

Development of a Capillary Isoelectric Focusing Method for an Acidic Therapeutic Protein

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Abstract

Capillary isoelectric focusing (cIEF) is becoming an established methodology for the quantitative analysis of charged variants in therapeutic proteins. cIEF method development has increased at Genentech and the technique is routinely used to support process development and clinical lot release. Recently, a new method was required for an extremely acidic molecule having ten major isoforms in the 3.0 to 4.5 pI range. An acidic method was developed for both the Convergent Bioscience iCE280 and the Beckman Coulter PA800 platforms. Development of the acidic method for the iCE280 had several challenges. The first challenge was preventing protein precipitation during focusing. Second, the 2.85 pI marker was lost from the separation window while optimizing narrow range ampholytes. The last challenge was refining the ampholytic conditions needed to adequately resolve the ten major isoforms. In addition, Beckman-Coulter recently developed a new method for acidic molecules that was tested and optimized here at Genentech. The work presented in this poster shows that these two new methods allow the quantitative analysis of the charge heterogeneity for such an extreme acidic molecule.

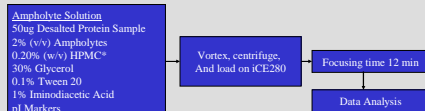
Materials and Methods

Hardware:

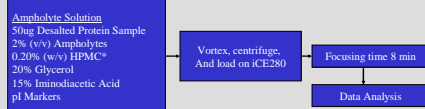
Convergent Bioscience: iCE280 Analyzer
5cm 100um Fc-coated Capillary

Beckman Coulter: Proteomlab PA800
20cm Neutral Coated Capillary

iCE280 icIEF Acidic Method

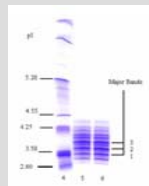


PA800 cIEF Acidic Method



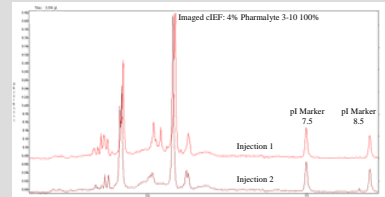
Transition from Gel method to cIEF Method

Scan of Coomassie Stained IEF Gel

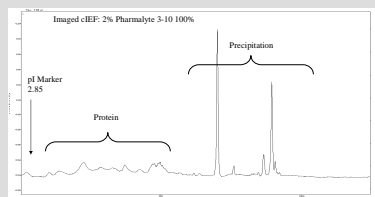


Issues with IEF Gel:
Not Quantifiable
Poor Reproducibility
Non-Automated Operation

Initial evaluation with generic icIEF conditions provided poor performance. The protein precipitated into irreproducible spikes.

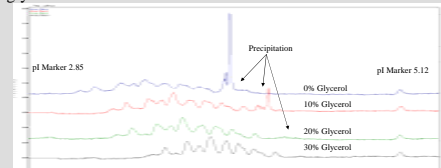


Initial investigation found limited benefit to traditional solubilizers such as urea and detergents. Urea, even at low concentrations, completely denatured the protein and worsened the separation. After considerable investigation, it was determined that the percentage of ampholyte greatly effected the amount of precipitation of the protein. Lowering the percentage of ampholyte provided the starting point of this proteins optimization.

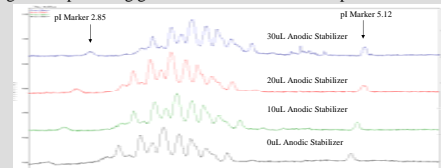


Optimization of icIEF Method

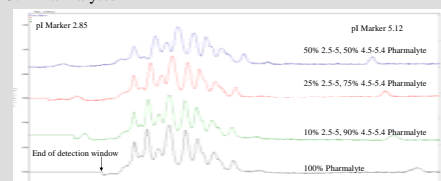
Precipitation was reduced by the addition of the solubilizer glycerol.



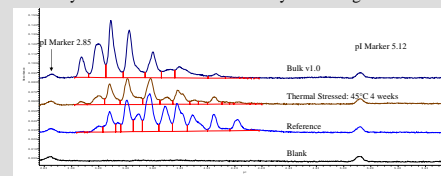
The iminodiacetic acid stabilizes the acid portion of the pH gradient providing greater resolution of the 2.85 pI marker.



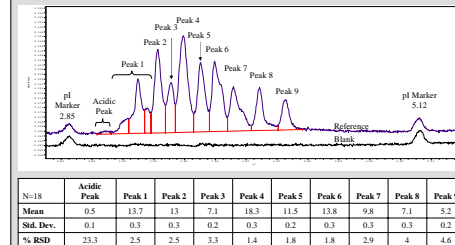
The ampholyte mixture was optimized at 25% 2.5-5 in 75% 4.5-5.4 Pharmalytes



This assay has been shown to be stability indicating



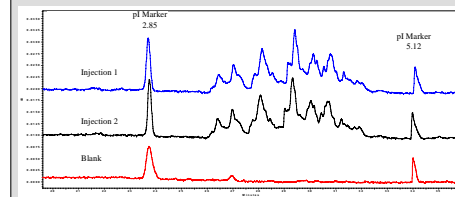
icIEF Method Reproducibility



N=18	Acidic Peak	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9
Mean	0.5	13.7	13	7.1	18.3	11.5	13.8	9.8	7.1	5.2
Std. Dev.	0.1	0.3	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.2
% RSD	23.3	2.5	2.5	3.3	1.4	1.8	1.8	2.9	4	4.6

Assessment of Beckman Coulter Acidic cIEF Method

This method is reproducible on the Beckman PA800



Conclusions

The development of an acidic method requires the consideration of several factors. The solubility of the analyte under focusing conditions needs to be addressed before further optimizing can be considered. Solubility factors included ampholyte percentage, ampholyte range and the addition of solubilizers. The addition anodic stabilizer can provide the full pH gradient for analysis. This allows for the optimization of ampholytes without the loss of the profile from being in the detection window. Balancing these requirements can provide an automated, quantifiable, reproducible and stability indicating assay.

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[®]Hydroyl propyl methyl cellulose