Biotech

Application Note

USD 3350a

Evaluation of Visible and Sub-Visible Particle Generation in Pall Mixing Technologies

Pall® Magnetic Mixer and LevMixer® 50 L Systems

Contents

1 Introduction

The presence of particles is a significant concern in single-use bioprocessing due to the potential impact on product quality and patient safety, especially in process steps after which filtration is not possible such as final filling.

The purpose of this study was to compare particle generation over time using two of the most common single-use mixing technologies in the bioprocessing industry: The Pall Magnetic Mixer system and Pall LevMixer system.

The LevMixer system features an impeller which levitates in operation, and does not experience any friction as it rotates. In contrast, the Magnetic Mixer system has an impeller which is coupled to its drive by magnetic downforce, with a bearing assembly situated below the impeller to facilitate rotation. Friction in this bearing assembly may cause problems in some downstream applications by generating particles and/or impacting products that are sensitive to stresses.

2 Materials and Method

2.1 Materials

- 50 L Magnetic Mixer system and LevMixer system cubical tank (Part number: LM50NCN-B4N)
- 50 L Magnetic Mixer single-use biocontainer (Part number: 730473, Allegro™ film)
- 50 L LevMixer single-use biocontainer (Part number: 730467, Allegro film)
- Magnetic Mixer system drive unit (Part number: DU006-EU)
- LevMixer system drive unit (Part number: LT-DBTL300)
- Type-2 ultrapure water (UPW)
- Proprietary automated image analysis system for particle counting

Table 1.

Experiment conditions

Mixers	LevMixer and Magnetic Mixer 50 L systems
Liquid	Type-2 ultrapure water (pre-filtered 0.1 µm)
Temperature	Room temperature: 21.5 °C ± 0.5 °C
Mixing Speed	Maximum: LevMixer system = 210 rpm; Magnetic Mixer system = 300 rpm
Mixing Time	6 hours
Liquid Volume	25 L
Inflation	Yes
Sampling Time/Location	T_0; $T_{0.5h}$; T_{2h}; T_{6h}. From the drain port of the mixer in 500 mL 2D biocontainers
2D Biocontainer Flushing	3 flushes of 200 mL to maximize particle extraction
Analytical Method	Microscopic particle count test (based on USP<788> Method 2)

Note: 3 biocontainers of each mixer type were tested. Each biocontainer was made with different lots of single use components.

2.2 Method

The mixer hardware was set up, and the single-use mixing biocontainer was installed, according to the procedures outlined in Pall's published Instructions for Use (IFU). The biocontainer was then inflated via a 0.2 µm vent filter (KA1V002PV2G) and filled by weight with 25 ± 0.05 kg of ultrapure water via a 0.1 µm liquid filter (KA02DJLP2S, pre-flushed with 2 L of UPW) mounted on the filling line, and connected via a sanitary quick connector.

After the mixing biocontainer was filled, a custom sampling manifold (as shown on Figure 1) was connected to the drain of the mixing biocontainer using a Pall Kleenpak® Presto sterile connector.

Mixing was performed at maximum impeller speed for 6 hours in a temperature-controlled room.

2.2.1 Sampling

A 500 mL sample of filtered UPW was tested to characterize the baseline particle level (blank).

Samples from each mixing biocontainer were then taken in 500 mL biocontainers via the closed system sampling manifold at defined time points as follows:

- Sample 1 = T_{0h} 500 mL (after filling and mixing for 1 minute)
- Sample 2 = $T_{0.5h}$ 500 mL
- Sample 3 = T_{2h} 500 mL
- Sample 4 = T_{6h} 500 mL

Figure 1.

The sampling assembly

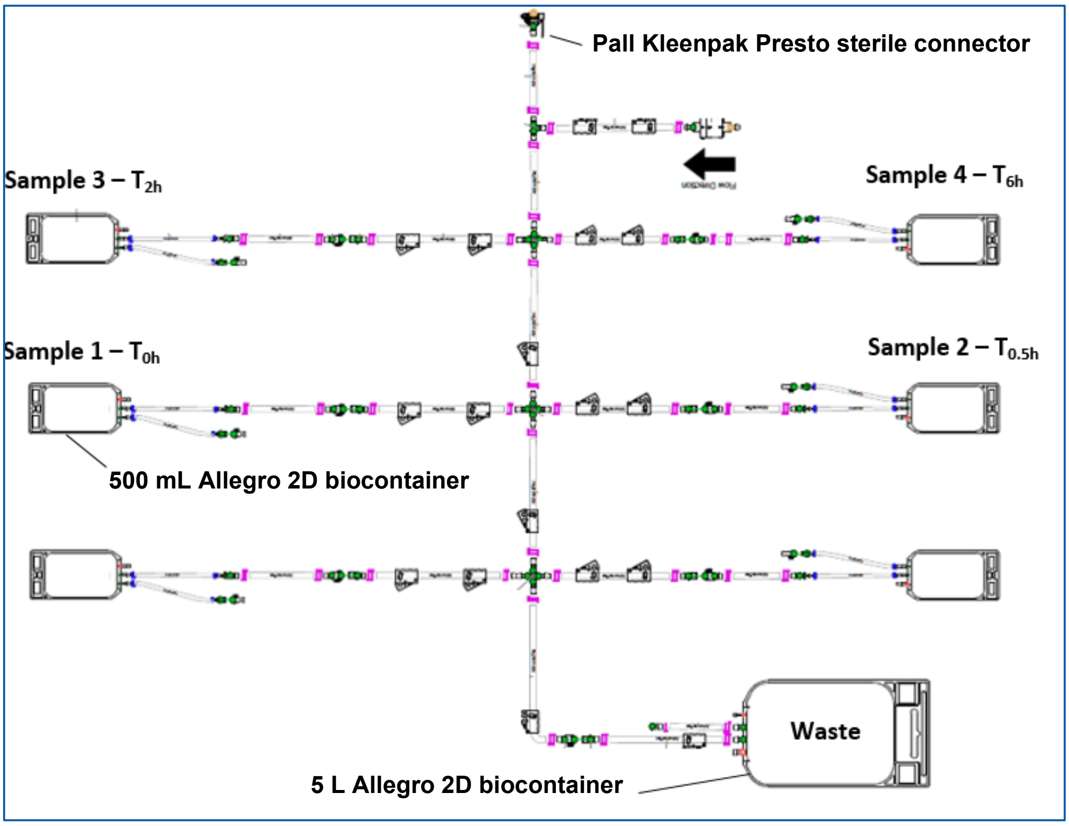
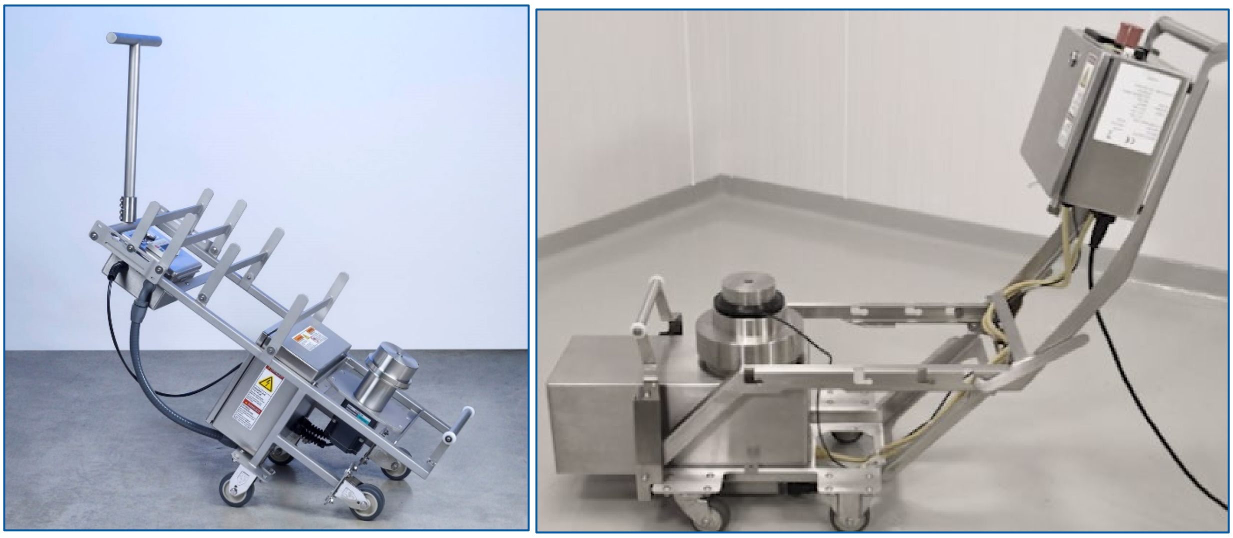


Figure 2.

The Magnetic Mixer drive unit (left) and the LevMixer drive unit (right)



Before each new sample was taken, a 300 mL volume of water (corresponding to three times the tubing volume from the mixing biocontainer to the farthest sampling biocontainer) was drained from the mixing biocontainer into a 5 L waste biocontainer by gravity with the drain valve opened, to eliminate potential contamination from previous samples.

2.2.2 Analysis

Water samples, including the blank, were tested for sub-visible and visible particles using the USP<788> Method 2 (microscopic particle count test).

2.2.3 Data Processing and Calculations

Particles were recovered from the sampling biocontainers by emptying them and additionally rinsing with 3x 200 mL of pure filtered water.

A blank value was obtained by calculating the mean particle count of the 6 blank samples taken (one during each mixing biocontainer run). For each time point, this blank value was subtracted from the total count recorded according to calculation below:

$$\text{Total particles in sample} = [(\sum \text{sample} + \text{Flush } 1,2,3) - \text{Average } (\sum \text{blanks} + \text{Flush } 1,2,3)]$$

For ease of comparison between mixing technologies, absolute particle counts were normalized based on the maximum count for the T_{6h} samples of the Magnetic Mixer system, which was deemed to represent the highest particle count at conclusion of the process. Normalization was performed as shown in the calculation below:

$$Normalization\ (\%) = [\frac{\text{Particles Count } (T_{0h}\ ; T_{0.5h}; T_{2h}; T_{6h})}{(\text{Maximum Particles } T_{6h} \text{ Magnetic Mixer})}] \text{ x } 100$$

3 Results

Figure 3.

Evolution of sub-visible and visible particles in the LevMixer (LM) system and Magnetic Mixer (MM) system over time calculated in % and normalized based on the particles level in the MM samples T_{6h}

MMAV: Magnetic Mixer Average; LMAV: LevMixer Average.

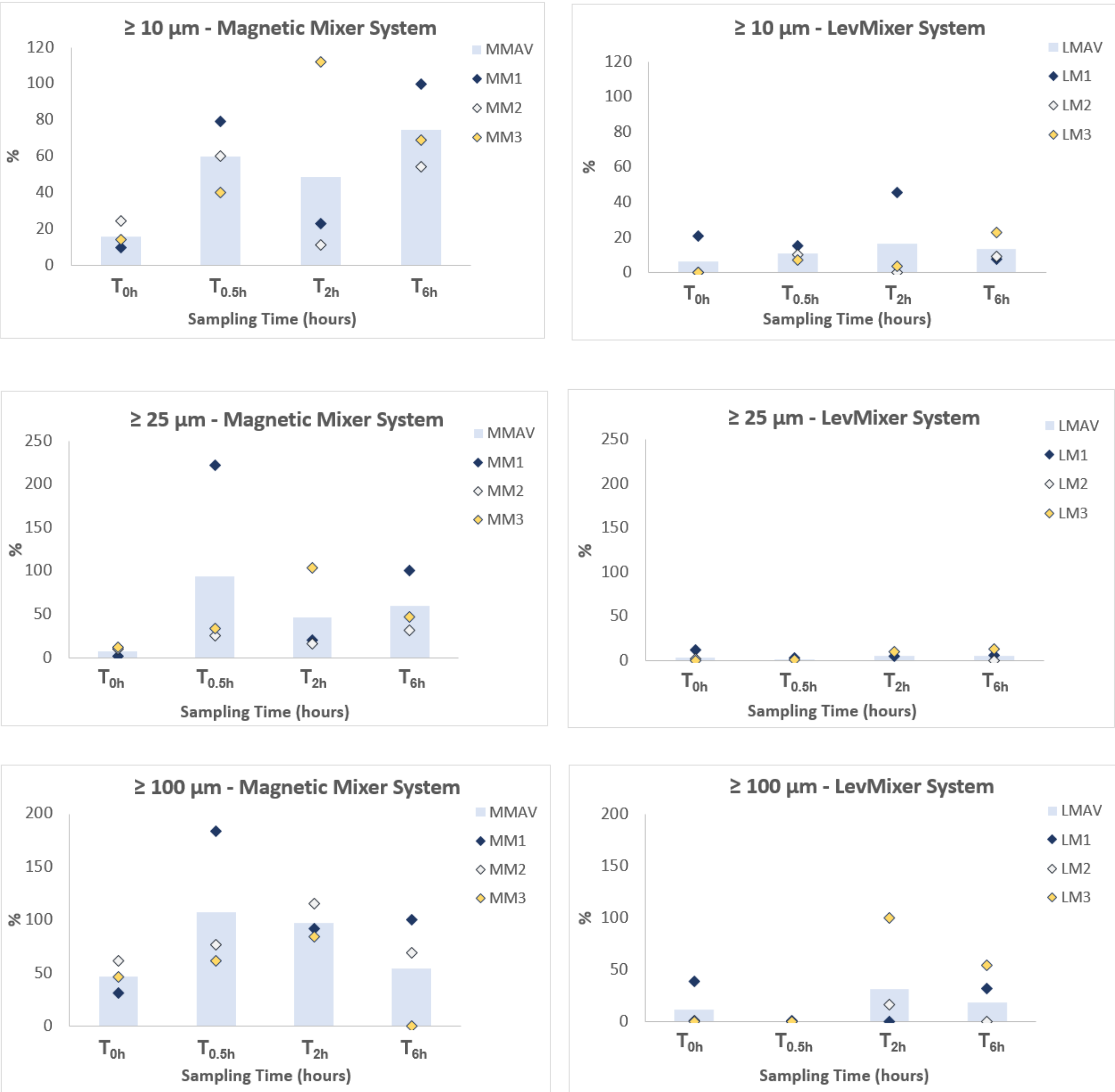
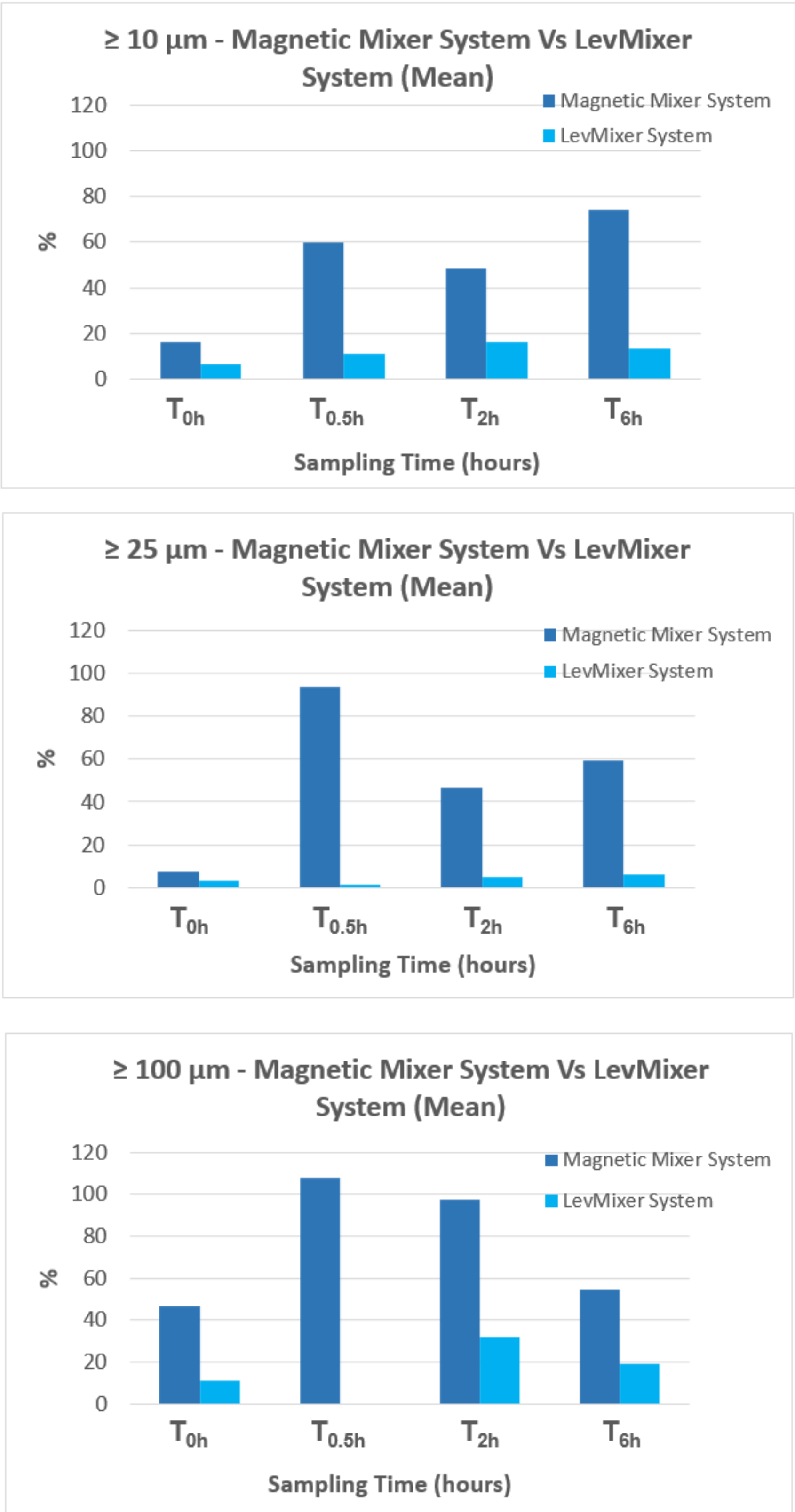


Figure 4.

Evolution of sub-visible and visible particles in the LevMixer (LM) and Magnetic Mixer (MM) over time (mean only).



4 Conclusion

When used for mixing under comparable process conditions (25 L liquid in a 50 L mixing system, impeller running at maximum speed for up to 6 hours), the LevMixer system was found to generate substantially lower particle levels compared with the Magnetic Mixer system. In some particle size ranges, LevMixer system particle counts were found to be an order of magnitude lower than with the Magnetic Mixer system.

In general, the particle levels in the Magnetic Mixer single-use biocontainer at T_{0h} and $T_{0.5h}$ were significantly higher compared with those in the LevMixer system. This is not unexpected, and can be attributed to differences in the Magnetic Mixer systems impeller design and manufacturing processes, and to the increased friction forces within the Magnetic Mixer system bearing assembly during mixing.

Despite the disparity in particle generation, this study demonstrates that the particle levels tend to stabilize at $T_{0.5h}$ with both the Magnetic Mixer system and the LevMixer system, meaning that there was little or no particle generation by either mixer subsequent to this time point. All results remained well below Pall's supply specifications for sub-visible particles in single-use systems, corresponding to USP <788> limits for Large Volume Parenterals: The number of particles ≥10 µm did not exceed 12 particles/mL, and the number of particles ≥25 µm did not exceed 2 particles/mL.

For applications where powerful, high-shear mixing is required, and where particle generation is not a leading concern (e.g. buffer and media preparation) due to downstream filtration, Pall recommends Magnetic Mixer system as the technology of choice. However, for applications that are sensitive to particles (e.g. downstream product processing, processes without subsequent filtration etc.), Pall instead recommends LevMixer system as the preferred technology.

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