Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

MOBIUS® POWER MIX 2000, 2500 and 3000

Performance Characterization and Scalability

Executive Summary:

Mobius® Power MIX 2000, Mobius® Power MIX 2500, and Mobius® Power MIX 3000 are high performance, high volume, single use mixing systems for GMP biopharmaceutical manufacturing.

The novel extension designs for Mobius® Power MIX 2500 and Mobius® Power MIX 3000 allow for flexibility in adjusting to high volumes, while maintaining the excellent mixing capabilities proven in the entire Mobius® Power MIX family. This note presents performance data for these high capacity Mobius® Power MIX systems for applications including mixing of high concentration buffers, cell culture media, and high viscosity solutions. Scalability for liquid-liquid, sinking powder, floating powder mixing, and temperature control is demonstrated for the full range of Mobius® Power MIX volumes from 100L to 3000L.

Figure 1:

Mobius® Power MIX 2000, Mobius® Power MIX 2500 and Mobius® Power MIX 300

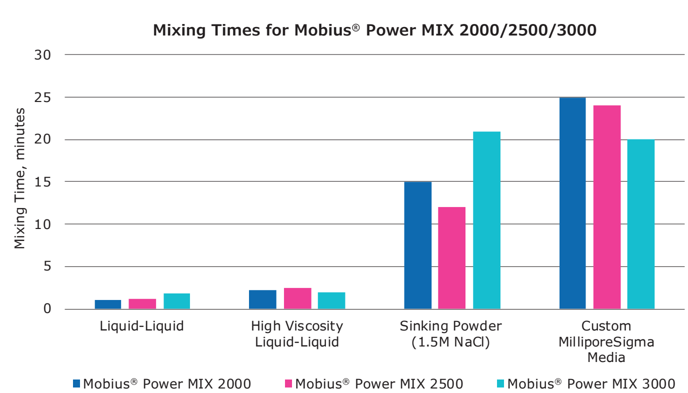


Figure 2:

Mixing Performance for Mobius® Power MIX 2000, Mobius® Power MIX 2500, Mobius® Power MIX 3000

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Introduction:

The challenges in mixing biopharmaceutical ingredients tend to increase with increasing volume. Creating mixing systems with a strong vortex and abundant surface movement is key to successful mixing of buffers (sinking powders), media (floating powders) and high viscosity solutions. The powerful magnetically coupled impeller in the Mobius® Power MIX 2000 produces this strong vortex and provides flow patterns to quickly incorporate both sinking and floating powders into solution. The novel design of the height extensions for the Mobius® Power MIX 2500 and 3000 enhance vortex formation and thereby improve mixing efficiency even with increased volume. To understand the capabilities of the Mobius® Power MIX 2000, Mobius® Power MIX 2500, and Mobius® Power MIX 3000, a series of characterization trials (outlined below) are performed, including trials to quantify mixing performance, vortex size, and temperature control functionality. The results of these characterizations are shown to fit into scalability models for the entire line of Mobius® Power MIX from 100L to 3000L.

Characterization Trials:

Category	Solute / Process Variables	Monitor
Liquid-Liquid Mixing	1.5M NaCl solution 80% nomonal volume, impeller speeds from 25% to 100% maximum	Conductivity
High Viscosity Liquid-Liquid Mixing	1.5 M NaCl solution into PEG 20000 solution 80% nominal volume, impeller speeds at 50% and 100% maximum	Conductivity
Sinking Powder Mixing	NaCl (to final concentration of 90 g/l) 80% nominal volume, maximum impeller speed	Conductivity, Particle Count
Floating Powder Mixing	MilliporeSigma custom CHO media powder 80% nominal volume, maximum impeller speed	Conductivity, Particle Count, pH
Vortex Size	80% nominal volume, impeller speeds from 25% to 100% maximum	Height, diameter, intensity
Temperature Control	Ramp temperature from 25°C to 60°C to 4°C, nominal volumes, impeller speed at 50% maximum	Rate of temperature change, temperature uniformity

Methods and Results:

Equipment:

- Mobius® Power MIX 2000 stainless steel jacketed carrier with Omega RTD-809 temperature sensor and load cells
- One extension to create Mobius® Power MIX 2500
- Two extensions to create Mobius® Power MIX 3000
- Mixer Assemblies for Mobius® Power MIX 2000, Mobius® Power MIX 2500, and Mobius® Power MIX 3000
- Lauda T10000 TCU
- Hamilton OneFerm Single Use pH VP 70 probe in Mixer Assembly probe port
- Mettler Toledo InPro 7100 Conductivity probes, with integral temperature sensors
- Mettler Toledo Particle Track G400 FBRM probe
- Datalogging equipment for recording pH, conductivity, particle count, temperature
- Video Camera
- Brookfield Low-Range Viscometer LVCE115

Materials:	Solution Concentrations:
• Sodium Chloride (MilliporeSigma S9888)	90 g/l
• CHO Media (MilliporeSigma Custom)	20.3 g/l
• Polyethylene Glycol 20000 (MilliporeSigma 8188975000)	320 g/l

Mixing Characterization:

Mixing performance is often quantified by analyzing concentration of an additive as it mixes into the system over time. This concentration is typically detected by a change in conductivity, for example, or through a visual change (i.e. color change or visual dissolution of particles or solids). For powder-liquid mixing, in addition to traditional conductivity and pH sensors, the Mettler Toledo® FBRM (Focused Beam Reflectance Measurement) probe is used to track particle size and distribution over time. Analysis of the trace curves of conductivity and particle distribution to find stability is used to determine mixing time, matching this measured response to the visual data.

Mixing times for liquid-liquid, liquid-liquid at high viscosity, sinking powder (buffer) and floating powder (media) mixing are determined at maximum impeller speed and 80% nominal volumes (1600L for Mobius® Power MIX 2000, 2000L for Mobius® Power MIX 2500, and 2400L for Mobius® Power MIX 3000). This reflects typical protocols for buffer and media mixing describing powder addition into 80-90% final liquid volume and Q.S. to final volume after mixing.

Liquid-Liquid by Conductivity

Adding a small amount of 1.5M NaCl solution (as described in table below) to DI water in the mixer produces a change in conductivity of approximately 30-50 µS/cm. Mixing time is defined as the time for conductivity reading to reach a stable value at least 99% of final reading. Liquid-liquid mixing times are determined at the critical 80% volume and four speeds (25%, 50%, 75% and 100% of maximum rpm). Results are summarized in Figure 3, with an example of conductivity response curve in Figure 4.

Liquid Volume	Description of Volume	mL of 1.5M NaCl
1600	80% nominal volume in Mobius® Power MIX 2000	480
2000	80% nominal volume in Mobius® Power MIX 2500	600
2400	80% nominal volume in Mobius® Power MIX 3000	720

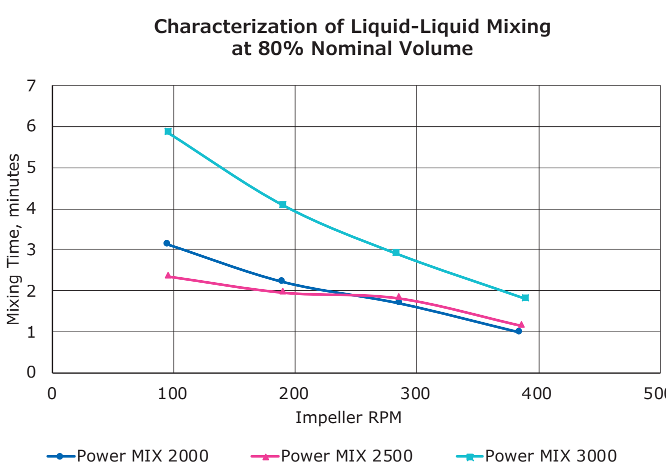


Figure 3:

Liquid-Liquid Mixing in Mobius® Power MIX 2000, Mobius® Power MIX 2500 and Mobius® Power MIX 3000

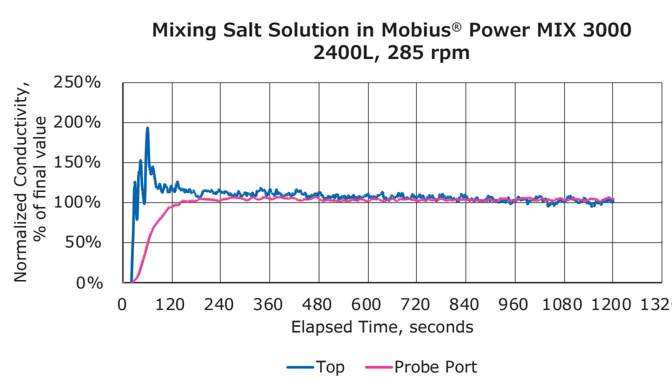


Figure 4:

Conductivity Response Curve for Liquid-Liquid Mixing in Mobius® Power MIX 3000

Liquid-Liquid at High Viscosity

To reach a target viscosity of 250 cps, 640 kg PEG 20000 is added to 2000L of DI water creating a final volume of 2400L in the Mobius® Power MIX 3000. Liquid-liquid mixing times are measured by the same conductivity method used for liquid-liquid mixing at 1 cps (in DI water). Mixing time is determined at two impeller speeds (maximum and half speed) at key volumes of 1600L for Mobius® Power MIX 2000, 2000L for Mobius® Power MIX 2500, and 2400L for Mobius® Power MIX 3000. The comparison of mixing time at 1 cps and 250 cps is shown in Figure 5. Mixing time at maximum rpm is similar for 1 cps and 250 cps solutions. Even at half speed, mixing time in a high viscosity solution is less than 5 minutes.

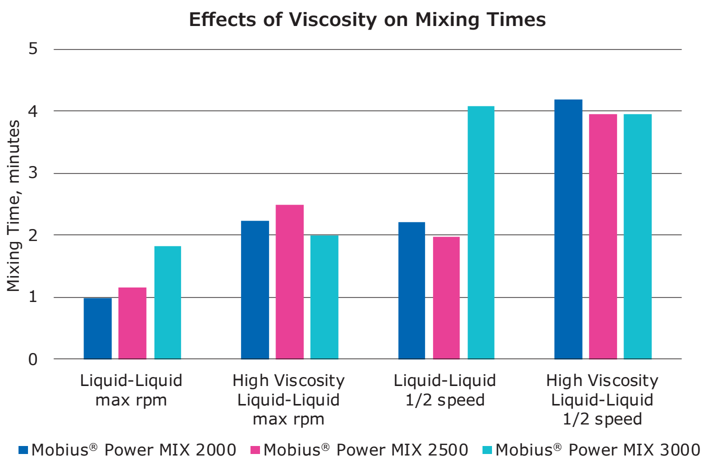


Figure 5:

Comparison of Mixing at 1 cps and 250 cps

Sinking Powder/Buffers

Buffer powders tend to sink quickly when added to solute (i.e. DI water). Buffer solutions show an increase in conductivity with dissolution of ions during mixing. Salts, such as NaCl, can be used to represent the sinking powder buffers. To characterize sinking powder mixing, NaCl solutions are made to concentration of 90 g/l, starting at 80% nominal volume. Although conductivity readings can appear stable before mixing is complete, mixing time is defined at the time when particle count reaches a steady state minimum value or when no particles are visible in the solution. Figure 6 gives the record of formulating 1.5M NaCl in Mobius® Power MIX 2000, Mobius® Power MIX 2500, and Mobius® Power MIX 3000 with mixing times of 15, 12, and 18 minutes respectively.

Sinking Powder Trials			Mixing Time, minutes			
Mixer	**Starting Volume, liters**	**NaCl added, kg**	**Time to add all powder**	**t99 conductivity**	**t99 particle count**	**Visually mixed**
Mobius® Power MIX 2000	1600	144	2	5	15	15
Mobius® Power MIX 2500	2000	180	2.5	9	12	12
Mobius® Power MIX 3000	2400	216	5	14	21	18

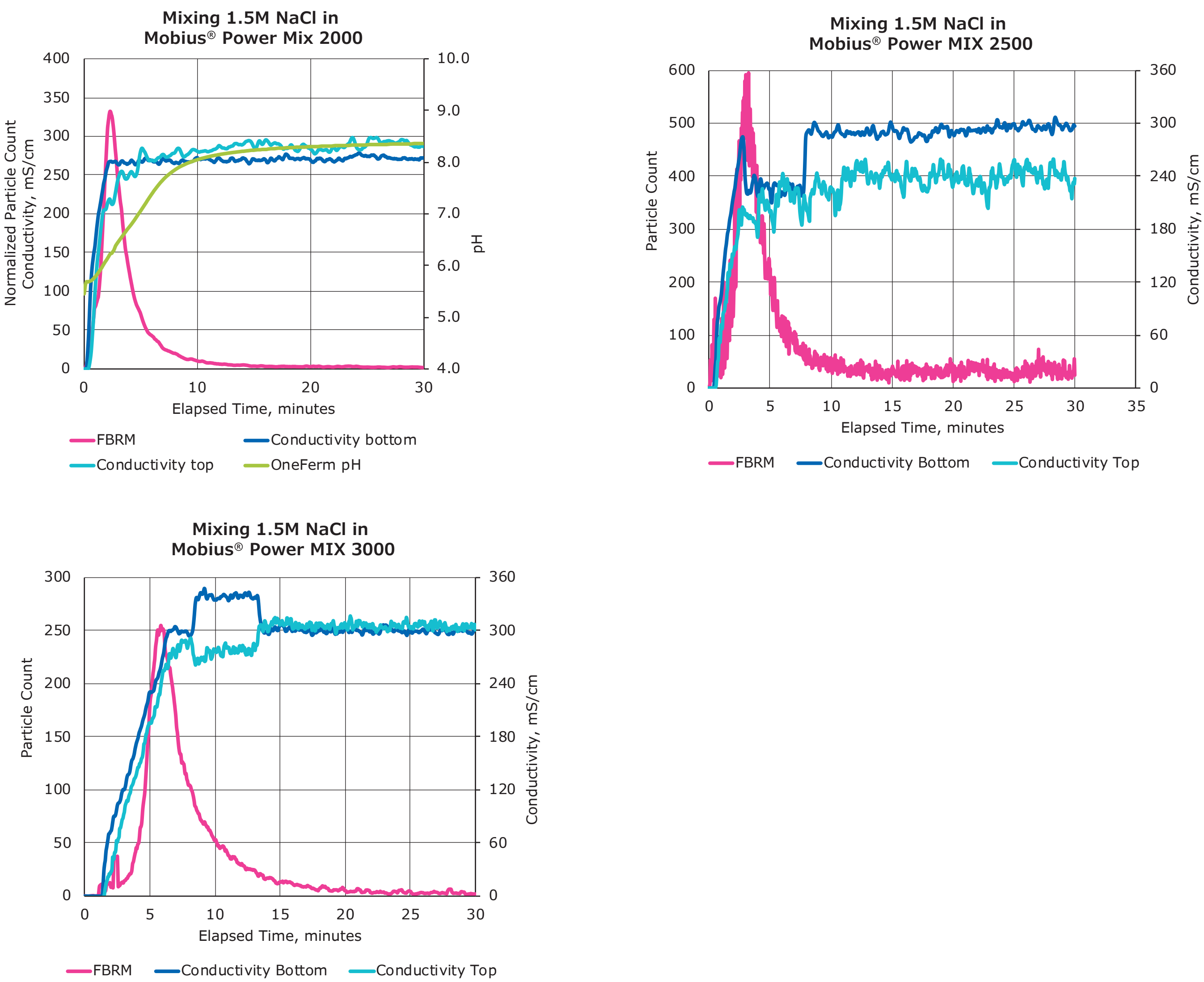


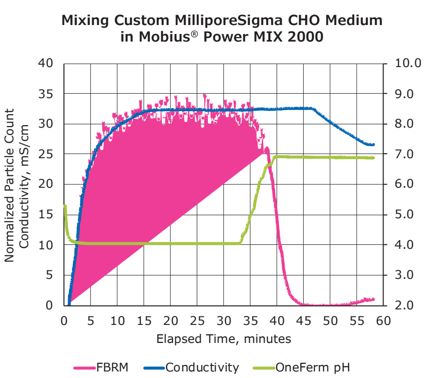
Figure 6:

Record of Sinking Powder Mixing Trials in Mobius® Power MIX 2000, Mobius® Power MIX 2500, Mobius® Power MIX 3000

Floating Powder/Media

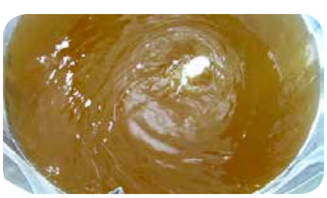
Media powders tend to float on the top of the water in the mixers, but are eventually incorporated in the liquid, with an accompanying rise in conductivity and particle count in the solution. Mixing time is defined at the time for conductivity and particle count readings to reach a stability at 99% of the final reading and by a visual record of the elimination of floating powder on the surface of the liquid, including agglomerates. Complete dissolution of component powders in chemically defined media sometimes occurs only after pH adjustment of the solution. A record of visual changes, along with drop in particle count, is used to quantify this step of media preparation. Records of media mixing trials in Figure 7 reflect mixing times of 25 minutes for Mobius® Power MIX 2000, 24 minutes for Mobius® Power MIX 2500, and 20 minutes for Mobius® Power MIX 3000. The decrease in mixing time for higher volume can be directly attributed to the increase in vortex size as the height of liquid is increased.

Floating Powder Trials			Mixing time, minutes			
Mixer	**Starting Volume, L**	**Media Powder added, kg**	**Time to add all powder**	**t99 conductivity**	**t99 particle count**	**Visually mixed**
Mobius® Power MIX 2000	1600	40.6	1	15	25	25
Mobius® Power MIX 2500	2000	50.75	3	9	28	24
Mobius® Power MIX 3000	2400	60.9	5	10	16	20

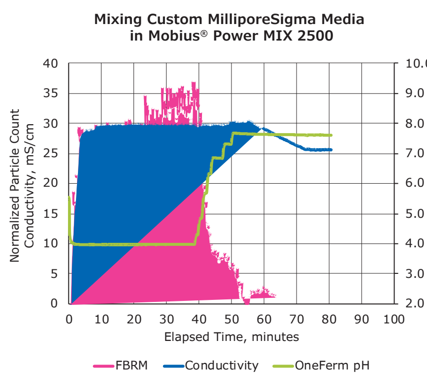


t99 conductivity

t99 particle count



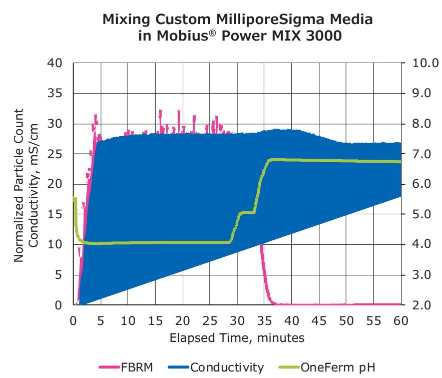
After pH adjustment



t99 conductivity

t99 particle count

After pH adjustment



t99 conductivity

t99 particle count

After pH adjustment

Figure 7:

Record of Mixing Chemically Defined Media, including pH Adjustment, in Mobius® Power MIX 2000, Mobius® Power MIX 2500, and Mobius® Power MIX 3000

Vortex Quantification

The size and intensity of the vortex is a key parameter in floating powder mixing. The addition of the height extensions to the Mobius® Power MIX 2000 to make Mobius® Power MIX 2500 and Mobius® Power MIX 3000 are done without any adjustment to impeller or motor. Even though power per volume is then reduced, mixing time for the media powder is not increased. Instead, the addition of liquid height results in a larger vortex that greatly enhances the mixing of the floating powder, moving the powder off the surface and into the bulk more quickly. Across the range of volumes and impeller speeds, a record can be made of the properties of the vortex; including vortex depth, width of vortex at top of liquid, and percentage of time vortex is present. These parameters are used to calculate relative vortex size. Examples of vortex characterization are given here with a plot of relative vortex size over multiple speeds (power per volume) at 80% nominal volumes shown in Figure 8.

Volume	RPM	W/m3	Observations on Vortex	Vortex Depth	% Vortex On	Vortex Width	Vortex Size	Relative Vortex Size
1600	385	128	Surface to impeller for 2-3 seconds, approximately every 10 seconds. Surface diameter approx. 4-6". Depth = approx. 0.5"	100%	20%	9%	1.87%	100%
2000	386	103	Nearly constant aggressive vortex. Intermittent moments without vortex last 1-3 seconds. Surface diameter 3-5". Depth = approx. 0.375".	100%	90%	9%	8.41%	450%
2400	390	86	Constant vortex, bottom stays within 1-2 feet of impeller. Surface diameter approx. 7-10".	75%	100%	19%	13.98%	748%

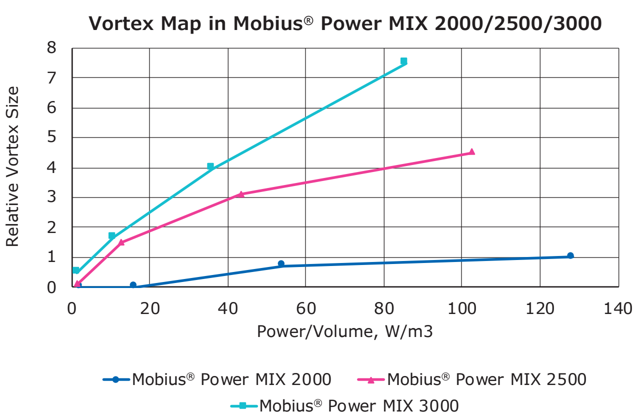


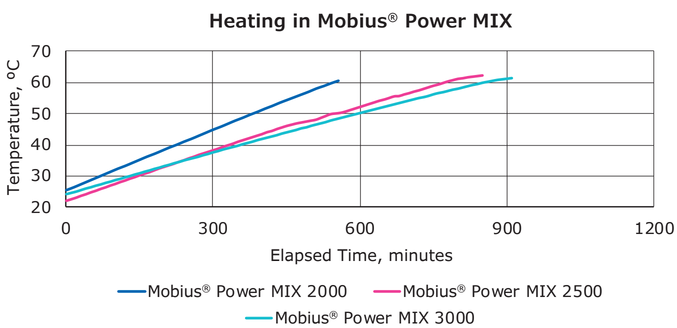
Figure 8:

Vortex Map for Mobius® Power MIX 2000, Power MIX 2500, Power MIX 3000

Temperature Control and Uniformity

The Mobius® Power MIX 2500 and Mobius® Power MIX 3000 are created by adding stainless steel extensions to the Mobius® Power MIX 2000 stainless steel vessel. The extensions are insulated stainless steel, while the Mobius® Power MIX 2000 vessel is jacketed for temperature control. Trials are performed in Mobius® Power MIX 2000, Mobius® Power MIX 2500, and Mobius® Power MIX 3000 at nominal volume to document the ability of the jacketed and insulated vessels to effectively increase and decrease temperature of liquid in vessel and maintain uniform temperature throughout the entire vessel. Temperature is monitored through an Omega RTD-809 sensor located in the bottom of the vessel, a temperature sensor in the probe port and a sensor hanging in the vessel at the top of the liquid. A record of temperature heating and cooling in each system is shown in Figure 9. Uniformity of temperature is documented in Figure 10, showing less than 1°C difference in temperature throughout the vessel for all volumes. This 1°C difference is within the calibration accuracy of the sensors.

Mixer	Heating	Cooling
	°C/min	
Mobius® Power MIX 2000	0.06	-0.05
Mobius® Power MIX 2500	0.05	-0.04
Mobius® Power MIX 3000	0.04	-0.03



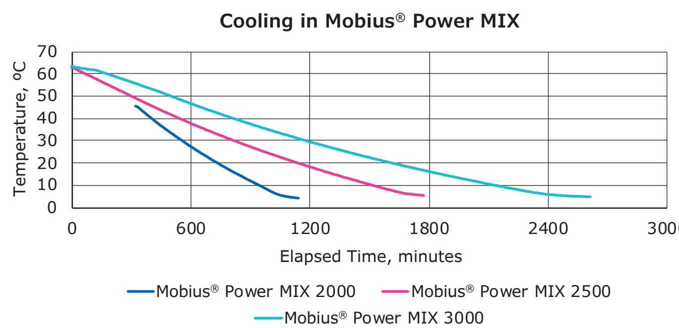


Figure 9:

Temperature Ramping in Mobius® Power MIX 2000, Mobius® Power MIX 2500, Mobius® Power MIX 3000

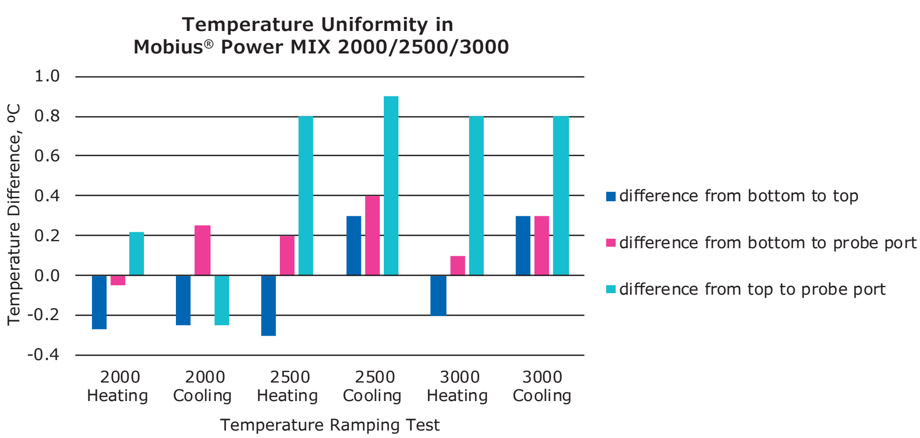


Figure 10:

Temperature Uniformity in Mobius® Power MIX 2000, Mobius® Power MIX 2500, Mobius® Power MIX 3000

Scalability:

Scalability from Mobius® Power MIX 100 to Mobius® Power MIX 3000 is demonstrated for liquid-liquid mixing, sinking powder mixing, floating powder mixing, and heating and cooling rates.

Liquid-Liquid Mixing

Characterization of mixing performance is often defined by the relationship between mixing time and power input for any given system. An example of theses characterization maps for Mobius® Power MIX 200 and Mobius® Power MIX 2000 are given in Figure 11, showing the typical power curve relationship between mixing time and power input.

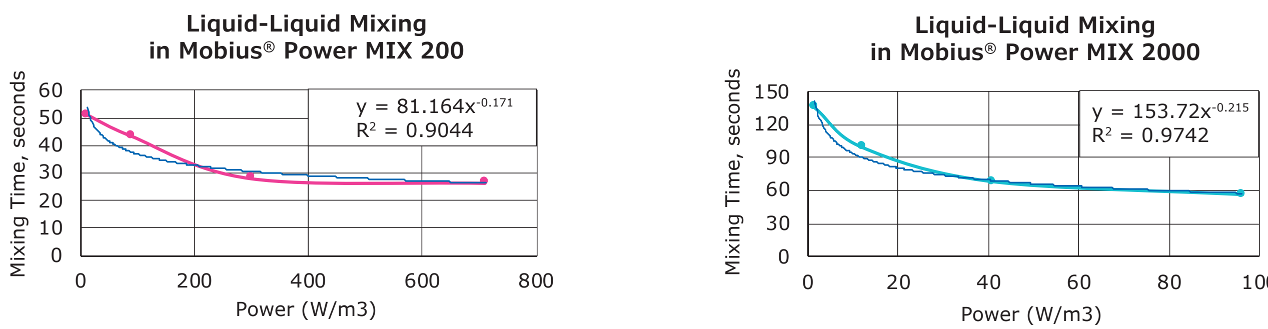


Figure 11:

Mixing Time as function of Power/Volume for Mobius® Power MIX 200 and Mobius® Power MIX 2000

To show scalability between sizes of Mobius® Power MIX, it is possible to relate mixing time at a key condition (i.e. 80% nominal volume, maximum rpm) to the power/volume at that same condition.

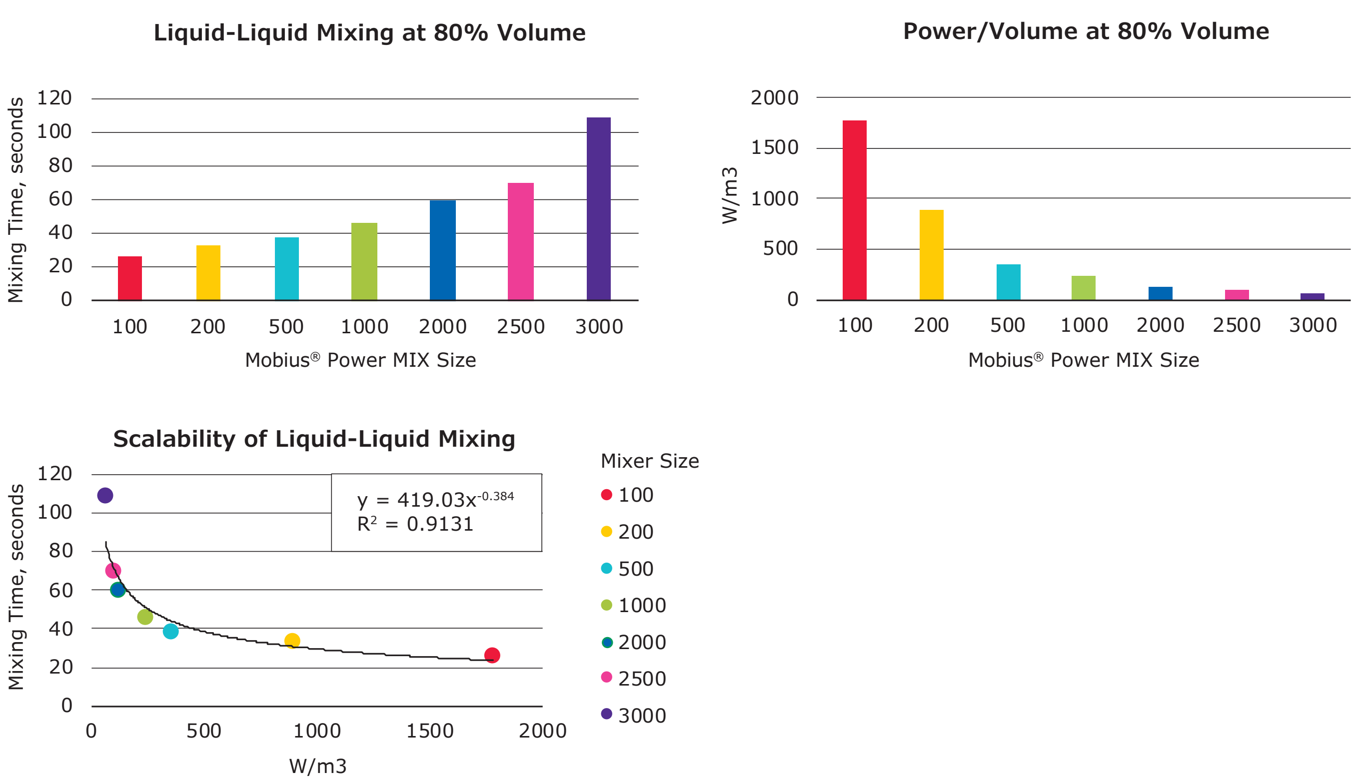
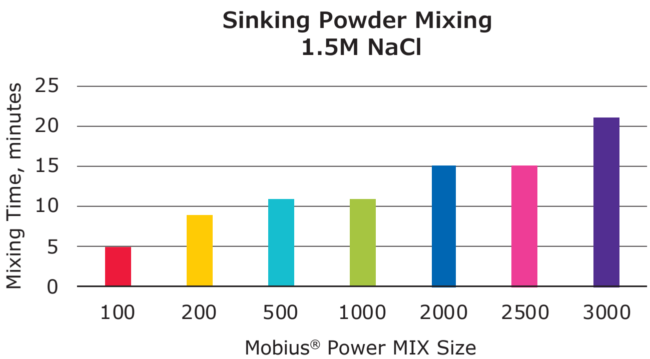


Figure 12:

Scalability in Liquid-Liquid Mixing across all volumes of Mobius® Power MIX from 100L to 3000L showing typical power curve relationship between mixing time and power input

Sinking Powder Mixing

A similar power curve relationship can be created for mixing sinking powders. In fact, sinking powder mixing time can be directly correlated to liquid-liquid mixing time at the same conditions.



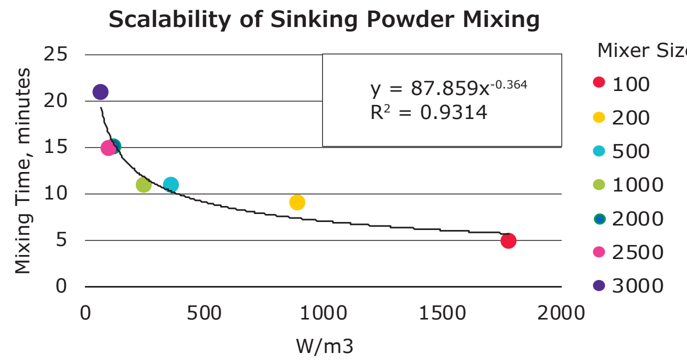


Figure 13:

Scalability in Sinking Powder Mixing across all volumes of Mobius® Power MIX from 100L to 3000L showing typical power curve relationship between mixing time and power input

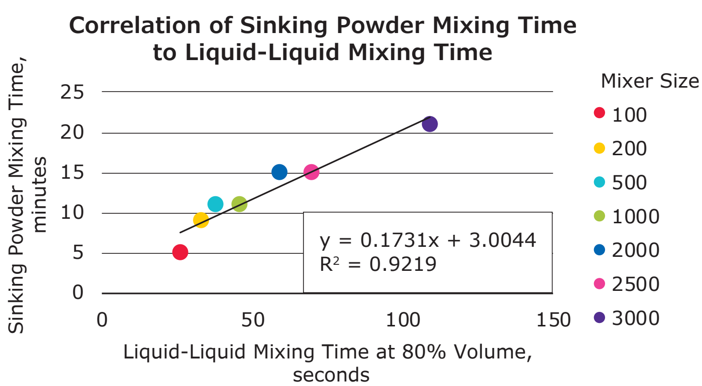
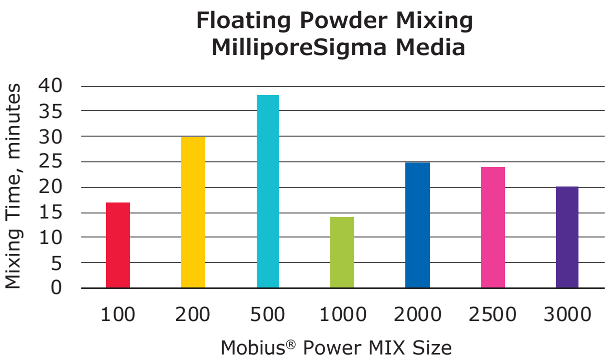


Figure 14:

Correlation between Liquid-Liquid Mixing Time and Sinking Powder Mixing Time across Mobius® Power MIX volumes from 100 to 3000L

Floating Powder Mixing

Using the same relationships for Floating Powder mixing appears to show two distinct power curves for mixing time versus power/volume. One curve provides a good model for the lower volume mixers (up to 500L), with a second curve for volumes from 1000L to 3000L.



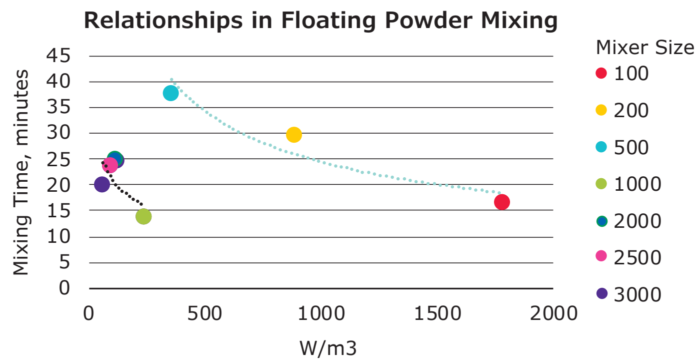
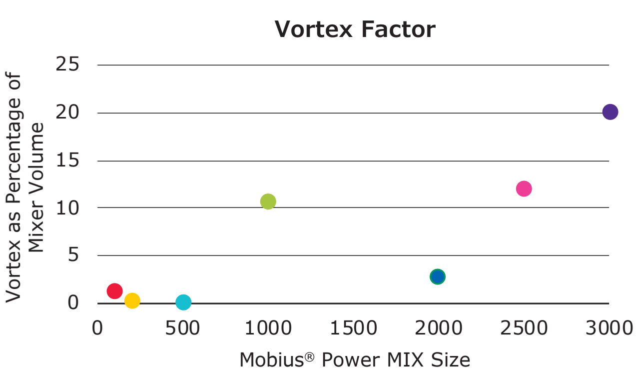


Figure 15:

Scalability in Floating Powder Mixing does not show typical power curve relationship between mixing time and power input

A secondary factor is needed to find a scalability relationship for floating powder mixing. It is observed in the mixing trials that the vortex in large scale systems (1000L to 3000L) is stronger and larger than in the systems from 100L to 500L. Characterization of vortex size is achieved by quantifying vortex depth, width and percentage of time the vortex appears. This allows for a relative vortex size (i.e. Vortex Factor) to be recorded for a range of speeds at key volumes in each mixer. The Vortex Factor is directly related to liquid height times power per volume.



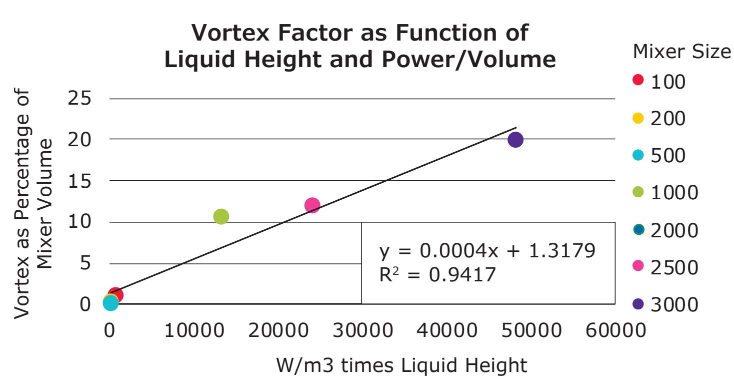
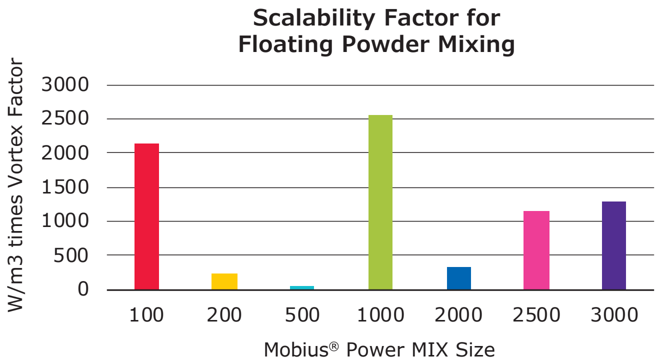


Figure 16:

Vortex Factor across all Mobius® Power MIX volumes from 100L to 3000L at maximum impeller speed and 80% nominal volume

It is then possible to relate mixing time to power per volume multiplied by the Vortex Factor as shown in Figure 17. Using this Scalability Factor once again produces the typical power curve relationship between mixing time and power input.



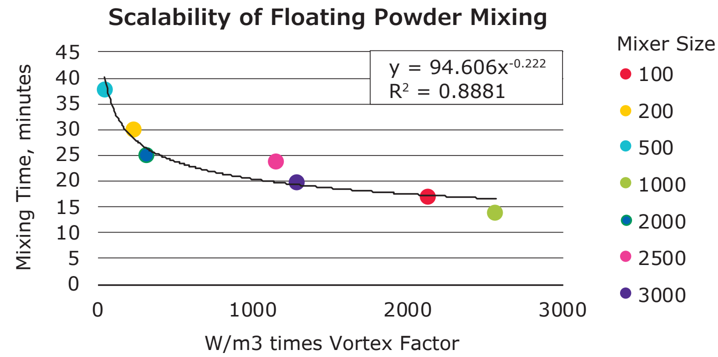
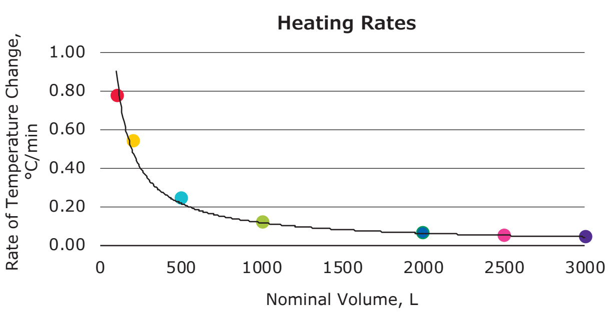


Figure 17:

Scalability in Floating Powder Mixing across all volumes of Mobius® Power MIX from 100L to 3000L showing typical power curve relationship between mixing time and Scalability Factor (power input multiplied by Vortex Factor).

Temperature Control

Trials to evaluate the heating and cooling rates for the full line of Mobius® Power MIX equipment are all conducted using the same Lauda T10000 Temperature Control Unit. Keeping the TCU constant allows for a comparison of the designs across all volumes. The graphs in Figure 18 show the heating and cooling rates from 100L to 3000L follow a typical power curve. There is no additional increase in heating or cooling time for the Mobius® Power MIX 2500 and Mobius® Power MIX 3000 systems with the insulated extensions on top of the jacketed Mobius® Power MIX 2000.



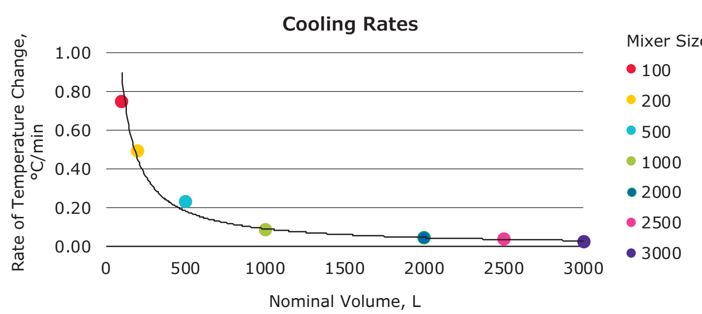


Figure 18:

Scalability for Heating and Cooling Rates from Mobius® Power MIX 100 to Mobius® Power MIX 3000

Conclusion:

The Mobius® Power MIX 2000, Mobius® Power MIX 2500 and Mobius® Power MIX 3000 provide effective clinical scale, single-use solutions for mixing of biopharmaceutical ingredients. Characterization of mixing performance across the range of processes from liquid-liquid mixing to media preparation demonstrate the outstanding capabilities of these systems. Scalability of performance is maintained across the entire line of Mobius® Power MIX systems from 100L to 3000L.

For more information on the Mobius® Power MIX, refer to Mobius® Power MIX Data Sheets and Specification Sheets and these Application Notes:

- Mobius® Power MIX 100 Mixing Characterization for Buffer and Media Preparation
- Mobius® Power MIX 200 Mixing Characterization for Buffer and Media Preparation
- Mobius® Power MIX 500 Mixing Characterization for Buffer and Media Preparation
- Mobius® Power MIX 1000 Mixing Characterization for Buffer and Media Preparation
- Mobius® Power MIX 2000 Mixing Characterization for Buffer and Media Preparation

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