Comparing the Performance of the Maurice and MauriceFlex Systems

for Charge Heterogeneity Analysis

The Maurice™ and MauriceFlex™ are capillary electrophoresis (CE) systems for the separation of proteins based on charge or size. The MauriceFlex system can also fractionate individual protein charge variants for offline characterization with mass spectrometry and binding potency analysis on SPR instruments. For charge heterogeneity analysis, both systems use whole capillary imaging, otherwise known as imaged capillary isoelectric focusing (icIEF). The study presented in this technical note compares the analytical icIEF performance between the Maurice and MauriceFlex systems across four different biotherapeutic modalities including a bispecific antibody (BsAb), monoclonal antibody (mAb), a fusion protein, and an adeno-associated virus (AAV).

Materials

Material	Vendor	Catalog #
Lunsumio® (Mosunetuzumab)	Genentech	NA
Benlysta® (Belimumab)	GlaxoSmithKline	NA
Orencia® (Abatacept)	Bristol Myers Squibb	NA
AAV9	Virovek	NA
Maurice System	Bio-Techne	090-158
MauriceFlex System		090-158
Maurice cIEF Cartridge		PS-MC02-C
Maurice cIEF Method Development Kit*		PS-MDK01-C
Phosphate Buffer Saline (PBS)	Sigma-Aldrich	P5493
Dithiothreitol (DTT)	Bio-Techne	042-251
DNase I Reaction Buffer (10X)	New England Biolabs	B0303S
Acetone	Millipore Sigma	100014
Iminodiacetic Acid (IDA)	Millipore Sigma	220000
Benzonase® Nuclease	Millipore Sigma	E8263-5KU

Table 1. Materials and reagents used in this study.
*The Maurice cIEF Method Development Kit contains all the necessary reagents for icIEF runs on Maurice/MauriceFlex systems.

Methods

Details for each sample are listed in the results section, along with their corresponding datasets. However, it should be noted that each prepared sample was split into two equal parts and loaded onto the Maurice and MauriceFlex systems respectively for parallel analysis. Data were generated using both absorbance and native fluorescence (NF) modes of detection (except AAV9, which was analyzed only with NF), and all data were analyzed with Compass for iCE Software.

Results

Mosunetuzumab (BsAb)

The BsAb sample was prepared at a final concentration of 0.1 mg/mL in an ampholyte solution containing 4% Pharmalytes 8-10.5 and 3-10 (3:1), 5 mM arginine, and pI markers 7.05 and 9.50. The sample was split into two parts, each of which was loaded onto the Maurice and MauriceFlex systems respectively for parallel analysis. A Maurice cIEF cartridge was inserted in each instrument. Samples were focused for 1 minute at 1500 V, then 12 minutes at 3000 V.

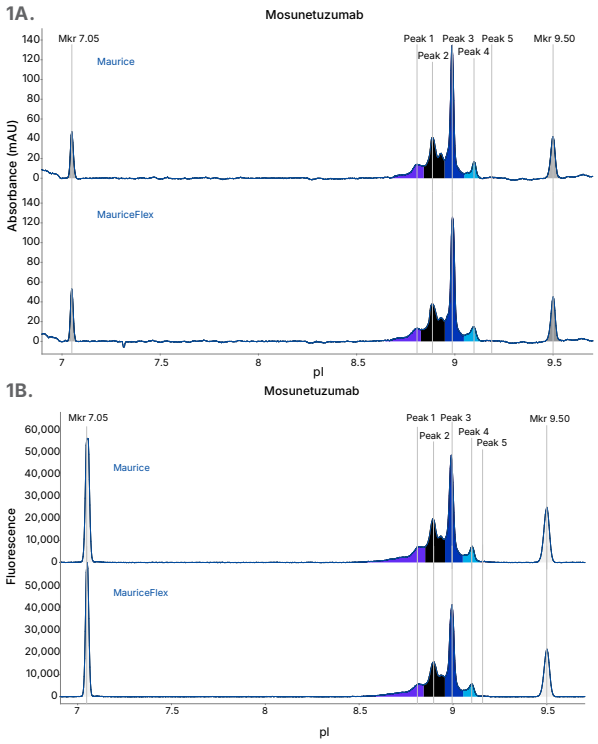


Figure 1. A comparison of charge profiles of Mosunetuzumab between Maurice and MauriceFlex systems, generated under **(A)** absorbance and **(B)** native fluorescence detection modes. The five major peaks detected are comparable between both instruments.

Figure 1A compares the charge profiles of Mosunetuzumab generated on the Maurice and MauriceFlex systems using absorbance detection, while **Figure 1B** compares the charge profiles between the two instruments using NF detection. In both modes, the data are highly comparable, generating near-identical percent peak area (%PA) values. **Figures 2A** and **2B** compare the average %PA values between both systems with absorbance and NF respectively, along with percent relative standard deviation (%RSD) values listed in **Table 2**.

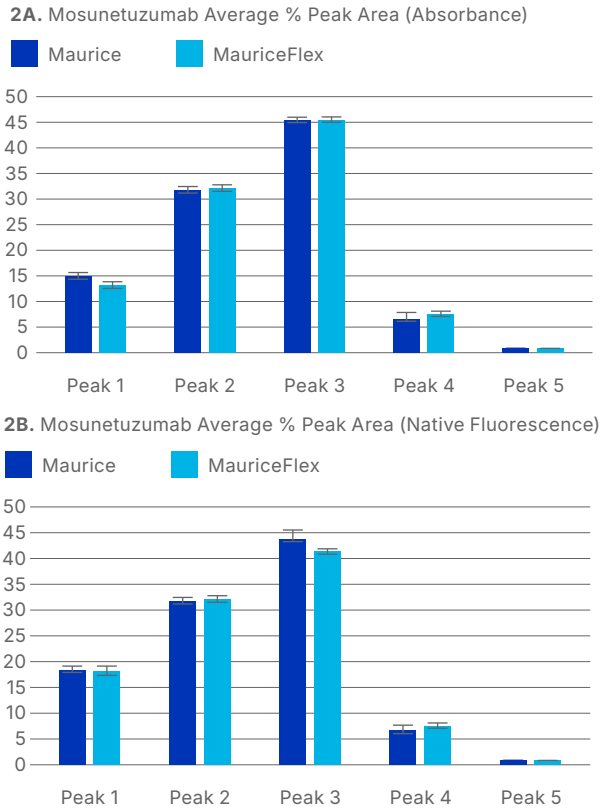


Figure 2. Graphical representation of average % peak areas for Mosunetuzumab charge variants (Peaks 1–5) analyzed on Maurice and MauriceFlex systems. Analysis was with **(A)** absorbance and **(B)** native fluorescence detection modes. Error bars indicate standard deviations, demonstrating comparable system performance.

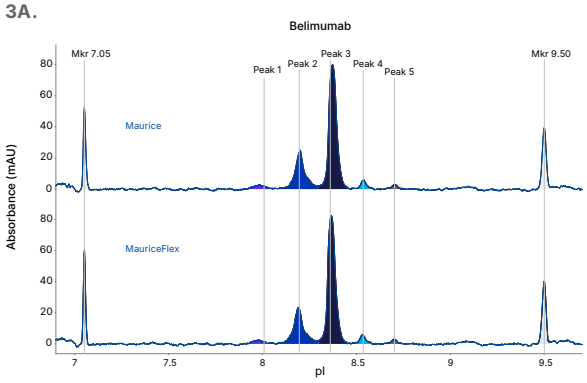
Mosunetuzumab Percent Peak Area (Absorbance); n=8										
	Maurice					MauriceFlex				
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**
%RSD	6.43	2.17	1.78	21.88	10.91	5.36	2.20	1.16	3.23	14.41
Mosunetuzumab Percent Peak Area (Native Fluorescence); n=8										
%RSD	2.67	2.79	4.34	11.84	21.76	4.41	1.68	1.12	2.84	14.28

Table 2. %RSD values of Mosunetuzumab across both systems are within acceptable ranges, demonstrating consistent performance between the two instruments.

Belimumab (mAb)

Belimumab (brand name Benlysta®) was prepared at a final concentration of 0.1 mg/mL in an ampholyte solution containing 4% Pharmalytes 8-10.5 and 3-10 (4:1), 20% SimpleSol, 5 mM arginine, and Maurice pI markers 7.05 and 9.50. The samples were loaded onto the instruments along with the Maurice cIEF cartridge and focused for 1 min at 1500 V, then 12 min at 3000 V.

3A.



3B.

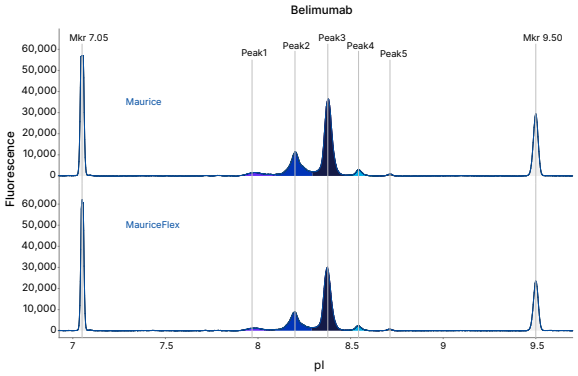
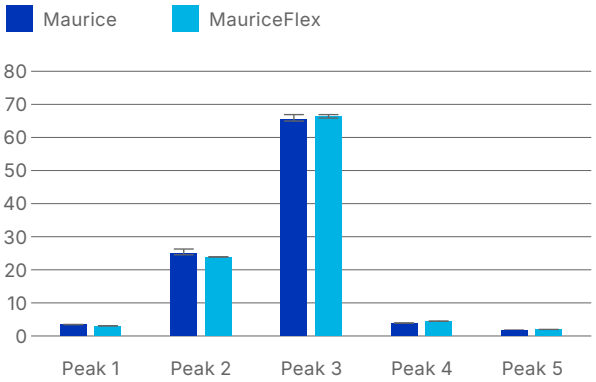


Figure 3. Comparison of Belimumab charge profiles between the Maurice and MauriceFlex systems, shown with **(A)** absorbance and **(B)** native fluorescence detection modes, demonstrating comparable results.

Figures 3A and **3B** show the charge profile of Belimumab detected with absorbance and NF modes respectively, and each figure compares data generated from the Maurice and MauriceFlex systems. Bar graphs supplementing comparative data are shown in **Figure 4**. For each detection mode, the data from both instruments are in alignment with each other, further evidenced by the summary in **Table 3**.

4A. Belimumab Average % Peak Area (Absorbance)



4B. Belimumab Average % Peak Area (Native Fluorescence)

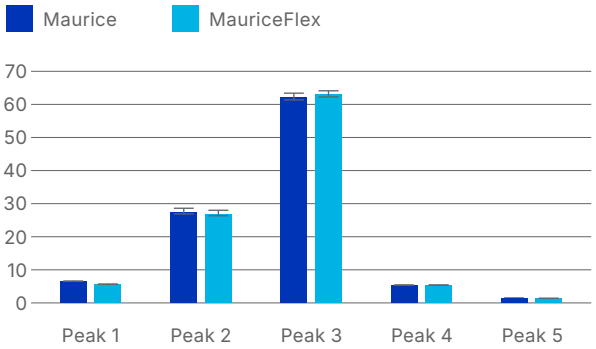


Figure 4. Comparison of average % peak areas for Belimumab (Peaks 1–5) analyzed on Maurice and MauriceFlex systems. **(A)** Absorbance and **(B)** native fluorescence detection modes were used. Error bars show standard deviations, indicating consistent results across platforms.

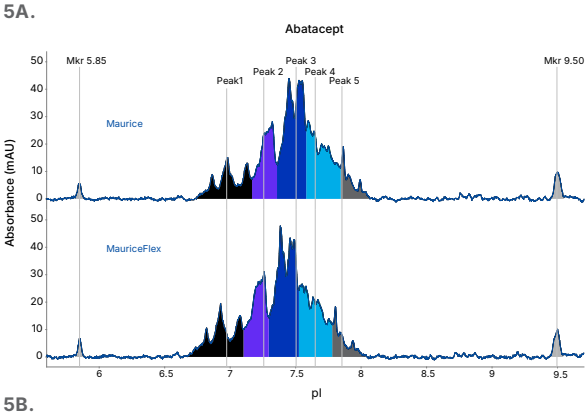
Belimumab Percent Peak Area (Absorbance); n=8										
	Maurice					MauriceFlex				
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**
%RSD	3.79	1.55	0.98	3.36	5.63	4.72	0.61	0.46	4.13	3.22
Belimumab Percent Peak Area (Native Fluorescence); n=8										
%RSD	4.08	1.95	1.41	2.75	6.29	4.07	1.87	1.32	4.37	5.52

Table 3. %RSD values for Belimumab show strong alignment and reproducibility between the two systems.

Abatacept (Fusion Protein)

250 mg of Abatacept was dissolved in 2 mL dH2O and stored at -80°C in aliquots of 125 mg/mL. Abatacept was prepared at a final concentration of 0.5 mg/mL in an ampholyte mixture containing 4M Urea, 0.35% MC, 1% SERVALYT (2-9), 5 mM IDA, pI Marker 5.85 and pI Marker 9.5. The samples were loaded onto the Maurice and MauriceFlex systems respectively, and separated for 1 min at 1500 V, then 6 min at 3000 V.

5A.



5B.

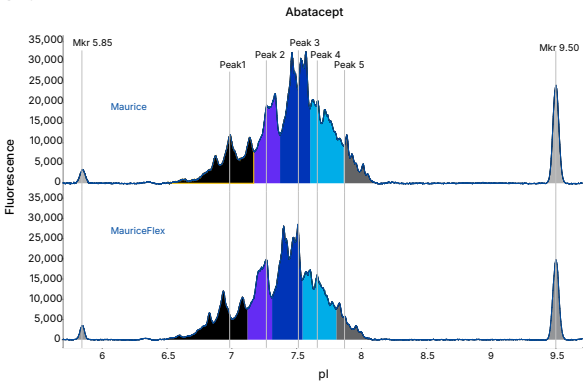
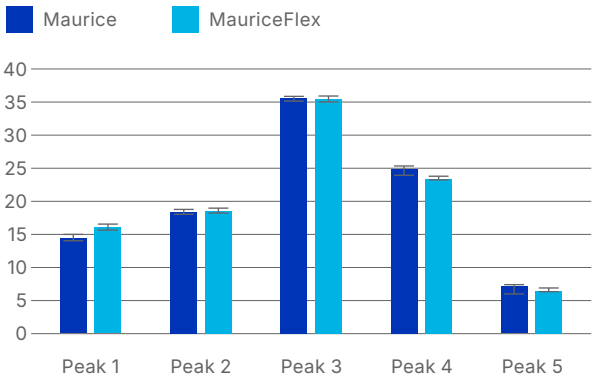


Figure 5. Charge profile comparison of Abatacept between the Maurice and MauriceFlex systems, displayed using **(A)** absorbance and **(B)** native fluorescence detection modes, showing consistent results between both systems.

Figure 5A compares the charge profiles of Abatacept between the two instruments using absorbance detection, while **Figure 5B** shows results when analyzed with fluorescence detection. Under each mode, the data from both instruments are in close alignment, as shown in **Figures 6A** and **6B** with %RSD values in **Table 4**.

6A. Abatacept % Peak Area (Absorbance)



6B. Abatacept % Peak Area (Native Fluorescence)

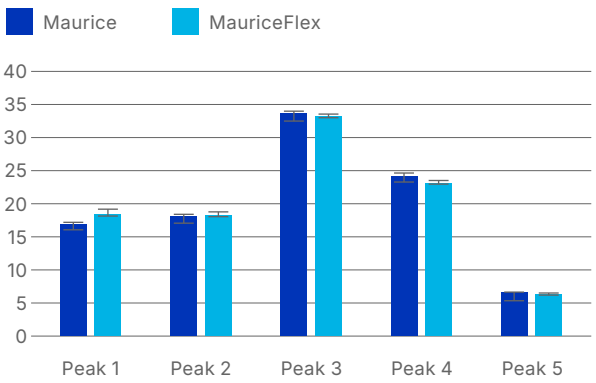


Figure 6. Visualization of average % peak areas for Abatacept (Peaks 1–5) using Maurice and MauriceFlex systems with **(A)** absorbance and **(B)** native fluorescence detection modes. Error bars denote standard deviations, confirming comparability between the systems.

Abatacept Percent Peak Area (Absorbance); n=8										
	Maurice					**MauriceFlex**				
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**
%RSD	2.57	1.95	0.90	1.58	2.34	2.02	1.87	1.29	0.89	2.25
Abatacept Percent Peak Area (Native Fluorescence); n=8										
%RSD	1.14	1.60	1.21	1.26	1.12	1.65	1.33	0.91	1.24	2.99

Table 4. Analysis of % peak area for Abatacept on Maurice and MauriceFlex Systems resulted in %RSD values comparable between each peak on both systems.

AAV9

Intact AAV Analysis: 10 µL of AAV sample was combined with 1 µL of DNase (diluted 1:5) and 1 µL of 10X DNase Buffer, followed by incubation for 30 minutes at 37°C. After incubation, the sample volume was adjusted to 25 µL with H_2O. The sample was then mixed with an ampholyte mixture (100 µL) containing 35% methylcellulose, 5% Pharmalyte 3-10, arginine (5 mM), IDA (5 mM), 22% SimpleSol, 22% formamide, pI Markers 5.85 and 9.5, and DI water. The samples were loaded onto the Maurice and MauriceFlex systems respectively, and separated for 1 min at 1000 V, 1 min at 2000 V, and finally 9 min at 3000 V. Data were generated through NF detection.

Denatured AAVs Analysis: 10 µL of AAV sample was combined with 1 µL of DNase (diluted 1:5) and 1 µL of 10X DNase Buffer, then incubated for 30 minutes at 37°C. After incubation, cold acetone (5X the sample volume) was added, followed by incubation for 1 hour at -80°C. The sample was centrifuged at 13,200 rpm for 10 minutes, and the resulting pellet was air-dried. The pellet was then resuspended in a 10 µL solution containing DMSO, 20 mM histidine/ 30 mM acetate buffer, and DTT in a 7:2:1 ratio. The mixture was incubated at 70°C for 5 minutes. Finally, 10 µL of the prepared sample mixture was added to an ampholyte mixture (50 µL) containing 0.35% MC, 3% Pharmalyte: 3-10, DTT (5 mM), IDA (5 mM), Arg (4.5 mM), pI Markers 4.05 and 8.4, Urea (4M) and 17% Formamide. The samples were loaded onto the Maurice and MauriceFlex systems respectively, and separated for 1 min at 1000 V, 1 min at 2000 V, and finally 12 min at 3000 V. Data were generated through NF detection.

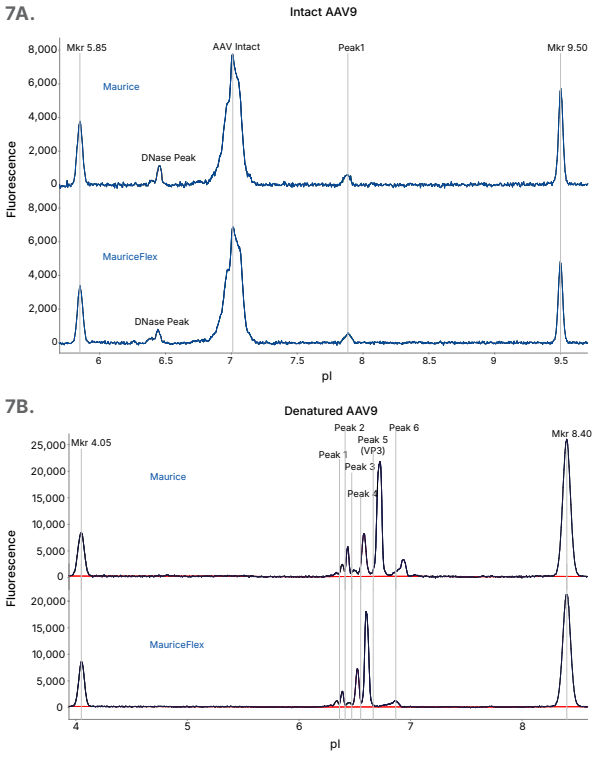


Figure 7. Charge profile comparison of AAV9 between the Maurice and MauriceFlex systems, using **(A)** the intact icIEF method and **(B)** denatured icIEF method. Using native fluorescence detection, the data are consistent across both systems.

Figure 7A compares the representative electropherograms of intact AAV9 generated from the Maurice and MauriceFlex systems, while **Figure 7B** compares the data for denatured AAV9. The denatured method allows for detection of AAV viral proteins – namely VP1, VP2, and VP3, the ratio of which is known to differ between serotypes and can impact transduction efficiency. Both the intact and denatured samples yielded consistent results between the instruments, also compared in Table 5, and graphically represented in **Figure 8A** and **8B**.

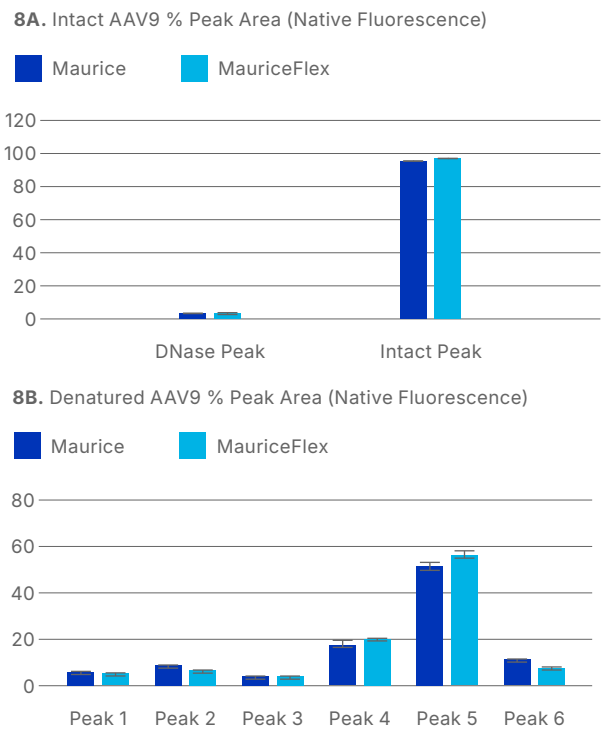


Figure 8. Graphical representation of average % peak areas for AAV9 charge variants analyzed using native fluorescence detection. **(A)** Intact AAV9 compares the %PA of the intact peak, while **(B)** denatured AAV9 compares sis different peaks. Error bars indicate standard deviations, highlighting consistent system performance.

Intact AAV9 Percent Peak Area (Native Fluorescence); n=8												
	Maurice						MauriceFlex					
	DNase Peak			Intact Peak			DNase Peak			Intact Peak		
%RSD	5.54			0.21			4.20			0.12		
Denatured AAV9 Percent Peak Area (Native Fluorescence); n=8												
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
%RSD	2.20	0.58	4.05	6.33	2.00	1.31	5.21	2.50	7.27	1.28	1.58	9.02

Table 5. %RSD values presented for the intact method (single peak) and denatured method (viral proteins).

Summary of Comparative icIEF Results

The bar graphs shown below in **Figure 9** and **Figure 10** summarize the percent peak area and pI value comparisons for all four biotherapeutic molecules—Mosunetuzumab, Belimumab, Abatacept, and AAV9—measured on both the Maurice and MauriceFlex systems. As demonstrated in the data throughout this tech note, the results are comparable across all molecules, highlighting the consistency and reliability of both systems in characterizing charge variants.

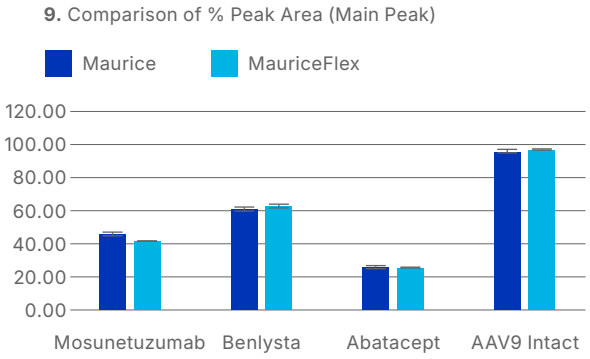


Figure 9. An overall analysis of performance on the Maurice and MauriceFlex systems. Percent peak area values for the most abundant peak of each molecule are displayed, with the blue and orange bars representing data from the Maurice and MauriceFlex system, respectively. Error bars indicate standard deviation, illustrating consistency in %peak area measurements between the two systems across all molecules analyzed.

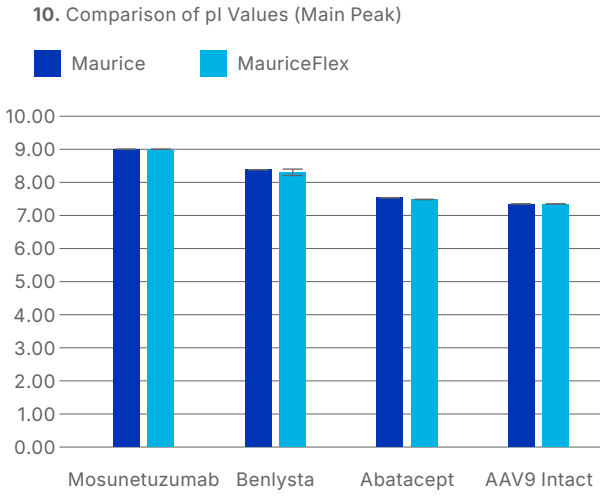


Figure 10. An overview of pI values measured between both systems for four different molecules. The bar graph displays the main peak pI values for each molecule, with the blue and orange bars representing Maurice and MauriceFlex, respectively. Error bars represent standard deviation, highlighting the close agreement in pI measurements between the two systems across all molecules.

Conclusion

This study demonstrates that icIEF data generated with the MauriceFlex system are comparable to those obtained with the Maurice system for all four molecules analyzed: Mosunetuzumab, Benlysta, Abatacept, and AAV9. Measuring the percent peak area, a key metric for icIEF instrument comparison, revealed consistent results between both systems. Data comparability was further corroborated by apparent pI values, thus underscoring the suitability of Maurice and MauriceFlex systems for charge variant analysis. These findings emphasize that either instrument can be effectively utilized across various phases of biotherapeutic development, including process development, quality control (QC), and other process stages. Whether for analytical development or QC release testing, both the Maurice and MauriceFlex systems provide high-quality, reproducible data for charge heterogeneity analysis, emphasizing their value as a key analytical tool in the biopharma labs.

Learn More About
Maurice and MauriceFlex