Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Mobius® MIX200 Disposable Mixing System

200 L Mixing Study

Introduction

Biopharmaceutical companies are increasingly making the change from stainless steel systems to disposable technologies. The reasons for this change are clear: lower risk of contamination, reduced cleaning and validation requirements, and increased flexibility in multi-product facilities and biotech start-ups.

We offer a disposable mixer that is currently available in a 200 L format. The Mobius® MIX200 Disposable Mixing System includes a 200 L single-use container with an integrated, magnetically driven impeller, motor, and carrier. This study investigates the use of the disposable mixing system for solutions commonly used by customers as well as the efficacy of the mixer to put a buffer, protein, and media into solution. Successful mixing was determined by visual confirmation, the homogeneity of the solution (similar measurements at the top and bottom of the process container), as well as the necessary time-to-mix.

Experimental Design

Three different solutions were tested in the disposable mixer. One-liter sample volumes were mixed on a stir plate to establish general baseline pH, conductivity, and absorbance measurements. The details of the test solutions as well as the baseline measurements are in **Table 1**.

The disposable mixing system was bottom-filled with 200 L of RO-quality water and the mixer was set to 400 RPM. The powdered component was introduced into the system via a bung port at the top of the process container. Samples were taken from the process container via a pipette from the top and a sample port at the bottom of the container. The hold-up volume of the bottom sample port was drained prior to each measurement, to eliminate false measurements from a dead-leg in the tubing. Measurements were taken for at least 20 minutes and continued until at least 10 consecutive, stable, and matching measurements were recorded from the top and bottom. Visual inspection also confirmed that all of the powder went into solution. A more detailed experimental protocol can be found at the end of the document.

Table 1. Test Solutions

Component	Concentration	Target pH	Target Conductivity (mS/cm)	Target Absorbance A_{280}
Phosphate-Buffered Saline	10 mM	7.22	15.98	—
Hank's Balanced Salts (Cell Culture Grade)	1X (9.8 g/L)	6.18	15.09	—
Bovine Serum Albumin	1 g/L (in 10 mM of PBS)	—	—	0.688

Results

The figures in this section graph the pH, conductivity, and absorbance results from the three mixing trials.

Figures 1 and **2** plot the pH and conductivity values for the top and bottom measurements for 1X Phosphate-Buffered Saline and the 1X Hank's Media.

Figure 3 plots the absorbance values and calculated concentration of the Bovine Serum Albumin samples taken from the top and bottom of the bag. The concentration was calculated using Beer's Law:

A = ε*c*l

Where:

A = absorbance at 280 nm

ε = molar extinction coefficient for BSA: 0.677 g/L

c = concentration in the units corresponding to ε

l = length of light path (cuvette) = 1 cm

Discussion

The data for all three solutions shows that the Mobius® MIX200 Disposable Mixing System is able to homogeneously mix powder-liquid solutions at the tested concentrations in under 20 minutes. The data for the 10 mM PBS shows a decline in pH values after 30 minutes. This is most likely due to chemical changes in the solution (e.g. dissolution of atmospheric carbon dioxide) that can be attributed to the weak buffering capacity of the solution. The drop in pH is not a mixing phenomenon and the mixture remained completely mixed, even during the pH change within the solution.

Visual inspection of the process containers during mixing and draining of the system confirmed that for the tested concentrations, the powders did not settle on the bottom or attach to the sides of the container.

Figure 1. 10 mM Phosphate-Buffered Saline: Mixing Profile

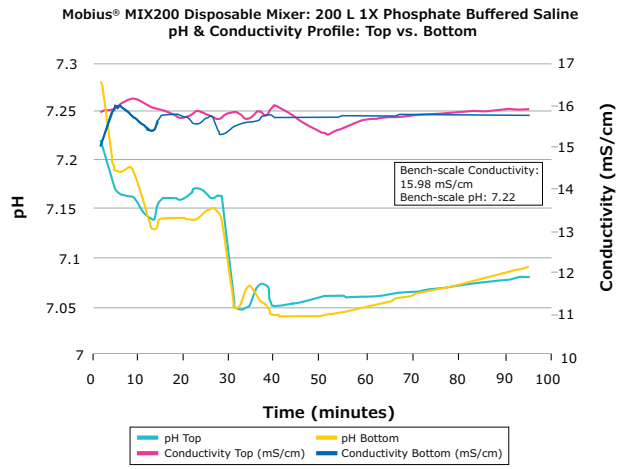


Figure 2. 1X Hank's Buffered Salts: Cell Culture-Grade Media

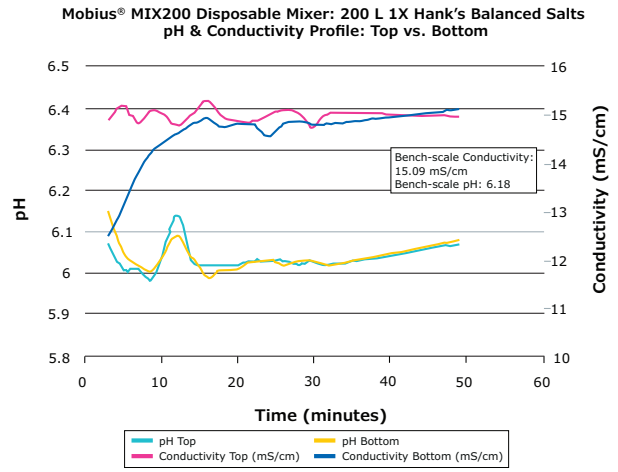
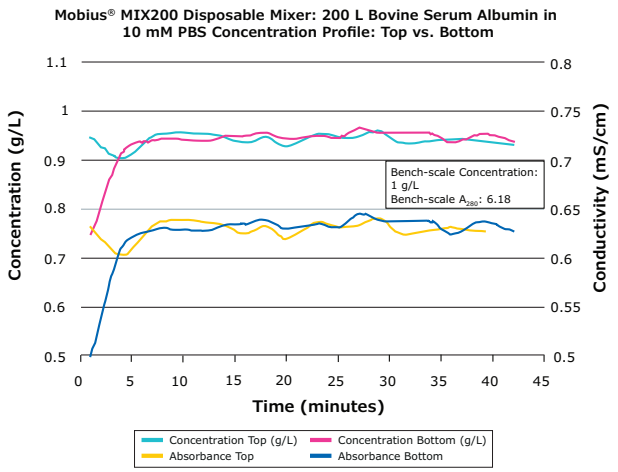


Figure 3. 1 g/L BSA on 10 mM Phosphate-Buffered Saline



Recommendations

Based on this study, we confirm that the Mobius® MIX200 Disposable Mixing System is an efficient and effective solution for mixing. The information presented in this document can be used as a reference for various buffer, media, and protein solutions. However, due to the variation in mixing applications, we recommend that each specific application be evaluated individually prior to implementation. Our application specialists will work with customers to help develop effective and efficient Mobius® disposable mixing solutions to suit specific needs.

Experimental Protocol

Objective

The objective of this procedure is to characterize the time-to-mix and uniformity of mixing of fluids in Mobius® disposable mixing systems using the specific solutions listed in **Table 1**.

Specific Applications

- Phosphate-buffered Saline
- Hank's Buffered Salts Media: Cell Culture Grade
- Bovine Serum Albumin

Materials

The following materials were used:

- 200 L disposable mixer
- 200 L process container with top bung port and bottom drain
- pH probe
- Conductivity probe
- UV spectrophotometer
- Pipettes and pipettor
- Sampling containers
- Funnel for transferring solids into the process container

Success Criteria

Success was defined as:

- Complete mixing of the target solution, as indicated by:
  - Visual confirmation (no powder remaining in container)
  - pH measurement
  - Conductivity measurement
  - Absorbance measurement for protein

Experimental Method

1. Fill process container with water (from the bottom of the process container)
   - For BSA: 200 L of 10 mM PBS was used as the suspension fluid
2. Start mixer at 400 RPM (selected as a midpoint speed)
3. Add solid components to process container through bung port. Start timer
4. Perform measurements at regular intervals by sampling with a pipette from the top and the bottom of the process container
   - For buffer/media: Measure pH and conductivity at regular intervals
   - For BSA: Measure A_{280} at regular intervals
5. Continue mixing for at least 20 minutes until complete mixing is achieved, as indicated by:
   - Visual confirmation
   - Stability of 10 consecutive measurements
   - Convergence of all measurements (all locations read equally)*

*Small samples of each material will be mixed at bench scale, using a magnetic stirrer to establish target values for each measurement

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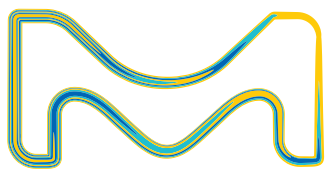

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