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Application Note

Optimizing buffer mixing and preparation

Using the Mobius® Single-Use Mixing Solution with Process Chemicals and Filters

Introduction

This study develops optimal process conditions for mixing and filtration of 1x PBS buffer using the single-use Mobius® MIX solution and Millipore Express® filters.

The Mobius® MIX solution delivers advanced technology for mixing pharmaceutical ingredients from intermediate to final drug products and for the preparation of process solutions, such as buffers and media. Each single-use mixing container includes a magnetically-driven levitating impeller for improved mixing consistency and efficiency. The Mobius® MIX solution includes a powder delivery system, 10 L, 50 L, 100 L, 200 L, 500 L, and 1000 L single-use mixers.

Millipore Express® SHC (0.5/0.2 µm), and Millipore Express® SHF (0.2 µm) sterilizing-grade filters were used in this study. These sterilizing-grade polyethersulfone (PES) membrane filters are offered in capsule and cartridge formats. The capsules are gamma and autoclave compatible and can be purchased gamma sterilized.

Many of our buffer products bear the Emprove® Expert and Emprove® Essential trademark. The Emprove® product lines have been developed to make work safer, faster and more cost-efficient, and offer regulatory documentation that strengthens the quality section of registration dossiers.

Experimental Methods

Mixing Study

Three batches of 1x PBS were prepared at 50 L, 200 L, and 1000 L volumes using the following methodology:

1. The bag was installed into the mixer container and inflated prior to use.
2. The scale/load cells were tared with the empty mixer vessel. A floor scale was used for the 50 L and 200 L mixer vessels. The 1,000 L vessel comes with on-board load cells.
3. Water was added to approximately 90% of the final target volume.
4. The mixer vessel was turned on and the impeller was set to the maximum setting:
   - Mobius® MIX 50: 1000 RPM
   - Mobius® MIX 200: 500 RPM
   - Mobius® MIX 1000: 250 RPM
5. The Emprove® Expert chemicals used to prepare 1x PBS buffer were weighed into a powder delivery bag, the bag was connected to the mixer bag and the powder delivery valve was opened to transfer the powder to the mixer vessel as one bulk addition. For the 1,000 L scale preparation, the Mobius® hoist was used to lift the powder bag above the mix vessel and hold it in place while the powder bag was connected to the mix bag. The hoist was not needed to connect the powder bag to the 50 L and 200 L mixer bags. **Table 1** details the chemical materials needed to prepare individual batches of PBS, including the final concentration and final total powder masses.

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6. Samples were taken from the top of the mixer vessel and from the needle-free sample ports beginning immediately after adding the powder into the mixer vessel. Samples were returned back to the mixer vessel after each measurement.
7. The pH and conductivity of the buffer were measured every 2 minutes. When the measurements at the top and bottom were equal and stable for 10 minutes, the buffer was considered mixed. At this point, water was added to 100% of the final target with the impeller still running.

Table 1. Emprove® Expert Chemical Requirements for Individual Batches of 1x PBS buffer

Chemical	Conc [g/L]	Weight [g]		
		50 L	200 L	1000 L
Sodium Chloride	8	400	1600	8000
di-Sodium Hydrogenphosphate, Dihydrate	1.78	89	356	1780
Potassium Dihydrogen Phosphate	0.27	14	54	270
Total Powder Weight [kg]	**N/A**	**0.503**	**2.01**	**10.05**

Filtration Study

After the 1x PBS buffer mixing study was performed at 50 L scale, the filterability of the buffer was tested using OptiScale® 25 devices (3.5 cm^2) and the Vmax™ method at a constant pressure of 10 psi. The Vmax™ method is an accelerated screening test based on the gradual pore size plugging model, and estimates scale up filter area requirements. Three replicates were performed for each filter to ensure data was repeatable and reliable. **Table 2** outlines the filters that were evaluated in this study.

Table 2. Filter Evaluation

Filter	Chemistry	Pore Size
Millipore Express® SHC	Hydrophilic PES	0.5/0.2 µm
Millipore Express® SHF	Hydrophilic PES	0.2 µm

Results and Discussion

Mixing Study

Table 3 details the mixing times required to get the first stable pH and conductivity readings prior to filling to 100% of the final volume (i.e. when the readings of samples taken from the top of the carrier and from the sample ports matched each other). They were 12, 6, and 10 minutes for the 50 L, 200 L, and 1000 L scales, respectively. This demonstrates that the mixer technology is effective and efficient across a wide range of volumes. Once the final water addition is complete, it is recommended to continue mixing and verify that the pH and conductivity values remain stable for a minimum of 10 minutes before filtering or using the buffer.

Table 3. Mixing Time Requirements for 1x PBS Buffer Preparation at 50 L, 200 L, and 1000 L Scales

Step	Step Duration		
	50 L	200 L	1000 L
PBS buffer addition	< 3 seconds	< 3 seconds	9 seconds
Mixing time to get the first stable value of pH and conductivity	12 minutes	6 minutes	10 minutes

In this study no attempt was made to match the mixing times of the two different scales of preparation by adjusting the impeller speed or initial fill volume. Instead, the maximum impeller speed available at each scale was used with a fixed initial volume based on experience and ease-of-use as described above. Separate studies have been performed with the Mobius® single-use mixing products to determine a scaling algorithm for both floating and sinking powders. Please contact your local representative for more information.

Filtration Study

The optimal filter choice and area requirement for sterile filtration of the 1x PBS buffer was determined using the Vmax™ method at a constant pressure of 10 psi. This method is an accelerated screening test based on the gradual pore size plugging model and it estimates scale up filter area requirements. Vmax™ testing was performed in triplicate for all filters shown in **table 2** using the freshly prepared 50 L batch of 1x PBS buffer. Since PBS buffer is non-plugging, the Vmax™ results were then used to project filtration area requirements for a range of batch sizes and process times using the following equation:

Equation 1: $Amin = V_B / (Jmean*t_B)$

Where:

Amin: minimum membrane area of the filter [m^2]
V_B: batch volume [L]
Jmean: mean flux [L / (m^2.h)]
t_B: batch process time [h]

In addition, the K_{Buf} method of determining sterile filter requirements for non-plugging buffers was also used and these results were compared to the filter recommendations generated by the Vmax™ method. K_{Buf} is an efficient and simplified filter sizing tool for buffer filtration which is based on well characterized membrane permeability values. The K_{Buf} sizing method has been shown to closely approximate sterile filter sizing requirements for buffer filtration applications generated through the more laborious Vmax™ sizing method. Filter sizing via the K_{Buf} method is determined using the following equation:

$\#$ of 10” Filter Cartridges = $V_B \times K_{Buf} / t_B \times \Delta P$

Where:

V_B: batch volume [L]

K_{Buf}: sizing constant for a specific filter type which can be applied to water-like (viscosity = 1cP), non-plugging buffers

t_B: batch filtration time [h]

ΔP: filtration process differential pressure [psid]

In all cases, the filter sizing recommendations resulting from the Vmax™ and the K_{Buf} methods were within 10% of each other, supporting the continued use of K_{Buf} as a simpler alternative sizing method for non-plugging buffers.

Millipore Express® SHF membrane filters exhibited better overall filtration performance, than Millipore Express® SHC filters, which required 33% additional filtration area to process the same buffer volume in equivalent time.

Table 4 summarizes the optimal filter capsule recommendations for 0.2 µm sterile filtration of 1x PBS buffer batches ranging from 10 L to 1,000 L. In all cases, the final filter products chosen are gamma-compatible capsules that can be incorporated directly into single-use assemblies, resulting in sterile, fully-closed flowpaths for the final buffer processing. In keeping with best practice guidance, a minimum 50% safety factor was applied to all minimum areas and the filter capsules with the closest membrane area at or above this safety factor were selected.

Table 4. Recommendations for sterile filtration of 10–1,000 L batch sizes of 1x PBS buffer

Batch Size [L]	Filtration Time [min]	Recommended Filter
10	10	Opticap® XL 150 with Millipore Express® SHF membrane
50	15	Opticap® XL 300 with Millipore Express® SHF membrane
100	20	Opticap® XL 600 with Millipore Express® SHF membrane
200	30	Opticap® XL 600 with Millipore Express® SHF membrane
500	60	Opticap® XL 600 with Millipore Express® SHF membrane
1000	120	Opticap® XL 600 with Millipore Express® SHF membrane

Since 1x PBS buffer is a non-plugging solution, filter sizing is dependent on flux and batch time, not volumetric capacity. Therefore, the selected filtration time determines the required membrane area. If a smaller device is desired, this can be achieved by increasing the batch filtration time.

Conclusions

This study highlights an efficient way to prepare and filter 50 L, 200 L, and 1000 L batches of 1x PBS buffer using Mobius® MIX solution and sterile filters. Millipore Express® SHF filters were the optimal choice for sterile filtration of the 1x PBS buffer for all batch sizes.

Best Practices for Mixing PBS

- It is recommended to inflate the mixer bags with air prior to the addition of water to ensure proper alignment with the mix vessel.
- The clamp on the bottom outlet port should be placed as close as possible to the bag to minimize dead leg volume where salts could accumulate.
- Filling the mixer with water to approximately 90% of the target final volume prior to adding powders is recommended.
- In order to achieve lowest mix times, it is recommended that the impeller be set at the maximum RPM setting. However, if scalable mix times is a concern, contact your local representative for information.
- The chemical components should be added to the mix bag at a rate which prevents the accumulation of large amounts of powder at the bottom of the mix bag, in order to avoid settling in the outlet port.
- Once all of the components are visually dissolved, the pH can be adjusted, if necessary. Finally the solution can be brought to 100% of final target volume by adding water with the impeller still running.

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