

Mobius® Power Mix 500

Mixing Characterization for Buffer and Media Preparation

Executive Summary

Mobius® Power MIX 500 is a 500 liter high performance, single-use mixing system targeted for mixing in biopharmaceutical product/process development and manufacturing. Leveraging the proven technology of magnetically coupled NovAseptic® mixers, traditionally used with stainless steel tanks, the 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 500 for mixing of these sinking and floating powders. A characterization map of liquid-liquid mixing for a range of volumes and speeds is also included.

Introduction

Mixing of buffers (sinking powders) and media (floating powders), 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 500 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 speed up to 370 rpm, complete mixing for even the most difficult powders can be achieved in less than 40 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® (Focused Beam Reflectance Measurement) probe is used to track the distribution of particles over time. The FBRM® probe from Mettler Toledo® uses a focused laser beam directly into the process, determining the backscatter of light to measure and count particles. 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.



Figure 1. Mobius® Power MIX 500 stainless steel and plastic carriers

Mixing times demonstrated in the Mobius® Power MIX 500

Mixing Type	Final Product	Mixing Time
Liquid-Liquid	NaCl solution	30 seconds
Sinking Powder, low concentration	1X DPBS Buffer	2 minutes
Sinking Powder, high concentration	1.5M NaCl	11 minutes
Floating Powder, basal medium	DMEM	17 minutes
Floating Powder, chemically defined medium	Ex-Cell® Advanced™ Medium	16 minutes
Floating Powder, chemically defined medium	Custom CHO Medium	38 minutes

Materials and Methods:

Mobius® Power MIX 500 jacketed carrier with temperature sensor and load cells

- 500L Mixer Assembly
- Hamilton OneFerm® Single Use pH VP 70 probe in Mixer Assembly probe port
- Mettler Toledo® InPro® 7100 Conductivity probes
- Mettler Toledo® Particle Track G400 FBRM® probe

Buffers and Medias were prepared according to manufacturers' data sheets, as outlined in table below. The general procedure included:

1. Fill bag to recommended volume with DI water.
2. Run impeller at maximum speed (370 rpm).
3. Add appropriate amount of solute. Additions made above impeller, with open-top liner for better visual record.
4. Powders added as quickly as possible, to fully tax mixing capability. Record addition time.
5. Record of process made through several sensors. Two conductivity sensors, one at the top of the liquid and the second installed in the probe port at minimum volume. Hamilton OneFerm® Single-Use pH sensor installed in second mixer bag probe port. The FBRM® probe also installed at minimum volume, in place of a sample port. Video camera set up at the top of the vessel.
6. Mixer run for at least 2 minutes past time when no visible powders were present.

7. For chemically defined media mixes, pH adjustment was made to fully dissolve all particles and then DI water added to reach final volume. pH adjustment was made with titration of 5N NaOH for Custom Merck CHO medium and Sodium Carbonate powder for Ex-Cell® Advanced™ medium.
8. Data analysis on conductivity and particle distribution traces to find time to reach t99 (99% of final value) or $\pm 1\%$ process stability.
9. Excerpts from video records compiled to show distribution and dissolution of powders and quality of mixing.

Solution	Starting Volume, liters	Solute	Powder added, kg	Final Concentration, g/l
1X DPBS Buffer	450 (90%)	Dulbecco's Phosphate Buffered Saline (Sigma D5773)	4.925	9.86
1.5M NaCl	400 (80%)	Sodium Chloride (Fisher S67110)	36.0	90
DMEM	400 (80%)	Dulbecco's Modified Eagle's Medium	6.0	12
Chemically Defined CHO Media	400 (80%)	Ex-Cell® Advanced™ Medium	115.0	23
Custom CHO Media	400 (80%)	Custom Merck CHO Medium	101.5	20.3

Results:

Solution	Time to add all powder	Time to t99 conductivity	Time to complete visual mixing	Total Mixing Time*
1X DPBS Buffer	0.2 min.	1 min.	2 min.	2 min.
1.5M NaCl	0.5 min.	7 min.	11 min.	11 min.
DMEM	0.5 min.	10 min.	17 min.	17 min.
Ex-Cell® Advanced™ CHO Medium	1 min.	11 min.	16 min.	27 min. ¹
Custom Merck CHO Medium	2 min.	22 min.	38 min.	55 min. ²

*Complete Particle Distribution and Dissolution

¹Includes pH adjustment

²Includes 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.

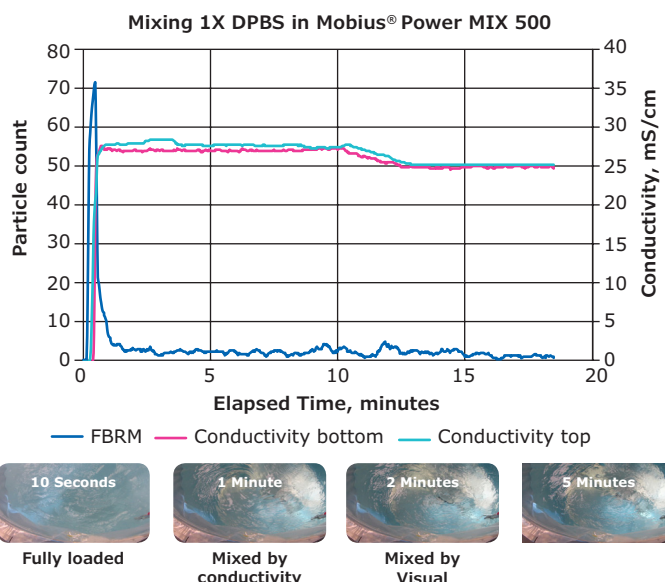


Figure 2. At 45-60 seconds, process data for mixing 1X DPBS in Mobius® Power MIX 500 shows conductivity reaching final value at both top and bottom of vessel. Particle count reaches minimum value at approximately 1-2 minutes. At same time, visual mixing is complete as the liquid becomes clear. As shown in the 4 minute picture, no further changes in visual were seen after 2 minutes, confirming complete mix at that time. At 10 minutes, Q.S. to 500L shows decrease in conductivity and no change in particle count.

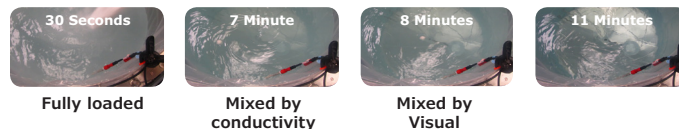
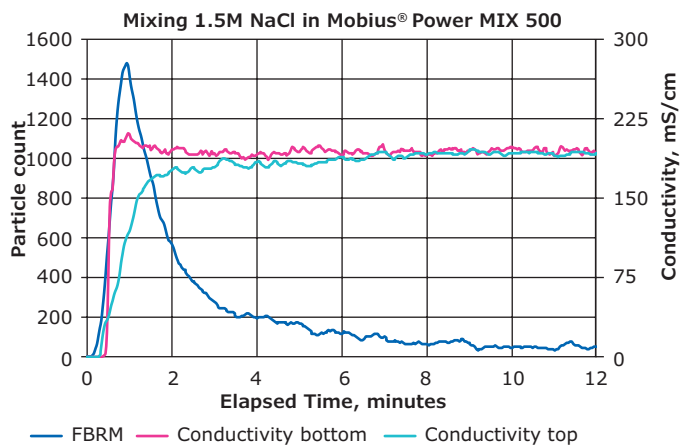


Figure 3. At 7 minutes, conductivity readings at both the top and bottom of the vessel reach a stable value, indicating homogeneity in mixing 1.5M NaCl in the Mobius® Power Mix 500. At this time, the solution is starting to become clear, with the impeller somewhat visible. Clarity improves greatly at 8 minutes and even more at 11 minutes, where visual mixing is deemed complete. Particle count also reaches a minimum at 9 minutes.

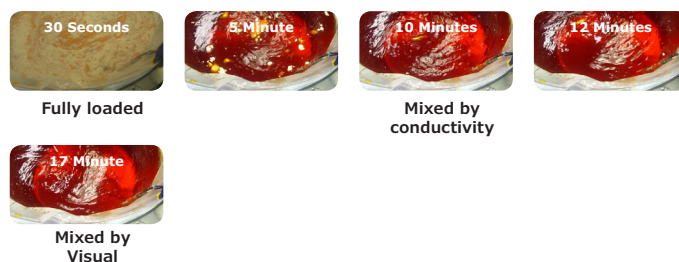
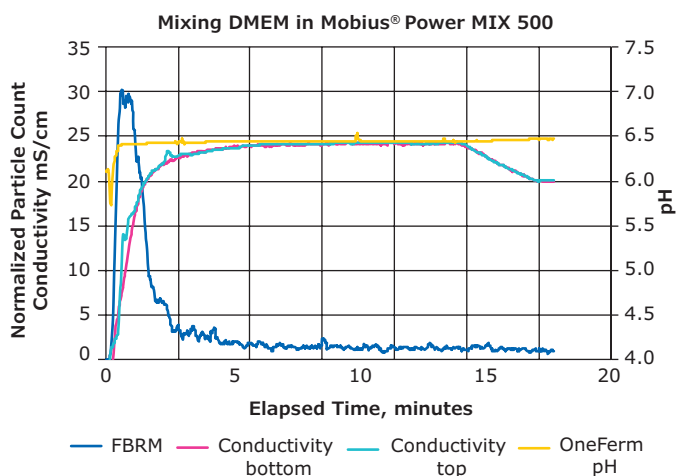


Figure 4. At 10 minutes, process data for mixing DMEM in Mobius® Power MIX 500 shows conductivity reaching final value. At this time, only a few small agglomerates of powder are visible. These last pieces are slowly dissolving. At 17 minutes, visual mixing is complete with no visible powder on the surface or in the liquid. Particle count appears to reach a minimum between 12 to 15 minutes. At 25 minutes, with Q.S. to 500L, there is a decrease in conductivity.

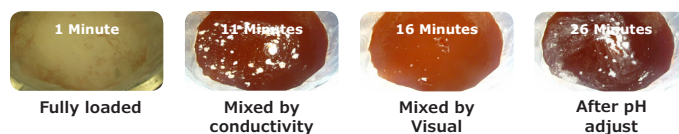
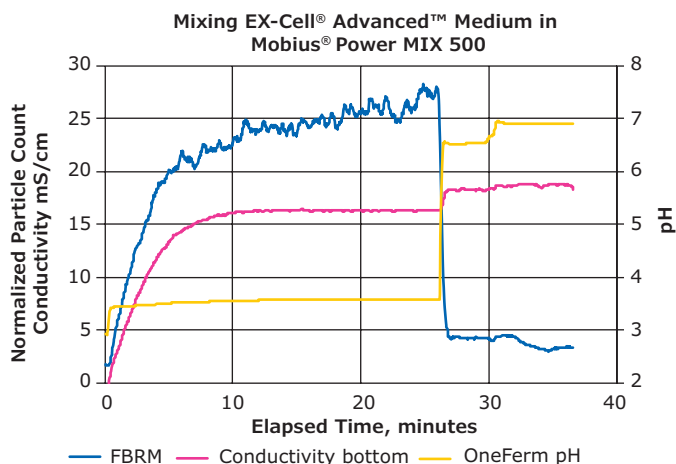


Figure 5. Ex-Cell® Advanced™ medium powder mixes similarly to DMEM powder. Conductivity reaches a stable value at 11 minutes. The last of the powder is incorporated at 16 minutes for complete visual mixing. Particle count appears to level out around 20 minutes, but does still increase some after that, most likely related to bubbles. After pH adjustment, particle count goes to minimum, but there is some foaming and so particle count does not reach zero.

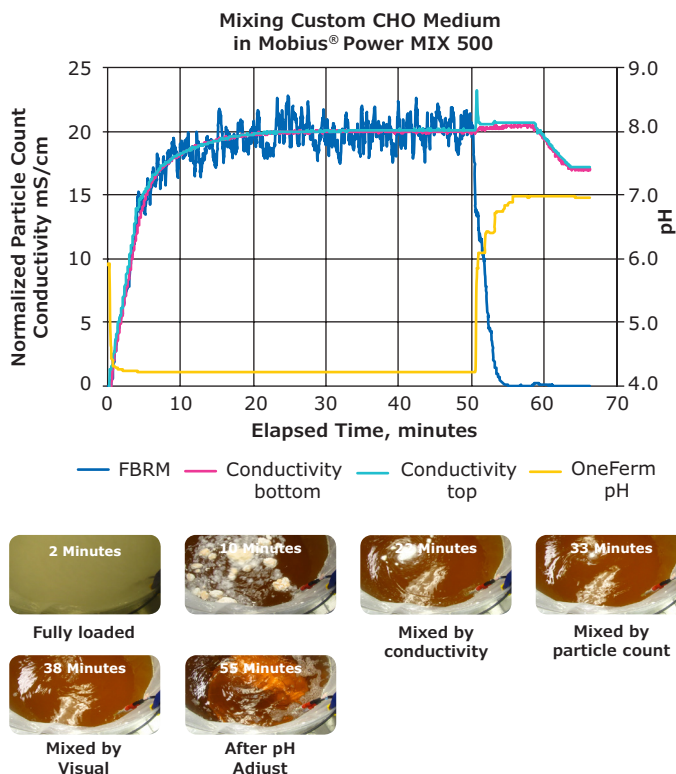


Figure 6. The Custom CHO medium is most challenging to mix. After 10 minutes in the Mobius® Power MIX 500, it appears that most of the material is mixed except for several clumps that remain dry. At 22 minutes, conductivity readings reach a stable maximum. At this time, there are only a few small clumps not mixed. Particle count reaches its maximum around 33 minutes. The final clumps are dissolved at 38 minutes. After pH adjustment, starting at 50 minutes, the solution becomes very clear, with particle count moving to zero.

Although primarily designed for mixing powder into liquid, the Mobius® Power MIX 500 is also efficient at liquid-liquid mixing, especially useful for titrations and pool blending. Liquid-liquid mixing time is determined by tracing the conductivity response after a small amount of concentrated salt solution (1.25M NaCl) is added to the mixer volume already at low concentration of salt. To fully characterize the system, trials are conducted at four volumes (25%, 50%, 75% and 100% of 500 L) and four speeds (25%, 50%, 75% and 100% of 370 rpm). The resulting characterization map provides a guideline to the effect of impeller speed on mixing time throughout the whole of the vessel.

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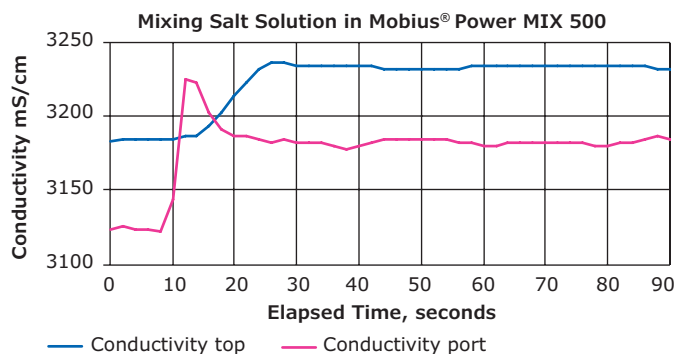


Figure 7. Example of conductivity trace for full speed and full volume, where mixing is complete in 30 seconds, as measured by reaching t99 (99% of final value).

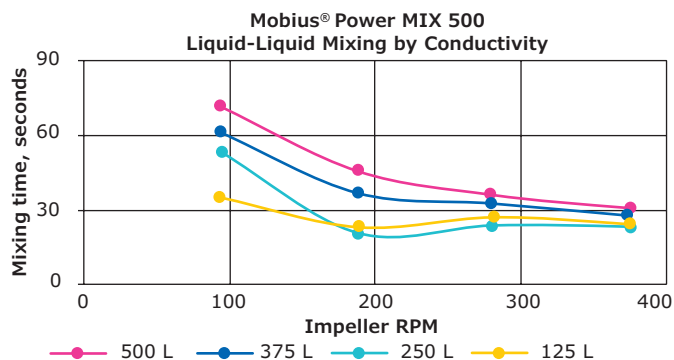


Figure 8. Mixing Characterization Map for Mobius® Power MIX 500

Conclusion

The Mobius® Power MIX 500 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 40 minutes. Efficient liquid-liquid mixing allows for effective pH titration, a critical process step in media preparation.

For more information on the Mobius® Power MIX 500, refer to Mobius® Power MIX 500 Data Sheet and Specification Sheet.

References:

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

