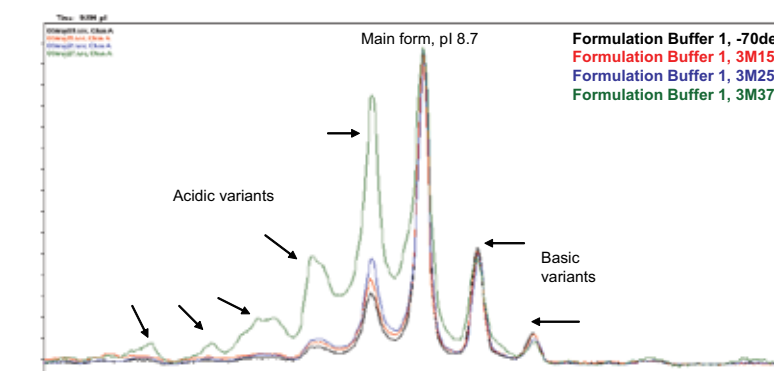
Therapeutic protein D produced from several CHO clones is evaluated for variations in charge heterogeneity



cIEF profile of therapeutic protein D. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10.5 (Pharmalyte), 0.25 mg/ml protein, 2mcl marker, pI 8.1, 2mcl marker, pI 9.5, 112 water, focusing time 1 min at 1500V, 8 min at 3000V

Accelerated Stability Studies to Support Product Characterization

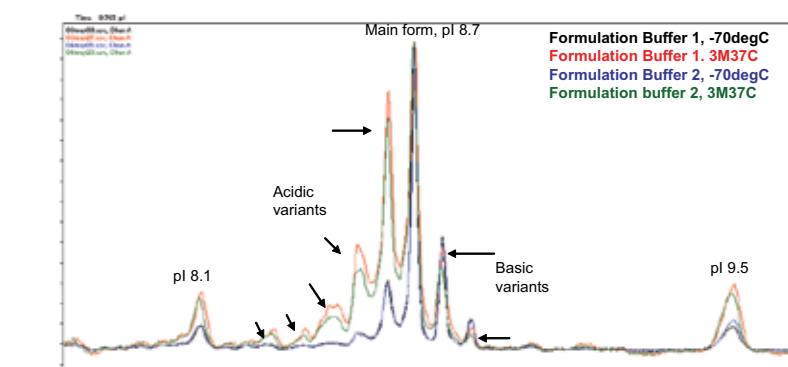
Significant changes in the cIEF profile are observed after storage at 37C for 3 months



cIEF profile of therapeutic protein B. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10 (Bio-Lyte), 0.25mg/ml protein, 2mcl marker, pI 8.1, 2mcl marker, pI 9.5, 112 water, focusing time 1 min at 1500V, 8 min at 3000V

Accelerated Stability Studies to Support Formulation Development

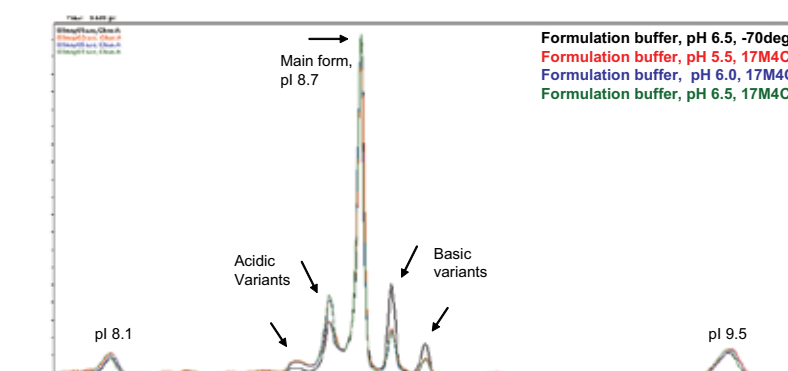
Therapeutic protein B is evaluated for charge heterogeneity during screening of formulation buffers



cIEF profile of therapeutic protein B. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10 (Bio-Lyte), 0.25 mg/ml protein, 2mcl marker, pI 8.1, 2mcl marker, pI 9.5, 112 water, focusing time 1 min at 1500V, 8 min at 3000V

Stability Studies to Support Formulation Optimization

Therapeutic protein B is evaluated for charge heterogeneity during optimization of the final formulation



cIEF profile of therapeutic protein B. Sample, 70mcl 1% methylcellulose, 8% carrier ampholytes, 1:2 pH 3-10 + pH 8-10 (Bio-Lyte), 0.25 mg/ml protein, 2mcl marker, pI 8.1, 2mcl marker, pI 9.5, 112 water, focusing time 1 min at 1500V, 8 min at 3000V

Conclusions

In bioprocess development for therapeutic proteins, various production cell clones, process conditions and formulations are evaluated for product quality attributes to aid in the selection of the most desirable clone, process and formulation. Capillary isoelectric focusing is one analytical tool routinely used for the examination of charge-based isoforms of therapeutic proteins. We have successfully implemented imaged cIEF methods for the pI profiling of various therapeutic proteins and for monitoring structural changes (deamidation, glycosylation, etc.) during the manufacturing process and upon storage. Specific applications of imaged cIEF in bioprocess development include clone selection, process development, formulation screening, stability monitoring and product characterization.