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Filtration, Separation
& Preparation

Application Note

Mobius® Power MIX 1000

Mixing Characterization for Buffer and Media Preparation

Executive Summary

Mobius® Power MIX 1000 is a 1000 liter high performance, single-use mixing system targeted for clinical scale 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 1000 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.

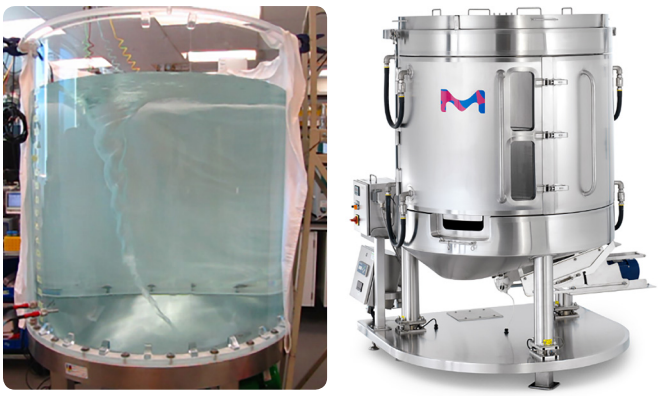


Figure 1. Mobius® Power MIX 1000 prototype tank, used in development, and final production vessel

Introduction

Mixing of buffers (sinking powders) and media (floating powders), presents substantial challenges in getting good dispersion and dissolution of particles, especially at high volumes. The creation of a strong vortex in the Mobius® Power MIX 1000 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 380 rpm, complete mixing for even the most difficult powders can be achieved in less than 15 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.

Mixing times demonstrated in the Mobius® Power MIX 1000

Mixing Type	Final Product	Mixing Time
Liquid-Liquid	NaCl solution	40 seconds
Sinking Powder, low concentration	1X DPBS Buffer	2 minutes
Sinking Powder, high concentration	1.5 M NaCl	11 minutes
Floating Powder, basal medium	DMEM	5 minutes
Floating Powder, soybean-casein digest medium	TSB (Tryptic Soy Broth)	11 minutes
Floating Powder, chemically defined medium	Custom CHO Medium	15 minutes

Materials and Methods:

Mobius® Power MIX 1000 jacketed carrier with temperature sensor and load cells

- 1000L 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 Media 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 -380 rpm. (One additional run with Custom CHO medium at 240 rpm.)
3. Add appropriate amount of solute. Additions made at location of powder port 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 10 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 CHO medium.
8. Data analysis on conductivity and particle distribution traces to find time to reach t99 (99% of final value) or ± 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	900 (90%)	Dulbecco's Phosphate Buffered Saline (Sigma D5773)	9.86	9.86
1.5M NaCl	800 (80%)	Sodium Chloride (Fisher S67110)	72.0	90
DMEM	800 (80%)	Dulbecco's Modified Eagle's Medium – high glucose (Sigma D5648)	13.71	13.7
TSB	800 (80%)	Becton, Dickinson & Co Select APS TSB (Ref 214887)	30.0	30
Custom CHO Media	800 (80%)	Custom CHO Medium	20.3	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.	2 min.	2 min.	2 min.
1.5M NaCl	1 min.	3 min.	9 min.	11 min.
DMEM	0.3 min.	2 min.	5 min.	5 min.
TSB	1 min.	5 min.	6 min.	7 min.
Custom CHO Medium (380 rpm)	2 min.	4 min.	12 min.	30 min.[1]
Custom CHO Medium (240 rpm)	9 min.	15 min.	15 min.	40 min.[1]

*Complete Particle Distribution and Dissolution

[1]Includes pH titration

Figures 2 through **7** 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.

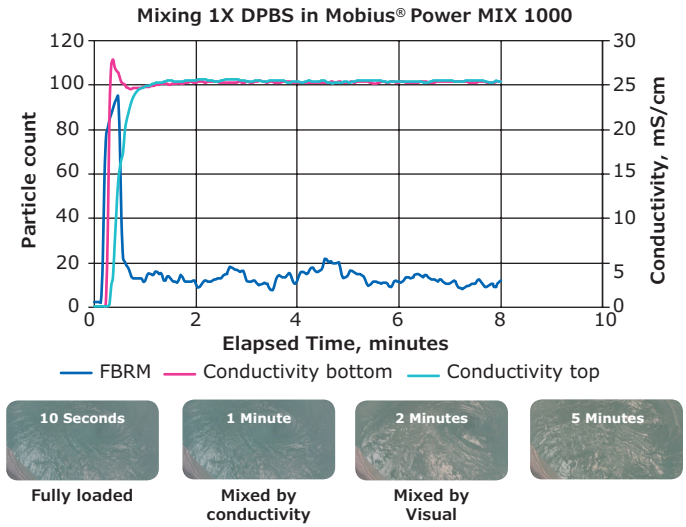


Figure 2. At 70-80 seconds, process data for mixing 1X DPBS in Mobius® Power MIX 1000 shows conductivity reaching final value at both top and bottom of vessel. Particle count reaches minimum value at approximately 1-2 minutes. At same time, visual mixing is complete as the liquid becomes clear. No further changes in visual were seen after 2 minutes, confirming complete mix at that time. After 1-2 minutes, particle count varies at low values as bubbles form and dissipate.

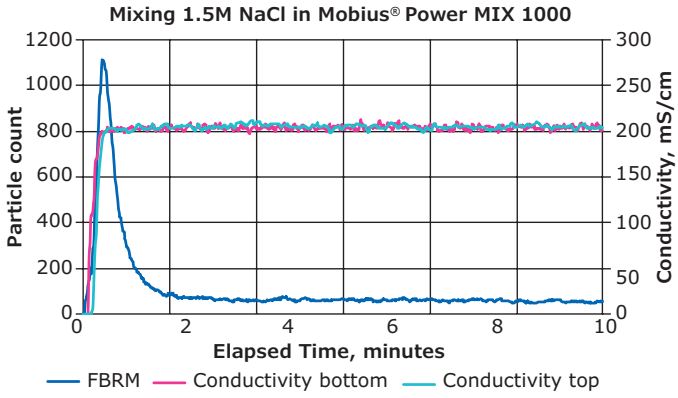
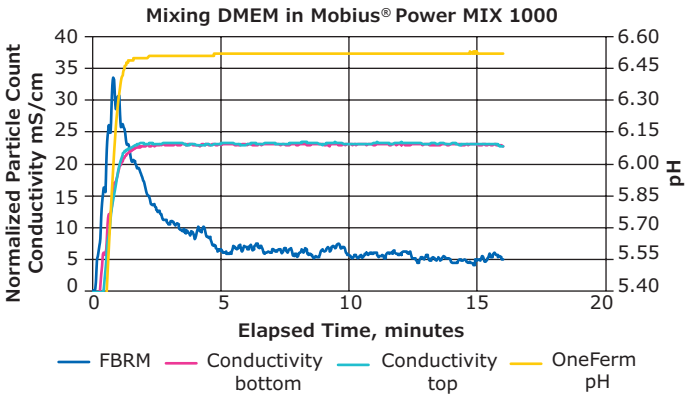


Figure 3. At 3 minutes, conductivity readings at both the top and bottom of the vessel reach a stable value, indicating homogeneity in mixing 1.5M NaCl in the Mobius® Power MIX 1000. Particle count reaches a minimum at 9 minutes. Mixing by visual is deemed complete at 11 minutes, as there is no further change in appearance after 11 minutes.



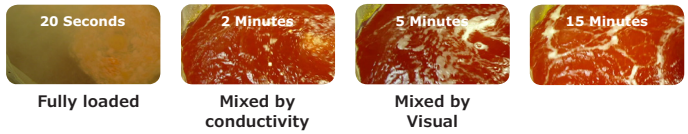
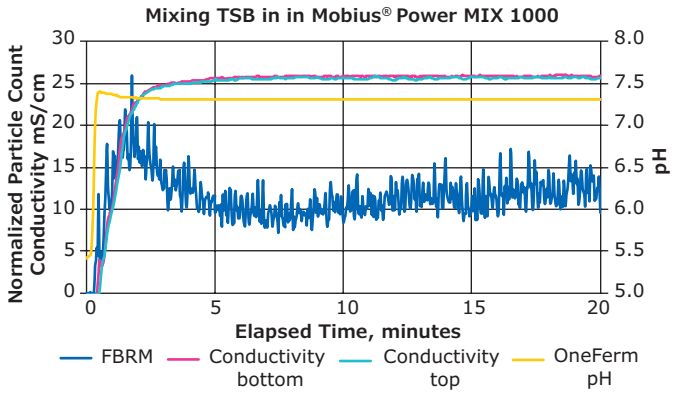


Figure 4. At 2 minutes, process data for mixing DMEM in Mobius® Power MIX 1000 shows conductivity reaching final value. At 5 minutes, visual mixing is complete with no visible powder on the surface or in the liquid. By 5 minutes, the measure of particles in solution reaches 99% of the final value. The system is run for 10 minutes past the visual mixing time, with no change in visual, pH, conductivity or particle count, establishing the mixing time at 5 minutes. At 15 minutes, with Q.S. to 1000L, there is a slight drop in conductivity. Minimum (not zero) particle count is indicative of small bubbles.



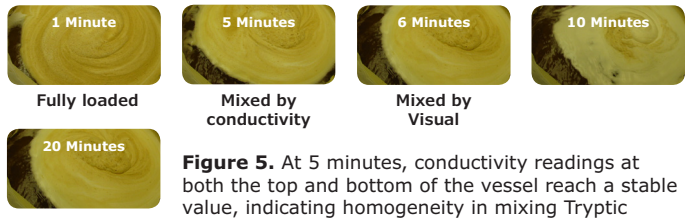


Figure 5. At 5 minutes, conductivity readings at both the top and bottom of the vessel reach a stable value, indicating homogeneity in mixing Tryptic Soy Broth in the Mobius® Power MIX 1000. However visual mixing is not complete until 6 minutes when there is no powder visible on the surface or in the liquid. Particle count reaches a minimum between 5 to 7 minutes, where not only distribution, but dissolution is complete. The data from the FBRM never reaches zero, not because of particles, but as a measure of foam/bubbles in the system, which increases as the system continues to run past homogeneity.

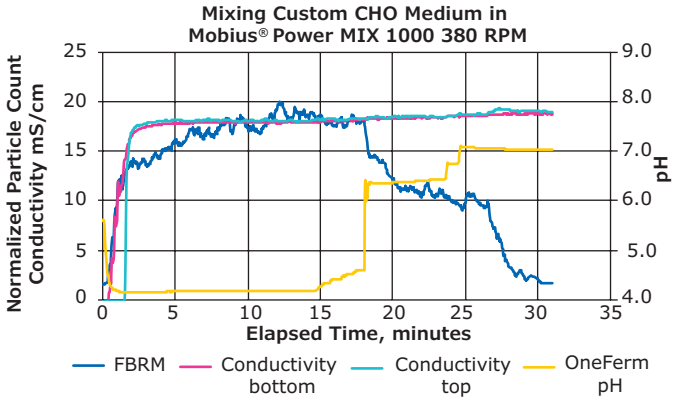
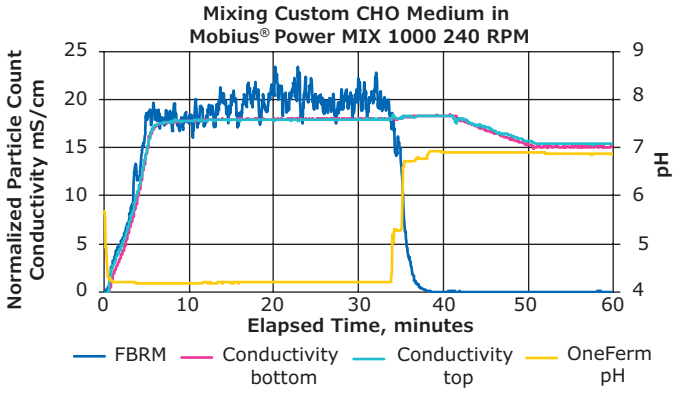


Figure 6. Mixing Custom CHO media at 380 rpm in the Mobius® Power MIX 1000 is dominated by dust and foaming, making it difficult to judge visual mixing. Data analysis shows mixing by conductivity stability at 4 minutes. Particle count reaches a maximum at 12 minutes, where no particles are visible on the liquid surface. pH adjustment, performed at 15-25 minutes, is necessary to bring all components into solution, slightly raising conductivity and reducing particle count to minimum, indicating a fully blended and dissolved mixture. Minimum (not zero) particle count is indicative of small bubbles and foaming.



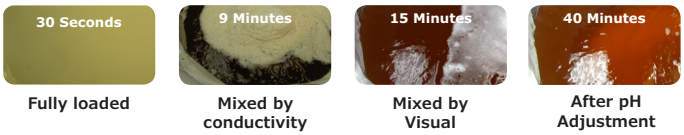


Figure 7. With impeller rpm reduced to 240 rpm, mixing time for Custom medium is increased, but foaming is essentially eliminated. Conductivity reaches a stable value at 9 minutes. The last of the powder is incorporated at 15 minutes for visual mixing complete. At the same time, particle count reaches 99% of the maximum value. After pH adjustment, particle count goes to zero, without any foaming or bubbles.

Although primarily designed for mixing powder into liquid, the Mobius® Power MIX 1000 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 1000L) 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.

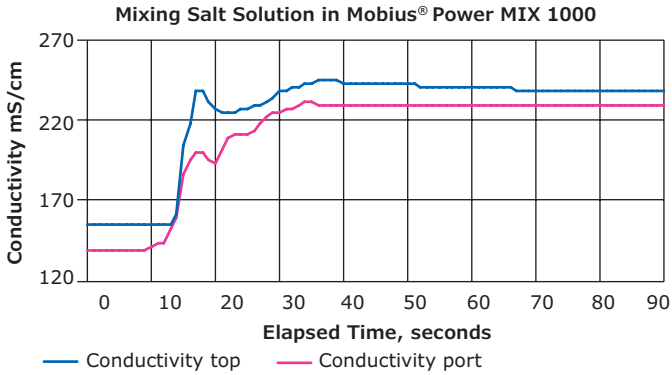


Figure 8. Example of conductivity trace for full speed and full volume, where mixing is complete in 40 seconds, as measured by reaching t99 (99% of final value).

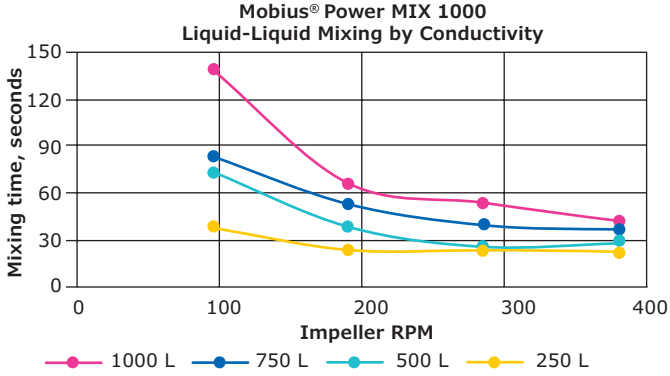


Figure 8. Mixing Characterization Map for Mobius® Power MIX 1000

Conclusion

The Mobius® Power MIX 1000 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 less than 10 minutes. Even with reduced speed to reduce foaming in the most challenging floating powder media, wetting out and effective dispersion of powder has been demonstrated in under 15 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 1000, refer to Mobius® Power MIX 1000 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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