# HyPerforma Single-Use Mixer (S.U.M.) performance evaluation

## Measurement of particulate size and quantity generated during operation

### Introduction

Disposables have become increasingly popular in bioproduction, from media storage bags and filling manifolds to mixing vessels and bioreactors. Simple designs such as 2-dimensional “pillow” bags on rocking platforms and recirculation loops were early attempts to meet this need. However, this style of agitation is similar to the industry standard used in traditional stainless steel systems. Other types of mixing devices were later developed, including oscillating agitators and magnetically coupled impellers.

The stirred tank is the most commonly utilized style of mixing vessel for both liquid–liquid and powder–liquid mixing applications. The Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) was designed to meet this industry standard but also to provide the unique advantage of a completely disposable and portable system. The S.U.M. BioProcess Container (BPC) is supplied in either contained or open-top formats. The contained system allows for sterile liquid–liquid mixing or dust-free powder–liquid mixing. The powder–liquid BPCs contain a 3-inch tri-clamp port for powder addition that can be coupled to another powder-dispensing Thermo Scientific™ Powdertainer™ II BPC. Mixing applications in downstream

processes require a high level of scrutiny into factors such as container integrity, leachables, extractables, and generation of particulates. This study evaluated particulates generated during a mixing operation. Analyses were performed on samples obtained at regular time intervals to characterize the size and quantity of particulates generated.

## Materials and methods

A Thermo Scientific™ 50 L S.U.M. stainless steel outer support container with drive and controller and a Thermo Scientific™ liquid–liquid mixing 50 L S.U.M. BPC (Cat. No. SH30767.01) were used in this evaluation. Since all S.U.M.s and single-use bioreactors (S.U.B.s) currently utilize the same bearing-and-seal assembly, which is integral to the BPC, and since the surface-to-volume ratio of the 50 L S.U.M. is higher than that of larger systems, this setup is considered to be a worst-case scenario with regard to particulate concentration. Water for injection (WFI) was used to fill the S.U.M. to 50% nominal volume (25 L). Each trial was performed at room temperature (approximately 25°C).

To assess particulate generation over time during a mixing operation, multiple samples were obtained. Samples were obtained by aliquoting a 500 mL sample from the S.U.M. BPC using a 500 mL Thermo Scientific™ Nalgene™ polyethylene bottle and cap (Cat. No. 34240-0650). Initially, the mixer was operated at 350 rpm for 3 minutes and then sampled for the first time point. Thereafter, samples were collected at 2, 4, 8, 12, 16, and 24 hours, with the mixer operating at 350 rpm. After 24 hours of mixing, the bearing-and-seal assembly was submerged in WFI and agitated again at 350 rpm for 2 hours. A final sample was then collected. For confirmation, second and third trials were performed similarly, with a new 50 L S.U.M. BPC (with integrated bearing-and-seal assembly) and fresh WFI, but samples were obtained less frequently at 0, 12, 24, and 26 (following bearing-and-seal assembly submersion) hours.

A Multisizer™ 4 counter (Beckman Coulter) was used to determine particle size and quantity. This analyzer uses electrical sensing zone (ESZ) and digital pulse processing (DPP) methods to analyze particle size and quantity. Samples were prepared for analysis by adding 10 mL of 20% NaCl in $H_2O$ (filtered with a 20 nm filter) to 200 mL of each sample, to make the samples as conductive as the electrolyte (0.9% NaCl in $H_2O$). The analyses were performed with an aperture setting of 1 µm to 30 µm using volumetric control mode and background subtraction.

## Results and discussion

The ESZ method allows determination of the distribution of the number of particles, based on their size, over a very large range of sizes. Table 1 lists the sample identification information, particle size, and quantity for that sample.

**Table 1. Sampling protocol used during a 24-hour mixing evaluation to determine particle size and quantity, and results of particle analysis.**

| Sample time (hours) | Sample description | Sample ID | Particle count (particles/mL) | Mean particle size (µm) | Count of >10 µm particles | Count of >25 µm particles |
|---|---|---|---|---|---|---|
| 0 | 3 minutes after initiation of agitation | 1.1 | 1,868 | 1.69 | 1 | 0 |
| | | 2.1 | 2,498 | 1.68 | 8 | 0 |
| | | 3.1 | 1,093 | 1.61 | 3 | 0 |
| 2 | | 1.2 | 3,732 | 1.58 | 3 | 0 |
| 4 | | 1.3 | 5,212 | 1.50 | 0 | 0 |
| 8 | | 1.4 | 8,087 | 1.44 | 1 | 0 |
| 12 | | 1.5 | 10,435 | 1.42 | 0 | 0 |
| | | 2.2 | 7,059 | 1.55 | 5 | 0 |
| | | 3.2 | 7,803 | 1.38 | 0 | 0 |
| 16 | | 1.6 | 12,236 | 1.41 | 0 | 0 |
| 24 | | 1.7 | 16,574 | 1.37 | 0 | 0 |
| | | 2.3 | 11,878 | 1.47 | 0 | 0 |
| | | 3.3 | 12,090 | 1.38 | 0 | 0 |
| 26 | Submerged bearing-and-seal assembly | 1.8 | 15,100 | 1.35 | 0 | 0 |
| | | 2.4 | 12,195 | 1.41 | 0 | 0 |
| | | 3.4 | 12,459 | 1.36 | 0 | 0 |

It is apparent from all three trials that the mean particle size decreased with increased mixing time. The samples taken at 3 minutes after initiation of agitation had a total average size of 1.66 µm, whereas the samples taken at 26 hours had a total average size of 1.37 µm. This reduction in size is most likely due to particles breaking apart through agitation. The total average number of particles 1–30 µm in size from the first samples taken in the three trials was 1,819, while the total average from the final samples was 13,251, supporting the idea that the particles were breaking apart.

United States Pharmacopeia (USP) Chapter 788, Particulate Matter in Injections, requires that injectable solutions of <100 mL per injection (small-volume injections) do not exceed a maximum particle count of 6,000 particles/container of ≥10 µm in size and 600 particles/container of ≥25 µm in size. For injectable solutions of >100 mL (large-volume injections), USP 788 requires not exceeding a maximum particle count of 25 particles/mL of ≥10 µm in size and 3 particles/mL of ≥25 µm in size as determined by light obscuration. Results from this evaluation show the total average number of particles ≥10 µm in size to be 3.3 particles/mL and particles ≥25 µm in size to be 0 (none found). The S.U.M. mixing system, even when evaluated at a high surface-to-volume ratio with the bearing-and-seal assembly rotating at maximum speed for 24 hours, meets USP 788 requirements for particle count.

The methods stated for use in USP 788, however, are light obscuration or microscopic particle counting. In this evaluation, we utilized the ESZ method to determine the distribution of particle sizes within a greater range than required by USP 788. These results also meet the requirements under USP 788 for microscopic particle count (small-volume injections: 3,000 particles/container of ≥10 µm in size and 300 particles/container of ≥25 µm in size; large-volume injections: 12 particles/mL of ≥10 µm in size and 2 particles/mL of ≥25 µm in size).

## Summary

Based upon the ESZ method for particle and size determination, the HyPerforma S.U.M. meets the particle size and quantity requirements of USP 788. The evaluation included a worst-case scenario of a high surface-to-volume ratio, maximum rotational speed (350 rpm) of the bearing-and-seal assembly (including impeller), and a 24-hour mixing time with an additional 2 hours of mixing at maximum rotational speed while the bearing-and-seal assembly was submerged. Multiple samples were obtained during three trials in order to assess particle size and quantity. In each of the three trials, the highest counts of particles of ≥10 µm in size were found in the first samples taken. These samples contained an average of 3.3 particles of ≥10 µm. No particles of ≥25 µm were found in any samples collected throughout the evaluation.