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# 1,500 L and 2,500 L Buffer and Media Preparations With Flexel<sup>®</sup> for Magnetic Mixer

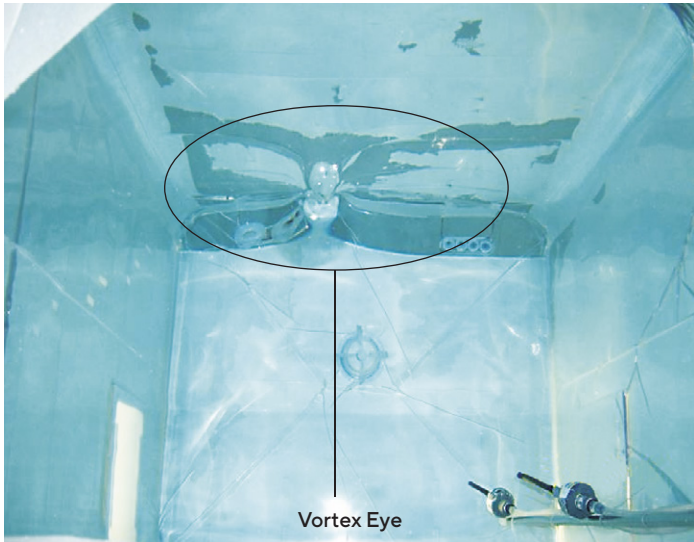
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## Executive Summary

Buffer and Media preparation are an important component of many bioprocess applications. At large scale, these processes remain dominated by multiple use, stainless steel vessels. Single-use, disposable solutions have lacked adequate mixing technology at larger volumes. Development of the Flexel<sup>®</sup> for Magnetic Mixer<sup>1</sup> technology with compatible large scale mixing Palletank<sup>®</sup> provides a unique single-use alternative to traditional stainless steel technology. This application note presents data supporting this technology to make Buffers and Media at volumes of 1,500 L and 2,500 L, as well as demonstrating liquid – liquid mixing at a volume of 2,500 L.

<sup>1</sup> This product uses Pall patented Magnetic Mixer technology. All information on patents can be found at [www.Pall.com/patents](http://www.Pall.com/patents).



2,500 L

## Introduction

Large scale Buffer and Media preparation presents a significant challenge due to the high mixing action needed to dissolve powders into solution. This application study investigates use of the Flexel® for Magnetic Mixer technology with:

- a standard 2,000 L Palletank® with 2,000 L Flexel® Bag for Magnetic Mixer for buffer and media preparation at 1,500 L,
- a custom 2,500 L Palletank® with 2,500 L Flexel® Bag for Magnetic Mixer for buffer and media preparation, as well as liquid-liquid mixing at 2,500 L.

Two SAFC® ready to use powders were used in the study, 1X DPBS and Ex-Cell™ CD CHO Fusion Media. Both of these are commonly used in bioprocess applications. A high concentration salt solution was used for the liquid – liquid portion of the study. Conductivity measurement was used to determine mixing times.

## Materials and Methods

The list of materials and equipment used for this application is:

1. 2,000 L and 2,500 L Palletank® with load cells
2. Magnetic Mixer Drive Unit (LT-DU-006-EU)
3. Eurotherm Chessell Data Logger
4. Dascor 4 – 20 mA Transmitter for conductivity
5. Sensorex conductivity probe CS200K10-TNR
6. Custom 2,500 L Flexel® Bag for Magnetic Mixer (FMB116246)
7. Standard 2,000 L Flexel® Bag for Magnetic Mixer (FMB116245)
8. Floor Scale
9. SAFC® media:
  - Ex-Cell™ CD CHO Fusion – Product Number 44075
  - Sodium Bicarbonate
  - DPBS – Product Number 56064C
10. Morton Iodized Salt
11. Conductivity Probe Calibration Standard:
  - Oakton conductivity solution, 12,880 mS/cm – P/N EW-00606-10
  - Oakton conductivity solution, 1413 mS/cm – P/N EW-00653-18



## Method Used:

Two batches of each solution, 1X DPBS and Ex-Cell™ CD CHO Fusion media, were made; for each test volume. 1,500 L batches were made in a 2,000 L Flexel® Bag for Magnetic Mixer while 2,500 L batches were made in a 2,500 L Flexel® Bag for Magnetic Mixer. A conductivity probe was scaled once using 0 mS/cm in air and a 12,880 mS/cm standard prior to all experiments taking place. The sensor was mounted on a metal pole and suspended in the solutions approximately 3 feet from the bottom of the bag. Data was recorded using a Chessel Data Logger at 1 second intervals. Each batch was prepared using the following general procedure.

1. Bag was filled to 90% of the final volume with water
2. Magnetic Mixer drive unit was set to 300 rpm.
3. Appropriate amount of DPBS or Media powder was added to the bag
4. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.
5. For Media batches, the appropriate amount of Sodium Bicarbonate powder was added.
6. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.
7. Bag was diluted (QS) to the final volume.
8. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.

Once the final Media batch was complete, the bag was drained to a volume of 2,250 L and the liquid – liquid mixing study was performed using the following procedure.

1. A solution of 32 lbs (14.5 kg) Morton Iodized Salt in 250 L of DI water was prepared in a secondary container.
2. Magnetic Mixer drive unit was set to 300 rpm.
3. Salt solution was added to the bag as quickly as possible.
4. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.

## Results and Discussions

The table below shows the masses for each powder added and the resulting concentrations.

Solution	Final Solution Volume [L]	PBS Powder Added [kg]	CHO Media Powder Added [kg]	Bicarb Powder Added [kg]	Concentration PBS or CHO Media [kg/L]	Concentration BiCarb [kg/L]
1X DPBS	1,500	14.33			0.010	
1X DPBS	2,500	23.88			0.010	
CHO Media	1,500		30.14	1,860	0.020	0.001
CHO Media	2,500		50.23	3,130	0.020	0.001

Preliminary study has proven the linearity between conductivity and concentration of DPBS and Ex-Cell™ CD CHO Fusion as per the two following graphs:

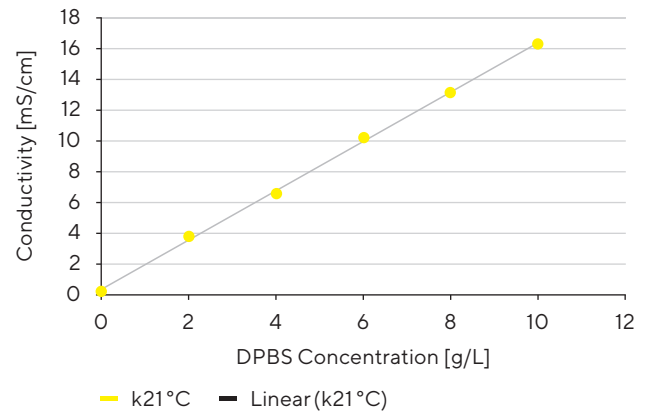


Figure 1: Conductivity vs Concentration: DPBS

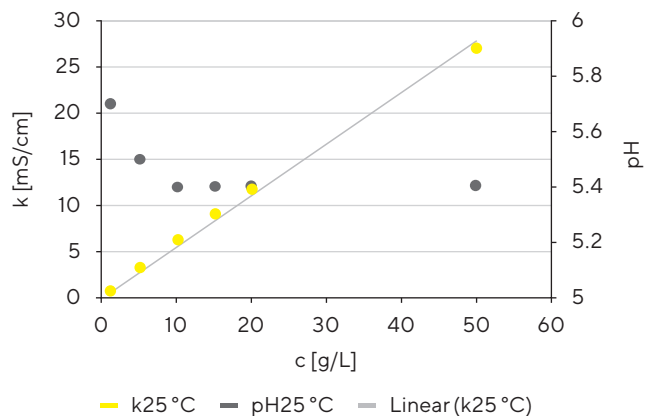


Figure 2: Conductivity and pH measurement vs Concentration of Ex-Cell™ CD CHO Fusion

## Conclusion

The tables below show powder addition times, Mixing times, and Post QS mixing times for the four solutions made. Detailed graphs can be seen in the appendix. Powder volumes were not added as one bulk addition, but spread out over time based on visually observing dissolving efficiency. Mixing time was determined to be when the conductivity probe remained constant within a 2% margin.

Volume [L]	DPBS Powder Addition [min]*	Mixing Time after powder addition [min]	Dilution Time [min]	Total Preparation [min]
1,500	4	9	6	19
2,500	6	13	10	29

\* Multiple manual powder transfer steps

Volume [L]	CHO Powder Addition [min]*	Mixing Time after powder addition [min]	Bicarbon-ate Addition + Mixing time [min]	Dilution Time [min]	Total Preparation [min]
1,500	29	4	10	6	49
2,500	60	5	18	10	93

\* Multiple manual powder transfer steps

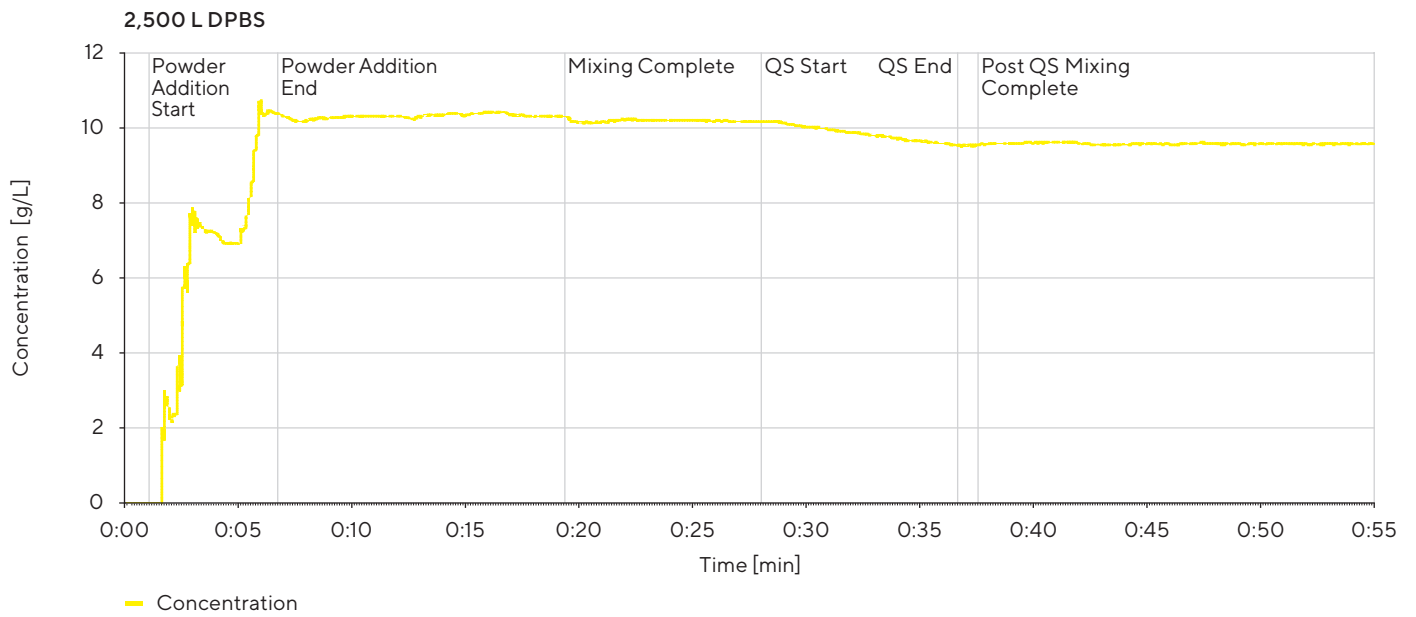
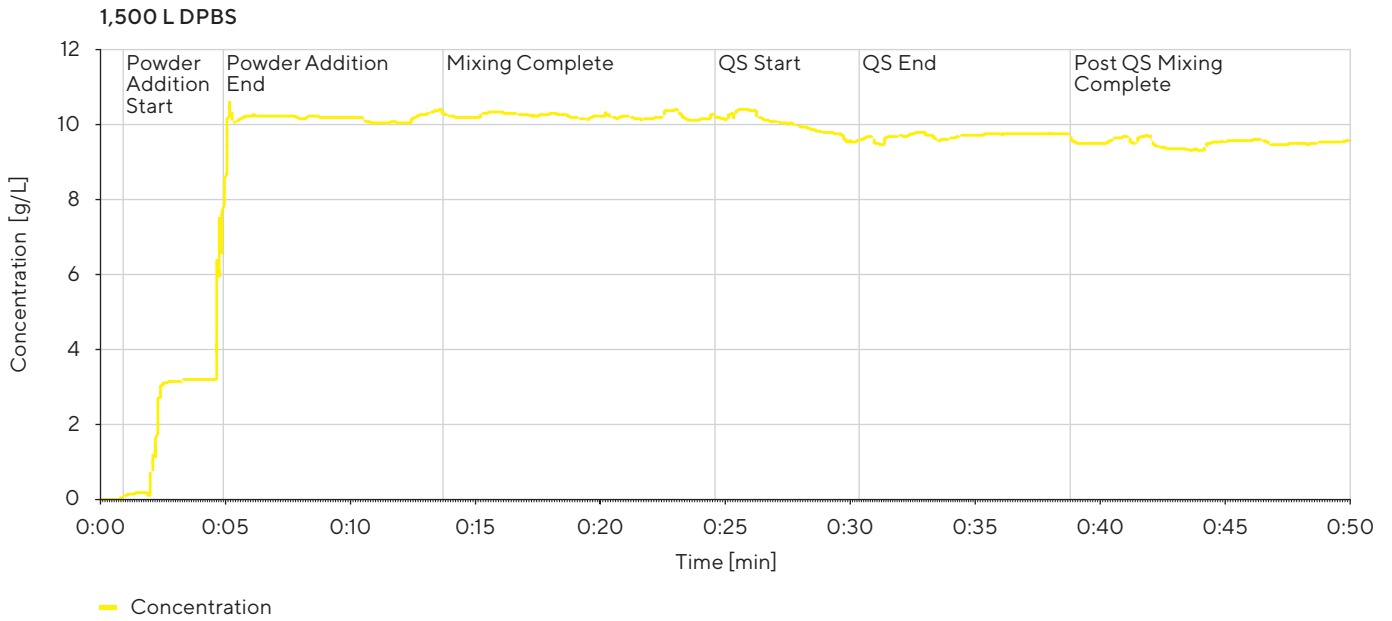
Vortexes were observed at all volumes, and DPBS powder visually dissolved almost instantaneously.

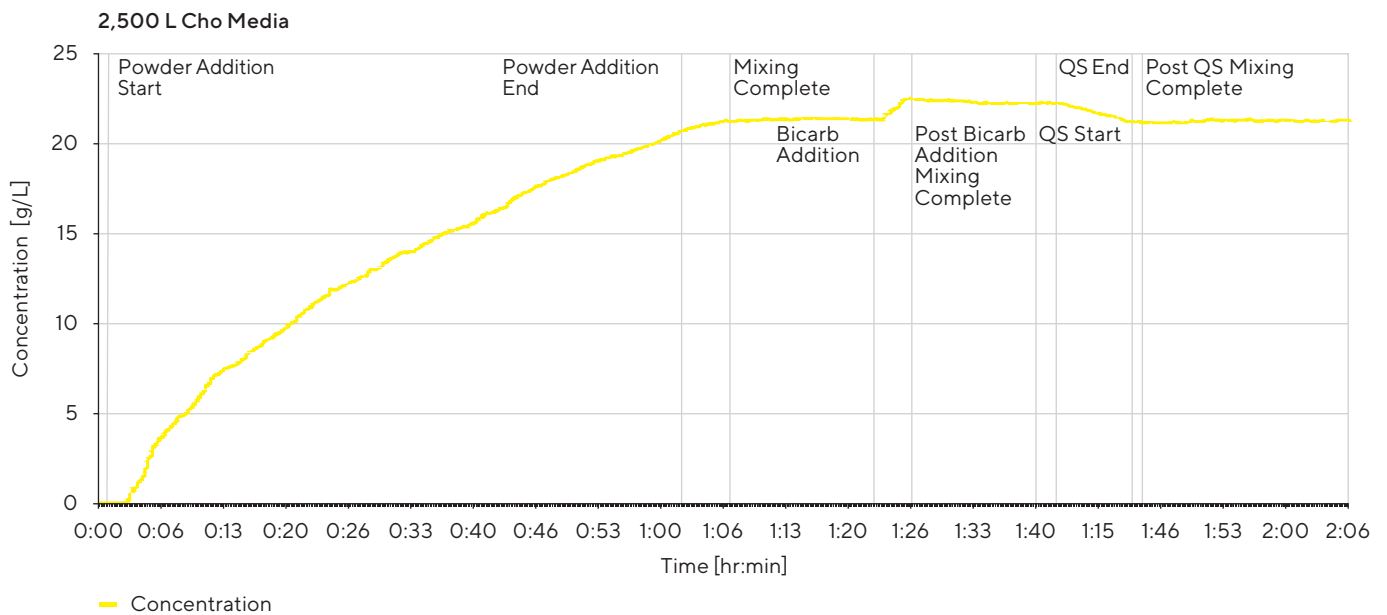
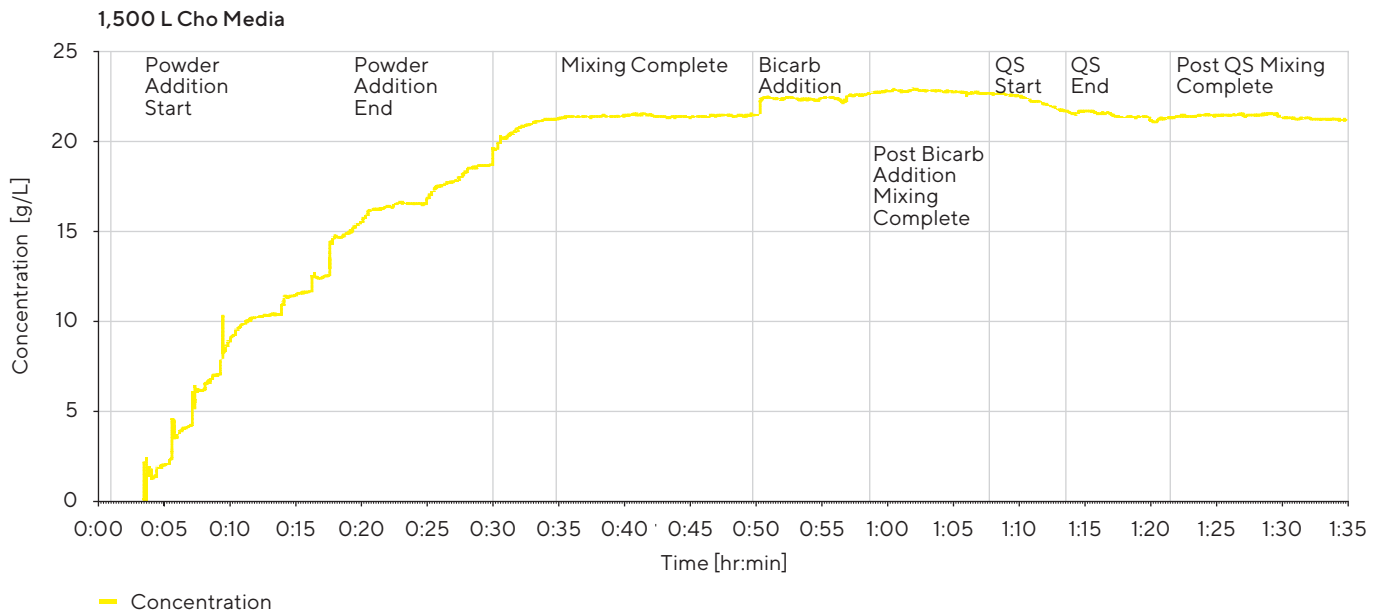
The salt solution used in the liquid – liquid portion of the study was added manually over a three minute period. Mixing time was determined to be less than 4 minutes. A detailed graph can be seen in the appendix.

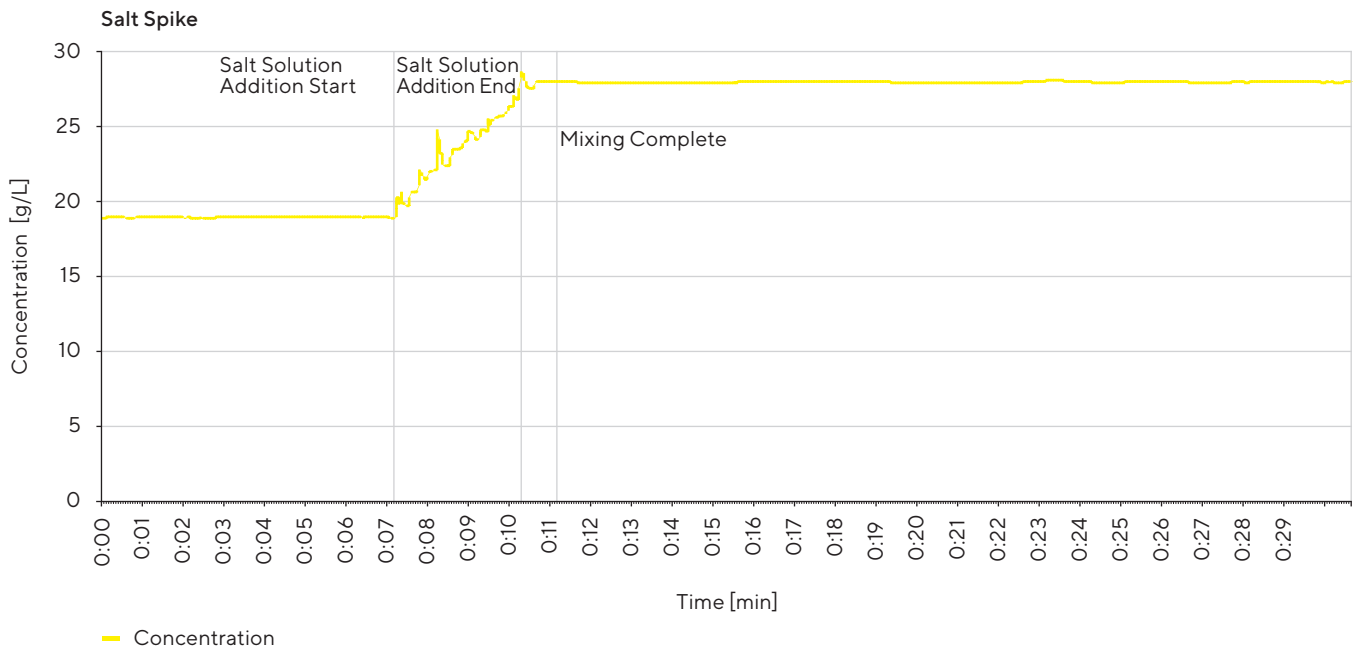
Mixing of both 1X DPBS solutions and Ex-Cell™ CD CHO Fusion media solutions using the Magnetic Mixer technology at volumes up to 2,500 L is viable. DPBS powder dissolves quickly at these volumes and complete mixing is achieved in less than 20 minutes. For Ex-Cell™ CD CHO Fusion media, mixing time at 1,500 L is less than 35 minutes, and less than 70 minutes for a 2,500 L liter batch.

Liquid – Liquid mixing using the Magnetic Mixer Palletank® system at volumes up to 2,500 L is also viable. 250 Liters of liquid were completely mixed in less than 4 minutes.

# Appendix







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