Millipore®
Filtration, Separation
& Preparation

Application Note

Mobius® Power MIX 2000

Mixing characterization for buffer and media preparation.

Executive Summary

Mobius® Power MIX 2000 liter high performance, single-use mixing system meets the increasing demands for single-use systems in GMP biopharmaceutical manufacturing. Leveraging the proven technology of MilliporeSigma's magnetically coupled NovAseptic® mixers (traditionally used with stainless steel tanks) the new Mobius® Power MIX platform offers solutions for a wide range of mixing applications, including high concentration buffers and hard-to-mix cell culture media. This application note presents performance data from the Mobius® Power MIX 2000 for mixing of several sinking and floating powders. A characterization map of liquid-liquid mixing for a range of volumes and speeds is also included.

Table 1. Mixing times demonstrated in the Mobius® Power MIX 2000

Mixing Type	Final Product	Mixing Time
Liquid-Liquid	NaCl solution	1 minute
Sinking Powder, low concentration	1X PBS Buffer	2 minutes
Sinking Powder, high concentration	1.5M NaCl	15 minutes
Floating Powder, basal medium	DMEM	5 minutes
Floating Powder, soybean-casein digest medium	TSB (Tryptic Soy Broth)	20 minutes
Floating Powder, chemically defined medium	Custom MilliporeSigma CHO media	25 minutes

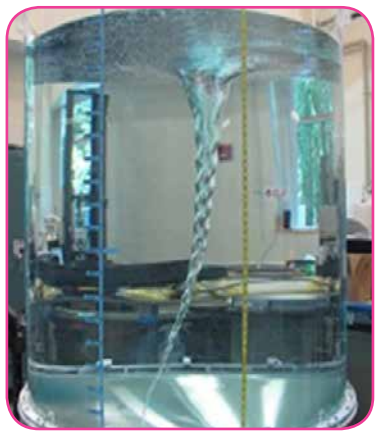

Figure 1.
Mobius® Power MIX 2000 final production vessel (top image) and prototype tank, used in development (bottom image)

Introduction

Mixing of buffers (sinking powders) and media (floating powders), especially at high volume, presents substantial challenges in getting good dispersion and dissolution of particles. The creation of a vortex and abundant surface movement in the Mobius® Power MIX 2000 is key to the success in these processes. The axial and radial flow patterns allow for quick distribution of sinking powders, minimizing settling at the bottom of the vessel. Floating powders are drawn into the vortex, allowing for effective wetting and distribution throughout the entire vessel volume. With impeller speeds up to 380 rpm, mixing time for even the most difficult to mix powders can be reduced to less than 30 minutes.

Quantification of mixing time is traditionally accomplished by tracing the response of pH and/or conductivity, along with visual observations of powder dissolution. In these trials, conductivity and pH sensors are in two locations within the vessel: in the probe port at minimum volume and at surface of the liquid. Video records of the mixing process provides the visual data.
In addition to conductivity and pH measurements, an FBRM® probe is used to track the distribution of particles over time. The FBRM® (Focused Beam Reflectance Measurement) probe from Mettler Toledo® uses a focused laser beam directly into the process, utilizing the backscatter of light to measure and count particles.[1] 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.

Materials and Methods

The Mobius® Power MIX 2000 includes:

- Mobius® Power MIX 2000 jacketed carrier with temperature sensor and load cells
- 2000L Mixer Assembly
- Hamilton OneFerm® Single-Use pH VP 70 probe in Mixer Assembly probe port

Additional Equipment:

- Mettler Toledo® InPro® 7100 Conductivity probes
- Mettler Toledo® Particle Track G400 FBRM® probe

The general procedure for buffer and media preparation includes:

1. Fill bag to recommended volume with DI water.
2. Run impeller at maximum speed (380 rpm).
3. Add appropriate amount of solute. (Additions made at location of powder port above impeller, although open-top liner was used for better visual record.)
4. Add powder as quickly as possible to fully tax mixing capability. Record addition time.
5. Record process using several sensors: two conductivity sensors, one at top of the liquid and the second installed in probe port at minimum volume; one Hamilton OneFerm® Single-Use pH sensor, installed in second probe port; one FBRM® probe, installed at minimum volume, in place of a sample port; and a video camera set to record at the top of the vessel.
6. Run mixer for at least 10 minutes past time when no visible powders are present.
7. For media mixes, titrate with NaOH (5N for high volume) to reach recommended pH value and then add DI water to reach final volume.
8. Perform data analysis on conductivity and particle distribution traces to find time to reach t99 (99% of final value) and ± 1% process stability.
9. Identify excerpts from video records compiled to show distribution and dissolution of powders and quality of mixing.

Table 2. Buffer and media preparation specifications

Solution	Starting Volume liters	Solute	Powder added, kg	Final Concentration g/l
1X PBS Buffer	1800 (90%)	Dulbecco's Phosphate Buffered Saline (Sigma D5773)	19.72	9.86
1.5M NaCl	1600	Sodium Chloride (Fisher S67110)	144.00	90
TSB	1800 (90%)	Becton, Dickinson & Co Select APS TSB (Ref 214887)	60.00	30
DMEM	1800 (90%)	Dulbecco's Modified Eagle's Medium – high glucose (Sigma D5648)	27.42	13.7
Custom MilliporeSigma CHO Media	1800 (90%)	Custom MilliporeSigma CHO Media	40.63	20.3

Table 3. Results

Solution	Time to add all powder	Time to t99 conductivity	Time to complete visual mixing	**Total Mixing Time** Complete Particle Distribution and Dissolution
1X PBS Buffer	30 seconds	2 minutes	2 minutes	2 minutes
1.5M NaCl	2 minutes	5 minutes	15 minutes	15 minutes
DMEM	2 minutes	5 minutes	5 minutes	5 minutes
TSB	3 minutes	10 minutes	20 minutes	20 minutes
CHO Medium	1 minute	15 minutes	25 minutes	35 minutes (includes pH titration)

Figures 2 through 6 provide a record of each mixing trial, with images at key milestones in the process and traces of conductivity, pH or particle count to quantify the mixing progression.

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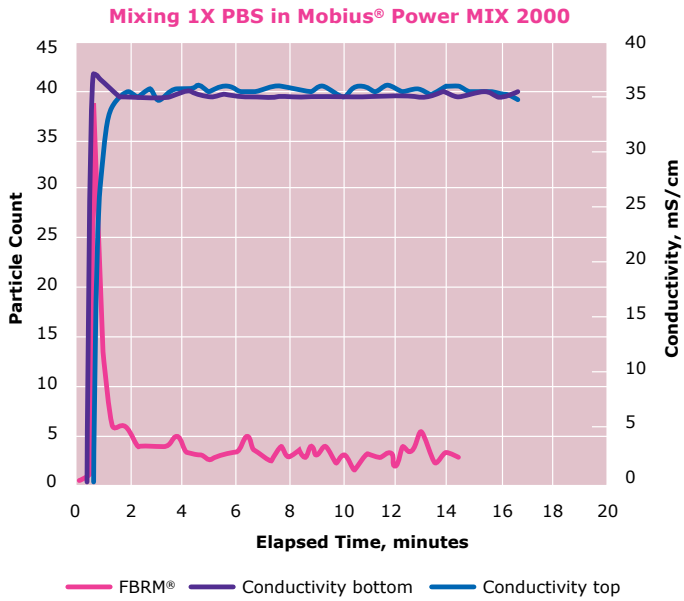


Figure 2.
At **2 minutes**, process data for mixing **1X PBS** in Mobius® Power MIX 2000 shows conductivity reaching final value at both top and bottom of vessel, and particle count reaching minimum. At same time, visual mixing is complete as it is once again possible to see through to the bottom of the vessel. No further changes in measured values or visual were seen after 2 minutes, confirming complete mix at that time.

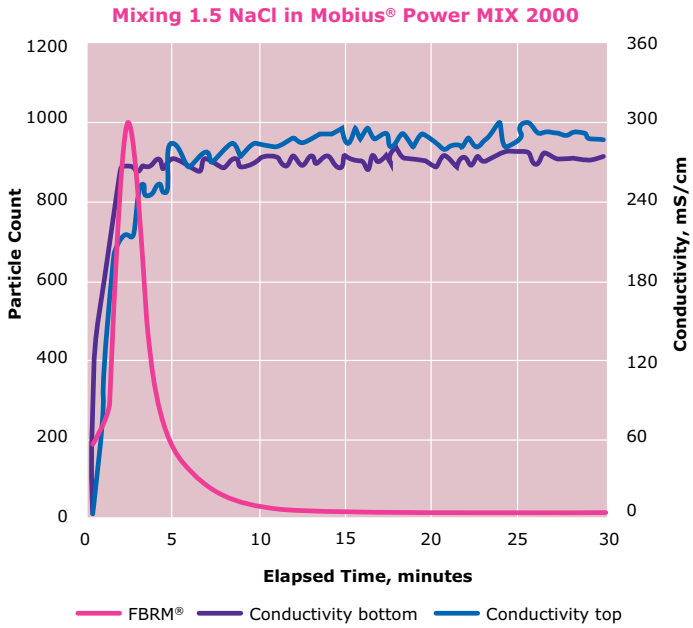


Figure 3.
At **5 minutes**, conductivity readings at both the top and bottom of the vessel reach a stable value, indicating homogeneity in mixing **1.5M NaCl** in the Mobius® Power Mix 2000. However visual mixing is not complete until **15 minutes** when it is once again possible to see through to the bottom of the vessel. Particle count reaches a minimum at 15 minutes, where not only distribution, but dissolution is complete. After 15 minutes, there is no change in visual or measured values.

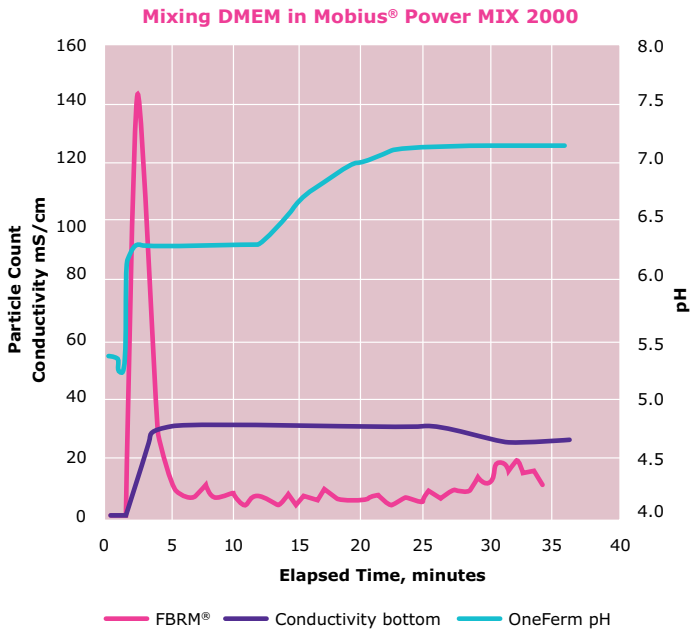
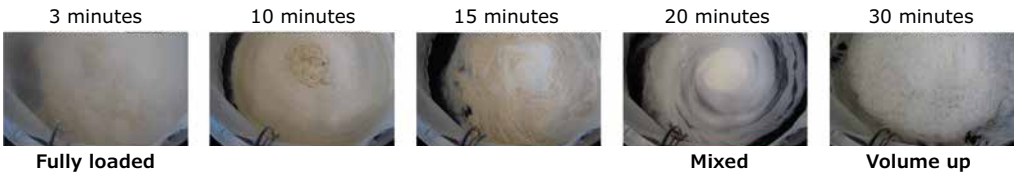


Figure 4.
At **5 minutes**, process data for mixing **DMEM** in Mobius® Power MIX 2000 shows conductivity reaching final value and particle count reaching minimum. At same time, visual mixing is complete with no visible powder on the surface or in the liquid. After pH adjustment at 12 minutes, there is no further dissolution of powder, but the liquid does change color slightly. At 25 minutes, volume is brought up to 2000L, with small decrease in conductivity and slight increase in particle count, as FBRM® probe starts recognizing bubbles with the increased vortex at higher liquid volume.



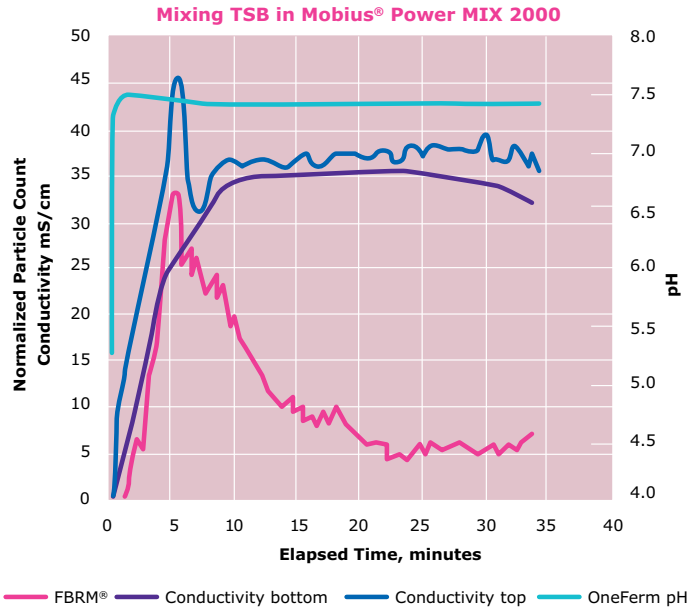
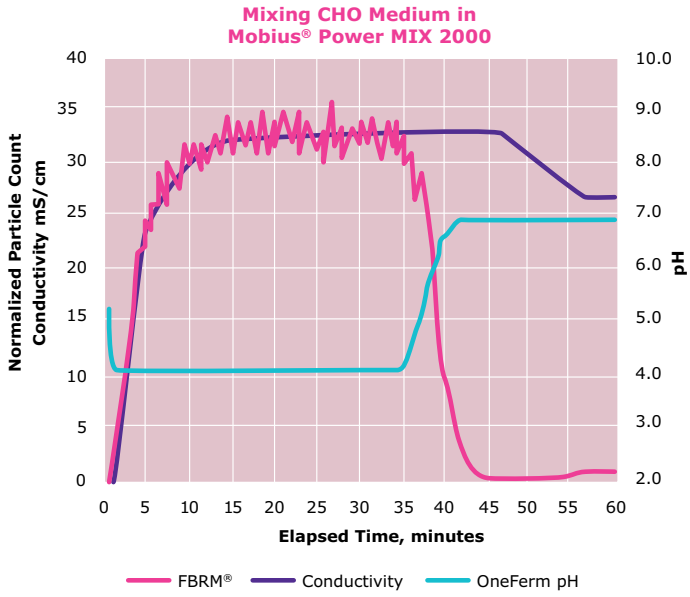


Figure 5.
At 10 minutes, conductivity readings at both the top and bottom of the vessel reach a stable value, indicating homogeneity in mixing **Tryptic Soy Broth** in the Mobius® Power Mix 2000. However visual mixing is not complete until **20 minutes** when there is no powder visible on the surface or in the liquid. Only a small quantity of foam is present. Particle count also reaches a minimum at **20 minutes**, where not only distribution, but dissolution is complete. The addition of water at 25 minutes reduces conductivity slightly, while the expansion of foam is reflected by an apparent increase in particle count.

Figure 6.
At **15 minutes**, process data for mixing chemically defined CHO Medium in Mobius® Power MIX 2000 shows conductivity reaching stable value and particle count reaching maximum, with only a small amount of powder on the surface.
At **25 minutes**, visual mixing is complete with no visible powder on the surface and almost complete dissipation of foam. After safety factor of 10 minutes, pH adjustment, performed at **35 minutes**, is necessary to bring all components immediately into solution, slightly raising conductivity and reducing particle count to zero, indicating a fully blended and dissolved mixture.
At **45 minutes**, DI water is added to bring volume up to 2000L, resulting in a decrease in conductivity. Particle count appears to increase, but only as FBRM® probe measures bubbles forming in solution with increased vortex at higher liquid volume.



Liquid-liquid mixing

Although primarily designed for mixing powder into liquid, the Mobius® Power MIX 2000 is also efficient at liquid-liquid mixing, especially useful for titrations and pool blending. Liquid-liquid mixing time was determined by tracing the conductivity response after a small amount of concentrated salt solution (1.25M NaCl) was added to the mixer volume already at low concentration of salt. To fully characterize the system, trials were conducted at four volumes (25%, 50%, 75% and 100% of 2000L) and four speeds (25%, 50%, 75% and 100% of 380 rpm). The resulting characterization map provides a guideline to the effect of impeller speed on mixing time throughout the whole of the vessel.

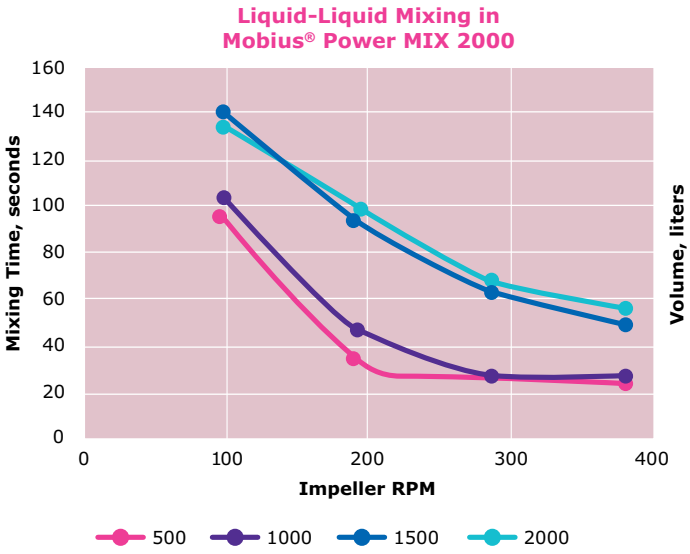


Figure 7.
Mixing Characterization Map for Mobius® Power MIX 2000.

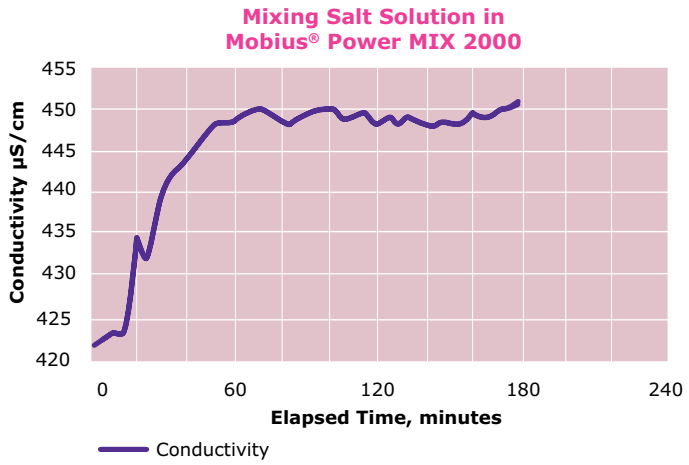


Figure 8.
Example of conductivity trace for full speed and full volume, where mixing is complete in 60 seconds, as measured by reaching t99 (99% of final value and +/-1% stability).

Conclusion

The Mobius® Power MIX 2000 has proven effective in meeting the challenges of buffer and media preparation in a single-use system. Complete distribution and dissolution of high concentration sinking powders can be accomplished in 15 minutes or less. For the most challenging floating powder media, wetting out and effective dispersion of powder has been demonstrated in under 35 minutes. Efficient liquid-liquid mixing allows for effective pH titration, a critical process step in media preparation.

For more information on CHO media, refer to the Cellvento™ CHO Platform Flyer.

For more information on the Mobius® Power MIX 2000, refer to the Mobius® Power MIX 2000 Data Sheet and Specification Sheet.

References

1 http://us.mt.com/us/en/home/supportive_content/specials/Lasentec-FBRM-Method-of-Measurement.html

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