Mixing of Various Dry Powdered Media and Buffers with Entegris Flex Mixing System

INTRODUCTION

Mixers play an essential role during both upstream and downstream processing, specifically in the formulation of media and buffers. Media preparation feeds into various upstream steps. These solutions contain nutrients like amino acids, sugars, and basal solids to support cell growth. These materials have different densities and properties which may present mixing challenges. Cell culture media powders are generally comprised of floating powders that form low-density clumps at the surface of the liquid; Buffer solutions are generally comprised of salts are used in downstream processing to maintain a physiological pH range during protein purification, filtration, or final formulation. Buffers typically have higher densities and tend to sink to the bottom of the tank.

GOAL

To demonstrate the efficacy of the Entegris mixing system for both buffer and media mixing, several different solutions were prepared by mixing appropriate powders with sterile water. DPBS, DMEM, and HyCell CHO media were solubilized and measured. The results of these mixing parameters were collected to show the effective uses of the Entegris Flex Mixing System.

MATERIALS AND METHODS

Reverse Osmosis Deionized water was utilized as a sterile process fluid to dissolve all powders in this study. The recorded temperature of the RODI water was 22.8°C. Materials used in each step are described in Table 1.

The Entegris Flex Mixing System was set up according to the Quick Start Guide and connected to an appropriately-sized peristaltic pump (Cole Parmer). Conductivity (Mettler-Toledo) was measured periodically by two probes placed on opposite ends of the top of the mixing vessel throughout the mixing process. When the powdered media was visually solubilized and conductivity results between both probes were consistent and stable, the mixing process was considered complete.

Table 1. Materials Utilized for Mixing Studies

200L Entegris Mixing Bag (FCB02326.01)
200L PE drum and dolly
Peristaltic pump (Cole Parmer, MasterFlex B/T EW77111-60)
RODI process water
DPBS (Cytiva, SH30013)
DMEM (Cytiva, SH30003) HyCell CHO medium (Cytiva, SH30933)
Conductivity transmitter with sensors (Mettler Toledo)

Table 2. Operating Conditions

Working volume: 200 L*
Water temperature 22.8°C
Position two calibrated conductivity probes in the single-use mixer at opposing sides of the bag.
Fill the mixing bag with DI water to 90% of the nominal volume.
Start pump at 100% rpm (20 L/min) and allow 5 minutes to reach steady state mixing.
Start conductivity recording.
Add sufficient powder (DPBS, DMEM, or HyCell CHO) for a 200 L 1X solution followed by QS to 200 L total using the density determined in the benchtop preparation.
Record the conductivity levels and mixing time until the conductivity reaches at least 95 – 99% of the benchtop preparation.

RESULTS AND DISCUSSION

200 L Scale 1X DBPS Mixing Evaluation

The conductivity levels and mixing time were recorded until the conductivity reached at least 99% of the benchtop preparation (see Figure 1). This mixing time (T99) of the average conductivity to reach 99% of the benchtop control was found to be approximately 3 minutes, with standard deviation observed between the two conductivity probes greater than 10% until approximately 5 minutes after powder addition.

200 L Scale 1X DPBS Mixing Evaluation
Parallel Conductivity Measurements *in situ*

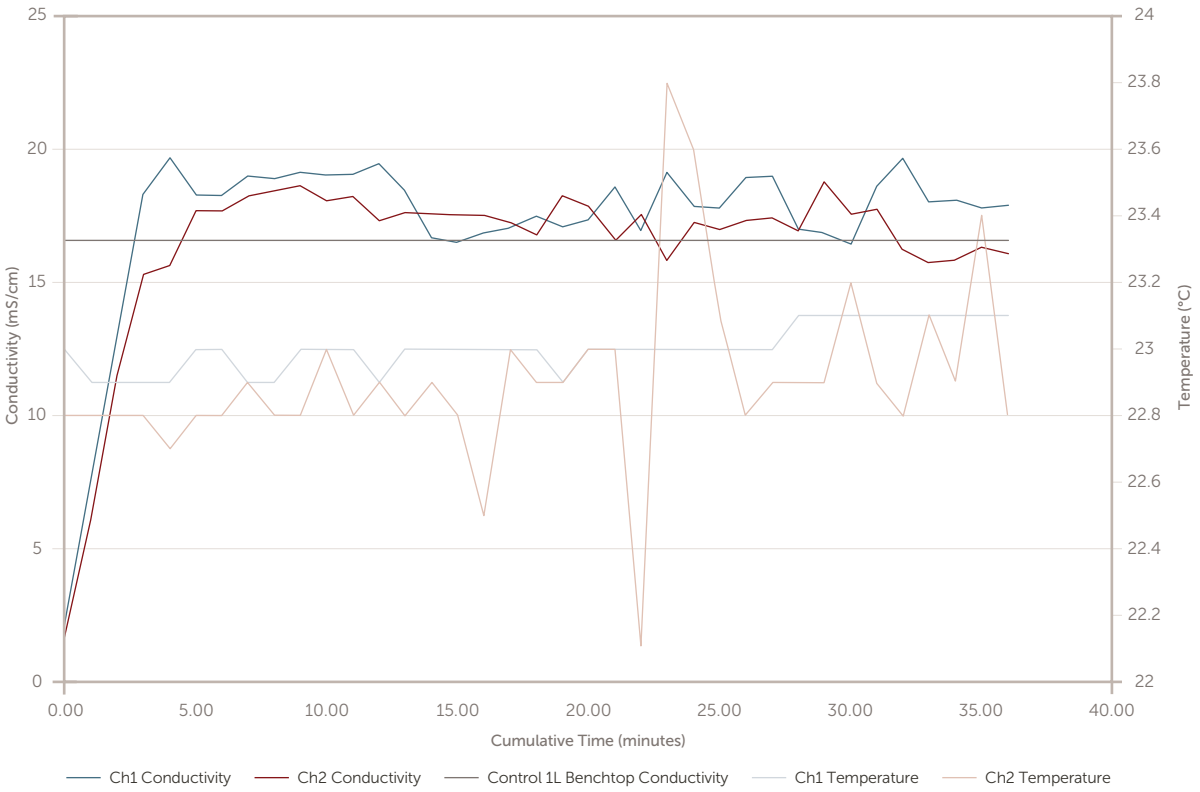

Figure 1. 1X DPBS.

200 L Scale 1X DMEM Mixing Evaluation

The mixing time to reach a conductivity within 1% of the benchtop preparation (T99) and relative standard deviations within 10% was observed to be 14 minutes (see Figure 2).

200 L Scale 1X DMEM Mixing Evaluation
Parallel Conductivity Measurements *in situ*

Figure 2. 1X DMEM.

200 L Scale 1X CHO Chemically-Defined Medium Mixing Evaluation

The mixing time to reach conductivity most similar to the benchtop preparation (95%) was 24 minutes, with relative standard deviations were above 10% until 28 minutes.

200 L Scale 1X CHO Chemically-Defined Medium Mixing Evaluation
Parallel Conductivity Measurements *in situ*

Figure 3. 1X CHO Chemically-Defined Media.

CONCLUSION

The Entegris mixing system can mix simple buffers and media within 5 – 15 minutes and more complex serum-free media like HyCell CHO in ~30 minutes. The results of the testing are summarized below:

Entegris Single-Use Mixer Study Summary

APPLICATION	SOLUTION	MIXING TIME
Buffer	1X DPBS	T99: ~5 minutes
Media	1X DMEM	T99: ~14 minutes
	1X HyCell CHO	T95: ~28

FOR MORE INFORMATION

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