An Even Faster iCE Method

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Introduction

Platform methods, high throughput and ease of use have made the iCE system the gold standard for protein charge heterogeneity characterization for biopharmaceuticals. At 15 -18 minutes per sample, iCE methods are fast and simple. However, biopharmaceutical companies are always looking for higher throughput.

In this poster, we investigate a new rapid iCE method that utilizes a new column coating, eliminating the need for high viscosity polymer additives. By eliminating the viscous polymer additives, the column rinsing and sample injection cycles can be reduced from 2.5 minutes to 25 seconds. The required focusing time can also be reduced by 2 -3 minutes for a high resolution monoclonal antibody platform method. The peak pattern and resolution of the new column coating is compared to the FC coating in the current iCE cartridge. The new column coating provides high resolution while increasing sample throughput to 10 minute per sample.

Experimental Info

Instrument:
- iCE3 IEF Analyzer from ProteinSimple

IEF Cartridges:
- cIEF Cartridge FC-Coated (p/n 101701)
- New Cartridge

Detection:
- Whole column detection at 280 nm

Molecules:
- Hemoglobin standard
- mAb 1
- mAb 2

Methods
- Hemoglobin System Suitability Method
  3-10 Pharmalytes
- mAb1 Method
  1% 3-10, 3% 5-8 Pharmalytes, 4 M Urea
- mAb 2 Method
  4% 3-10 Pharmalytes

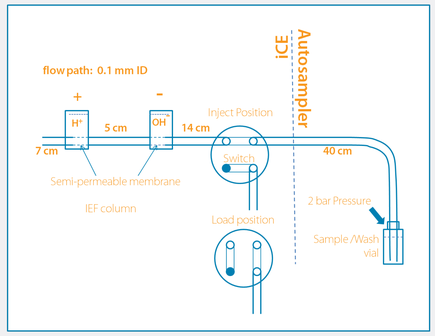

Figure 1: Flow path of the iCE IEF Analyzer.

Results and Discussion

1. New coating saves 7 minutes per sample injection

The iCE3 flow path is shown in Figure 1. During sample injection, the valve between the IEF column and the autosampler is in the Inject position. Once the column is completely rinsed and filled with the sample solution, the valve turns to the Load position to close the flow path between the IEF column and the autosampler. Separation voltage is then applied across the electrolyte tanks to start the IEF separation. At the end of the separation, the IEF column is rinsed with a wash solution to prepare the column for the next injection. The FC coated iCE column requires 0.35% methylcellulose in the sample solution and 0.5% methylcellulose in the wash solution. The pressure applied by the autosampler during sample/wash injection is 2 bar. The methylcellulose viscosity requires a longer injection step and wash cycle to completely rinse the column. For a reproducible and robust separation, it is critical the IEF column be completely rinsed with the sample solution and the wash solution. The current FC coated method requires a 2 minute sample injection and a 3 minute wash cycle.

The new coating eliminates the methylcellulose requirement in both the sample and wash solutions. Both solutions have much lower viscosity, reducing the time needed to rinse the IEF column. The sample injection time is now 25 minutes and the wash cycle is approximately 40 seconds.

The low viscosity of the sample solution also reduces the focusing time. Under the same chemical conditions (mixture of Pharmalytes pH 8-10.5 and pH 5-8), the focusing time using the new coating is reduced from 11 minutes to 9 minutes.

The total time savings with the new coating is about 7 minutes. A 10 minute per injection, high resolution platform mAb method is now a possibility.

2. Identical peak pattern with equal or better resolution

The peak patterns of the three tested samples are identical on the new column and the FC column.

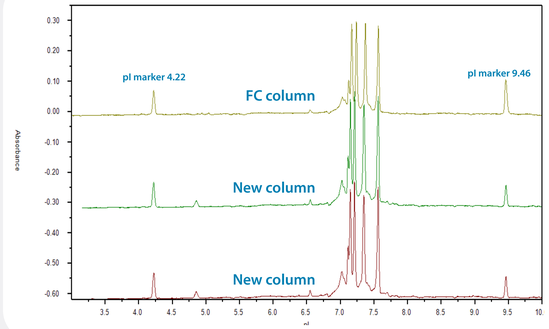

Figure 2: Hemoglobin System Suitability Standard.

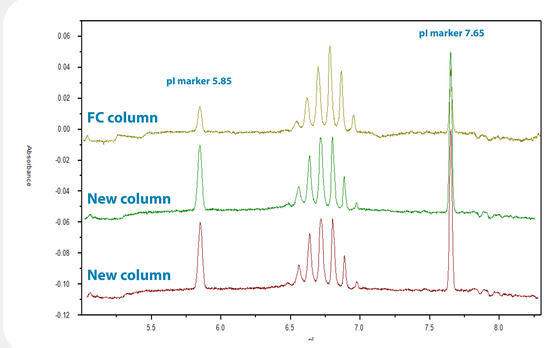

Figure 3: mAb1 in 1% 3-10, 3% 5-8 Pharmalytes with 4 M Urea.

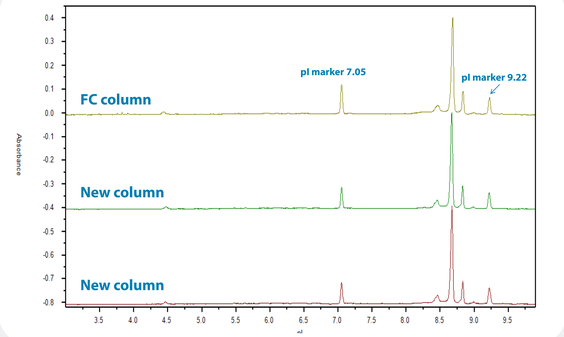

Figure 4: mAb2 in 4% 3-10 Pharmalytes.

3. Durability of the new column

To investigate column coating durability, 75 sample injections were performed in a overnight batch on the new column using the hemoglobin system suitability method. A compare plot of the first and last injection is shown in Figure 5. The results are reproducible across the entire batch.

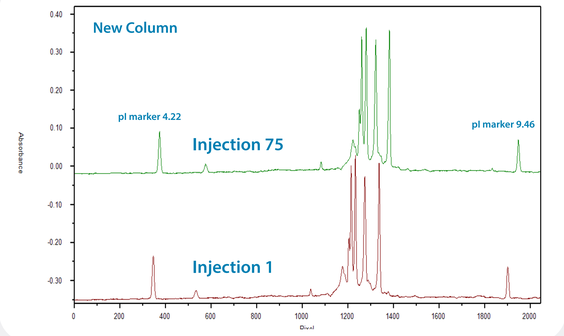

Figure 5: A compare plot of the first and 75th injection on the new column.

Conclusions

Initial experiments indicate a 10 minutes/sample throughput is possible with a new capillary column. These experiments show that for these three molecules, the new column provides an equivalent peak pattern and appears to be durable and reproducible. Further work is needed to confirm the initial results of these experiments.