Enhancing Peak Pattern Stability and Reproducibility in Capillary Isoelectric Focusing (cIEF) by Plugging Capillary Column during Focusing

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

Introduction

In isoelectric focusing (IEF), at the end of the focusing process, all components in a sample are focused and stop at their pI points. In order to perform IEF in a capillary column (cIEF), two forces within the column that interfere with the focusing process have to be eliminated: electroosmotic flow (EOF) and hydrodynamic flow. In commercial cIEF instruments, the EOF is substantially reduced by column coatings and the hydrodynamic flow is eliminated by placing both ends of the column at the same level during the IEF process.

In ProteinSimple's iCE280 IEF Analyzer, the hydrodynamic flow within the separation column is eliminated by using a specially designed, constant fluid level waste vial at the outlet of the column and a balancing vial at the column inlet in the autosampler that has the same fluid level as the waste vial. This design constantly provides equal fluid levels at both ends of the column regardless of the waste volume dumped into the waste vial.

However, some high concentration additives could generate an unbalancing force within the column during IEF for some unknown reasons. One example is high concentration urea. When these high concentration additives are used, the peak pattern sometimes is pushed back or forth within the separation column during the IEF process, making the peak pattern unstable. This can reduce separation resolution and reproducibility.

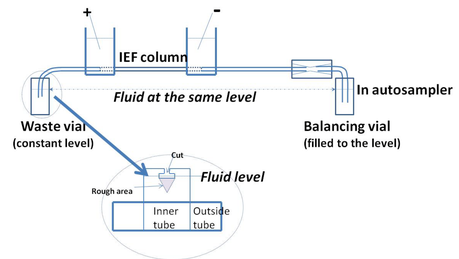
We found that this problem could be solved by plugging one end of the column during the IEF process with a micro switch valve. The new design will be adapted in ProteinSimple's new model iCE instrument.

In this presentation, we will compare the peak pattern stability of a monoclonal antibody on the iCE of the new design and the existing design when 8 M urea is used as the additive.

The Current way of Eliminating Hydropdynamic Flow within the Column in the iCE280 IEF Analyzer

As shown in the figure below, the hydrodynamic flow in the capillary column in the iCE280 Analyzer is eliminated by a well designed balancing system.

At the waste end (the outlet of the column), a specially designed waste vial ensures constant fluid level at ±1 mm range. This fluid level is unaffected by the volume of the waste dumped into the vial.

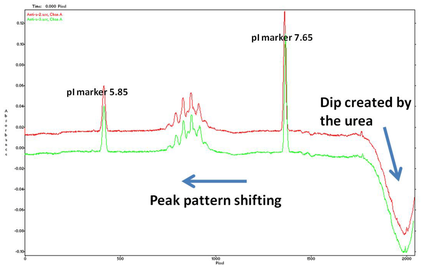


As shown in the above figure, the waste vial has a special cut at the top of its inner tube. Combined with the rough area beneath the cut, this structure ensures that the fluid inside the inner tube flows into the outside tube at a fixed level when the inner tube is filled higher than this fixed level (as indicated in the figure). The actual fluid level in this waste vial is within ±1 mm of the designed level.

At the inlet side of the column, the constant fluid level is provided by a balancing vial inside the autosampler that is always filled to a fixed level.

When some high concentration additives are used in the sample, an unknown hydrodynamic force is created within the capillary even in the well balanced plumbing system as shown above. This force may be caused by the volume changes during the IEF process.

8 M urea is an example. When the sample solution contains 8 M urea, the peak pattern shifting can be observed from time to time as shown below (e-grams of a monoclonal antibody under the condition of 8 M urea)

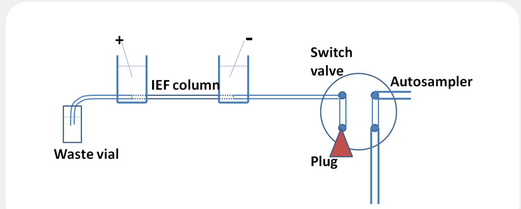


The dip observed in the above figure is caused by urea. Since urea has weak absorption at 280 nm (iCE's detection wavelength) during the IEF process, when the peak pattern shifts, electrolytes in the two electrolyte tanks at both ends of the capillary column may invade the column. The electrolytes have no absorption at 280 nm, thus creating a dip in the baseline.

The peak pattern shifting makes the peak pattern unstable and sometimes destroys resolution.

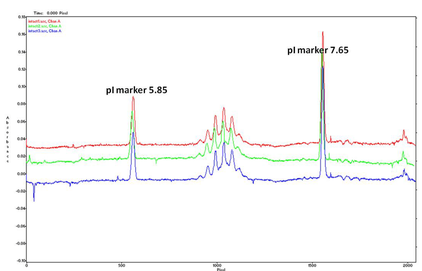
Solution to the Problem

Instead of keeping the plumbing system well balanced, plug the capillary column during the IEF process with a micro switch valve, as shown below.



In the above configuration, the sample can be injected from the autosampler into the IEF column when the column is connected to the autosampler. During the IEF process, the switch valve turns to another position cutting off the connection between the column and the rest of the plumbing system.

Below is the e-gram of the same monoclonal antibody running under 8 M urea condition. No peak pattern shifting is observed.



Conclusions

Plugging the capillary column during the IEF totally eliminates the peak pattern shifting problem when high concentration additives are used. Overall, the new design ensures better peak pattern stability and reproducibility even without additives.