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APPLICATION NOTE

iCE3 AND MAURICE DATA COMPARABILITY EVALUATED USING THREE BIOMOLECULES

INTRODUCTION

The iCE platform has been the gold standard for monitoring charge heterogeneity of biological products for more than 20 years. When we introduced Maurice, the next-generation iCIEF instrument that leverages the iCE imaged cIEF technology, we ensured that our customers achieved the same performance and data quality. Designed to simplify the workflow, Maurice significantly decreases instrument setup time and minimizes potential sources of error through its pre-assembled cartridge that contains the capillary and associated system fluidics. This application note is intended to help assure our customers of the data comparability between iCE3 and Maurice and showcases the charge isoform characterization of three molecules: erythropoietin (EPO), monoclonal antibody 11 (mAb11), and anti-α1-anti-trypsin. Each of these molecules was run on both iCE3 and Maurice to compare charge isoform peak quantitation and pI reproducibility using absorbance detection. In another published study, Maurice was used as a platform method for iCIEF analyses of a biosimilar, recombinant EPO, and a fusion protein[1].

HOW IS MAURICE DIFFERENT FROM iCE3?

Maurice simplifies your charge heterogeneity analysis workflow by packaging all the valves and fluidics into a ready-to-use cIEF cartridge, whereas iCE3 requires a manual process. For further simplicity, you also get an integrated autosampler. Your run time is considerably shortened as there's no transfer line, capillary, and switch valve maintenance or tedious cartridge install procedures. Simply 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.

Importantly, the advantages of Maurice don't end there. You can now save precious samples, because Maurice also offers a native fluorescence detection mode that is three times as sensitive as absorbance. Using either detection mode, you'll get high-quality data in 10 minutes, and maintain the compliance you require with either the Compass for iCE software or Empower®.

MATERIALS AND METHODS

Maurice typically uses 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 both systems.

To test data comparability, we ran three different molecules on iCE3 and Maurice in 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 were 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 both systems.

Lyophilized EPO prepared for physicochemical tests 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. Vials were vortexed for at least 30 seconds until all solid material was dissolved. 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.

The reconstituted sample was mixed with a cIEF master mix (as described in the cIEF Method Development Guide) so that the final sample contained 4M 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. Samples were vortexed for 10 seconds and centrifuged for 3 minutes at 10,000 rpm to pellet any insoluble particles. 150 µL and 330 µL of sample was transferred to a Maurice and iCE vial respectively, taking care to avoid touching the bottom of the centrifuge tube. Samples were pre-focused on both systems at 1,500 V for 1 minute followed by separation at 3,000 V for 6 minutes.

10 mg/mL of the mAb11 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 before centrifuging for 3 minutes at 10,000 rpm. 150 µL and 330 µL of sample was transferred to a Maurice and iCE vial respectively while taking care to avoid touching the bottom of the centrifuge tube. Samples were pre-focused on both systems at 1,500 V for 1 minute followed separation at 3,000 V for 6 minutes.

The anti-α1-anti-trypsin molecule was used in the iCE3 intercompany collaboration study[2], therefore we also ran it on iCE3 and Maurice as a part of this study. 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, 4M 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 and centrifuged for 3 minutes at 10,000 rpm. 150 µL and 330 µL of sample was transferred to a Maurice and iCE vial respectively, taking care to avoid touching the bottom of the centrifuge tube. Samples were pre-focused on both systems at 1,500 V for 1 minute followed by separation at 3,000 V for 12 minutes.

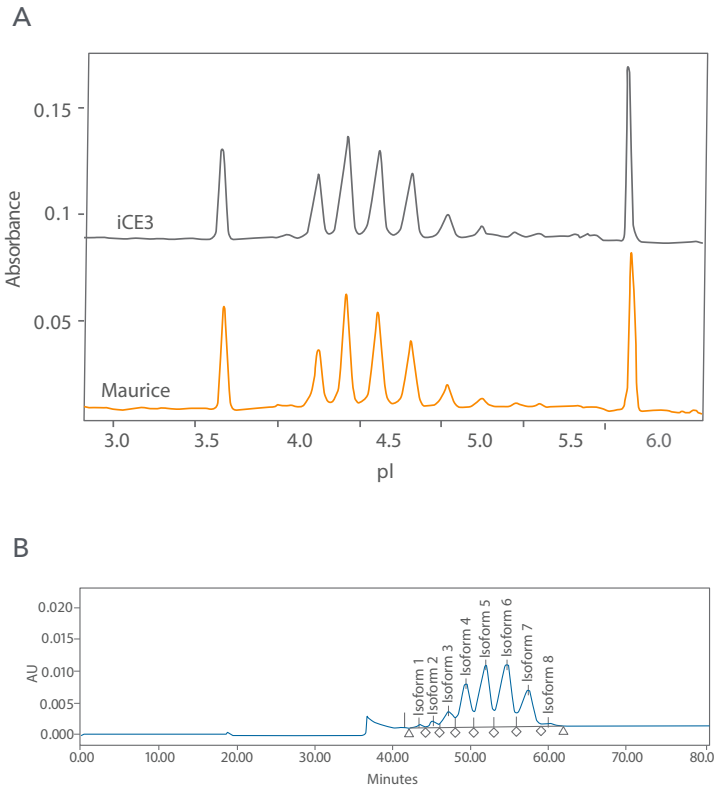

FIGURE 1. Comparable EPO profiles generated on iCE3 and Maurice systems (A) Profiles are comparable to CZE data generated by EDQM (B).

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

TABLE 1. EPO pI values with % CVs from iCE3 and Maurice systems.

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

TABLE 2. EPO average peak % composition with % CVs on iCE3 and Maurice.

RESULTS

ERYTHROPOETIN (EPO) ASSAY

The iCE3 and Maurice systems gave us consistent EPO profiles that contained eight baseline-resolved peaks (**FIGURE 1A**). 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 found to be comparable to the CZE data provided by EDQM (**FIGURE 1B**). Furthermore, the pI positions on both systems were all within 0.1 pI units and were consistent with CVs of ≤0.1% (**TABLE 1**). Peak composition percentages were also equivalent across both systems: ≤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**).

MONOCLONAL ANTIBODY 11 (mAB11) ASSAY

The data was comparable on both systems. Six peaks were detected with a main peak at a pI of 7.2 around 0.15 absorption units (**FIGURE 2**). The pI values were highly 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 comparability on both systems (**TABLE 4**). The iCE3 and Maurice systems 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.

ANTI-α1-ANTI-TRYPSIN ASSAY

Both instruments generated comparable 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) was greater than 0.05 absorbance units on both iCE3 and Maurice. Reported pI values were also the same, and with consistent CVs of ≤0.4% (**TABLE 5**). Peak composition percentages were comparable on both 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**).

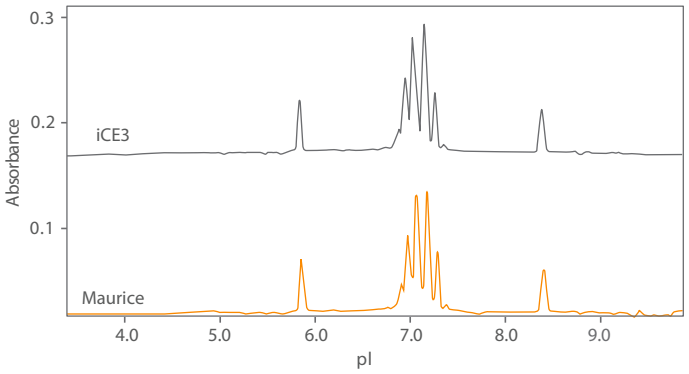

FIGURE 2. Comparable mAb11 profiles generated on iCE3 and Maurice systems.

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

TABLE 3. mAb11 pI values with % CVs on iCE3 and Maurice.

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

TABLE 4. mAb11 average % peak composition with %CVs on iCE3 and Maurice.

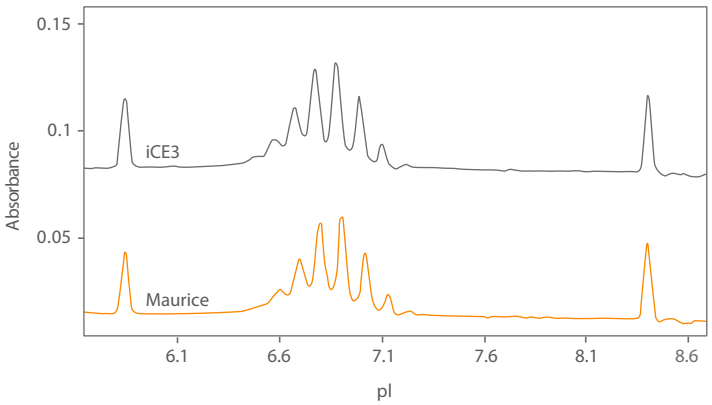

FIGURE 3. Comparable anti-α1-anti trypsin profiles generated on iCE3 and Maurice.

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

TABLE 5. Anti-α1-anti trypsin pI values with %CVs for iCE3 and Maurice.

AVERAGE % PEAK COMPOSITION, (n=36)		
PEAK	iCE3 (% CV)	MAURICE (% CV)
1	3.3% (10.1%)	3.2% (13.0%)
2	8.8% (3.8%)	8.8% (6.9%)
3	17.0% (4.3%)	17.4% (2.2%)
4	25.6% (2.2%)	25.3% (1.6%)
5	25.0% (1.8%)	24.9% (1.9%)
6	14.7% (2.2%)	14.8% (1.9%)
7	4.7% (3.7%)	4.6% (8.1%)
8	0.9% (10.5%)	1.0% (14.1%)

TABLE 6. Anti-α1-anti trypsin average % peak composition with % CVs on iCE3 and Maurice.

CONCLUSION

On comparing cIEF absorbance assays using iCE3 and Maurice, we achieved comparable data for erythropoietin (EPO), mAb11, and anti-α1-anti-trypsin. pI values from both systems were all within 0.1 pI units, with CVs ≤0.4% and peak composition percentages were highly consistent for peaks at >2% composition. Our replicate data demonstrated the robustness of both systems. 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 on both systems was 2.4%.

icIEF with Maurice has already been heralded as a reference method for characterizing biopharmaceuticals[3] and has also been used in the development of other therapeutic modalities such as gene therapies, ADCs, mRNA vaccines[4], etc. It has been established that Maurice performs comparably to iCE3, plus it provides significant other benefits. In addition to the same great data, you'll experience unparalleled ease-of-use that minimizes setup time and sources of user error. Maurice gives the iCE platform a further boost by adding cIEF native fluorescence detection for improved sensitivity and decreased sample amounts. Maurice also provides the option of CE-SDS analysis to confirm purity of your biomolecules in the same instrument. 21 CFR compliance is also assured with the Compass for iCE or Empower software. Even if you're only interested in absorbance data for charge heterogeneity, Maurice will accelerate your therapeutic's charge analysis, and we've ensured that you can transfer methods seamlessly between iCE3 and Maurice when the time comes. Learn more at https://www.bio-techne.com/p/imaged-capillary-electrophoresis-ice/maurice_090-000.

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