Millipore®
Filtration, Separation
& Preparation

Application Note

Mobius® Power Mix 200

Mixing Characterization for Buffer and Media Preparation

Executive Summary

Mobius® Power MIX 200 is a 200 liter high performance, single-use mixing system targeted for small 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 200 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. Power MIX 200 stainless steel and plastic carriers

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 200 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 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® (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 200

Mixing Type	Final Product	Mixing Time
Liquid-Liquid	NaCl solution	25 seconds
Sinking Powder, low concentration	1X DPBS Buffer	2 minutes
Sinking Powder, high concentration	1.5M NaCl	9 minutes
Floating Powder, basal medium	DMEM	7 minutes
Floating Powder, chemically defined medium	MilliporeSigma Ex-Cell® Advanced™ Medium	14 minutes
Floating Powder, chemically defined medium	Custom MilliporeSigma CHO Medium	30 minutes

Materials and Methods:

Mobius® Power MIX 200 jacketed carrier with temperature sensor and load cells

- 200L 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).
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 MilliporeSigma 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 ± 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	180 (90%)	Dulbecco's Phosphate Buffered Saline (Sigma D5773)	1.97	9.86
1.5M NaCl	160 (80%)	Sodium Chloride	14.4	90
DMEM	160 (80%)	Dulbecco's Modified Eagle's Medium	2.40	12
Chemically Defined CHO Media	160 (80%)	MilliporeSigma Ex-Cell® Advanced™ Medium	46.00	23
Custom CHO Media	160 (80%)	Custom MilliporeSigma CHO Medium	40.60	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.	4 min.
1.5M NaCl	0.2 min.	2 min.	7 min.	9 min.
DMEM	0.5 min.	5 min.	7 min.	7 min.
Ex-Cell® Advanced™ CHO Medium	0.2 min.	12 min.	14 min.	20 min.[1]
Custom CHO Medium	0.3 min.	22 min.	30 min.	40 min.[2]

*Complete Particle Distribution and Dissolution

[1]Includes pH titration

[2]Includes pH adjustment

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.

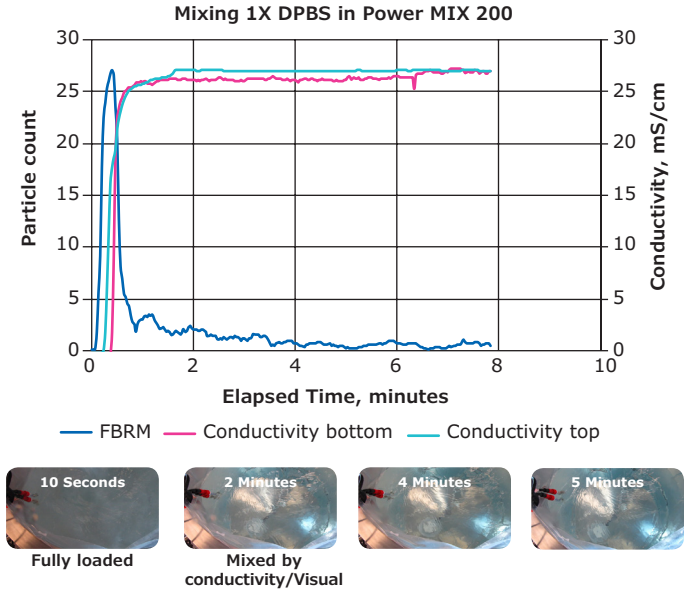
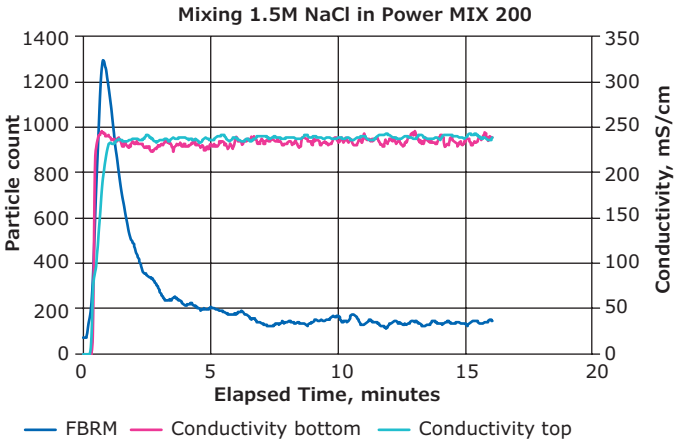


Figure 2. At 90 seconds, process data for mixing 1X DPBS in Mobius® Power MIX 200 shows conductivity reaching final value at both top and bottom of vessel. Visual mixing is complete as the liquid becomes clear between 2 and 4 minutes. Particle count reaches minimum value at approximately 4 minutes. No further changes in visual were seen after 4 minutes, confirming complete mix at that time.



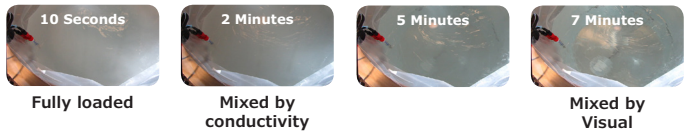
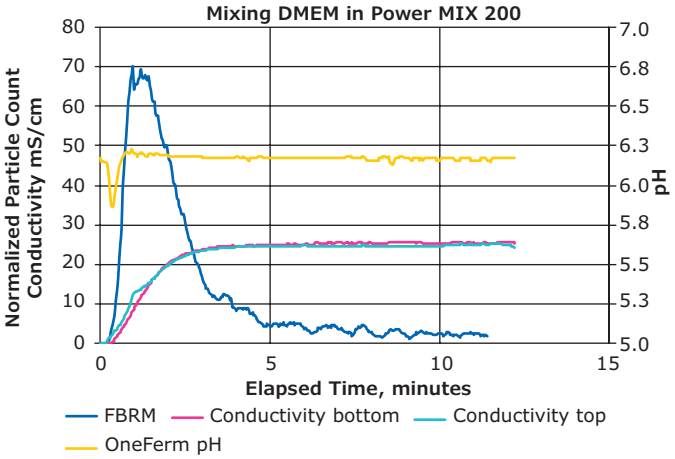


Figure 3. At 2 minutes, conductivity readings at both the top and bottom of the vessel reach a stable value, indicating homogeneity in mixing 1.5M NaCl in the Mobius® Power MIX 200. The solution begins to become clear at 5 minutes, with complete clarity to the bottom of the vessel at 7 minutes. At 9 minutes, particle count reaches a minimum, stable reading.



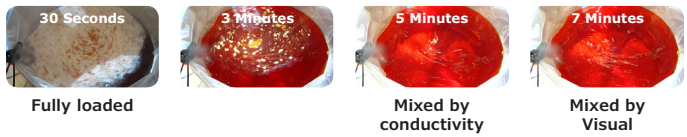
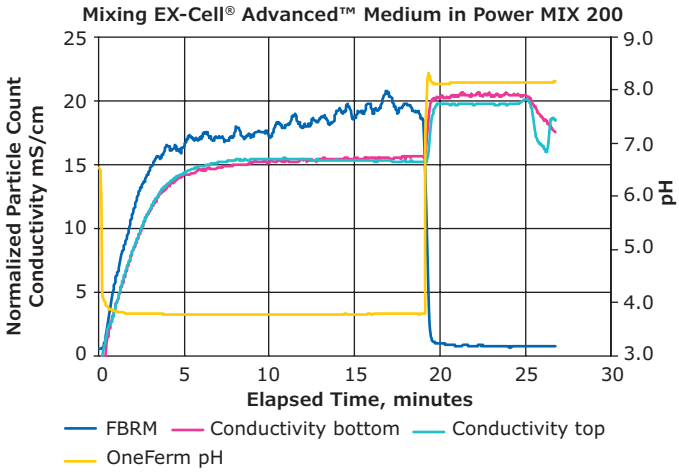


Figure 4. At 5 minutes, process data for mixing DMEM in Mobius® Power MIX 200 shows conductivity reaching final value. At this time, only a few small agglomerates of powder are visible. These last pieces are slowly dissolving. At 7 minutes, visual mixing is complete with no visible powder on the surface or in the liquid. The solution appears very clear. Particle count reaches a minimum between 6-9 minutes.



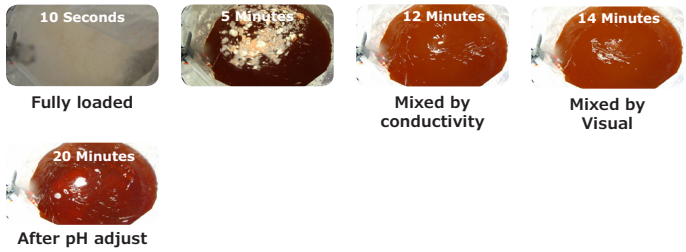


Figure 5. While mixing Ex-Cell® Advanced™ medium in Mobius® Power MIX 200, conductivity reaches a stable value at 12 minutes. The last of the powder is incorporated at 14 minutes for complete visual mixing. Particle count reaches maximum at 14 to 16 minutes. After pH adjustment, particle count goes to minimum, with an associated increase in conductivity. Q.S. to 200L at 26 minutes decreases conductivity.

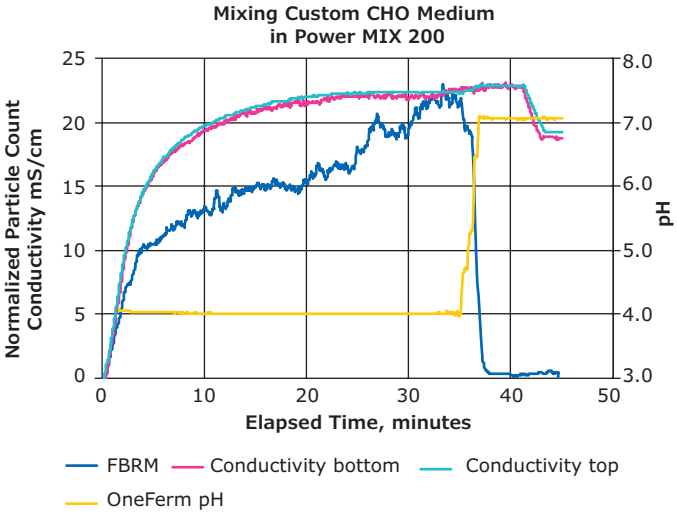


Figure 6. The addition of Custom CHO medium to the Mobius® Power MIX 200 is accompanied by a great deal of dust. By 10 minutes, it appears that most of the material is mixed except for several clumps that remain dry. Conductivity reaches 99% at 22 minutes. The final clumps are mixed in by 30 minutes. At that time, particle count appears to be at maximum. After pH adjustment, starting at 35 minutes, the solution becomes very clear, with particle count moving to zero. Conductivity increases slightly with pH adjustment. Final Q.S. to 200L starting at 41 minutes brings conductivity down.

Although primarily designed for mixing powder into liquid, the Mobius® Power MIX 200 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 200L) 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.

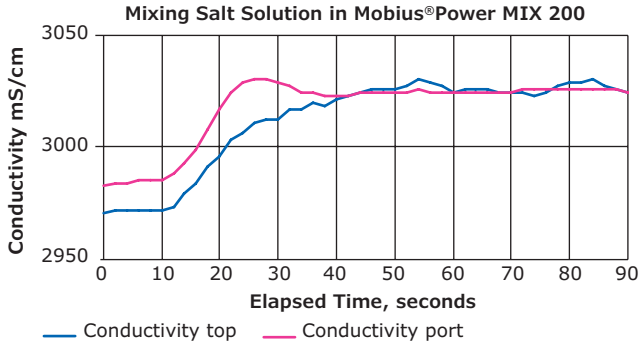


Figure 7. Example of conductivity trace for full speed and full volume, where mixing is complete in 25 seconds, as measured by reaching t99 (99% of final value).

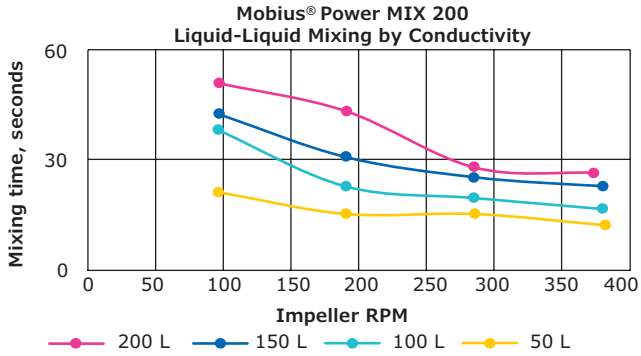


Figure 8. Mixing Characterization Map for Mobius® Power MIX 200

Conclusion

The Mobius® Power MIX 200 has proven effective in meeting the challenges of buffer and media preparation in a single-use system at small scale. Complete distribution and dissolution of high concentration sinking powders can be accomplished in 10 minutes or less. For the most challenging floating powder media, wetting out and effective dispersion of powder has also been demonstrated in under 30 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 200, refer to Mobius® Power MIX Data Sheet and Mobius® Power MIX 100/200/500 Specification Sheet.

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References:

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

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