Rapid and reproducible parallel processing of charge heterogeneity analysis of protein therapeutics by multi-capillary isoelectric focusing

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Rapid characterization of protein therapeutic charge variants can be challenging for the growing number and wider variety of new modality drug candidates. Automated capillary electrophoresis instruments offer fast separation times and high reproducibility for capillary isoelectric focusing. Currently, single capillary cIEF systems have limited throughput for such assays. To address this issue, SCIEX has introduced the BioPhase 8800 system, a multi-capillary electrophoresis platform. This new system enables parallel isoelectric focusing analysis for up to 8 samples at a time.

Therapeutic proteins are a rapidly growing class of biologics. The introduction of monoclonal antibody (mAb) drugs has transformed the pharmaceutical landscape to combat a number of clinical conditions, including cancer and autoimmune disease.[1] The introduction of new modalities, including bi- and tri-specific antibodies, antibody-drug conjugates, and fusion proteins, is increasing.[2,3] Protein therapeutic charge variant assessment is essential at different manufacturing stages as they are subjected to instability, causing alterations in their primary amino acid sequence and variable post-translational modification (PTM).[4,5] These changes can often be detected by changes in the protein isoelectric point.[6] Therefore, robust and reproducible techniques for charge variant characterization are of high importance. A frequently used high precision technique to characterize charge heterogeneity of recombinant protein therapeutics is isoelectric focusing.[7] In this technical note, we demonstrate parallel, highly reproducible capillary isoelectric focusing analysis using a multi-capillary separation approach to accelerate analysis time.

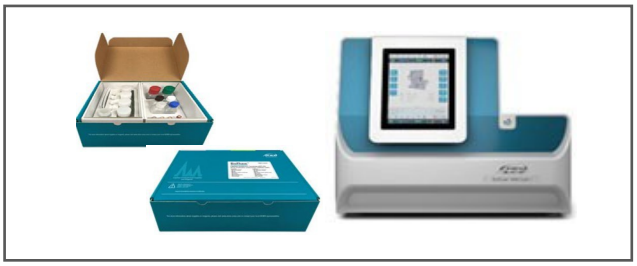

Figure 1. The BioPhase 8800 system equipped with consumable/ reagents kits and both UV and LIF detectors.

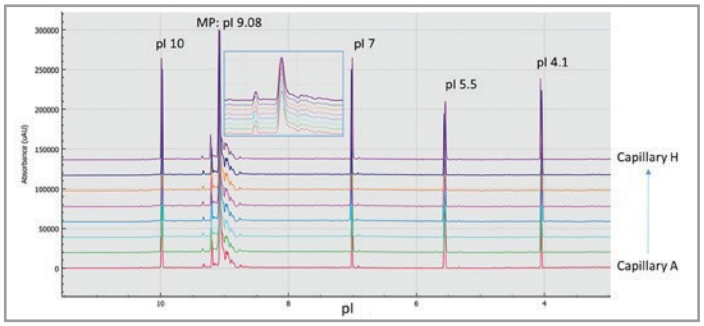

Figure 2. Multi-capillary isoelectric focusing of the NIST mAb. Traces A to H represent the simultaneous cIEF analysis of the same sample loaded in eight sample wells using eight individual capillaries of the BioPhase 8800 system. The X axis represents the calibrated pI values. Peaks: pI 10, 7, 5.5 and 4.1 – co-injected pI markers, MP – main peak (pI = 9.08). The inset shows the enlarged part of the NIST mAb peaks. Conditions: 50 µm I.D., 30 cm total (20 cm effective) neutral coated capillary; focusing condition: 25 kV for 15 min; mobilization condition: 30 kV for 30 min; normal polarity; capillary temperature 20°C.

Key features

- The BioPhase 8800 system offers the ability to multiplex batch analysis and data reduction to support high throughput charge heterogeneity screening of therapeutic proteins
- Isoelectric focusing in a multi-capillary format significantly cuts method development and optimization time
- Seamless method transfer from the conventional single to the new multi-capillary format
- Provision of kitted reagents/consumables and pre-assembled cartridge simplifies the operation with minimized operator error
- The improved algorithm in the BioPhase analysis enables data analysis and peak reporting based on pI, translating into decreased data processing time and less analyst intervention.
- The total analysis time for a 96-well plate sample set was ~12 hours with the BioPhase 8800 system in comparison to 5 days on the single capillary system

Experimental

Chemicals: The BioPhase 8800 system cIEF kit (Part # C30101) with CE grade water, cathodic stabilizer, anodic stabilizer, cIEF anolyte, cIEF catholyte, cIEF chemical mobilizer, cIEF urea, cIEF gel, cIEF neutral capillary conditioning solution, cIEF formamide, sample, and reagent plate kit were from SCIEX (Framingham, MA). The NIST mAb (RM 8671) reference material 8671 was purchased from NIST (Gaithersburg, MD) and the Pharmalyte IEF Carrier Ampholytes (Part # 17-0456-01 Cytiva) was from VWR.

Sample preparation: All buffers and reagents were prepared following the BioPhase 8800 cIEF kit user guide. For assay linearity evaluation, a series of two-fold dilutions of the NIST mAb was prepared with CE grade water to a final concentration of 10 (undiluted), 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078 mg/mL respectively. In all other instances, the NIST mAb was diluted to 5 mg/mL with CE grade water. For each sample, a master mix containing 200 µL urea-cIEF gel, 25 µL cathodic stabilizer, 3 µL anodic stabilizer, 12 µL pharmalyte, and 2 µL of each pI markers (pI 10.0, 7.0, 5.5, and 4.1) was mixed with 8 µL of diluted NIST mAb (all concentrations). The sample mixture was mixed thoroughly at room temperature. 100 µL aliquots were placed across the 8 injecting well positions in the injection sample plate. Another 100 µL was transferred to the sample vial of the single capillary system.

Single capillary isoelectric focusing: All single capillary cIEF measurements were accomplished using a PA 800 Plus Pharmaceutical Analysis system (Part # A74603, SCIEX) with UV absorbance detection at 280 nm. A cartridge with a 20 cm effective length (30 cm total length) with an eCap neutral capillary (Part # A80976) was assembled. The detailed methods can be found in the SCIEX cIEF reference guide.[8]

Multi-capillary cIEF analysis were performed using the BioPhase 8800 system. Sample preparation and detection methods were the same as those used in the PA 800 Plus analyses. Samples were separated using a neutral multi-capillary cartridge. The detailed method can be found in the reference guide. The BioPhase software package was used for data acquisition and processing.

Results and discussion

Multi-capillary isoelectric focusing was performed using the BioPhase 8800 system. With the new data analysis software for BioPhase, and peaks are reported based on pI, translating into decreased data processing time and less analyst intervention. pI and peak area % reproducibility for the NIST monoclonal antibody was compared in several aspects to a single capillary electrophoresis unit (PA 800 Plus). Figure 2 compares eight isoelectric focusing electropherograms simultaneously obtained by the BioPhase 8800 system. Peaks of pI 10.0, 7.0, 5.5, and 4.1 correspond to the individual components of the co-injected pI marker set, while MP represents the main peak of the NIST monoclonal antibody with its pI determined as 9.08. The inset shows the enlarged part of the NIST mAb peaks.

Capillary	Total Corrected Area		% Basic Corrected Area		% Main Corrected Area				Calibrated Main pI	
	Avg	%RSD	Avg	%RSD	Avg	%RSD	Avg	%RSD	Avg	%RSD
A	19595.9	2.7	11.6	0.8	69.5	0.2	18.9	0.8	9.08	0.1
B	20016	4	11.7	1.7	68.6	0.4	19.6	1	9.08	0.0
C	20769.4	2.9	11.7	1	68.8	0.8	19.5	3.1	9.08	0.1
D	20908	0.2	11.8	2	67.9	0.3	20.3	0.2	9.08	0
E	22005.1	2.4	11.7	1	68.6	0.5	19.7	1.8	9.08	0
F	21339.3	1.7	11.7	0.7	68.6	0.6	19.6	1.8	9.08	0
G	20622.4	3.9	11.6	1.7	68.8	0.3	19.6	1.3	9.08	0.1
H	20010	3.7	11.5	1.1	68.4	0.9	20.1	3.7	9.08	0.1
Avg	20658.3		11.7		68.7		19.7		9.08	
%RSD	3.8		0.8		0.7		2.1		0.02	

Table 1. Assay repeatability. Average of six injections for each capillary in the array (n=6).

The total corrected peak area% reproducibility of NIST MAb charge variants using a multi-capillary electrophoresis approach was RSD=3.8%, (Table 1). The basic, main, and acidic peak variant subsets across the 8 capillaries showed a corrected peak area% RSD values of 0.8%, 2.1%, and 0.7%, respectively. The pI value for the main peak was 9.08 and 0.02% RSD value over the assay repeatability exercise.

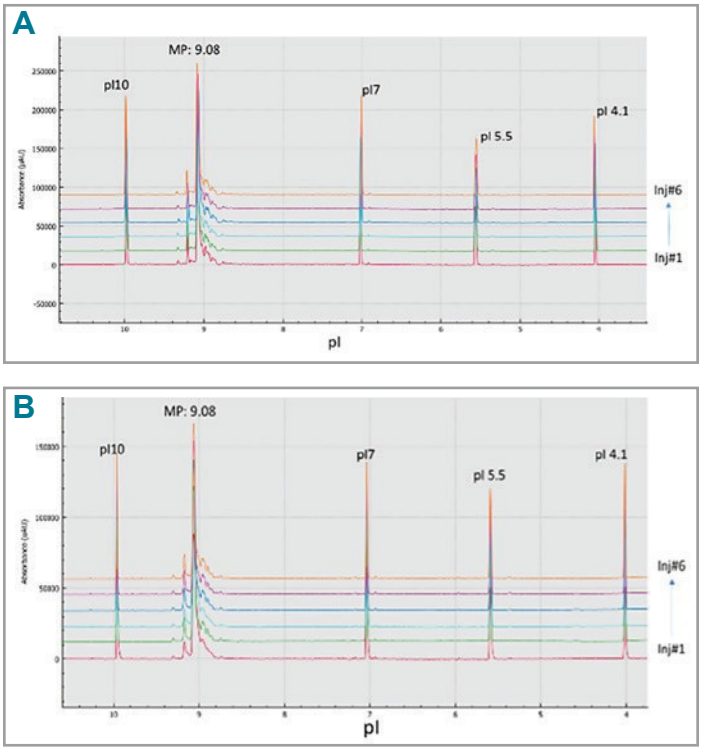

Figure 3. Reproducibility of six consecutive cIEF separations of the NIST monoclonal antibody chare variants on capillary A of the BioPhase 8800 system (Panel A) and the single capillary PA 800 Plus Pharmaceutical Analysis system (Panel B), respectively. Peaks and conditions for the BioPhase 8800 system are the same as in Figure1. Conditions for the PA 800 Plus analysis: 50 µm I.D., 30 cm total (20cm effective) neutral coated capillary; focusing condition: 25 kV for 15min; mobilization condition:30 kV for 30 min; normal polarity; capillary temperature 20° C.

The pI reproducibility for six consecutive injections was evaluated on both the BioPhase 8800 system and the single capillary PA 800 Plus Pharmaceutical Analysis system (Figure 3). Excellent total corrected peak area reproducibility was obtained with both systems: RSD=0.03%. (n=6), for the multi-capillary unit and RSD=0.04% (n=6) for the single capillary system. The calibrated pI values were 9.08 and 9.06 for the BioPhase 8800 and PA 800 Plus systems, respectively, and in both instances with <0.001% RSD. Considering the 60 min cIEF analysis time per run for the eight capillary array, the total analysis time for a 96-well plate

Instrument	Total Corrected Area		% Basic Corrected Area		% Main Corrected Area		% Acidic Corrected Area		Calibrated Main pI	
	Avg	%RSD	Avg	%RSD	Avg	%RSD	Avg	%RSD	Avg	%RSD
BioPhase 8800	19595.95	0.03	11.59	0.01	69.52	0	18.89	0.01	9.08	0.001
PA 800 Plus	12876.01	0.04	10.55	0.04	69.83	0.01	19.61	0.01	9.06	0

Table 2. Comparative assay repeatability with the BioPhase 8800 and PA 800 Plus systems (n=6).

sample set is approximately 12 hours with the BioPhase 8800 system compared to 5 days for the same number of samples with the PA 800 Plus system.

In order to investigate sample stability while awaiting analysis, NIST mAb samples were stored in the sample compartment of the BioPhase 8800 system for stability analysis. Samples were held for up to 27 hours in the temperature-controlled compartment at 10° C after which no change was detected in the charge isoform profiles. Therefore, no degradation of the samples was detected for at least 24 hours, even for the analysis of the last row of the 96-well plate.

Direct comparison of the isoelectric focusing separation of the two systems illustrates similar profiles with slightly better resolution and sensitivity on the BioPhase 8800 system (Figure 4).

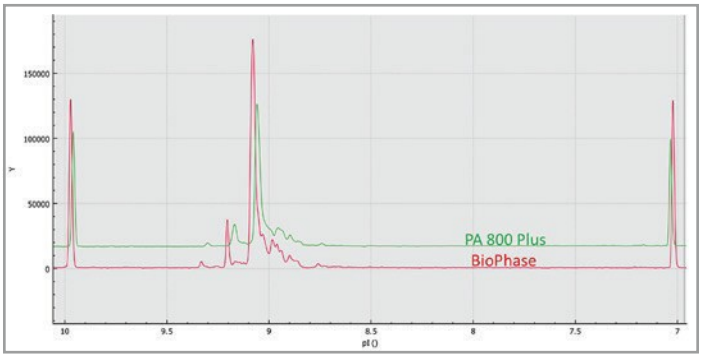

Figure 4. Comparison of the cIEF separation profiles of the NIST monoclonal antibody charge variants between capillary A of the BioPhase 8800 system (lower trace) and the single capillary PA 800 Plus Pharmaceutical Analysis system (upper trace). Peaks and conditions are the same as in Figure 2 and Figure 3.

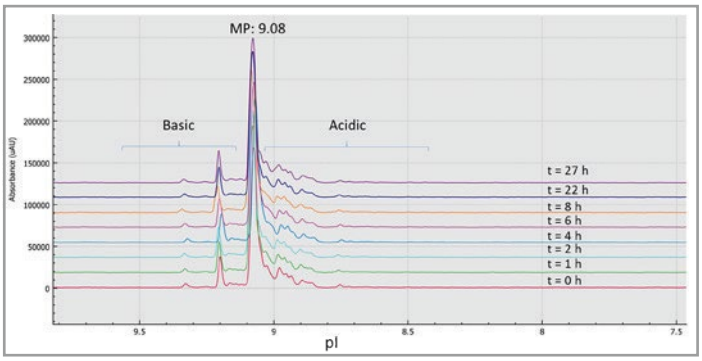

Figure 5. Effect of the storage time in the sample compartment of the BioPhase 8800 system on the cIEF profile of the NIST monoclonal antibody. Traces: 0 to 27 hours of storage at 10° C in the sample compartment of the unit. Peaks and conditions are the same as in Figure 3.

Corrected peak area was very reproducible with RSD=1.9% and the subsets of the basic, main, and acidic regions with the corresponding RSD values of 1.6%, 0.8%, and 3.4%, respectively, for the average of the eight capillaries in the array at each time point (Table 3). The pI value for the prominent peak was 9.08 with 0.02% RSD.

Assay linearity was assessed by injecting a serial dilution of the NIST mAb sample covering the concentration range of 0.08 to 10.0 mg/mL, using 280 nm UV detection wavelength. Results illustrated excellent linearity for both linear-linear (Panel C, r2=0.9978) and log-log (Panel A, r2=0.9981) plots, the latter for a better assessment of the lower concentration range (Figure 6). The UV detection signal response was linear over two orders of magnitudes, despite a somewhat overloaded 10 mg/mL concentration point. Based on these results, confident quantification can be achieved for impurities as low as the 0.08% concentration level.

Hour	Total Corrected Area		% Basic Corrected Area	% Main Corrected Area		% Acidic Corrected Area	Calibrated Main pI	
	Avg	%diff w/T0	Avg	Avg	%diff w/T0	Avg	Avg	%diff w/T0
0	20704.9	0	11.7	69.2	0	19.1	9.08	0
1	20467.9	1.2	11.7	69	0.3	19.3	9.08	0.1
2	20601.5	0.5	11.7	68.8	0.7	19.5	9.08	0.1
4	20791.2	0.4	11.5	68.6	0.9	19.9	9.08	0
6	20521.8	0.9	11.6	68.5	1	19.9	9.08	0
8	20743.8	0.2	11.8	68.2	1.4	20	9.08	0
22	19850.3	4.3	11.2	68.2	1.5	20.6	9.08	0.1
27	19754.7	4.8	11.5	67.4	2.7	21.2	9.08	0
Avg all hour	20429.5		11.6	68.5		19.9	9.08	
%RSD	2.0		1.6	0.8		3.4	0.02	

Table 3. Storage time stability testing of the NIST mAB in the sample compartment of the BioPhase 8800 system (10° C, n=8). T0=time zero

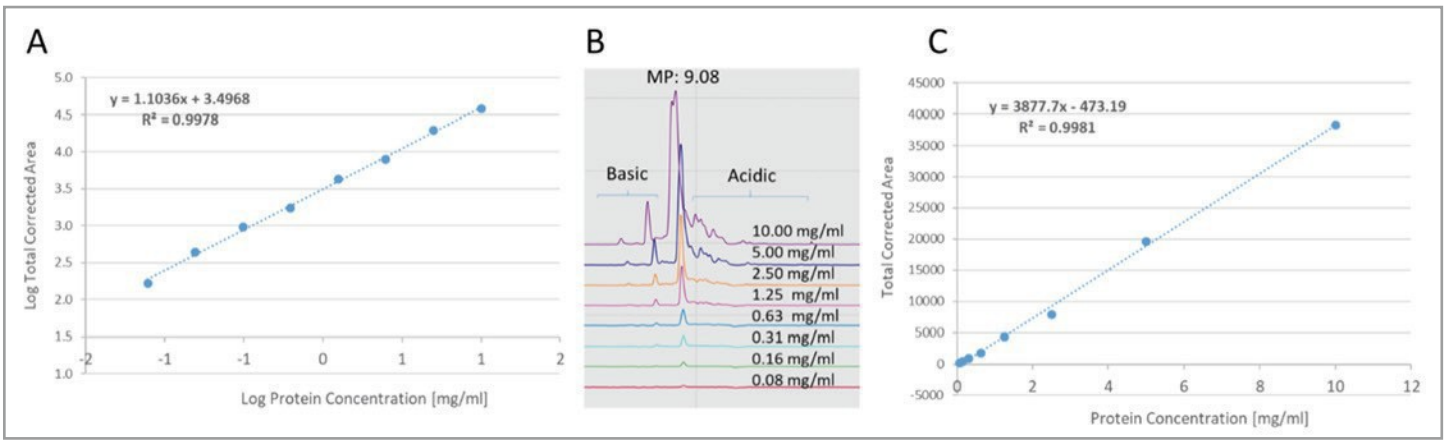

Figure 6. Capillary isoelectric focusing assay linearity study with the BioPhase 8800 system showing both log-log (A) and linear-linear log-log (C) interpretations. Panel B depicts the relevant electropherogram sections covering the concentration range from 0.08 to 10.00 mg/mL for the NIST monoclonal antibody. Peaks and conditions are the same as in Figure 3.

Conclusions

- Rapid and reproducible charge heterogeneity analysis of protein therapeutics was demonstrated by isoelectric focusing using the new BioPhase 8800 system.
- The results demonstrate excellent correlation of the BioPhase 8800 system to the single capillary PA 800 Plus Pharmaceutical Analysis system in respect to reproducibility but with increased throughput.
- Excellent quantitative performance was obtained for the NIST monoclonal antibody with UV 280 nm detection, resulting in confident quantification to as low as 80 µg/mL sample concentration.
- Good correlation of the isoelectric focusing parameters between the single capillary PA 800 Plus system can simplify the method transfer from the single to the multi-capillary system.
- The BioPhase 8800 system can be used to accelerate development of biologics using its parallel processing capability along with high quality CE separation capability.

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