Optimizing Parameters for Hands Free cIEF

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Introduction

cIEF analysis requires the protein sample to be pre-mixed with carrier ampholytes, pI markers, and other additives. Proteins can be sensitive to highly basic environments and can experience degradation when exposed to these conditions for extended periods. The iCE3 automates sample preparation minimizing protein sample degradation in IEF buffers and operator to operator variability. The sample is prepared immediately prior to injection limiting sample exposure to cIEF buffers and preventing degradation.

The development of a robust and repeatable automated sample preparation assay requires optimizing sample mixing parameters. During optimization, several factors need to be considered, such as the shape of the sample vial, the number of mixing strokes, air bubbles and sample dilution by the mixing action.

The Principle of the Automatic Sample Preparation

iCE3 Analyzer Configuration

The iCE3 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 10 ml vial positions at the back of the tray. One position is used for the column wash solution and the other three positions are available for automated sample preparation.

Two types of column cartridges are used in these experiments; FC and HT cartridges. Methyl cellulose (MC) in the sample solution is required for the FC cartridge. HT cartridge can be used with or without MC.

Figure 1. Alcott720NV Autosampler 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.

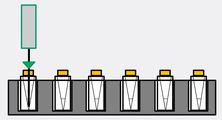


Figure 3: Needle dispenses IEF buffer into the sample vial

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.

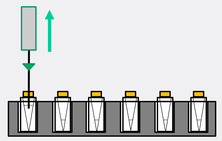


Figure 4. Sample is mixed when the needle aspirates the solution and dispenses it back in the vial.

Factors in the Sample Mixing

Protein Sample Volume

The sample volume in the well must be optimized for fast and complete mixing. If the desired final solution volume is 200 µl then the initial volume of the protein sample should be less than 40µl. This is because the autosampler aspirates 75% of the solution added to the vial. If the volume is too large, the mixing will be less efficient and require more mixing strokes for complete mixing. At the lower end of the volume range we recommend a volume greater than <5 µL. Volumes lower than 5µl may evaporate in the tray depending on the length of the run time. The optimal volume of the protein sample in the vial is 5 – 40 µL

Air Bubbles

The mixing action may create air bubbles in the final sample solution. This problem can be avoided by optimizing the "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.

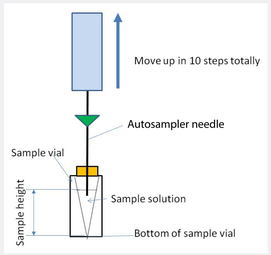


Figure 5. Needle mixing Depth.

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 above. This will ensure the dispensed solution is always mixed into the solution in the vial and bubbles are avoided. 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. In this way, the dispensed solution is always mixed into the solution in the vial and bubbles are avoided.

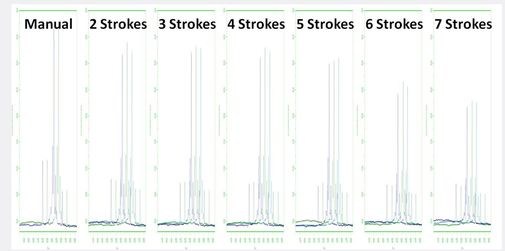
Sample Vial Type

Sample vials with the tapered insert or tapered bottom well in 96-well plates have the best efficiency in for the mixing action.

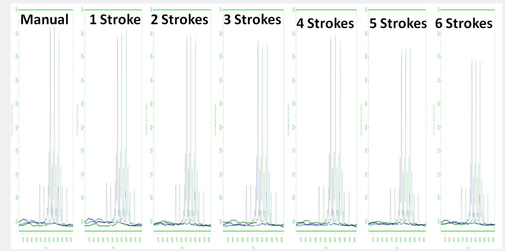
Mixing Strokes and Dilution Factor

In the mixing action, the sample solution is first aspirated from a sample vial into the autosampler needle and the tube connecting to the needle, and then is dispensed back to the sample vial. In the needle tubing a 20 µL air cushion is inserted to separate the sample solution and the DI water in the tubing. However if the number of the mixing strokes is large the final sample solution may be diluted. The number of mixing strokes should be minimized to limit dilution but support a fully mixed sample. The data below demonstrates the relation between the dilution effect and completeness of mixing. The sample is a mAb. After the mixing action, three injections are performed from the same vial to observe the difference in peak height. The reproducibility of these three injections is an indication of complete mixing. The three injections are overlaid below.

FC cartridge (with MC) results



HT cartridge (without MC) results



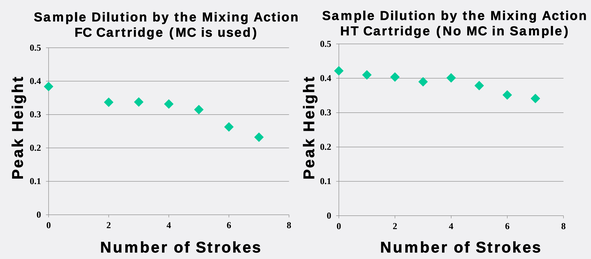


Figure 6. Dilution factor as a function of the number of mixing strokes

Conclusions

Selecting On-Board Mixing parameters:

Protein solution volume to be mixed: 5 µL – 40 µL
Mixing Depth: Set the depth such that the needle is below the liquid level in the sample vial or well.
Sample vial or 96-well plate: tapered bottom
Mixing strokes: For FC cartridge 4-5 mixing strokes is optimal, For HT cartridge, 1-4 mixing strokes.