Maurice, iCE3, and iCE280 Data Equivalency for cIEF Charge Heterogeneity Absorbance Assays

Introduction

iCE platform methods are the go-to technique when it comes to monitoring charge heterogeneity of your biological products. Maurice® gives the iCE platform a boost by adding cIEF fluorescence detection and CE-SDS capability on top of the same great absorbance data you've come to expect with iCE280™ and iCE3® systems. Maurice also simplifies your workflow by housing the capillary and associated system fluidics in a pre-assembled cartridge, decreasing instrument setup time and minimizing potential sources of user error.

In this application note, we demonstrate data equivalency across iCE instruments by running multiple molecules across all three systems. Data equivalency between iCE280 and iCE3 systems using Alcott and PrinCE autosamplers has been demonstrated before[1], so we focused on comparing system quantitation and reproducibility using absorbance mode on iCE280-PrinCE, iCE3-PrinCE, and Maurice systems.

Maurice – Simplifying the iCE workflow

Maurice simplifies your charge heterogeneity analysis workflow by packaging all the valves and fluidics you'd manually connect on iCE280 and iCE3 systems into a ready-to-use cIEF cartridge. Maurice also comes with an integrated autosampler for added simplicity. There's no transfer line, capillary, and switch valve maintenance or tedious cartridge install procedures at the beginning of your Maurice run. Just load your cartridge, samples and reagents into Maurice, set up your batch parameters, hit **Start** and you're done. At the end of the run, Maurice automatically cleans the cIEF cartridge for you. All you need to do is rinse the cartridge's electrolyte tanks, remove your samples and reagents and you're ready for the next run.

Seamless method transfer between systems

Absorbance method transfer between iCE280, iCE3, and Maurice systems is seamless as sample prep and system performance is the same. Maurice does use system specific pI standards that have been optimized for both absorbance and native fluorescence detection, but for this application note we used iCE pI standards since we're only comparing absorbance data. Sample preparation for each molecule tested was the same for all three systems.

To test data equivalency, we ran three different molecules on iCE280, iCE3, and Maurice systems in cIEF absorbance mode. A batch of six injections was run on three separate days by the same operator for a total of 36 injections for each molecule. Samples were prepared fresh each day and the data generated was analyzed to determine pI and % peak composition using ChromPerfect Software (v6.0.4). The analysis method for each molecule was kept consistent across the data generated by each of the three systems.

Erythropoeitin (EPO) Assay

RECONSTITUTING LYOPHILIZED EPO

We also ran erythopoeitin, a therapeutic protein known to be acidic, on all three systems. Lyophilized EPO prepared

for physicochemical tests CRS was purchased from the European Directorate for the Quality of Medicines and Healthcare (EDQM, PN Y0001725, batch 1). Each vial contained approximately 0.1 mg of lyophilized material and was reconstituted by adding 200 µL of DI water to the bottom of a room-temperature vial. Vials were vortexed for at least 30 seconds until all solid material was dissolved.

The EPO was then desalted and concentrated using a Vivaspin 500 spin column (Vivaspin, PN VS0101). Columns were spun at 10,000 rpm for 6 minutes or until there was approximately 25 µL of material left in the concentrator. Samples were then either mixed directly with master mix or stored at 4 °C.

SETTING UP THE RUN

The reconstituted sample was mixed with a cIEF master mix so that the final sample contained 4 M urea, 0.35% methyl cellulose, 3.5% 2.5-5 Pharmalyte, 0.5% 3-5 Servalyt and pI markers 3.59 and 5.85. This was all mixed in a 1.5 mL centrifuge tube for a final volume of 200 µL for Maurice and 400 µL for iCE3/iCE280 systems. Samples were vortexed for 10 seconds to mix and then centrifuged for 3 minutes at 10,000 rpm to pellet any insoluble particles. 150 µL or 330 µL of sample was transferred to a Maurice and iCE vial respectively, taking care to not touch the bottom of the centrifuge tube. Samples were pre-focused on all three systems at 1500 V for 1 minute followed by separation at 3000 V for 6 minutes.

EPO DATA IS EQUIVALENT

iCE280, iCE3, and Maurice systems all gave us consistent EPO profiles that contained eight baseline-resolved peaks (**Figure 1, top**). The EPO profile had four major peaks and three minor peaks with the highest peak at around 0.06 absorption units. The profiles were also equivalent to the CZE data provided by EDQM (**Figure 1, bottom**), giving us confidence in our iCE platform profile.

The pI positions across systems were all within 0.1 pI units and were very consistent with CVs of ≤0.1% (**Table 1**). Peak composition percentages were also equivalent comparable across systems with ≤2.1% variation for peaks with greater than 2% peak composition, and CVs for peaks with greater than 10% composition at ≤11.5% (**Table 2**).

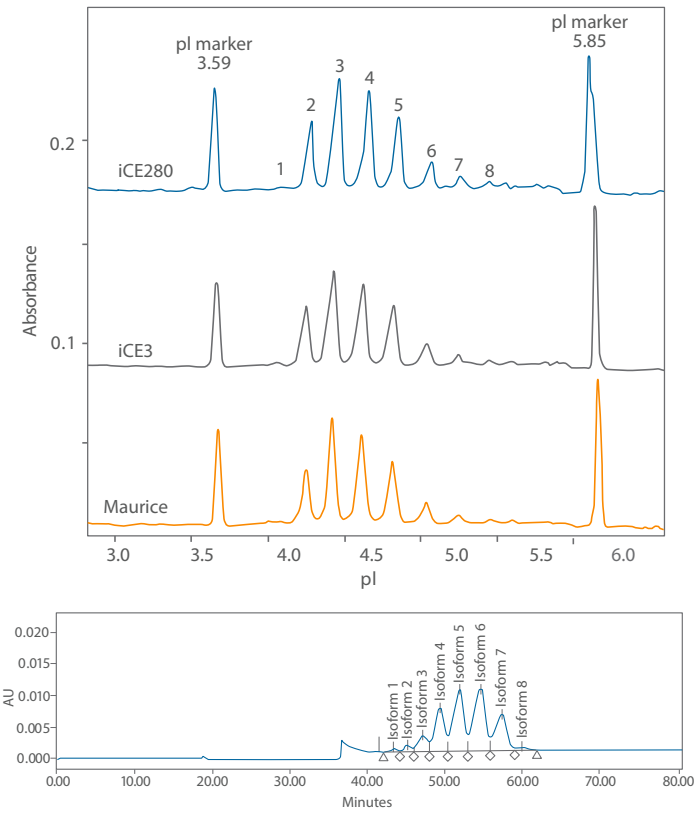

FIGURE 1. Equivalent EPO profiles generated on iCE280, iCE3, and Maurice systems (top). Profiles are comparable to CZE data generated by EDQM (bottom).

AVERAGE pI, (n=36)			
PEAK	**iCE280 (% CV)**	**iCE3 (% CV)**	**MAURICE (% CV)**
1	4.0 (1.1%)	4.0 (0.7%)	3.9 (0.3%)
2	4.1 (0.9%)	4.1 (0.8%)	4.1 (0.9%)
3	4.3 (0.9%)	4.3 (0.8%)	4.3 (1.1%)
4	4.5 (0.9%)	4.5 (0.8%)	4.5 (1.1%)
5	4.7 (0.9%)	4.7 (0.7%)	4.7 (1.0%)
6	4.9 (0.8%)	4.9 (0.6%)	4.9 (0.8%)
7	5.0 (0.8%)	5.0 (0.4%)	5.0 (0.8%)
8	5.2 (0.6%)	5.2 (0.3%)	5.2 (0.2%)

TABLE 1. EPO pI values, with % CVs in parenthesis, across all three iCE systems.

Monoclonal Antibody 11 (mAb11) Assay

SETTING UP THE RUN

We ran a monoclonal antibody (mAb11) on Maurice, iCE3 and iCE280 systems. 10 mg/mL protein stock was directly diluted into a cIEF master mix so that the final sample contained 0.35% methyl cellulose, 4% pH 3-10 Pharmalyte, and pI markers 5.85 and 8.40. Samples were vortexed for 10 seconds to mix before centrifuging for 3 minutes at 10,000 rpm. 150 µL or 330 µL of sample was transferred to a Maurice and iCE vial respectively taking care to not touch the bottom of the centrifuge tube. Samples were pre-focused on all three systems at 1500 V for 1 minute followed separation at 3000 V for 6 minutes.

mAB11 DATA IS EQUIVALENT

The data was equivalent across all three systems. Six peaks were detected with a main peak at 7.2 around 0.15 absorption units (**Figure 2**). The pI values were extremely consistent, with no variation regardless of the instrument used for analysis (**Table 3**). CVs for all systems came in ≤0.2%. Peak composition percentages were all within 2%, even for the minor peaks, demonstrating data equivalency across systems (**Table 4**). The iCE280 and iCE3 systems, and Maurice using cIEF absorbance mode were precise with CVs for peaks greater than 10% peak composition all under 11.5%. Data generated on Maurice was particularly precise with CVs ≤2.4% for peaks greater than 10% composition.

AVERAGE % PEAK COMPOSITION, (n=36)			
PEAK	iCE280 (% CV)	iCE3 (% CV)	MAURICE (% CV)
1	1.2 (17.4%)	1.4 (22.0%)	1.2 (19.2%)
2	15.7 (2.1%)	15.9 (1.7%)	15.7 (2.9%)
3	26.4 (2.0%)	26.8 (1.3%)	26.9 (1.6%)
4	25.8 (2.1%)	25.9 (1.6%)	26.2 (1.6%)
5	18.4 (2.9%)	18.5 (1.4%)	18.5 (1.5%)
6	6.9 (6.2%)	7.0 (6.2%)	6.8 (3.5%)
7	4.0 (11.9%)	3.3 (16.0%)	3.2 (6.9%)
8	1.6 (25.7%)	1.2 (20.6%)	1.4 (13.1%)

TABLE 2. EPO average peak % composition, with % CVs in parenthesis, across all three iCE systems.

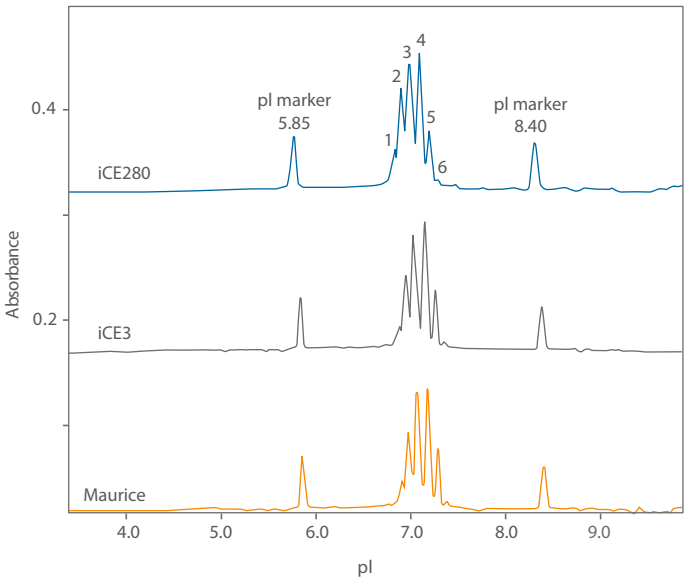

FIGURE 2. Equivalent mAb11 profiles generated on iCE280, iCE3, and Maurice systems.

AVERAGE pI, (n=36)			
PEAK	iCE280 (% CV)	iCE3 (% CV)	MAURICE (% CV)
1	6.9 (0.1%)	6.9 (0.1%)	6.9 (0.0%)
2	7.0 (0.1%)	7.0 (0.1%)	7.0 (0.0%)
3	7.1 (0.2%)	7.1 (0.1%)	7.1 (0.1%)
4	7.2 (0.1%)	7.2 (0.1%)	7.2 (0.0%)
5	7.3 (0.1%)	7.3 (0.0%)	7.3 (0.0%)
6	7.4 (0.2%)	7.4 (0.1%)	7.4 (0.0%)

TABLE 3. mAb11 pI values, with % CVs in parenthesis, across all three iiCE systems.

AVERAGE % PEAK COMPOSITION, (n=36)			
PEAK	iCE280 (% CV)	iCE3 (% CV)	MAURICE (% CV)
1	7.8% (14.5%)	6.2% (11.8%)	7.1% (9.0%)
2	20.5% (11.5%)	18.5% (4.0%)	18.4% (2.4%)
3	32.1% (6.0%)	32.7% (2.2%)	32.5% (1.4%)
4	28.2% (3.4%)	30.0% (1.9%)	29.5% (1.0%)
5	10.4% (7.1%)	11.5% (3.2%)	11.2% (1.3%)
6	1.0% (18.5%)	1.1% (10.0%)	1.3% (7.5%)

TABLE 4. mAb11 average % peak composition, with % CVs in parenthesis, across all three iCE systems.

Anti-α1-Anti-Trypsin Assay

SETTING UP THE RUN

Finally, we ran anti-α1-anti-trypsin on Maurice, iCE3, and iCE280 systems since it was the molecule used in the iCE3 intercompany collaboration study.[2] 5.87 mg/mL anti-α1-anti trypsin stock solution (EMD Calbiochem, PN 178260, LN 2638191) was directly diluted into a cIEF master mix so that the final sample contained 0.35% methyl cellulose, 4 M urea, 3% pH 5-8 Pharmalyte, 1% pH 3-10 Pharmalyte, and pI markers 5.85 and 8.40. Samples were vortexed for 10 seconds to mix then centrifuged for 3 minutes at 10,000 rpm. 150 µL or 330 µL of sample was transferred to a Maurice and iCE vial respectively, taking care to not touch the bottom of the centrifuge tube.

Samples were pre-focused on all three systems at 1500 V for 1 minute followed separation at 3000 V for 12 minutes.

ANTI-α1-ANTI-TRYPSIN DATA IS EQUIVALENT

iCE280, iCE3, and Maurice systems gave us equivalent anti-α1-anti-trypsin profiles that all contained seven distinguishable peaks and a very minor eighth peak around pI 7.2 (**Figure 3**). The major peak (peak 5) on all three systems was greater than 0.05 absorbance units.

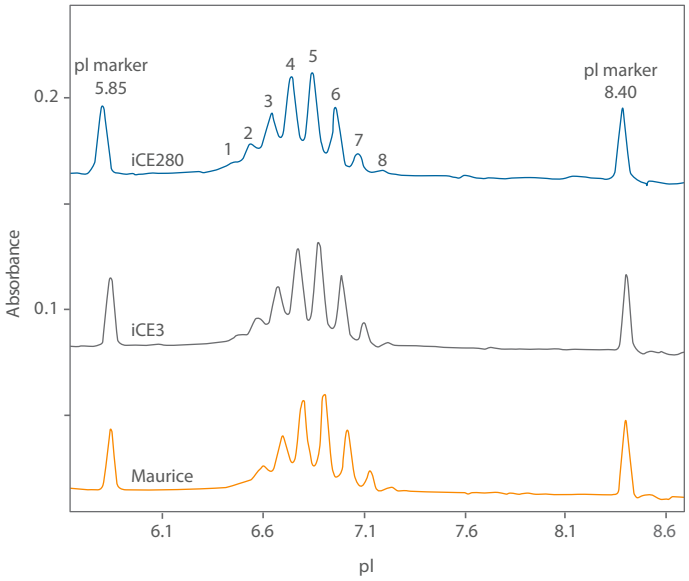

FIGURE 3. Equivalent anti-α1-anti trypsin profiles generated on iCE280, iCE3, and Maurice systems.

The data was again very consistent across all three systems. Reported pIs were the same and very consistent with CVs ≤0.4% (**Table 5**). Peak composition percentages were also equivalent across systems with ≤0.4% variation for peaks with greater than 2% peak composition, and single-digit CVs for all major peaks with ≥10% composition (**Table 6**).

AVERAGE pI, (n=36)			
PEAK	iCE280 (% CV)	iCE3 (% CV)	MAURICE (% CV)
1	6.5 (0.2%)	6.5 (0.1%)	6.5 (0.2%)
2	6.6 (0.2%)	6.6 (0.1%)	6.6 (0.1%)
3	6.7 (0.2%)	6.7 (0.1%)	6.7 (0.2%)
4	6.8 (0.2%)	6.8 (0.1%)	6.8 (0.2%)
5	6.9 (0.2%)	6.9 (0.1%)	6.9 (0.2%)
6	7.0 (0.2%)	7.0 (0.1%)	7.0 (0.2%)
7	7.1 (0.3%)	7.1 (0.1%)	7.1 (0.3%)
8	7.2 (0.3%)	7.2 (0.1%)	7.2 (0.3%)

TABLE 5. Anti-α1-anti trypsin pI values, with CVs in parenthesis, across all three iCE systems.

AVERAGE % PEAK COMPOSITION, (n=36)			
PEAK	iCE280 (% CV)	iCE3 (% CV)	MAURICE (% CV)
1	3.0% (25.8%)	3.3% (10.1%)	3.2% (13.0%)
2	8.4% (9.3%)	8.8% (3.8%)	8.8% (6.9%)
3	17.4% (2.6%)	17.0% (4.3%)	17.4% (2.2%)
4	25.5% (2.2%)	25.6% (2.2%)	25.3% (1.6%)
5	25.1% (2.7%)	25.0% (1.8%)	24.9% (1.9%)
6	14.8% (2.3%)	14.7% (2.2%)	14.8% (1.9%)
7	4.8% (4.5%)	4.7% (3.7%)	4.6% (8.1%)
8	1.0% (20.9%)	0.9% (10.5%)	1.0% (14.1%)

TABLE 6. Anti-α1-anti trypsin average % peak composition, with CVs in parenthesis, across all three iCE systems.

Conclusion

When it comes to monitoring the charge heterogeneity of your molecules, iCE3 and iCE280 systems have always given you the reproducibility needed to get your product to market quickly. Now with Maurice, you get the same great data plus unbeatable ease-of-use that minimizes setup time and sources of user error.

When we compared cIEF assays using Maurice's absorbance mode, the iCE280 and iCE3 systems, we got equivalent data for mAb11, erythopoeitin (EPO), and anti-α1-anti-trypsin. pI values across all systems were all within 0.1 pI units with CVs ≤0.4% and peak composition percentages were very consistent for peaks at >2% composition. Our replicate data also really demonstrated the robustness of iCE instruments. We ran six replicates per batch on three different days using samples prepared fresh each day for a total of 36 replicates, and all peaks with greater than 10% peak composition had CVs ≤11.5%. The average CV across all peaks greater than a 10% peak composition for all three molecules across all systems was 2.4%.

So when it comes to charge heterogeneity analysis in absorbance mode, iCE280, iCE3 or the Maurice system will give you equivalent data no matter which one you use — and you can transfer methods seemlessly between any of the three systems when the time comes.

References

1. iCE3 and iCE280 Analyzer System Performance Comparison, ProteinSimple Application Note.

2. Robustness of iCIEF methodology for the analysis of monoclonal antibodies: An interlaboratory study, O Salas-Solano, B Kennel, SS Park, K Roby, Z Sosic, B Boumajny, S Free, A Reed-Bogan, D Michels, W McElroy, P Bonasia, M Hong, X He, M Ruesch, F Moffatt, S Kiessig, B Nunnally, *J. Sep. Sci.* 2012; 35(22): 2124-2129.

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