Whitepaper

Assessment of Product Quality in Upstream Process Development Using Maurice™ CE-SDS and icIEF

Introduction

The global monoclonal antibody market is preparing itself for substantial growth in the coming years, with an expected compound annual growth rate (CAGR) of 12.6% from 2022 to 2027, resulting in a market of over $138.1 billion[1]. This increase in demand for monoclonal antibodies is driving a race among drug developers and manufacturers to be the first to deliver to patients. However, the drug development process is riddled with challenges, and inadequate analytical tools are among the biggest obstacles. Many of these tools are either manual and labor-intensive, or they are designed for a specific and limited type of analysis, which can cause significant bottlenecks as the therapeutic progresses from one stage to another. Method transfer requirements only exacerbate these bottlenecks, resulting in substantial costs in terms of time, labor, and capital.

The Case for a Multi-Attribute Analytical Tool

Advances in technology across various industries are rooted in the concept of "less is more", where a single solution is sought to address multiple problems. In the analytical instruments sector, there is a growing demand for versatile tools that can effectively analyze multiple attributes of drug substances or products. SDS-PAGE is still a widely used method to analyze product quality throughout the development of biotherapeutics[2], despite the availability of faster and automated alternatives such as capillary electrophoresis (CE)-based tools. Upstream stages usually require large numbers of samples to be analyzed, and since SDS-PAGE is a manual and time-consuming technique, a faster, more effective, and high throughput analytical tool for characterizing bioprocess samples would significantly shorten characterization timelines. The Maurice™ platform is an automated, multi-functional, capillary electrophoresis tool that offers capillary electrophoresis sodium dodecyl sulfate (CE-SDS) and imaged capillary isoelectric focusing (icIEF) capabilities for analyzing protein size and charge across various stages of biotherapeutic development. It offers the added benefit of being able to analyze multiple Critical Quality Attributes (CQAs) with just one instrument. By providing a comprehensive analysis of the protein and its characteristics in a short amount of time, scientists can obtain a more detailed understanding of the molecule.

Both CE-SDS and icIEF workflows on Maurice are simple, only requiring sample preparation and the insertion of the appropriate cartridge into the system for analysis. The cartridges are pre-assembled, thereby eliminating the need for any capillary loading, and instrument setup and clean up are automated. Today, icIEF is the gold standard for protein charge analysis, and the cIEF cartridge on Maurice generates reproducible, high-quality charge heterogeneity data in 10-15 minutes.

There are two cartridge choices available for CE-SDS analysis - Turbo CE-SDS and CE-SDS PLUS. Turbo CE-SDS, evocatively named, enables fast and high throughput analysis of samples, and is best suited for use in bioprocess and discovery stages. It provides high quality data in 5.5-8 minutes per sample, allowing the characterization of an array of samples and helping scientists make the right decision in early bioprocess stages. CE-SDS PLUS, on the other hand, provides superior resolution with an analysis time of 25-35 minutes and is an ideal characterization tool in analytical development and quality control (QC) release testing. Both icIEF and CE-SDS on Maurice are controlled using the same software, Compass for iCE, which is 21 CFR Part 11 compliant. Additionally, scientists have the option to use Waters™ Empower® software with the Maurice system.

This whitepaper demonstrates the rapid analysis of critical quality attributes of monoclonal antibodies (mAbs)—both cell supernatants and purified samples—in upstream bioprocess stages using icIEF and Turbo CE-SDS on Maurice.

Materials and Methods

TABLE 1 lists the materials and reagents used in this study, including the Maurice cIEF Method Development Kit and the Turbo CE-SDS Size Application Kit, which both contain the reagents necessary for their respective assays.

A HEK293EBNA clone producing a monoclonal human antibody (Hu x hCD4/30345-1) was grown in suspension with varying ratios of free L-Glutamine and L-Alanyl-L-Glutamine (GlutaMAX™) in the media.

In total, five conditions were evaluated, shown in **TABLE 2** in the next section. For each condition, 2 x 100 mL duplicate cultures were grown in 500 mL shake flasks for 7 days. After cell culture, the conditioned media were harvested for purification. Protein G-purified mAb samples and their corresponding cell supernatants were then analyzed on a Maurice system (PN 090-000) with the Turbo CE-SDS and cIEF cartridges.

For CE-SDS analysis with the Turbo CE-SDS cartridge, cell supernatants (30 µL) and purified mAb samples (0.5 mg/mL) were each mixed with the Maurice CE-SDS 1X PLUS Sample Buffer in a 1:1 ratio. The Maurice CE-SDS 25X Internal Standard (IS, 4%) was added to all samples, followed by the addition of 5% (V/V) of either iodoacetamide (IAM, 250 mM) for non-reduced analysis, or β-mercaptoethanol (β-ME, 14.2 M) for reduced analysis. All samples were then heated for 10 minutes at 70°C, cooled on ice for five minutes and finally subjected to centrifugation. The samples were loaded onto the Maurice instrument and injected for 8 seconds at 3500 V and separated for either 8 minutes at 4200 V (non-reduced), or 5.5 minutes at 4200 V (reduced).

For icIEF analysis, cell supernatants (15 µL, diluted in a 1:1 ratio with 0.5X phosphate-buffered saline) and purified samples (0.05 mg/mL) were prepared in a reagent mix containing Pharmalyte 3-10 (1%), Pharmalyte 5-8 (3%) urea (3.2 M) pI markers 5.85 and 8.4 (1% for purified samples and 0.2% for supernatants), methyl cellulose (0.35%), and deionized water. The samples were loaded onto the instrument and injected for 1 minute at 1500 V, followed by separation for 12 minutes at 3000 V.

All data generated with the Maurice system were analyzed using the Compass for iCE software.

Material	Vendor	Catalog #
Maurice Turbo CE-SDS Size Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK01-TS
Maurice Turbo CE-SDS Cartridge		PS-MC02-TS, PS-MC01-TS
Maurice cIEF Method Development Kit		PS-MDK01-C
Maurice cIEF Cartridge		PS-MC02-C
Maurice CE-SDS Molecular Weight Markers		046-432
Maurice CE-SDS IgG Standard		046-039
Maurice CE-SDS 25X Internal Standard		046-144
GlutaMAX™	ThermoFisher Scientific	35050061
L-Glutamine		21051024
Iodoacetamide (IAM)	Millipore Sigma	16125
β-mercaptoethanol (β-ME)		M-3148

TABLE 1. List of materials and reagents used in this study.

Results

Analysis of Cell Supernatants

Cell supernatants from five different flasks were analyzed with both Turbo CE-SDS and icIEF methods. Cells in each flask were cultured using a unique ratio of L-glutamine and GlutaMAX™, as shown in **TABLE 2**, which also shows the corresponding antibody yield measured after Protein G purification. L-glutamine is an essential amino acid that is required in cell culture media as a supplement to enable optimal clonal growth, as it is a precursor for the synthesis of amino acids, proteins, nucleotides and other molecules[3,4]. GlutaMAX™ plays a similar role in cell culture but is often used to supplant L-glutamine because it degrades much less compared to L-glutamine and therefore improves stability and cell viability[5,6]. While the data in **TABLE 2** indicate that there could be a correlation between the ratio of the two supplements and antibody yield, further experiments are required to draw indisputable conclusions.

FIGURES 1A and **1B** show the profiles of all five cell supernatants analyzed with Turbo CE-SDS under non-reduced and reduced conditions, respectively.

The expected peaks, namely intact monomer, heavy-heavy-light (HHL), heavy-heavy (HH), heavy-light (HL), heavy (H), and light (L) were detected under non-reduced conditions, and the heavy chain (HC), non-glycosylated heavy chain (NG HC), and light chain (LC) were detected under reduced conditions. While differences in peak intensity between profiles were observed under non-reduced conditions, quantification of the monomer's percent peak area resulted in comparable values (**TABLE 3**), indicating that the variation in the L-glutamine/GlutaMAX ratio did not impact the purity of the monoclonal antibody. Furthermore, triplicate injections were performed, and the relative standard deviation (%RSD) values for all samples were ≤1.15%, establishing the reproducibility of the method. Interestingly, quantification of the peaks detected under reduced conditions showed some differences. Although the LC and HC did not show any increase in their peak area percentages, a small increase in the percent peak area of NG HC by ~1% was observed (**FIGURE 2**), possibly correlating with changes in the L glutamine/GlutaMAX ratio. Further research is required to ascribe changes in antibody fragments to the impact of cell culture media supplements.

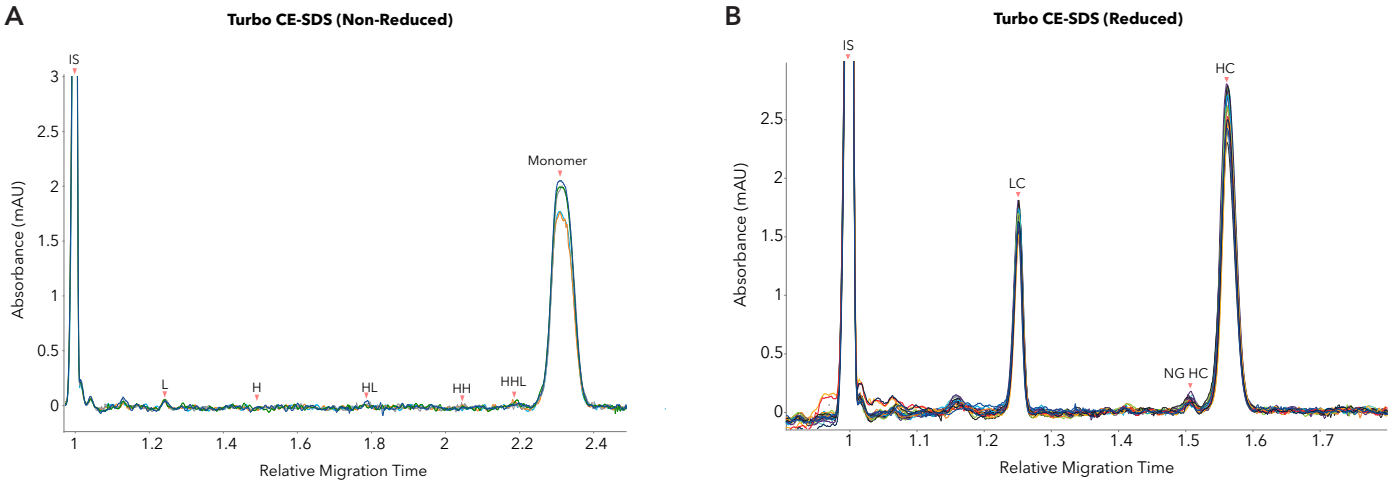

FIGURE 1. Variation of Glutamine/GlutaMAX content in the media does not impact purity of Hu x hCD4 in cell supernatants. **A.** Monomer data under non-reduced conditions are shown, along with % RSD values in Table 3. **B.** Under the same culture conditions, with reducing conditions, similar HC (~67%) and LC (~ 32%) percent peak areas were observed, with a maximal increase in percent peak area for NG-HC (1.1%) with 100% glutamine.

Flask	L-Glutamine (mM)	GlutaMAX (mM)	Yield (mg/L)
A	0	8	77.7
B	2	6	83.1
C	4	4	88.5
D	6	2	101.5
E	8	0	102.3

TABLE 2. The ratio of L-glutamine and GlutaMAX in each flask and the resulting antibody yield after harvesting and purification.

Percent Peak Area (Monomer)		
Flask	Average	%RSD
Flask A	96.83	0.96
Flask B	96.27	0.87
Flask C	96.17	1.15
Flask D	96.57	0.94
Flask E	96.97	0.69

TABLE 3. The average percent peak area of the monomer calculated for samples from each flask analyzed in triplicate under non-reduced conditions.

Cell supernatants from the same five flasks were also analyzed with icIEF using native fluorescence (NF) detection. One of the biggest advantages of NF is its low sample volume requirement by virtue of being four times more sensitive than absorbance[7]. **FIGURE 3** shows the charge profiles of all five cell supernatants, where heterogeneity is clearly seen with variations in the levels of the acidic and basic species. These data were quantified (**FIGURE 4**) to reveal subtle differences between the samples (**TABLE 4**).

Percent Peak Area (Native Fluorescence)			
Flask	**Peak**	**Average**	**%RSD**
A	Acidic	23.55	1.57
	Main	68.75	1.07
	Basic	7.73	11.98
B	Acidic	17.55	6.42
	Main	75.08	1.92
	Basic	7.38	10.58
C	Acidic	19.58	8.37
	Main	73.78	2.42
	Basic	6.68	9.74
D	Acidic	22.65	5.46
	Main	69.68	1.60
	Basic	7.68	7.99
E	Acidic	18.85	5.38
	Main	72.95	1.34
	Basic	8.20	5.72

TABLE 4. The average percent peak area and RSD values of the acidic, main, and basic peaks detected for Hu x hCD4/30345-1 from cell supernatants with Maurice icIEF.

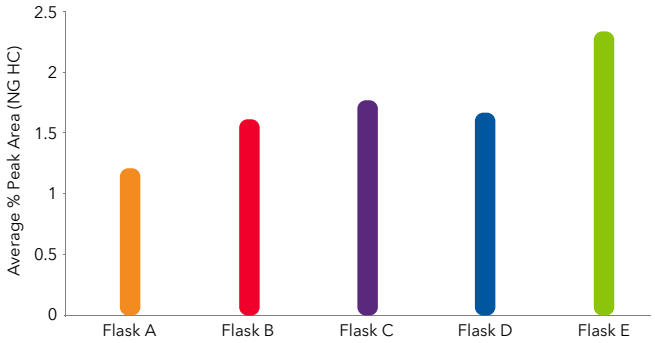

FIGURE 2. A graphical representation of the results from reduced CE-SDS analysis, showing a measurable change in the percent peak area of the NG HC.

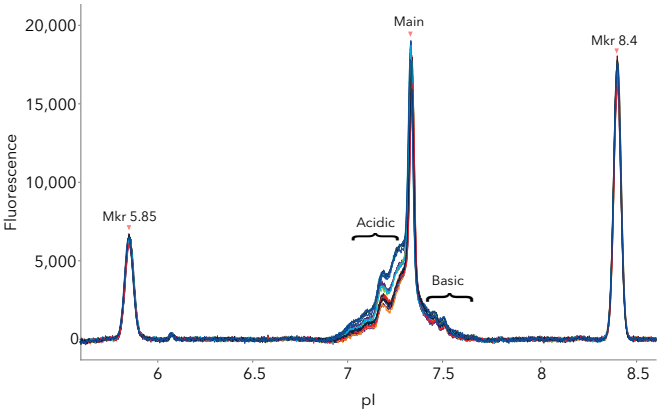

FIGURE 3. Variation of Glutamine/GlutaMAX content in the media resulted in changes in charge variants of Hu x hCD4. cIEF charge profiles (NF, 80s) of cell supernatants show differences in acidic, basic charge variants.

Analysis of Purified Monoclonal Antibodies

After cell expansion and achievement of sufficient titers, mAb samples were subjected to purification with Protein G, and the resulting five samples were analyzed with Maurice Turbo CE-SDS and Maurice icIEF to determine purity and charge heterogeneity similar as to described for the cell culture supernatants. **FIGURES 5A** and **5B** are results from non-reduced and reduced CE-SDS analysis, respectively. Fewer impurities were observed, as expected for purified samples, with peak purity summarized in **TABLE 5**.

The purity of the monomer was comparable across all five samples, whereas reduced samples demonstrated a variation in the percent peak area of NG HC, again close to an increase of ~1.1% (**FIGURE 6**). **FIGURE 7** shows the charge profiles of these five purified proteins analyzed with absorbance and native fluorescence detection modes, where slight differences in heterogeneity were observed, as summarized in **TABLE 6**.

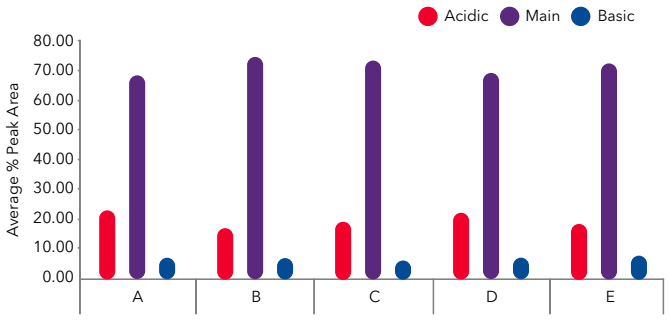

FIGURE 4. Quantification of the average percent peak area for the major icIEF peaks detected. The highest variations are seen for the acidic peaks between samples from each flask, denoted from A-E.

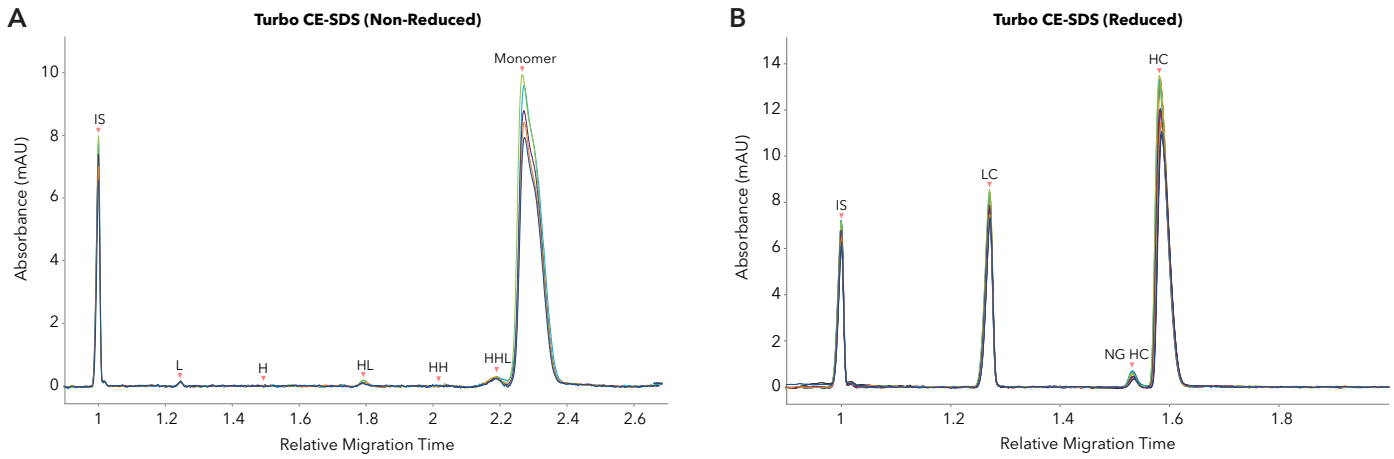

FIGURE 5. Variation of Glutamine/GlutaMAX content in the media does not impact purity of Hu x hCD4. **A.** Purified anti-CD4 mAbs under non-reducing and **B.** reducing conditions.

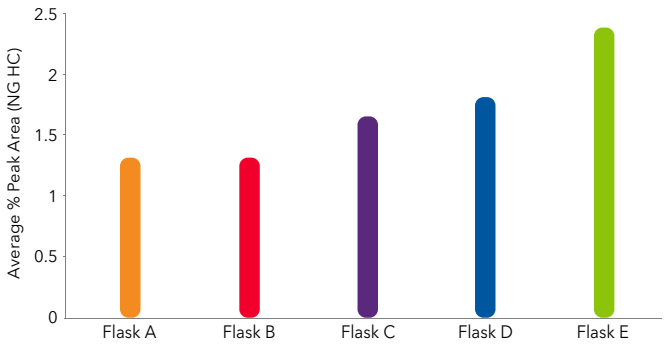

FIGURE 6. A graphical representation of the results from reduced CE-SDS analysis of purified Hu x hCD4/30345-1 samples, showing a similar change in the percent peak area of the NGHC (~1.1%) as observed among the cell supernatants.

Percent Peak Area (Monomer)		
Flask	**Average**	**%RSD**
Flask A	97.17	0.16
Flask B	96.50	0.37
Flask C	97.20	0.00
Flask D	96.73	0.12
Flask E	96.47	0.06

TABLE 5. The average percent peak area of the monomer calculated for purified samples from each flask, analyzed in triplicate.

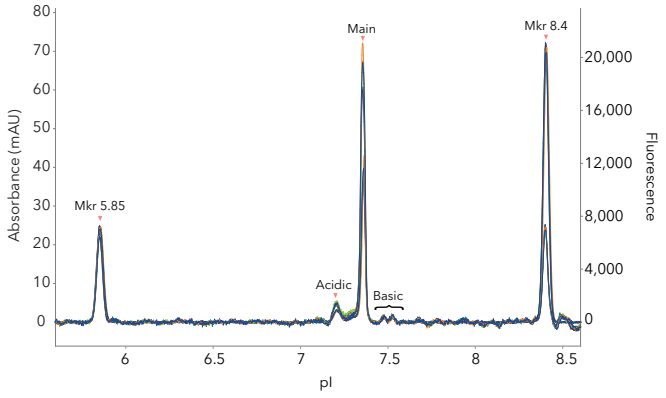

FIGURE 7. Variation of Glutamine/GlutaMAX content in the media resulted in slight changes in charge variants of Hu x hCD4. icIEF charge profiles of purified anti-CD4 mAb from five culture conditions show differences in acidic, main and basic percent peak area and exhibit heterogeneity.

Percent Peak Area					
		Absorbance		Native Fluorescence	
Flask	**Peak**	**Average**	**%RSD**	**Average**	**%RSD**
A	Acidic	14.30	5.07	15.03	2.57
	Main	82.23	0.65	80.03	0.39
	Basic	3.45	11.23	5.03	3.40
B	Acidic	14.40	6.68	14.98	1.92
	Main	82.58	0.96	80.38	0.31
	Basic	3.05	10.87	4.65	1.24
C	Acidic	14.88	3.93	15.58	6.51
	Main	81.35	1.46	79.08	1.08
	Basic	3.83	18.03	5.35	4.95
D	Acidic	17.73	2.96	16.98	8.60
	Main	78.65	1.16	79.08	1.94
	Basic	3.63	12.41	4.88	3.08
E	Acidic	16.90	2.90	17.28	5.52
	Main	79.90	1.10	77.88	1.47
	Basic	3.23	14.18	4.88	6.35

TABLE 6. The average percent peak area and RSD values of the acidic, main, and basic peaks for purified samples with Maurice icIEF using absorbance and native fluorescence detection.

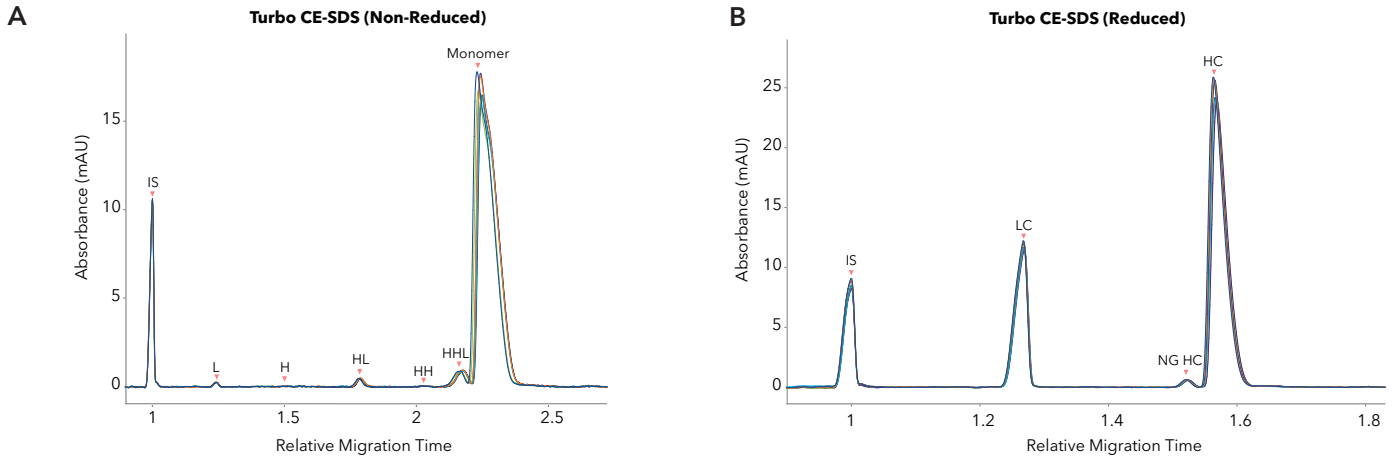

FIGURE 8. Two different clones of Hu x hCD4 demonstrate similar purity with Turbo CE-SDS. A. Triplicate injections run under non-reduced conditions and **B.** quadruplicate injections run under reduced conditions, with %RSD values listed in Table 7.

One of the biggest advantages of having fast CE-SDS and icIEF capabilities on a single instrument is the ability to quickly analyze an array of different clones, enabling scientists to quantitate the protein of interest and make better decisions faster. In this study, an additional clone (Clone 2) was analyzed along with the clone used in the current study (Clone 1), grown in varying concentrations of Glutamine/GlutaMAX. Both clones of the antibody were analyzed with Turbo CE-SDS and icIEF and their profiles were compared. **FIGURES 8A** and **8B** show the CE-SDS profiles generated under non-reduced and reduced conditions respectively, with the results summarized in **TABLE 7**. A comparison of the icIEF profiles of the two clones is shown in **FIGURE 9**, with the bar graph in **FIGURE 10** quantifying the percent peak area of the acidic, main, and basic peaks. In all three analyses in this study, the results were comparable between the two clones. However, it is important to note that purity and charge profiles between clones can be drastically different, which in turn depend on a variety of cell culture conditions such as nutrients, temperature, and pH to name a few.

Percent Peak Area					
		Non-reduced		Reduced	
Clone	Injection	Monomer	HC	NG HC	LC
1	1	95.3	67.1	1.1	31.8
	2	95	67.2	1.1	31.7
	3	95.1	67.2	1.1	31.7
	4	-	67.2	1.1	31.7
%RSD		0.16	0.07	0	0.16
2	1	95.4	67.1	1.1	31.9
	2	95	67.1	1.1	31.8
	3	95.3	67.1	1.1	31.8
	4	-	67.2	1.1	31.8
%RSD		0.22	0.07	0	0.16

TABLE 7. A summary of results from the CE-SDS analysis of the antibody from two clones. Both antibody samples were comparable to one another under non-reduced and reduced conditions, respectively.

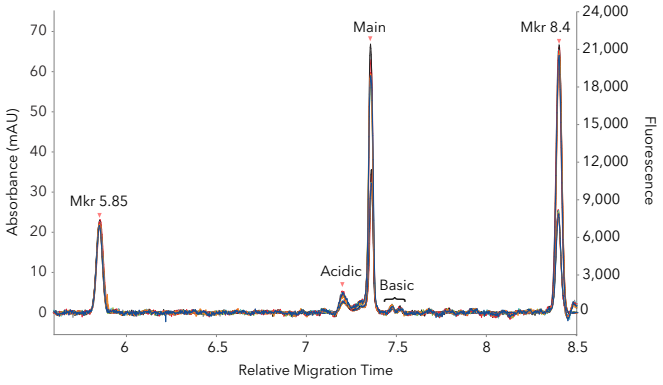

FIGURE 9. icIEF analysis of two clones of Hu x hCD4. Representative electropherograms of Clone 1 and Clone 2, each run in quadruplicate injections. Similar charge heterogeneity was observed between both clones.

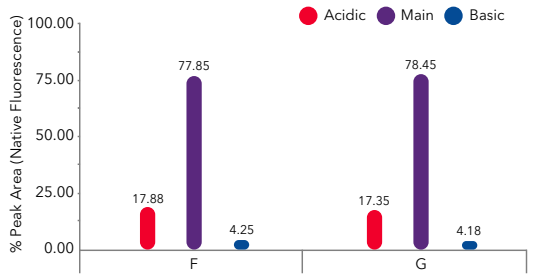

FIGURE 10. A bar graph quantifying the percent peak area of the three major peaks detected with Maurice icIEF using NF detection. The data show that the two clones in this case were highly comparable.

Conclusion

The upstream stages of biotherapeutic development require analytical tools that are both fast and reliable, capable of analyzing many samples quickly and accurately. This whitepaper demonstrated the use of both icIEF and CE-SDS on a single Maurice platform as an effective way of characterizing proteins in both cell supernatants and purified samples, delivering high-quality data with sensitivity, reproducibility, and precision. icIEF detects and quantifies small acidic and basic peak heterogeneity changes across different growth conditions, and Turbo CE-SDS provides quantitative high throughput purity data. Together, these methods provide insights into CQA assessment during upstream bioprocess development.

The Maurice system has already been used in the analytical development and QC release testing of over 100 commercial biotherapeutics, making it a trusted and established solution for protein characterization. With the introduction of Turbo CE-SDS in 2022, the Maurice system's capabilities have been expanded for use in upstream analysis, making it a cost-effective solution for protein characterization that can be used seamlessly in any stage of drug development. Visit our website to learn more about the Maurice system.

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