Hands-Free Capillary Isoelectric Focusing (cIEF)

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Introduction

The well-characterized biopharmaceutical requires an assessment of charge heterogeneity. Techniques like IEF gels, ion exchange chromatography, and traditional capillary IEF all have benefits, but each one has its own set of challenges. Imaged cIEF (ProteinSimple's iCE IEF Analyzer) combines the best of these three worlds by providing rapid analysis, platform methods, and simple method development.

For cIEF analysis, samples are pre-mixed with carrier ampholytes, pI markers, and other additives. Although iCE IEF Analyzer offers rapid analysis and high throughput, some proteins can still experience degradation when exposed to these conditions for extended periods. On-Board Sample Preparation with the new iCE3 IEF Analyzer solves this problem. The system prepares the sample immediately prior to injection limiting sample exposure to cIEF buffers and preventing degradation. As an additional benefit, automated sample preparation eliminates tedious pipetting. Simply load your samples and go.

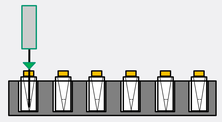
This poster presents the application of On-Board Sample Preparation to the analysis of proteins.

Instrument and Its Principle

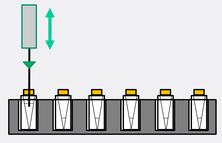
The sample tray of the iCE3's autosampler is shown below:

As shown in the previous picture, the sample tray can hold forty eight 1.5-mL sample vials or in the 96-well microplate version, 96 sample wells. The big vial positions in the back of the tray can hold up to three10-mL IEF buffer vials. The buffer vials are also cooled in the tray.

During sample analysis, protein samples in their original formulations are loaded in the sample vials as shown above. Sample vials are placed in the chilled autosampler sample tray.



Just prior to injection of a sample in the sample tray, the autosampler needle aspirates the IEF buffers stored in the 10-mL vials and dumps the buffers into the bottom of the sample vial.



The dumped buffers are mixed with protein sample solution in the sample vial by the autosampler needles. In each mixing stroke, the needle aspirates 70% of the total solution in the vial and dumps the solution back into the vial while the needle moves up and down to mix the solution in the vial.

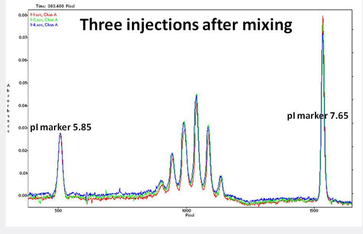
The needle performs 3-4 mixing strokes to mix the solution. The total sample mixing process takes about 2-5 minutes. After mixing, the sample in the vial is injected into the iCE Analyzer for analysis.

Results and Discussion

In the sample preparation method development, the most important parameters are mixing depth (movement range of the autosampler needle in the sample vial during the mixing action) and number of mixing strokes in the mixing action.

Mixing depth is determined by height of the sample solution in a sample vial. The ideal mixing depth should be slightly smaller than the height of solution in the sample vial.

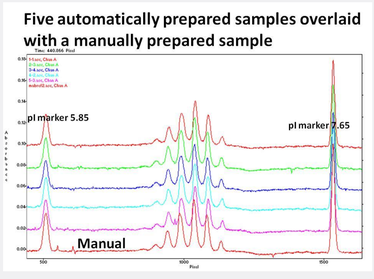
The required number of mixing strokes for a sample is determined by thoroughness of sample mixing at a given mixing depth. Thoroughness of mixing can be observed by the overlay of three consecutive injections from the sample vial after the sample is prepared.



The figure above shows results of three consecutive injections from a sample vial after the sample is prepared by the autosampler. The protein sample is a monoclonal antibody and is mixed with 1% Pharmalytes 3-10, 3% Pharmalytes 5-8, 0.35% methylcellulose, 4 M urea and two pI markers.

Three mixing strokes were performed in this automatic sample preparation. The total time of sample preparation was about 2.5 minutes in this example. The sample is well-mixed as shown in the previous figure.

Results of automated sample preparation are also compared with the sample prepared manually. The figure below shows the comparison. The protein sample is the same monoclonal antibody. Five automated sample preparations were performed and the results are overlaid with the result of a sample prepared manually. The figure below shows the overlaid e-grams. Results of the automated preparation are consistent with results from the manual sample preparation.



Conclusions

A cIEF sample can be prepared automatically by the iCE3 IEF Analyzer in less than 3 minutes immediately prior to its injection. Results of the automated sample preparation are consistent with the same sample prepared manually.

Simply load your samples (in their formulation buffers) into the iCE3 autosampler sample tray and go!