Optimizing Hands Free Capillary Isoelectric Focusing (cIEF)

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Introduction

In cIEF analysis, samples are pre-mixed with carrier ampholytes, pI markers, and additives. Although imaged cIEF (iCE3 IEF Analyzer) offers rapid analysis and high throughput, proteins can still experience degradation when exposed to carrier ampholytes and additives for extended periods. Automated 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.

There are several important method parameters such as Mixing Rate, Mixing Strokes and Mixing Depth that require optimization. This poster describes an optimization strategy for the on-board sample preparation and provides relevant examples.

The Principle of the Automated Sample Preparation

Autosampler Trays

The iCE autosampler tray is available in two configurations, 48 individual vials or 96-well plate. The 48-vial tray is shown below. Both trays include four large vial positions at the back of the tray that hold 10 mL vials. One position is used for the column wash solution and the other three positions are available for automated sample preparation.

Figure 1: Autosampler with 48 + 4 vial sample tray.

Automated sample preparation

During automated sample preparation the protein samples in their original formulations are loaded in the sample vials. The sample vials are placed in the chilled autosampler sample tray as shown in Figures 1 and 2.

Figure 2: Sample vials in sample tray.

Just prior to sample injection the autosampler needle aspirates the IEF buffers stored in the 10-mL vials and dispenses the buffers into the bottom of the sample vial as shown in Figure 3.

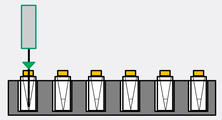

Figure 3: Needle dispenses IEF buffer into the sample vial

The dispensed buffers are mixed with the protein sample solution in the sample vial by the autosampler needle. In each mixing stroke the needle aspirates 75% of the solution in the vial. The aspirated solution is dispensed back into the vial as the needle moves up, as shown in Figure 4.

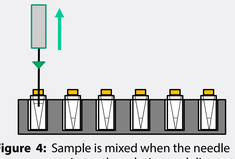

Figure 4: Sample is mixed when the needle aspirates the solution and dispenses it back in the vial.

Optimizing Mixing Parameters

Buffer Dispensing Rate

During automated sample preparation the autosampler needle aspirates the IEF buffers stored in the 10-mL vials and dispenses the buffers into the bottom of a sample vial.

Dispensing speed impacts mixing efficiency. A higher dispensing speed provides better mixing efficiency. The highest speed of the fluid delivery of the autosampler is **100 µL/s** and should be used as the **Dispensing Rate.**

Mixing Rate

After the buffer is dispensed into the sample vial the buffer and sample is mixed by the needle. In each mixing stroke, the needle aspirates 75% of the liquid in the vial and dispenses it back into the vial.

Mixing Rate also impacts mixing efficiency. A higher mixing rate provides better mixing efficiency. The optimal **Mixing Rate** is **100 µL/s**.

Mixing Depth

In each mixing stroke, the needle dispenses the aspirated solution from the sample vial back into the sample vial at the Mixing Rate while the needle moves up in 10 steps, as shown in Figure 5.

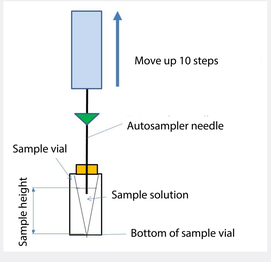

Figure 5: Needle mixing Depth.

When the needle dispenses solution back to the sample vial, it starts at the bottom of the vial (**Needle Depth**) and moves up as it dispenses. When it is finished dispensing, the needle stops at a position higher than the bottom of the vial as shown in Figure 5.

The distance between the vial bottom and the needle stopping point at the end of a mixing stroke is the **Mixing Depth** (total distance of the 10 steps). The optimal Mixing Depth should be slightly less than the sample height as shown in Figure 5. This will ensure the dispensed solution is always mixed into the solution in the vial. To determine the Mixing Depth, first measure the sample height of the final sample volume in the vial as shown in Figure 5. Subtract 1 – 2 mm from the sample height and enter this number as the **Mixing Depth**. This parameter is dependent on the final sample solution volume in the vial and the type of vial used in the experiments.

Number of mixing strokes

Thorough sample mixing depends on the parameters already discussed and the Number of Mixing Strokes. Once the above parameters and Number of Mixing Strokes are set, tests need to be done to see if the mixing is complete and the sample is reproducible. Starting value for **Number of Mixing Strokes is 4.**

To test the mixing parameters, perform three injections from the same sample vial after mixing. The peak heights of the three injections should be reproducible as shown in Figure 6.

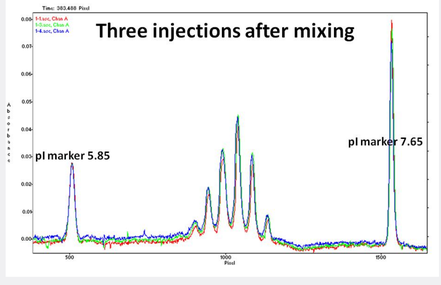

Figure 6: Three injections of an mixed sample will confirm if the sample and solution are completely mixed.

If the results are not reproducible, the **Number of Mixing Strokes** should increased.

Conclusions

Automated sample preparation on the iCE3 requires optimization of multiple parameters to ensure reproducible sample preparation.