

# Application of Capillary Isoelectrofocusing for protein analysis

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## Introduction

Capillary isoelectric focusing (cIEF) is an analytical method that allows detection of heterogeneity in the sample related to glycosylation and impurities, and offers many advantages over gel-based isoelectric focusing (IEF) such as automation, quantitation and faster analysis speed. The analysis of antigen's stability and consistency is a critical component of the manufacturing process that requires a variety of characterization methods capable to determine similarities and differences related to process. To expand characterization package cIEF method was developed to analyze the native, homologous, and chemically modified protein antigens.

## Methods

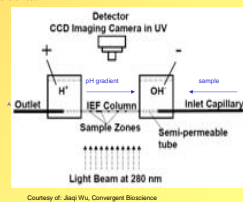
### Principle and Purpose of the Method

IEF is an electrophoretic method that separates proteins according to their isoelectric points (pI). In a pH gradient, under the influence of an electric field, a protein will move to the position in the pH gradient where its net charge is zero. A protein with a positive net charge will move to the cathode, becoming progressively less positively charged as it moves through the pH gradient until it reaches its pI. A protein with a negative net charge will on the other hand move towards the anode until it reaches its pI. This focusing effect concentrates proteins at their pI and allows proteins to be separated on the basis of very small charge differences. Capillary isoelectrofocusing provides a higher degree of resolution compared to gels due to a higher focusing voltage and shorter time of focusing

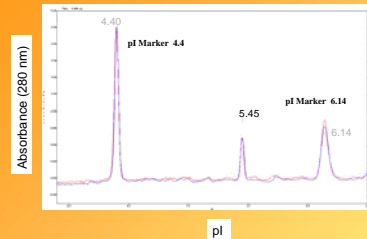
### Procedure

Isoelectrofocusing was done using a capillary imaged analyzer iCE280 (Convergent Biosciences, Toronto, Canada). iCE280 incorporates whole-column detection to monitor the IEF peak pattern of a protein within a capillary column. The IEF capillary column was conditioned using 0.5 % methylcellulose (MC) for 2 hours as described in iCE280 Analyzer Operator's Guide. Test samples were mixed with 0.5 % MC and injected into the capillary column using autosampler. For each run, the injected sample volume was 40 µL. The sample focusing times were 3 to 5 min. The analyte solution was 0.08 M H<sub>3</sub>PO<sub>4</sub> with 0.1 % MC. The catholyte solution was 0.1 M NaOH with 0.1 % MC. To measure pI a mixture of synthetic pI markers (pI 4.4 and 7.2) were injected with the sample (Bio Rad, Hercules, CA, USA) as internal reference standards. iCE280 CFR software 3.0 was used to acquire and analyze the spectra.

Figure 1 : Schematic diagram

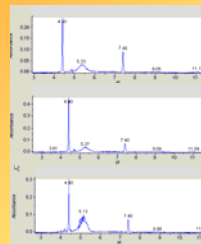


## Protein N lot-to-lot consistency



Three lots of Protein N were analyzed by cIEF to determine process consistency. DSC was used in parallel to monitor thermal stability which was similar for all three lots

## cIEF spectra of chemically treated Protein Q



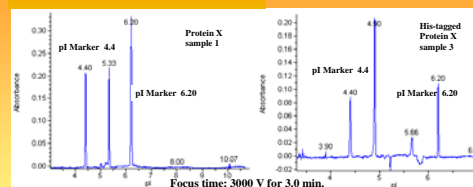
Process 1

Process 2

Process 3

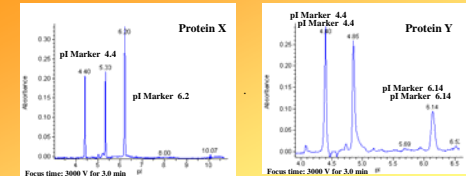
Protein Q produced using three different processes was compared by CD, DSC, and cIEF. All three samples had similar helical profile. No difference was detected by DSC for Protein Q obtained by processes 1 and 2, however showed lower transition temperature for that of process 3. cIEF detected subtle differences between the samples

## Protein X non-tagged and His-tagged



His-tagged Protein X appears to be more acidic than a non-tagged one. This observation was consistent with lower thermal stability of His-tagged protein shown by DSC. CD and AUC showed no differences in secondary structure and stoichiometry of the proteins.

## cIEF distinguishes between homologous proteins



Homologous proteins X and Y have helical secondary structure, monomeric form, and similar thermal stability, as shown by DSC, CD, and AUC. However, they can be distinguished by their pI values

## Summary

cIEF allows to:  
Determine pI of protein in solution  
Distinguish between homologous proteins  
Monitor process consistency and stability  
Obtain signature profile for complex mixtures

## References

### Citations

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