Mixing it Up with Maurice's cIEF On-Board Mixing

Introduction

Maurice®, the newest iCE family member, takes our gold-standard imaged cIEF technology to the next level by giving you unmatched ease-of-use and features like fluorescence detection for increased sensitivity.[1] And you'll still get the same great absorbance cIEF data quality and reproducibility that you've come to expect in just 10 minutes.

Maurice serially analyzes over 95 injections per batch so you can get a lot done at once. But what happens if your molecule isn't stable sitting in the instrument mixed with ampholytes at a concentration that gives you linear charge heterogeneity data? Maurice has that problem solved! On-board mixing lets you add stable stocks of your molecule into Maurice at the beginning of your batch. The IEF master mix is mixed in with your sample right before sample injection, so you won't ever have to worry about your sample degrading before it's analyzed again. Plus, if you're running a lot of samples with a platform method, on-board mixing saves you some hands-on time and minimizes user-to-user variability too.

In this application note, we compare the data collected on Maurice using both hand-mixed samples and samples mixed using the on-board feature.

On-board mixing basics

iCE FAMILY MEMBERS AND CONSUMABLES

Several iCE instruments have on-board mixing capabilities (**Table 1**). If you're currently using on-board mixing with the iCE3™ and the Alcott 720 Autosampler, just remember you'll only be able to detect proteins using absorbance. Maurice C. gives you cIEF absorbance and fluorescence detection, and Maurice does that plus CE-SDS separation[2] too. On-board mixing is available on Maurice – OBM (PN 090-153) and Maurice C. – OBM (PN 090-154).

When you run a batch on Maurice using on-board mixing, you'll need a few extra consumables compared to what you use when you mix your samples manually. You'll need 6 mL vials (PN 046-379) to hold the IEF master mix and

INSTRUMENT	SEPARATION	DETECTION MODE	ON-BOARD MIXING
iCE3 – PrinCE Next Autosampler	icIEF	Absorbance	No
iCE3 – Alcott 720 Autosampler	icIEF	Absorbance	Yes
Maurice C.	icIEF	Absorbance, fluorescence	Yes
Maurice	icIEF + CE-SDS	Absorbance, fluorescence	Yes

TABLE 1. Detection mode and on-board mixing availability for iCE family members.

deionized (DI) water, and either the sample vial or 96-well plate insert that holds 6 mL vials on the left.

BATCH PREPARATION

Setting up a Maurice batch with on-board mixing is pretty similar to setting up a batch with manually mixed samples. Just prepare the same batch reagents you'd normally use. Then add a 2 mL glass vial of DI water with a clear cap to position N2, and an empty 2 mL glass vial with a clear cap to position N3 (**Figure 1**).

Next, add your IEF master mix with your ampholytes and pI standards to a 6 mL vial that goes in position M1. You'll need 100 µL of master mix for each sample Maurice is mixing. Each vial can hold enough master mix for 48 samples, so add a second vial of IEF master mix to position M2 if you're going to do on-board mixing on 49 or more samples in one batch. Then, prepare two more 6 mL vials with 6 mL of DI water for positions M3 and M4 and you're ready to start your batch.

Sample preparation is just a matter of transferring your stock material to a sample vial or 96-well plate. You only need 25 µL of stock material per sample. Just make sure your stock material is ~5X more concentrated than the analysis concentration and that the salt concentration is <15 mM.

STARTING YOUR BATCH

Compass for iCE version 1.1 or above makes setting up your batch with on-board mixing easy. Simply select a new cIEF batch from the **File** menu, then enable on-board mixing by clicking its icon in the Layout window (**Figure 2**). Next, setup your batch parameters like you would for any other Maurice run — Compass for iCE automatically assigns master mix vials to your sample. Then just hit **Start**.

Your batch can have a mixture of both manually mixed samples and samples for Maurice to mix during the batch. On-board mixing will just take an extra eight minutes per injection. To turn on-board mixing off for a particular sample, right click the well or vial in the Layout window and select **Mixing Off**.

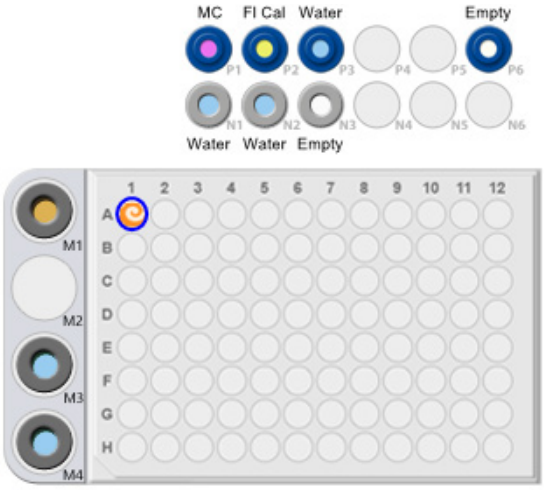

FIGURE 1. Reagent vial placement when setting up a Maurice cIEF batch with on-board mixing.

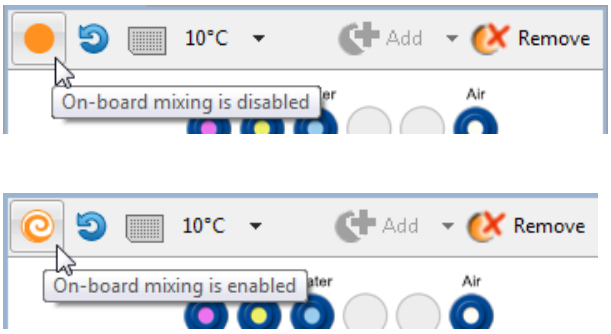

FIGURE 2. Enabling on-board mixing in Compass for iCE.

No sample carry-over

We wanted to be sure there was no sample carry-over with Maurice's on-board mixing. So we compared signal between blank samples injected after a manually mixed sample (where there's no carry-over concern) and after on-board mixing a 1000 µg/mL monoclonal antibody (mAb) sample. The signal between the two blanks using a 30-second fluorescence exposure were equivalent and neither had contamination peaks from the 1000 µg/mL sample (**Figure 3**). So that means you can do on-board mixing without worrying about sample carry-over.

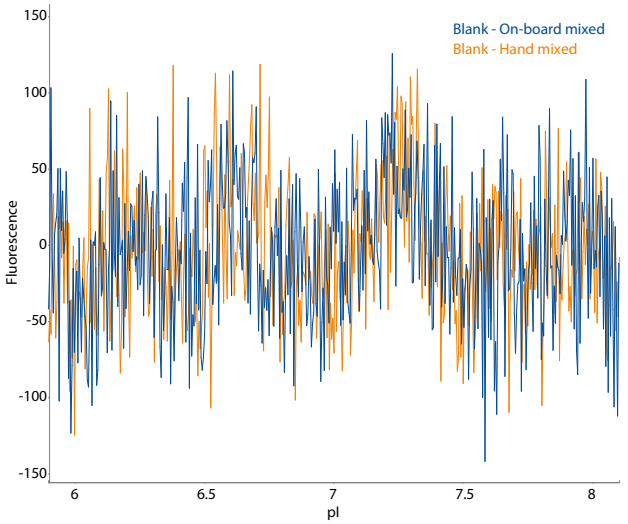

FIGURE 3. Comparison of blank samples run right after a 1000 µg/mL sample prepared with on-board mixing or hand mixing shows there's no carry-over between samples.

Mixing is consistent

When you're using on-board mixing with Maurice, the IEF master mix and sample need to be thoroughly mixed to generate consistent results. So we looked at absorbance and fluorescence profiles of a mAb prepared with both on-board mixing and thorough hand mixing. Samples were run in the same batch and the mAb was serially titrated 1:2 from 1000 µg/mL down to 0.488 µg/mL. 100 µL of IEF master mix containing 5% 3 to 10 Pharmalyte with the 4.05 and 9.99 pI marker was hand mixed with 25 µL of mAb. Samples were vortexed to make sure they were mixed thoroughly, then centrifuged at 10,000 x g for 3 minutes. The same IEF master mix and stock mAb were also placed in Maurice to be mixed during the batch. All samples were pre-focused for 1 min at 1500 V followed by focusing for 6 min at 3000 V.

The samples mixed on-board and the ones that were hand mixed gave highly comparable results using absorbance and fluorescence detection with a 20 second exposure (**Figure 4**). Linear titration curves were nearly identical and mixing efficiencies (on-board mixing signal/hand mixed signal x 100) were all between 98 and 104% for the samples mixed on-board. We took a closer look at sample concentrations near the LOQ and LOD and found that the profiles were equivalent. So now you can run samples that you couldn't pre-mix with IEF master mix before and be confident that they're mixed sufficiently too.

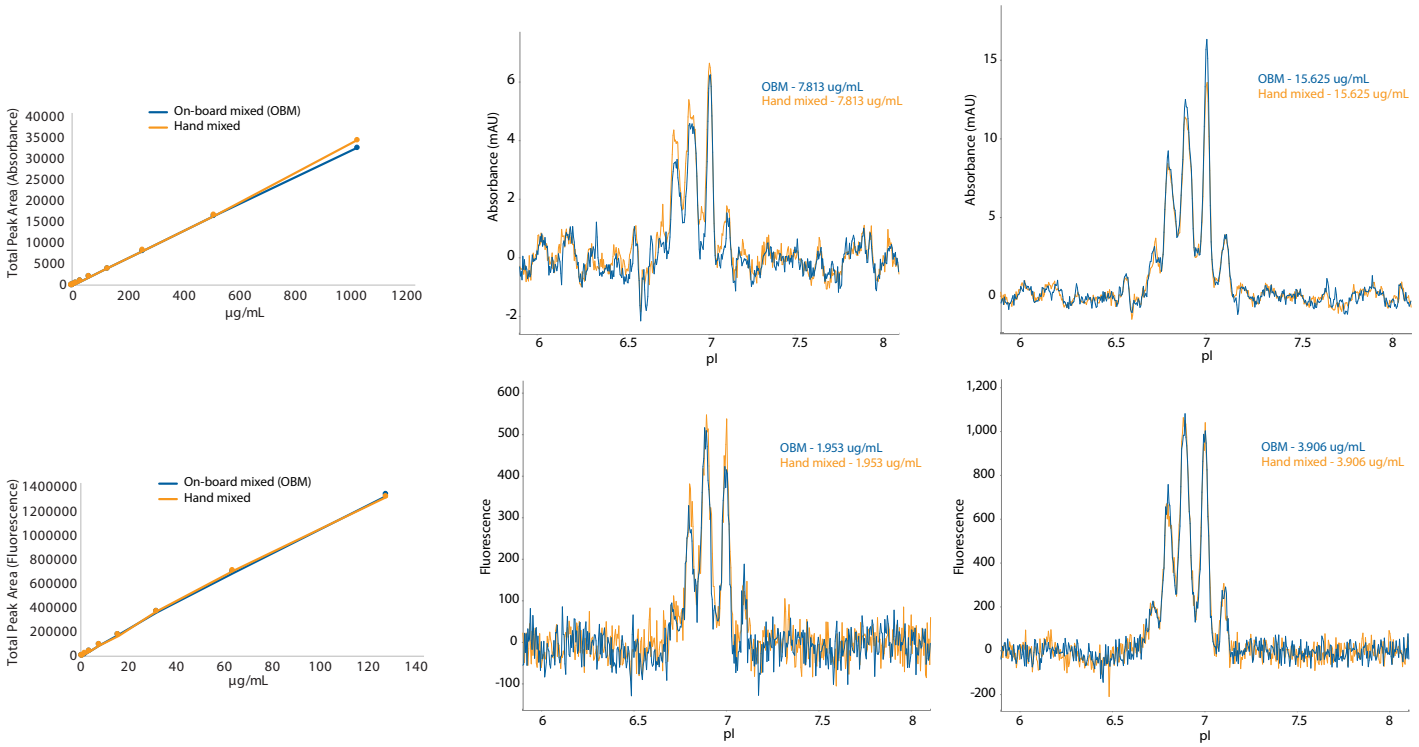

FIGURE 4. Linearity (left) of mAb from 0.488 to1000 µg/mL, signal at LOD (middle), and LOQ (right) for absorbance (top) and fluorescence (bottom) detection is equivalent between samples that were mixed on-board and hand-mixed.

Same data all the time

To test for sample mixing consistency, we ran the same mAb on multiple instruments using both vials and plates. 25 µL aliquots of 25 and 250 µg/mL mAb stock were prepared using on-board mixing and compared to hand-mixed controls on five different instruments for a total of 60 injections. The 250 µg/mL sample was evaluated using absorbance and fluorescence detection using a 20-second exposure time (**Table 2**). The lower 25 µg/mL sample was analyzed using fluorescence detection using 20- and 30-second exposure times (**Table 3**). The average percent mixing efficiencies across all experiment were between 93 and 107%, even though different instruments and different sample formats were used. RSDs were all below 5% too, showing just how robust Maurice's on-board mixing is.

Conclusion

Maurice lets you monitor the charge heterogeneity of your molecules with native fluorescence that improves your sensitivity 3-5X and gives you a simple workflow that cuts your hands-on time and minimizes user error. His self-contained on-board mixing automates sample preparation right before each injection, which lets you reproducibly monitor charge heterogeneity for your unstable protein plus saves you hands-on time.

We compared samples prepared with Maurice's on-board mixing to those that were hand mixed and got equivalent data. We also tested his mixing efficiencies which came in at +/- 10% compared to hand-mixed samples and saw no detectable sample carry-over. Day-to-day mixing was really consistent too with RSDs at less than 8.7%.

References

1. Improving Charge Variant Analysis with Maurice Native Fluorescence, ProteinSimple Application Note.

2. Sizing up IgG with Maurice CE-SDS, ProteinSimple Application Note.

	ABSORBANCE		FLUORESCENCE (20-SECOND EXPOSURE)	
Instrument	**Average % Efficiency**	**%RSD**	**Average % Efficiency**	**%RSD**
1	101.5%	1.1%	100.7%	1.0%
2	93.3%	0.7%	93.6%	1.0%
3	93.9%	3.3%	93.7%	3.0%
4	94.2%	0.2%	94.9%	0.3%
5	94.2%	0.2%	94.2%	0.2%
Inter-assay	95.4%	3.2%	95.4%	2.8%

TABLE 2. Maurice efficiently mixes a 250 µg/mL mAb compared to hand-mixed samples. Samples tested across multiple instruments and sample formats demonstrates on-board mixing robustness in both absorbance and native fluorescence. Inter-assay n = 60.

	FLUORESCENCE (20-SECOND EXPOSURE)		FLUORESCENCE (30-SECOND EXPOSURE)	
Instrument	**Average % Efficiency**	**%RSD**	**Average % Efficiency**	**%RSD**
1	106.5%	0.1%	106.3%	0.2%
2	96.0%	1.8%	95.6%	1.6%
3	95.7%	0.8%	96.1%	0.9%
4	94.2%	0.2%	100.3%	2.4%
5	94.2%	0.2%	94.2%	0.2%
Inter-assay	97.3%	4.8%	94.5%	4.5%

TABLE 3. Maurice efficiently mixes a 25 µg/mL mAb compared to hand-mixed samples. Samples tested across multiple instruments and sample formats using two different fluorescence exposures demonstrates on-board mixing robustness even at low sample concentrations. Inter-assay n=60.

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Toll-free: (888) 607-9692
Tel: (408) 510-5500
info@proteinsimple.com
proteinsimple.com

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