Accelerating icIEF and Fractionation Workflows with SupersonicIEF

A Guide to Assay Optimization on Maurice and MauriceFlex Systems

Maurice and MauriceFlex Systems both deliver charge heterogeneity results in approximately 10-15 minutes when the standard icIEF protocol is followed. While reasonably fast, this method can be further optimized to achieve even faster results, providing them as quickly as 5 minutes. Such a method has been developed and validated in a publication in *Electrophoresis;* titled *Development of the SupersonicIEF Method for High-Throughput Charge Variant Analysis*[1].

The SupersonicIEF method is based on the following principles:

1. Use of ampholytic blockers

- Equimolar concentrations of iminodiacetic acid (IDA) and arginine are used to compress the ampholytic gradient. The resulting compression accelerates the focusing process and increases peak sharpness without elevating current levels.

2. Implementing stepped voltage ramps

- A three-step voltage ramp (1500 V for 0.3 min, 2500 V for 0.2 min, and 3000 V for 0.2 min) is followed by a final focusing voltage of 4300 V for 3.5 minutes. This approach enables faster focusing while avoiding the high initial currents that can compromise capillary performance.

3. Lower ampholyte concentration

- Reducing the total ampholyte concentration to 2.5% helps mitigate Joule heating, allowing the method to operate at higher voltages. This ensures consistent performance across a range of sample types.

Benefits of the SupersonicIEF method

Reduces the total run time by 2-3x compared to standard icIEF protocols. For example, a full 96-well plate batch using SupersonicIEF can be completed in approximately 12 hours.

Provides excellent reproducibility, with demonstrated %RSD values for peak area consistently below 1.5% for main, acidic, and basic peaks.*

Works across a variety of sample types and sample concentrations.

Suitable for high-throughput applications and supports both early- and late-stage development workflows.

Reduces the time taken to verify charge isoform fractions with analytical icIEF.

*%RSD values may vary depending on the type of molecule.

General Guide for the SupersonicIEF Method for icIEF Assays

Table 1 lists the materials and reagents required for this method. The concentrations and ratios provided in the method are based on experiments that have been conducted, but further optimization may be necessary based on sample type.

Material	Vendor	Catalog #
Sample (e.g. mAb, fusion protein, ADC)	–	NA
Maurice or MauriceFlex System	Bio-Techne	**090-000/090-158**
Maurice cIEF or Maurice icIEF 400 Cartridge		**PS-MC02-C**
Maurice cIEF Method Development Kit*		**PS-MDK01-C**
NDSB195	Millipore Sigma	480001
Iminodiacetic acid (IDA)	Millipore Sigma	220000

TABLE 1.

Materials and reagents needed for the SupersonicIEF method.
*The Maurice cIEF Method Development Kit contains other necessary reagents, ampholytes, pI markers, and solubilizers for this method.

Prepare your samples to 1 mg/mL, then add to a master mix containing 3% Pharmalytes 8-10.5 and 3-10 (3:1 ratio), 400 mM NDSB195, 12.5 mM arginine, 12.5 mM IDA, pI markers 6.14 and 10.17. Load the samples and cartridge into the instrument.

Running conditions: Focusing for 0.3 min at 1500 V, 0.2 min at 2000 V, 0.2 min at 3000 V, and 3.5 min at 4300 V. Using native fluorescence detection, set the exposure time to 10 seconds for standard samples and 60 seconds for low-concentration samples.

Results

Figure 1 shows stacked profiles of USP mAb002 and USP mAb001, generated using the standard Maurice icIEF method and the optimized SupersonicIEF method, respectively. For each molecule, the profiles are highly comparable between both methods, suggesting that peak resolution is maintained even with gradient compression and stepped voltage increase.

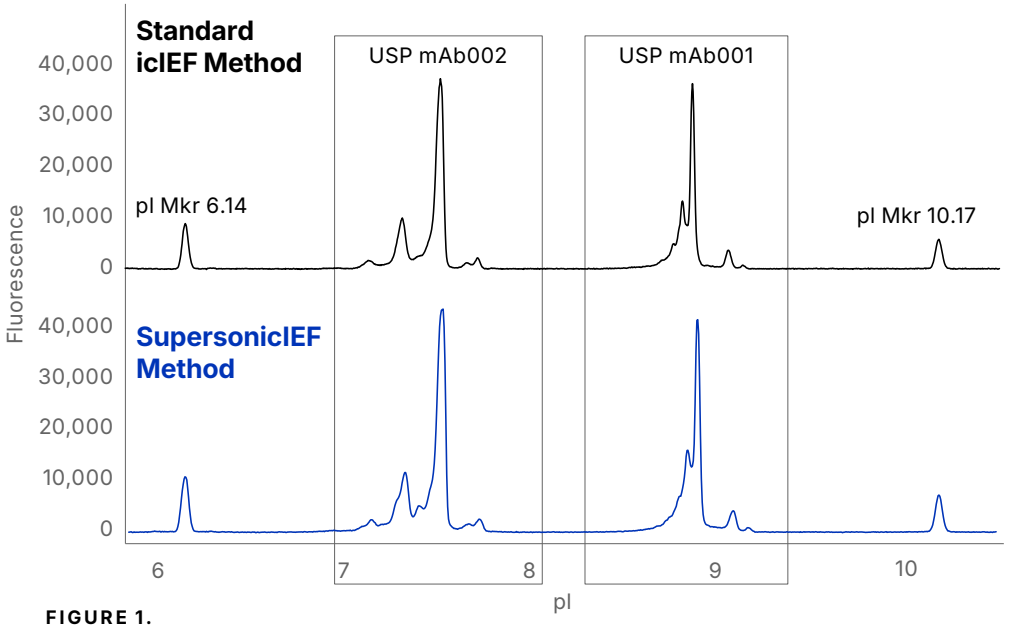


FIGURE 1.

A comparison of the standard icIEF method (black) and SupersonicIEF method (blue) on Maurice.
For each sample tested, the profiles between both methods are highly comparable.

Testing the SupersonicIEF Method on the MauriceFlex Cartridge

Designed for charge separation and mobilization of charge isoforms, the MauriceFlex Fractionation Cartridge features a larger diameter capillary. Lower voltages are required to keep the current low for this larger capillary. Furthermore, the mobilization step increases the run time. To accelerate the fractionation workflow, the method was optimized as follows:

1. **Applying the SupersonicIEF method to the focusing step during fractionation**
   - For better resolution, the MauriceFlex fractionation assay calls for additional arginine compared to the SupersonicIEF method discussed above, for increased resolution.
2. **Decreasing the mobilization current**
   - While ammonium acetate (CH3COONH4) is the standard mobilizer used in the MauriceFlex fractionation assay, replacing it with acetic acid (CH3COOH) and adding 20% formamide helps in suppressing current.
3. **Increasing the mobilization rate**
   - The mobilization voltage is increased from 1000 V to 1400 V.

Implementing these changes to the standard fractionation method accelerates focusing by 20 minutes, and accelerates mobilization time by another 20 minutes, resulting in a 40-minute faster method overall.*

*A 40-minute faster fractionation method has been documented based on experiments conducted on specific molecules, therefore it should be noted that the speed can vary depending on the type of molecule.

General Guide for the SupersonicIEF Method for MauriceFlex Fractionation Assays

Table 2 below lists the materials and reagents required for this method. The concentrations and ratios provided in the method are based on experiments that have been conducted, but further optimization may be necessary based on sample type.

Material	Vendor	Catalog #
Sample (e.g. mAb, fusion protein, ADC)	–	NA
MauriceFlex System	Bio-Techne	**090-158**
MauriceFlex cIEF Fractionation Cartridge		**PS-MC02-F**
MauriceFlex cIEF Fractionation Method Development Kit*		**PS-MDK01-F**
pI Standard 7.0 and 9.7		**040-031**, **040-790**
Acetic acid	Millipore Sigma	A6283
NDSB195	Millipore Sigma	480001
Iminodiacetic acid (IDA)	Millipore Sigma	220000

TABLE 2.

Materials and reagents needed for applying the Supersonic IEF method to fractionation assays.
*The Maurice cIEF Fractionation Method Development Kit contains other necessary reagents, ampholytes, pI markers, solubilizers, and mobilizers required for a typical fractionation assay.

Prepare your samples (0.5-3 mg/mL for mAbs, 1-3 mg/mL for ADCs), then add to a master mix containing 3% Pharmalytes 8-10.5 and 3-10 (3:1 ratio), 180 mM NDSB195, 16% SimpleSol, 25 mM arginine, 12.5 mM IDA, Maurice pI markers 7.05 and 9.5, and pI markers 7.0 and 9.72. Load the samples and cartridge into the instrument.

Running conditions: Focusing for 3 min at 300 V, 3 min at 600 V, 3 min at 800 V, 3 min at 2200 V, and 8 min at 2600 V. For mobilization buffer, use 10 mM acetic acid, 7.5 mM arginine, and 20% formamide in dH2O. Mobilize peaks for 20 min at 1100 V, followed by fraction collection for 45 seconds per fraction at 2600 V.

Results

Applying the SupersonicIEF Method to the Focusing Step of Fractionation

Figure 2 shows stacked profiles of USP mAb 001 focused using the standard MauriceFlex method (shown in blue, see **method development guide** for details), and using the SupersonicIEF method (shown in black). At this stage, standard mobilization conditions were used with ammonium acetate. The focusing time was reduced to 25 minutes with the SupersonicIEF method, compared to 45 minutes that is typical of the standard MauriceFlex method. Furthermore, elution times were brought down to 26 minutes from 37 minutes, likely due to the presence of IDA compressing the acidic portion of the gradient.

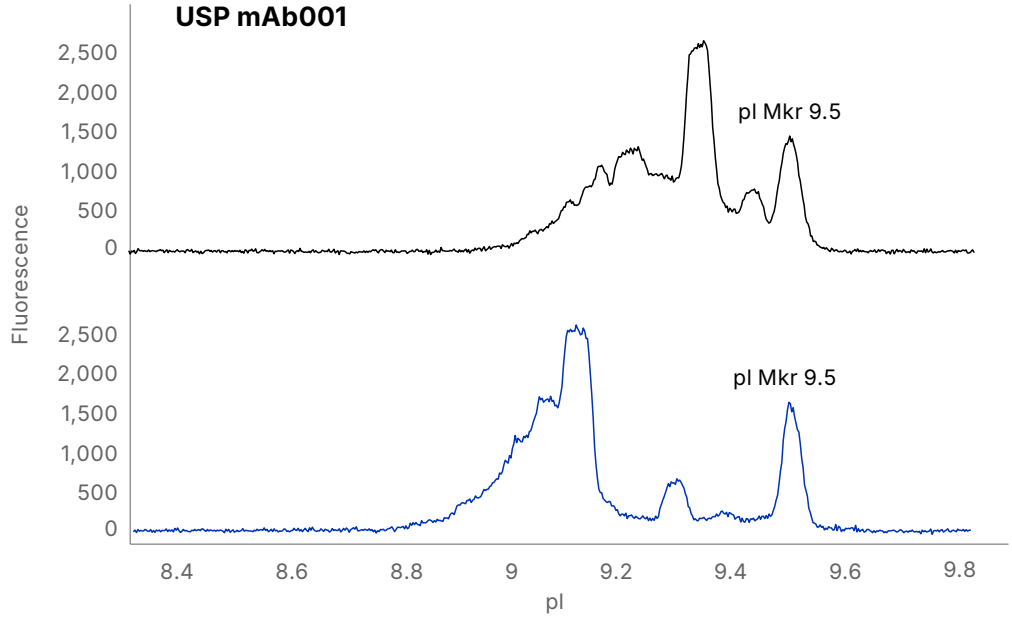


FIGURE 2.

A comparison of charge profiles of USP mAb 001 generated with the standard MauriceFlex method (blue) and the SupersonicIEF method (black). Using the SupersonicIEF method speeds up the focusing time but may differ in peak resolution compared to the standard method.

A decrease in resolution is possible during focusing with the SupersonicIEF method, but it improves during mobilization. As shown in **Figure 3**, mobilization causes sample stacking, which can recover or enhance resolution, thus making it easier to isolate and collect distinct charge variants.

Tip: Increasing the amount of narrow range ampholytes can also increase resolution if needed.

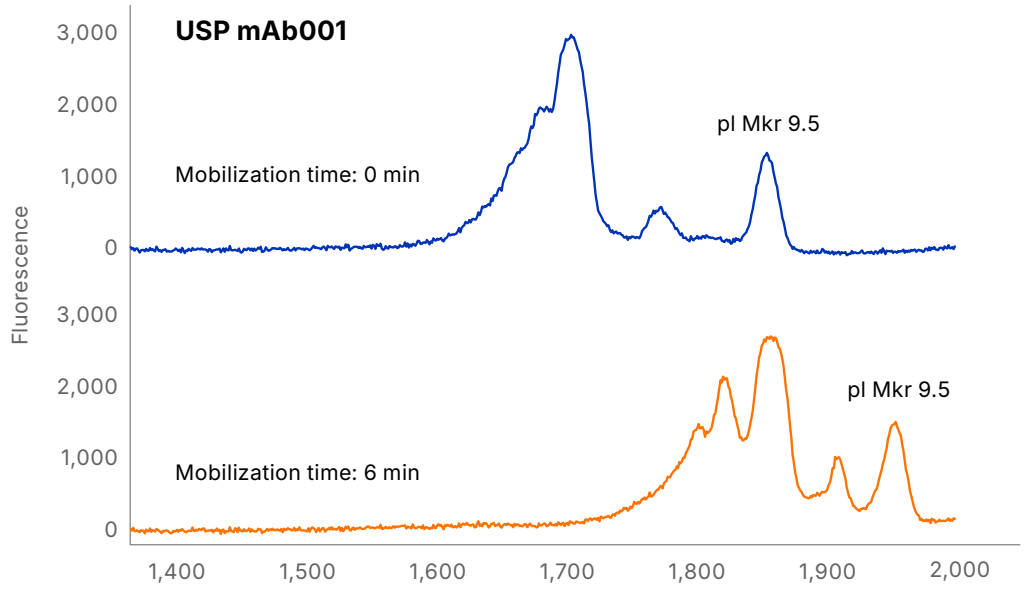


FIGURE 3.

Resolution recovery during mobilization of USP mAb 001 using the SupersonicIEF method.
The focused profile at time 0 (blue) shows broader peaks, while the profile at 6 minutes into mobilization (orange) shows improved resolution due to sample stacking. This effect enhances the separation of charge variants and enables better fraction collection.

Optimizing the Mobilization Step: The FlexsonicIEF Method for Faster Fractionation

Replacing ammonium acetate with acetic acid, buffered with additional arginine, accelerates the mobilization speed by approximately 20 minutes. **Figure 4** illustrates the difference between the mobilization speed of the standard MauriceFlex method versus the optimized method. Faster focusing with the SupersonicIEF method, combined with faster mobilization with the FlexsonicIEF™ method, results in fractionation that is at least 40 minutes faster!

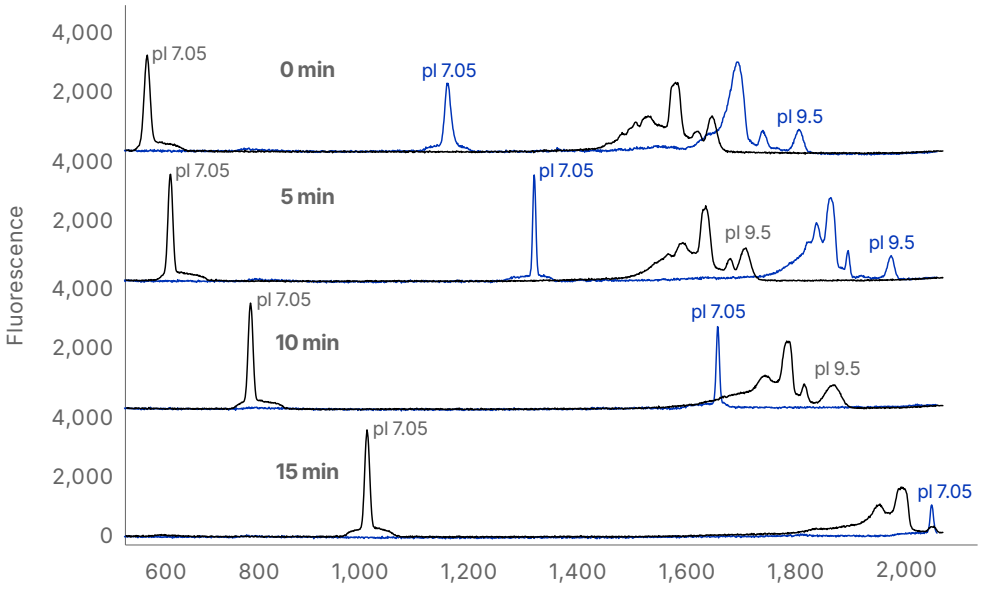


FIGURE 4.

Comparison of mobilization speed between the standard MauriceFlex method (gray traces) and the optimized FlexsonicIEF method (blue traces) at 0, 5, 10, and 15 minutes for the fractionation of USP mAb001. The FlexsonicIEF method uses acetic acid buffered with arginine and 20% formamide, enabling faster mobilization and reducing total fractionation time by approximately 40 minutes.

Increasing Sample Concentration for Greater Recovery

Higher sample concentrations allow for more material to be collected per fraction, which is particularly useful for downstream applications such as peptide mapping or mass spectrometry.

It is important to be aware of some challenges brought about by increasing sample concentration:

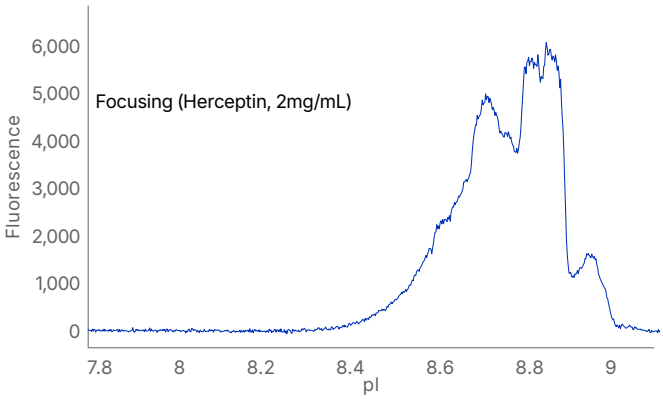
- **Elevated current:** Higher protein concentrations often correlate with higher salt content in the formulation buffer. This can lead to increased current, Joule heating, and in some cases, current loss during focusing or mobilization.
- **Sample aggregation:** Inadequate solubility at higher concentrations can result in protein precipitation, which may cause run failure or loss of acidic species.
- **Fluorescence quenching:** At higher concentrations, peak height may appear suppressed due to quenching, although this does not affect the actual fractionation.

The following optimizations can address the challenges listed prior:

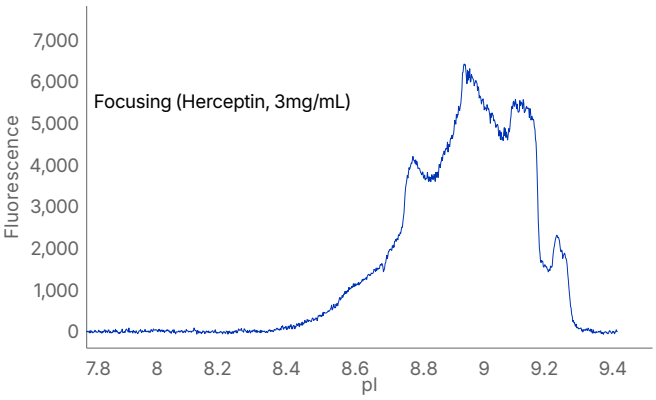
- **Desalting:** Reducing the salt content of the sample before analysis significantly lowers current and improves run stability. As shown in **Figure 5**, the desalted sample produces a more stable current profile and better recovery of acidic species compared to the non-desalted sample.
- **Solubilizer optimization:** A combination of urea, NDSB195, and SimpleSol enhances solubility and prevents aggregation. Urea is reconstituted in 0.5% methylcellulose to allow for higher concentrations and better compatibility with the icIEF matrix. Standard solubilizers and the recommended concentration ranges are listed in **Table 3**. Supplementing solubilizers with organic buffers (listed in **Table 4**) can help minimize sample loss.

To illustrate the success of this method, **Figure 5** shows profiles of Herceptin® (Trastuzumab) at 2 and 3 mg/mL. By optimizing the concentration of various solubilizers and desalting when required, the current was kept in check, leading to the fractionation of various charge isoforms of Herceptin.

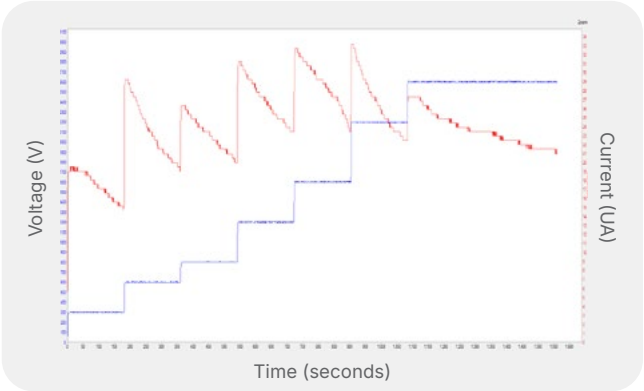
Herceptin, 2 mg/mL



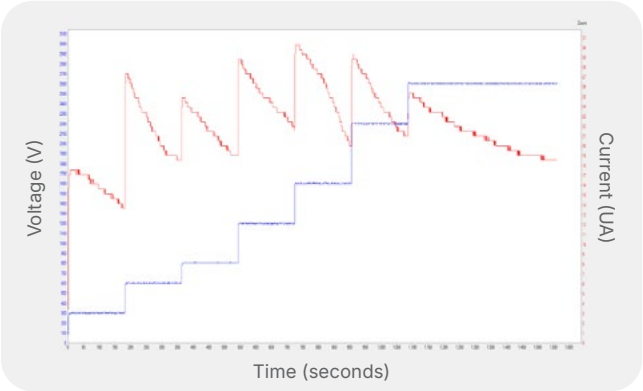
Herceptin, 3 mg/mL



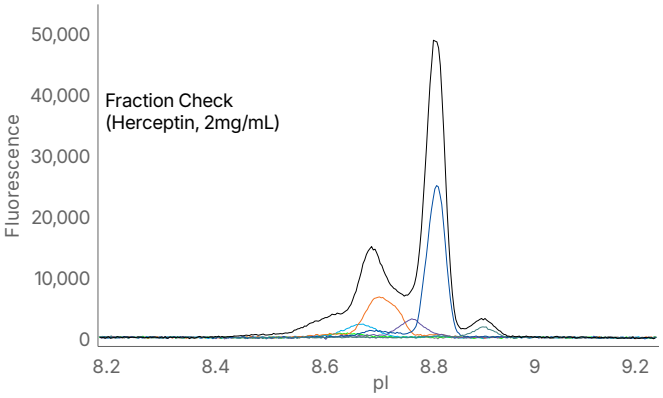
Herceptin, 2 mg/mL



Herceptin, 3 mg/mL



Herceptin, 2 mg/mL



Herceptin, 3 mg/mL

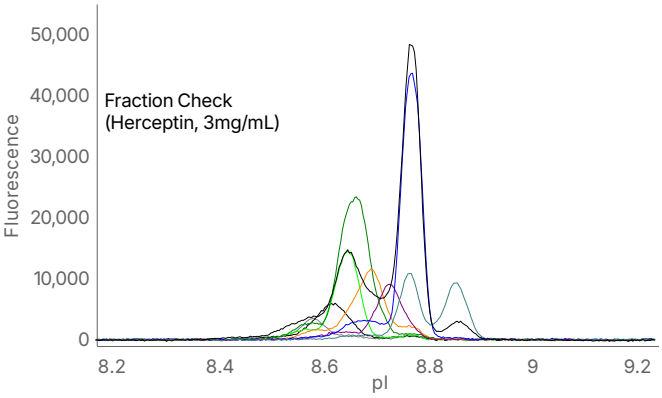


FIGURE 5.

Fractionation profiles of Herceptin® (Trastuzumab) at 2 mg/mL and 3 mg/mL final concentrations using the FlexsonicIEF method. The optimized solubilizer mix (3 M urea, 180 mM NDSB195, 20% SimpleSol) and desalting result in a stable current and successful mobilization of charge isoforms. Analytical icIEF overlay confirms that the collected fractions match the original sample profile (black trace, right-most figures), demonstrating reproducibility and resolution at elevated concentrations.

Solubilizers

Level	Urea	Formamide	NDSB195	DMSO	SimpleSol
Low	2	10	100	5	10
High	6	40	600	15	30
Unit	M	%	mM	%	%

TABLE 3.

A list of solubilizers typically used in icIEF and fractionation runs, along with their recommended ranges.
These solubilizers can be used individually or in combinations. Urea can be reconstituted with 0.5% methylcellulose instead of water.

Mobilizers

Level	Formamide	IPA	MeOH	ACN	Glycerol
Low	10	10	10	5	1
High	30	20	20	10	20
Unit	%	%	%	%	%

TABLE 4.

A list of mobilizers and their recommended ranges for charge variant fraction collection.

REFERENCES

1. McElroy, W., & Heger, C. D. (2024). Development of the SupersonicIEF Method for High-Throughput Charge Variant Analysis. *Electrophoresis*, 45(21-22), 1968–1975. **https://doi.org/10.1002/elps.202400117**
2. Technical Note: **Optimize Your icIEF Fractionation Method on the MauriceFlex System with Simple Western pI Markers**
3. **MauriceFlex cIEF Fractionation Method Development Guide**