High Throughput IEF Analysis Using Hands Free cIEF

proteinsimple™

Jiaqi Wu, Ph.D.
ProteinSimple, 27 Coronet Road, Toronto, Ontario, CANADA M8Z 2L8

Introduction

Sample preparation for cIEF analysis requires protein formulations to be modified with carrier ampholytes, pI markers, and other additives prior to injection. This can pose challenges, as some proteins are chemically unstable when exposed to highly basic environments, undergoing degradation reactions when stored under these conditions for extended periods. The onboard sample preparation feature of the new iCE3 IEF Analyzer can elevate preparative artifacts such as protein degradation by facilitating just-in-time (JIT) sample preparation. The automated sample preparation capability of the iCE 3 also has added benefit of reducing operator to operator variability. This feature can be implemented with either a 96 well plate or a 48 position standard vial tray. In this poster we demonstrate the results of automated sample preparation of 96 samples in a 96 well plate and compare them to the results using manual sample preparation.

Experimental

Instrument Configuration

The iCE3 Analyzer with Alcott 720NV equipped with a 96 well plate sample tray was used for these experiments. The autosampler configuration is shown in Figure 1. The 4 large vial positions in the back of the tray can hold up to three of 10-mL IEF buffer vials. The fourth vial position is dedicated to cartridge rinse solution, 0.5% methyl cellulose.

iCE Cartridge

Two types of column cartridges are available for the iCE3. The FC cartridge is the original iCE cartridge and requires , methyl cellulose (MC) in the sample solution. The HT cartridge can be used without MC in the sample solution which makes it ideal for automated sample preparation. The reduced viscosity of the IEF buffer increases mixing efficiency while reducing the formation of air bubbles during mixing. HT Cartridges were used for this study.

Samples Preparation

A mAb was used for this study. 5 µL of the mAb sample in its original formulation was spiked into each well of a 96 well plate.

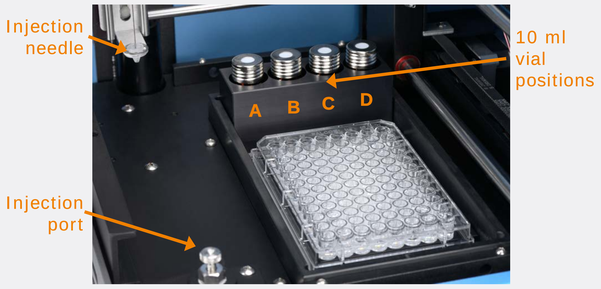


Figure 1. Alcott 720NV Autosampler with a 96 well plate sample tray.

cIEF Method Parameters

Two 10 mL vials were filled with a Master Mix containing 4% pH 3-10 Pharmalytes, 5 mM Arg, pI markers 7.65 and 9.50 and placed in tray positions A and B. 145 µL of the Master Mix was mixed with the sample in each well just prior to sample injection.

The sample was pre-focused at 1.5 kV for 1 min followed by focusing at 3 kV for 6 min.

Automatic Sample Preparation Parameters

Number of Mixing Strokes: 3
Mixing Depth: 44 mm (default for the 96 well plate)
Mixing rate: 100 µL/second (default)

The same sample was also manually prepared for comparison.

Results and Discussion

Mixing Stroke Optimization

The number of mixing strokes was optimized by observing mixing efficiency and the dilution factor created by the mixing action.

Mixing efficiency was tested by injecting three replicates after the mixing action. The result is show in Figure 2. A consistent peak height for the three replicates indicates complete mixing.

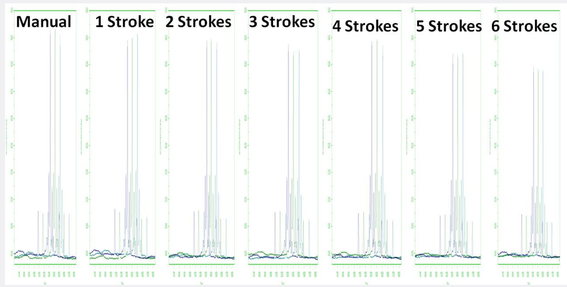


Figure 2. Alcott 720NV Autosampler with a 96 well plate sample tray.

The mixing was complete after two strokes with the current mixing parameters. The mixing strokes should be minimized to prevent sample dilution. The sample dilution effect is shown in Figure 3. The mixing strokes was set at three to ensure complete mixing and minimize sample dilution.

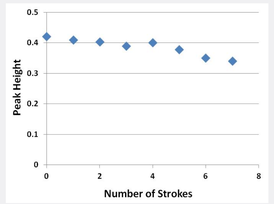


Figure 3. Sample dilution as a function of mixing strokes.

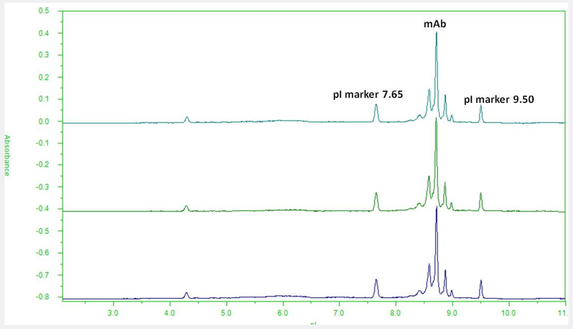
The FC cartridge requires 5 strokes for complete mixing. The elimination of methyl cellulose in the samples solution allowed the mixing strokes to be reduced to 3 with the HT cartridge, reducing the run time for each injection by about 1 min compared to FC cartridges.

Mixing Depth

Figure 4. Mixing Depth is dependent on the shape (depth) of the well and the solution height.

During sample mixing the needle moves up a specified distance after each stroke. The Mixing Depth is the distance between the needle stop at the bottom of the vial and the needle stop at the end of mixing. This is shown in Figure 4. The optimal Mixing Depth is a function of the shape of the well and the total volume of the final sample solution. If the needle moves out of the sample solution vial as it dispenses air bubbles will be created. For the conical well plate (Figure 4) and a final sample solution volume of 150 µl – 200 µL the optimal Mixing Depth is 44 mm.

Results



Automated Sample Preparation with 96 well Plate

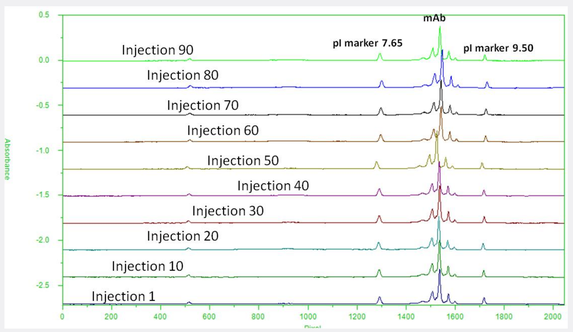


Figure 5. Comparison of Manual Sample preparation and Automated Sample Preparation.

The peak height, peak resolution and peak patterns are identical for both automated and manually prepared samples. Automated sample preparation with the HT cartridge will prepare and analyze 96 sample injections in 24.5 hours.

© 2011 ProteinSimple. ProteinSimple and the ProteinSimple logo are trademarks and/or registered trademarks of ProteinSimple.

Rev C.