Faster and Easier Charge Heterogeneity Analysis with the iCE3

Scott Mack, Senior Scientist, Erik Gentalen, Director of Applications and Susan Darling, Director of Product Marketing, ProteinSimple, Santa Clara, CA

Introduction

Three major usability improvements are now available for the iCE3 system. The new HT Cartridge improves resolution and run times by eliminating the need for methyl cellulose, saving up to 5 minutes per run when compared to the original FC cIEF Cartridge. Redesigned locking electrode arm hardware also reduces evaporation and minimizes cathodic drift, and updated software features allow automated pI calibration and data export.

Performance Overview

The HT Cartridge employs a new, proprietary column coating. This coating minimizes protein wall interactions, eliminating the need for methyl cellulose in the sample mixture. Eliminating methyl cellulose reduces the time needed for sample injection, while also reducing signal spiking. The user simply substitutes an equal volume of water for the methyl cellulose (**Table 1**). The focusing time can also be shortened by three minutes with no loss of peak resolution. On systems with the PrinCE Next MicroInjector, the sample and buffer load duration can be reduced from 60 to 30 seconds for additional time savings (**Figure 1**). The locking electrode arm further improves performance by reducing electrolyte evaporation and CO_2 absorption, a major source of cathodic drift, allowing uninterrupted batches of up to 100 sample injections. Finally, the automated pI calibration feature in iCE CFR Software version 4.0 eliminates the tedious task of manually assigning the two pI markers in each run.

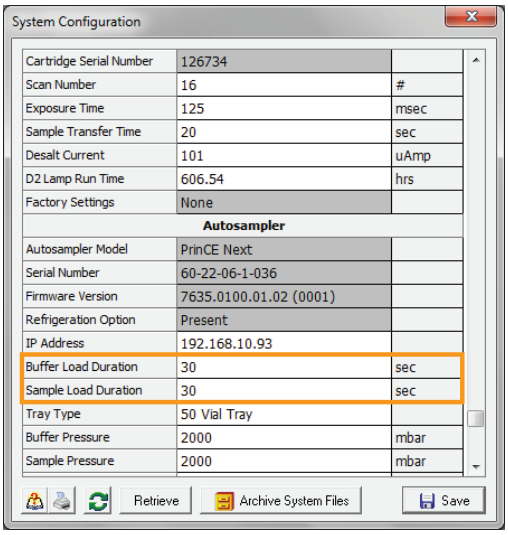

FIGURE 1. System Configuration window for PrinCE Next MicroInjector. Reduce Buffer Load Duration and Sample Load Duration to 30 seconds for use with HT Cartridge.

Analysis of Peak Clusters

Human IgG 1 Kappa was selected as a representative model of the biopharmaceutical iCE3 assay, and used to demonstrate performance of the HT Cartridge. 0.25 mg/mL IgG 1 Kappa was prepared with 2% v/v 3-10 Pharmalyte, 2.5% v/v 8-10.5 Pharmalyte, 4.0 M Urea, and 8.18 and 9.46 pI markers. This mixture was then run at 1500 V for 1 minute and 3000 V for 7 minutes. A typical profile is shown in **Figure 2**. Here peaks are assigned to clusters 1-5 by pI, and total area and peak area % within each cluster is calculated.

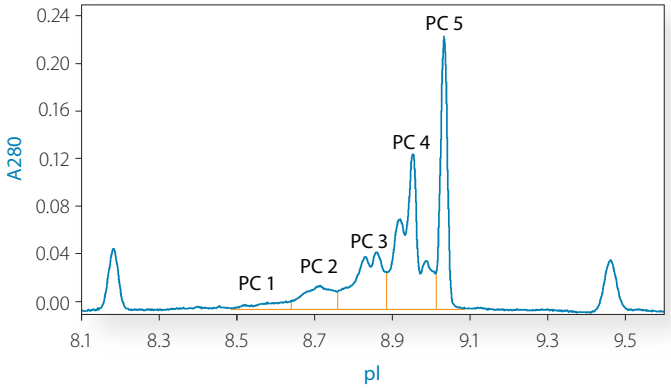

FIGURE 2. Peak cluster assignment. Five peak clusters were defined for the IgG 1 Kappa sample used in these experiments.

Table 2 shows the consistency of percent composition of the peak clusters across 12 HT capillaries run on two instruments. For all clusters that comprise greater than 5% of total peak area, the CV is less than 10%. Run time for 100 injections was 21.5 hours on the iCE3 with PrinCE Next MicroInjector as opposed to 29.5 with the original FC cIEF cartridge. With an Alcott 720NV Autosampler, 100 injections took 22.5 hours using the HT Cartridge, and 27.5 hours with the FC cIEF Cartridge. In Figure 3 A and B, we show the identical performance of the HT and FC cartridges using both the human IgG 1 kappa assay and our hemoglobin system suitability assay.

HT CARTRIDGE		FC CARTRIDGE	
Separation Method			
1 min	1500 V	1 min	1500 V
7 min	3000 V	10 min	3000 V
Sample Matrix			
DI Water	98 µL	DI Water 1% MethylCellulose	10 µL 88 µL
4M Urea (aq)	100 µL	4M Urea (aq)	100 µL
Pharmalyte 3 to 10	5.0 µL	Pharmalyte 3 to 10	5.0 µL
Pharmalyte 8 to 10.5	6.25 µL	Pharmalyte 8 to 10.5	6.25 µL
pI 8.18 Marker	0.63 µL	pI 8.18 Marker	0.63 µL
pI 9.46 Marker	0.63 µL	pI 9.46 Marker	0.63 µL
1.25 mg/mL IgG 1 Kappa	40 µL	1.25 mg/mL IgG 1 Kappa	40 µL

TABLE 1. Operational differences between HT and FC cartridge.

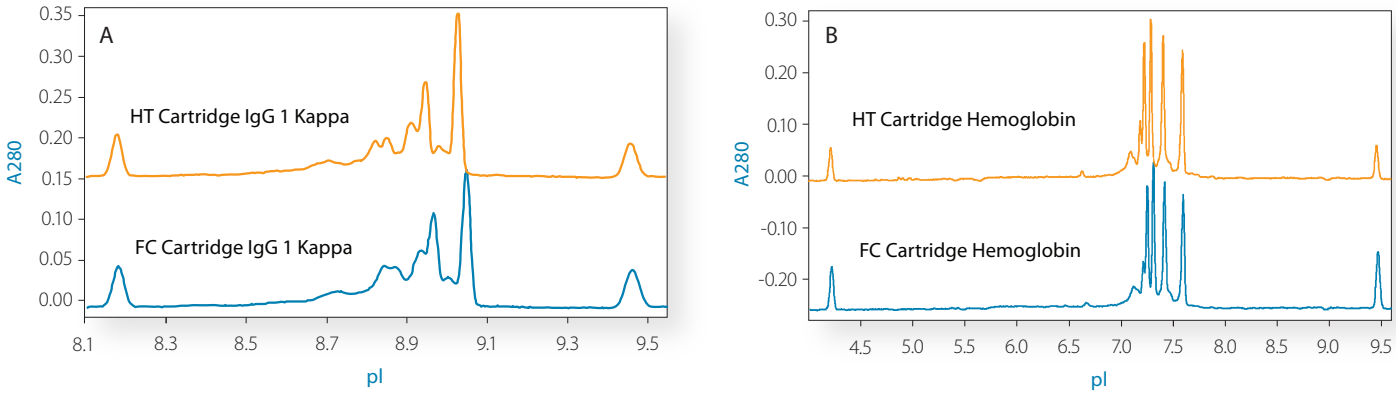

FIGURE 3. Comparison of HT and FC cartridge performance. (A) Focusing of IgG 1 Kappa in HT and FC cartridges, (B) focusing of Hemoglobin in HT and FC cartridges.

LOT	CARTRIDGE	INSTRUMENT	PEAK AREA % PC1	PEAK AREA % PC2	PEAK AREA % PC3	PEAK AREA % PC4	PEAK AREA % PC5
2U17A2	126641	1	3.242	9.734	21.452	41.431	24.134
2U17A2	126642	2	3.461	11.179	23.191	39.288	22.858
2U17A2	126644	1	3.361	10.583	23.224	39.443	23.379
3U05A1	126777	2	2.935	9.668	20.635	39.567	27.194
3U05A1	126779	1	3.295	11.041	20.542	39.492	25.629
3U05A1	126808	1	2.983	9.522	21.151	39.993	26.352
3U17A1	126961	2	2.529	10.029	19.988	40.517	26.937
3U17A1	126963	1	2.538	9.369	19.905	41.020	27.164
3U17A1	126965	2	2.588	9.340	21.109	38.334	27.860
3U25A1	127091	2	2.807	9.631	20.949	40.034	26.579
3U25A1	127093	1	2.974	10.010	21.215	39.912	25.809
3U25A1	127095	1	2.458	10.123	20.753	40.440	26.226
		Average	2.931	10.019	21.176	39.956	25.843
		Std Dev	0.338	0.592	1.012	0.794	1.520
		% CV	11.53%	5.91%	4.78%	1.99%	5.88%

TABLE 2. Peak cluster percent composition. Peak area percent for each of five peak clusters was calculated across 12 runs on two instruments. 12 different cartridges from four manufacturing lots were used.

Electrode Arm Upgrade

The new locking electrode arm for the iCE3 provides a tighter seal on the anolyte and catholyte wells, eliminating CO_2 -induced cathodic drift. This allows for 100 samples to be completed without replenishing electrolyte solutions or correcting for cathodic drift. During development and validation, up to 120 samples were tested in a single batch to verify the 100-sample specification with the required tolerance. In the example shown in **Figure 4**, pixel positions are graphed for the 8.18 and 9.46 markers over 120 consecutive runs on one instrument, showing minimal drift (<50 pixels). **Table 3** shows the data consistency over these 120 runs. Again, for peak clusters greater than 5% of the total area, CVs are well below 10%. **Figure 5** shows the peak profile consistency over the 120 runs, with pI automatically assigned by iCE CFR Software version 4.0. While we have demonstrated peak profile reproducibility on over 120 runs with the iCE3 system, improvements with the HT or FC cartridges are only guaranteed to 100 runs.

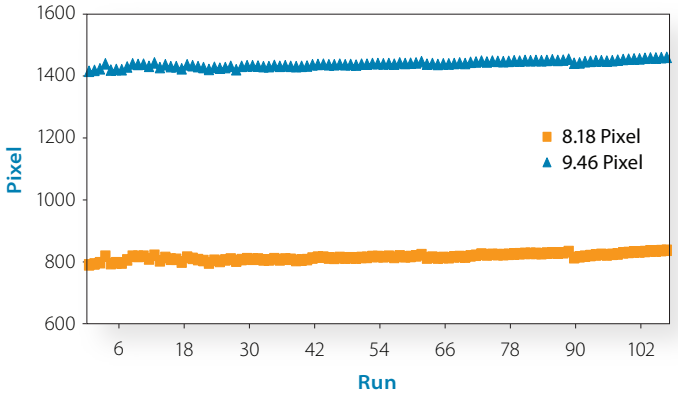

FIGURE 4. Pixel position of markers. Measured pixel position of 8.18 and 9.46 markers over 120 consecutive runs.

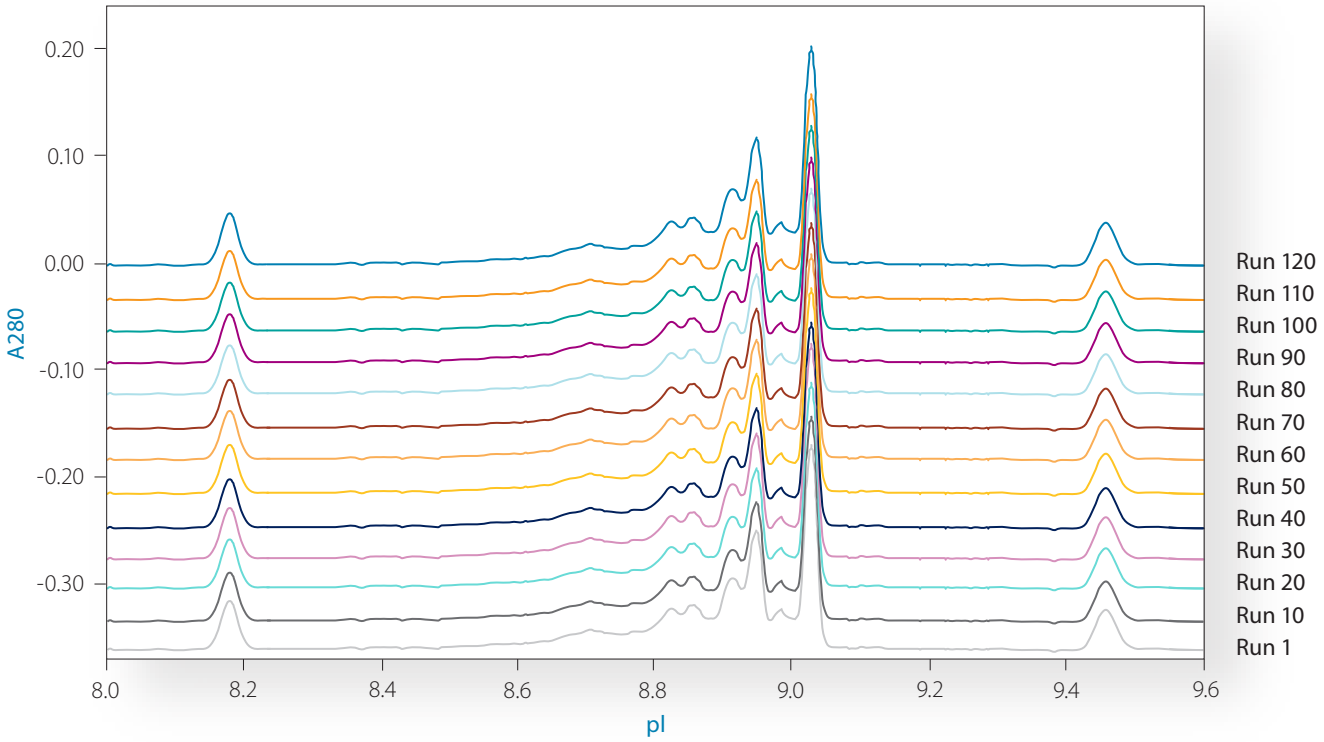

FIGURE 5. Overlaid electropherograms of data in Table 3.

CARTRIDGE	RUN	INSTRUMENT	PEAK AREA % PC1	PEAK AREA % PC2	PEAK AREA % PC3	PEAK AREA % PC4	PEAK AREA % PC5	pI PC5
126808	1	1	2.96	9.485	20.92	40.489	26.145	9.03
126808	10	1	3.136	9.316	21.773	39.531	26.244	9.03
126808	20	1	3.56	9.135	21.077	39.9	26.329	9.03
126808	30	1	3.375	9.675	20.833	40.005	26.112	9.03
126808	40	1	2.926	9.789	20.843	40.253	26.189	9.03
126808	50	1	3.463	9.516	21.5	39.39	26.131	9.03
126808	60	1	2.384	10.135	21.435	39.653	26.393	9.03
126808	70	1	3.03	9.393	21.322	39.891	26.365	9.03
126808	80	1	2.776	9.615	20.71	40.618	26.281	9.03
126808	90	1	2.417	9.664	21.53	40.231	26.158	9.03
126808	100	1	2.949	8.954	21.912	39.565	26.62	9.03
126808	110	1	2.848	9.911	20.929	39.704	26.608	9.03
126808	120	1	2.949	9.193	20.178	40.676	27.003	9.03
		Average	2.983	9.522	21.151	39.993	26.352	9.03
		Std Dev	0.338	0.315	0.463	0.412	0.247	0.000
		% CV	11.35%	3.31%	2.19%	1.03%	0.94%	0.00%

TABLE 3. Data consistency over 120 runs. Example data from 120 consecutive runs showing consistent peak area determination.

Automated pI Calibration and Data Conversion

Automated pI calibration and data conversion eliminates the need for tedious manual calibration. After the batch is complete, open the **Batch Review** window. In the **Raw Data** tab under **Injection Parameters**, simply click and drag a box around the low pI marker (**Figure 6**), right click and select **Set Low pI Window**. Repeat for the high pI marker and select **Set High pI Window** (**Figure 7**).

Once the high and low pI windows are set, click the **Process Settings** tool button (**Figure 7**). Next, in the **Process Settings** window (**Figure 8**) enable both the **Calibration** and **File Conversion** features if desired, select the output file format and save location, and click OK. For standard configurations, you can save these calibration parameters and reload for future batches through this window as well. Once this is complete, click the **Process All** tool button (**Figure 7**) and the selected pI marker windows will be applied to all data in the batch and the data exported if this option is enabled.

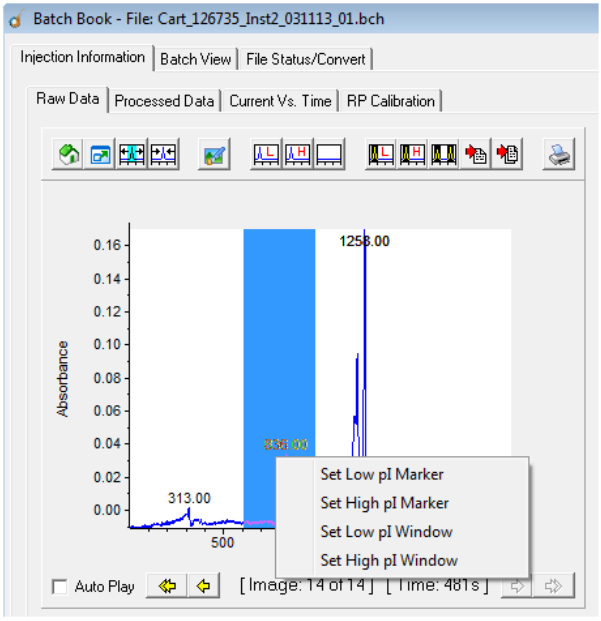

FIGURE 6. Automated pI assignment. Highlight to define window around low pI marker.

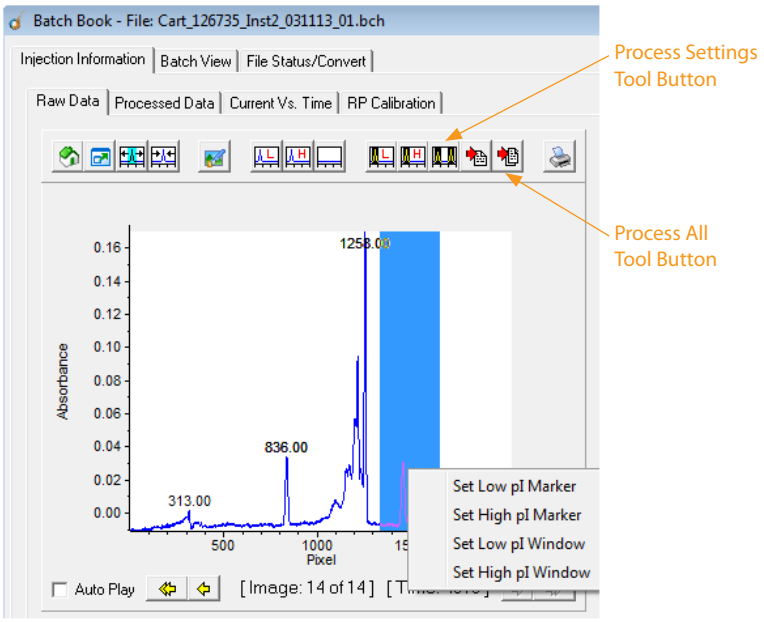

FIGURE 7. Automated pI assignment. Highlight to define window around high pI marker.

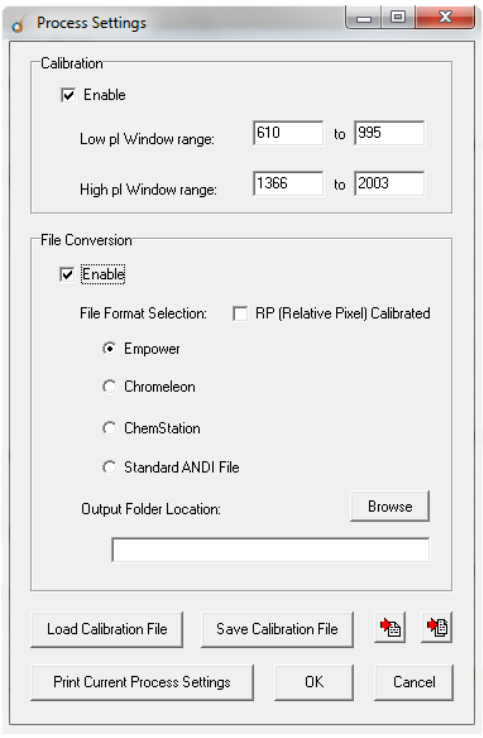

FIGURE 8. Set up Calibration and File Conversion in Process Settings window.

Conclusion

Coupled together, these iCE3 performance upgrades offer greater speed and improved ease of use. The HT Cartridge simplifies sample preparation, while saving precious time – up to 5 minutes per sample. The locking electrode arm and automated pI calibration further improve the iCE3 workflow by eliminating a major source of cathodic drift, and automating the time consuming manual task of pI calibration and data export.

proteinsimple

Toll-free: (888) 607-9692
Tel: (408) 510-5500
Fax: (408) 510-5599
orders@proteinsimple.com
proteinsimple.com

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