Faster and Easier Charge Heterogeneity Analysis with the iCE 3

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Introduction

Three major usability improvements are now available for the iCE3 system. A new HT cIEF Cartridge increases resolution while reducing run times by eliminating the need for methyl cellulose. A redesigned electrode arm assembly minimizes cathodic drift allowing robust analysis of 100 samples in a batch. The pI calibration and data export processes have been combined into a single automated procedure through the development of enhanced software features. These updates offer greater speed and improved ease of use. In this poster we present the results of these improvements on a model iCE method for analysis of a basic IgG1 mAb. The new HT cartridge reduces analysis time by five minutes, while still providing a highly resolved peak profile comparable to the original FC cartridge. An intermediate precision study demonstrated a %CV of less than 10% for peak all major peak clusters (>5% percent composition).

iCE3 Separations

Separations were performed on an iCE3 system equipped with either a PrinCE Next MicroInjector or an Alcott 720 NV Autosampler.

Results and Discussion

iCE 3 Enhancements 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. The removal of methyl cellulose reduces sample injection time and signal spiking. 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. The locking electrode arm further improves performance by reducing electrolyte evaporation and CO2 absorption, a major source of cathodic drift. This allows 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 streamlining data analysis.

HT cIEF Cartridge Performance

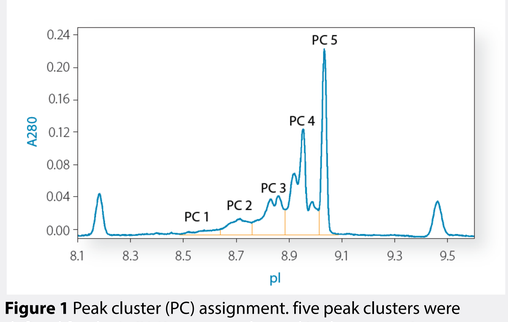

Figure 1 Peak cluster (PC) assignment. five peak clusters were defined for the IgG1 Kappa sample used in these experiments.

HT Cartridge		FC Cartridge	
Separation Method			
1 Minute	1500 V	1 Minute	1500 V
7 Minutes	3000 V	10 Minutes	3000 V
Sample Martix			
DI Water	98 µL	DI Water 1% Methycellulose	10 µL 88µL
10 M Urea	100 µL	10 M Urea	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 for IgG1 Kappa iCE separations between cartridge type.

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Cartridge	Instrument	Peak Area % PC 1	Peak Area % PC 2	Peak Area % PC 3	Peak Area % PC 4	Peak Area % PC 5
126641	1	3.24	9.73	21.45	41.43	24.13
126642	2	3.46	11.18	23.19	39.29	22.86
126644	1	3.36	10.58	23.22	39.44	23.38
126777	2	2.94	9.67	20.64	39.57	27.19
126779	1	3.30	11.04	20.54	39.49	25.63
126808	1	2.98	9.52	21.15	39.99	26.35
126961	2	2.53	10.03	19.99	40.52	26.94
126963	1	2.54	9.37	19.91	41.02	27.16
126965	2	2.59	9.34	21.11	38.33	27.86
127091	2	2.81	9.63	20.95	40.03	26.58
127093	1	2.97	10.01	21.22	39.91	25.81
127095	1	2.46	10.12	20.75	40.44	26.23
Average		2.93	10.02	21.18	39.96	25.84
Std Dev		0.338	0.592	1.012	0.794	1.52
% CV		11.53%	5.91%	4.78%	1.99%	5.88%

Table 2. Peak cluster percent composition analysis of IgG1 Kappa using the HT cIEF Cartridge. Peak area percent is reported as an average calculated from 12 replicates. The data series contains the results of 12 different cartridges, sourced from four manufacturing lots, tested using two iCE instruments.

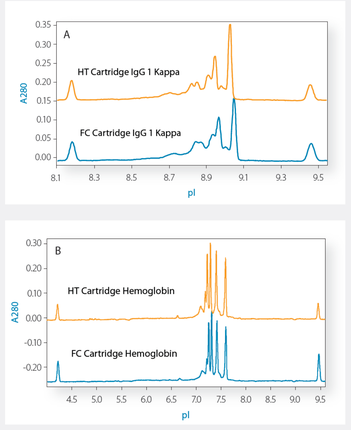

Figure 2. Comparisons of iCE separations on the HT and FC cartridges highlight the minimal effects of capillary coating on charge profile results. **(A)** The profile of IgG1 Kappa is slightly better resolved in the HT cartridge but there is no difference in the peak's pI points between the two cartridge types. **(B)** The same effects are observed in separations of Hemoglobin charge variants.

Improved Electrode Arm Assembly

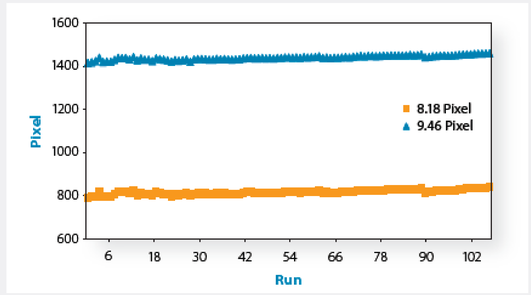

Figure 3. Plotting the position of the 9.46 and 8.18 markers from the IgG1 Kappa runs over 107 replicates shows the dramatic reduction in cathodic shift of the pH gradient resulting from proper electrolyte vial sealing.

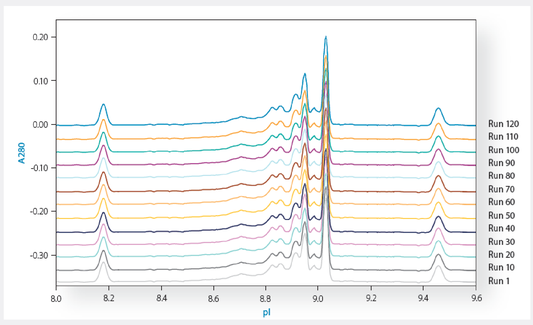

Figure 4. Overlay plots from each tenth IgG1 Kappa separation from a 120 replicate run set demonstrates the enhanced stability of charge heterogeneity profile and peak apparent isoelectric point produced on a iCE system equipped with the enhanced electrode arm. .

Cartridge	Run	Instrument	Peak Area % PC 1	Peak Area % PC 2	Peak Area % PC 3	Peak Area % PC 4	Peak Area % PC 5	pI PC5
126808	1	1	2.96	9.49	20.92	40.49	26.15	9.03
126808	10	1	3.14	9.32	21.77	39.53	26.24	9.03
126808	20	1	3.56	9.14	21.08	39.90	26.33	9.03
126808	30	1	3.38	9.68	20.83	40.01	26.11	9.03
126808	40	1	2.93	9.79	20.84	40.25	26.19	9.03
126808	50	1	3.46	9.52	21.50	39.39	26.13	9.03
126808	60	1	2.38	10.14	21.44	39.65	26.39	9.03
126808	70	1	3.03	9.39	21.32	39.89	26.37	9.03
126808	80	1	2.78	9.62	20.71	40.62	26.28	9.03
126808	90	1	2.42	9.66	21.53	40.23	26.16	9.03
126808	100	1	2.95	8.95	21.91	39.57	26.62	9.03
126808	110	1	2.85	9.91	20.93	39.70	26.61	9.03
126808	120	1	2.95	9.19	20.18	40.68	27.00	9.03
Average			2.98	9.52	21.15	39.99	26.35	9.03
Std Dev			0.338	0.315	0.463	0.412	0.247	0.00
% CV			11.35%	3.31%	2.19%	1.03%	0.94%	0.00%

Table 3. Percent composition and pI determination analysis from the runs contained in figure 3 are highly stable showing excellent lifetime repeatability of the HT cIEF cartridge

Automated pI Calibration and Data Conversion

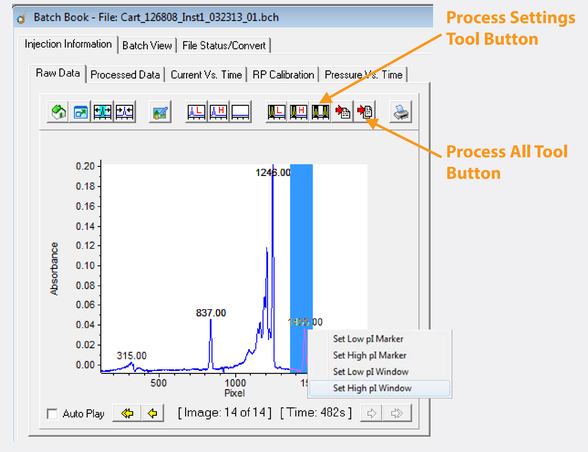

Figure 5. New pI marker calibration buttons have been added to Injection Information window of the batch book of iCE CFR Version. 4.0 software. They enable the software to search for low and high pI markers within a wide pixel range, and automatically calibrate pI for multiple analysis.

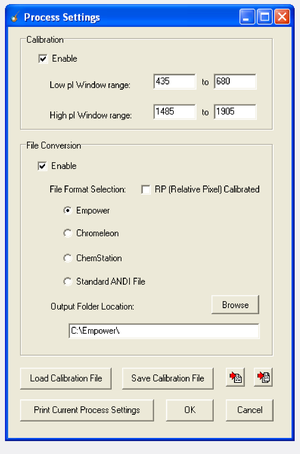

FIGURE 6. From the new Process Setting Window, pI marker calibration ranges and file conversion settings can be made and saved. Export destinations for converted files can also be assigned. These added features allow for complete process automation providing for enhance confidence in data security and traceability.

Conclusions

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.