Mixing efficiencies for the 200 L HyPerforma Single-Use Mixer

Introduction

The Thermo Scientific™ 200 L HyPerforma™ Single-Use Mixer (S.U.M.) provides rapid mixing for essential upstream and downstream processes. The addition of the Touchscreen Console to the 200 L HyPerforma S.U.M. adds real-time sensor visualization, data logging, and the ability to automate user-defined mixing recipes. To demonstrate the superior mixing capabilities for various applications and to determine optimal mixing parameters, five different mixing studies (Table 1) were performed with the 200 L HyPerforma S.U.M. at various working volumes and mixing speeds. The materials used in these mixing studies were chosen to model the way buffers (NaCl solution), complex media (Gibco™ AGT™ medium), and viscous liquids (corn syrup) are mixed in the 200 L HyPerforma S.U.M.

Materials and methods

A standard 200 L HyPerforma S.U.M. was used with a standard 200 L Thermo Scientific™ HyPerforma™ S.U.M. BioProcess Container (BPC) modified with sampling and probe ports at the bottom, middle, and top of the BPC.

To test the wide range of applications of the 200 L HyPerforma S.U.M., mixing studies were performed at various working volumes. The manufacturer-recommended maximum stirring speed—the point where maximum agitation occurs without excessive shaft wobbling or splashing—was determined through visual inspection (Table 2). The corresponding power was determined using Equation 1 [1], with the power number equal to 2.1 and the stirring speed observed.

$$P = N_p \rho N_i^3 D_i^5 \quad \text{(Equation 1)}$$

N_p: power number, ρ: density of mixture, N_i: stirring speed, D_i: impeller diameter.

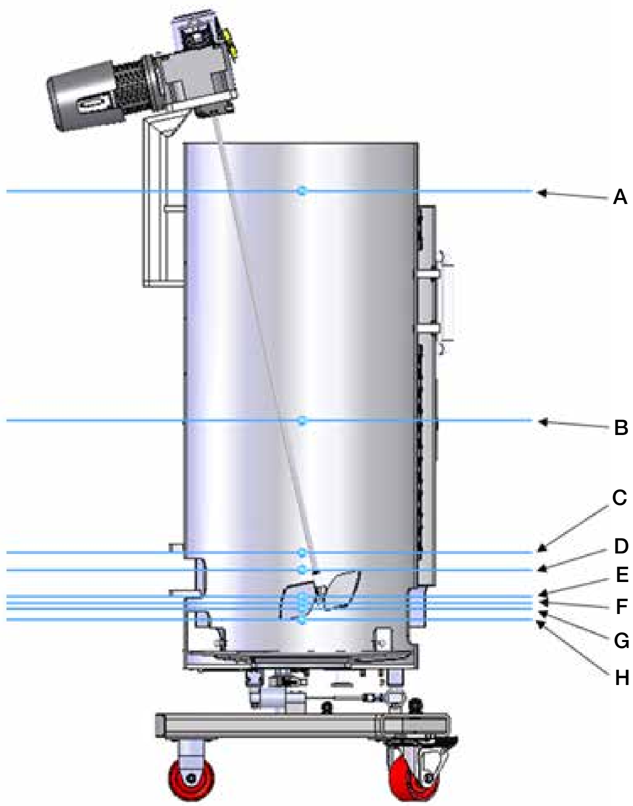
Table 2. The manufacturer-recommended maximum stirring speeds determined for the working volumes of 10:1, 5:1, 2:1, and full volume.

Working volume	Manufacturer-recommended maximum stirring speed
10:1 (20 L)	140 rpm (427 W/m^3)
5:1 (40 L)	170 rpm (382 W/m^3)
2:1 (100 L)	356 rpm (1,404 W/m^3)
Full volume (200 L)	356 rpm (702 W/m^3)

Table 1. Mixing studies performed on the 200 L HyPerforma S.U.M.

Mixing type	Mixing material	Concentration	Analytics
Liquid–liquid	NaCl solution	350 g/L NaCl in deionized (DI) H_2O	Conductivity
Liquid–solid	NaCl granules	1 M (final)	Conductivity, osmolality
Liquid–solid	AGT medium (granules)	1X (final)	Conductivity, osmolality, pH, glucose concentration, visual
Viscous liquid–liquid	Corn syrup with NaCl solution	80–88% corn syrup	Conductivity
Viscous liquid–solid	Corn syrup with NaCl granules	80–88% corn syrup	Conductivity

The impeller coverage at various locations on the 200 L HyPerforma S.U.M. was determined through visual inspection, and the results are given in Figure 1.



	Description	Height	Volume (L)
A	100% working volume	101.6 cm (40 in.)	200
B	50% working volume	50.8 cm (20 in.)	100
C	20% working volume	21.6 cm (8.5 in.)	40
D	Top of impeller	17.8 cm (7 in.)	32.5
E	Middle of impeller	12.1 cm (4.75 in)	21.6
F	10% working volume	10.8 cm (4.25 in.)	20
G	Probe belt covered	9.5 cm (3.75 in.)	17.4
H	Bottom of impeller	7.0 cm (2.75 in.)	12.6

Figure 1. The impeller coverage at various locations on the 200 L HyPerforma S.U.M., and the corresponding height and volume. The height was measured from the bottom center of the tank.

Liquid–liquid mixing: NaCl solution

Three conductivity probes were inserted into the BPC in the bottom, middle, and top ports. The BPC was filled with DI water to three working volumes—5:1, 2:1, and full volume. At each volume, the following power inputs were tested in triplicate: minimum (20 rpm), 20 W/m^3, 100 W/m^3, and maximum agitation (356 rpm). At 5:1 working volume, 356 rpm is above the manufacturer-recommended maximum stirring speed; however, it was tested for comparative purposes.

The predetermined agitation was set on the 200 L HyPerforma S.U.M. and was allowed to mix for 2 minutes to allow mixing flow patterns to stabilize. A small volume of concentrated NaCl solution (350 g/L in DI H_2O) was pipetted into the mixer as close to the surface of the water as possible, over a span of 3–4 seconds. Conductivity data were recorded for 5 minutes with a data logger. This process was repeated for each power input at each volume in triplicate. An average T95 mixing time—when the measured value reaches 95% of the final stable value—was calculated as the average of the slowest-responding sensor among the test replicates for each test condition.

Liquid–solid mixing: NaCl granules

Three conductivity probes were inserted into the BPC in the bottom, middle, and top ports. The BPC was filled with DI water to three working volumes—10:1, 5:1, and full volume. At each working volume, the manufacturer-recommended maximum stirring speed was used (see Table 2). The predetermined agitation was set and was allowed to mix for 2 minutes. Enough NaCl was quickly added to the mixer to make a 1 M NaCl solution. Samples were taken every minute for the first 20 minutes, every 5 minutes for the next 20 minutes, every 10 minutes for the next 20 minutes, and every minute for the last 10 minutes. Samples of 10 mL were taken from the bottom, middle, and top ports for full volume, and just the bottom sampling port for 5:1 and 10:1 working volumes. Conductivity was measured online, and osmolality was tested and recorded after samples were collected.

Liquid–solid mixing: AGT medium

A conductivity probe and pH probe were inserted into the BPC in the top and bottom ports. A camera was attached to the top of the mixer to observe the time when foam and powder from the medium were completely in suspension. The BPC was filled with DI water to 90% of three working volumes—10:1, 5:1, and full volume. At each working volume, the manufacturer-recommended maximum stirring speed was used (see Table 2).

The mixer was turned on to the predetermined agitation, and the manufacturer's recommended amount of AGT medium was quickly added to the top of the mixer. Samples were taken over a 70-minute time span using the same method described in the previous section. Samples of 10 mL were taken from the bottom, middle, and top ports for full volume testing and just the bottom port for low working volumes. The pH and conductivity were measured online with the Touchscreen Console. The osmolality and glucose concentration of the samples were tested and recorded after samples were collected.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

Conductivity probes were inserted into the top, middle, and bottom of the BPC. The BPC was filled initially with 200 L of 88% corn syrup/12% DI water (v/v). A solution of 350 g/L NaCl was made, and a 5 L mixture containing 12% of the salt solution and 88% corn syrup (v/v) was prepared. The agitation was set to 356 rpm, and 100 mL of the mixture of salt solution and corn syrup was quickly added. The viscosity of the 88% corn syrup solution in the HyPerforma S.U.M. was measured with a viscometer. The change in conductivity was monitored for 5 minutes. The process was repeated in triplicate at 84% corn syrup and 80% corn syrup. The T95 mixing times were calculated using the method described in the prior liquid–liquid mixing section.

Viscous liquid–solid mixing: corn syrup with NaCl granules

Conductivity probes were inserted into the top, middle, and bottom of the BPC. The BPC was filled initially to full volume with 89% corn syrup/11% DI water (v/v). A small sample was taken from the mixer to test viscosity and temperature. NaCl was added to the sample until complete saturation occurred and NaCl granules were visible. The agitation was set to 356 rpm, and the mixture of corn syrup and NaCl granules was added quickly to the top of the mixer. The change in conductivity was monitored and logged for 12 minutes, and the T95 mixing time was calculated using the same method described in the prior liquid–liquid mixing section. This process was repeated at several corn syrup concentrations ranging from 80% to 88%. The T95 mixing times and measured viscosities were averaged and plotted.

Results

Liquid–liquid mixing: NaCl solution

For liquid–liquid mixing of a salt solution, T95 mixing times were less than 3.5 minutes regardless of agitation or working volume, as shown in Figure 2. As working volumes were lowered, mixing times also decreased. With an increase in agitation, mixing times decreased as well. However, at the 5:1 and 2:1 working volumes there was not a significant decrease to already rapid mixing times when power was increased above 100 W/m^3.

Liquid–solid mixing: NaCl granules

For liquid–solid mixing using salt granules, the conductivity was measured for three working volumes, as shown in Figure 3. The T95 mixing times by conductivity were 5.5 minutes for the 10:1 working volume, 1 minute for the 5:1 working volume, and 2 minutes for full volume. This mixing trend is different from the liquid–liquid mixing results observed previously, where T95 mixing times decrease with lower working volumes. It was observed during this mixing study that at 10:1 working volume some of the salt granules temporarily sat at the bottom of the mixer for the first few minutes of mixing; this resulted in a longer T95 mixing time. A similar result was observed in the 50 L and 2,000 L HyPerforma S.U.M.s as well as the 2,000 L

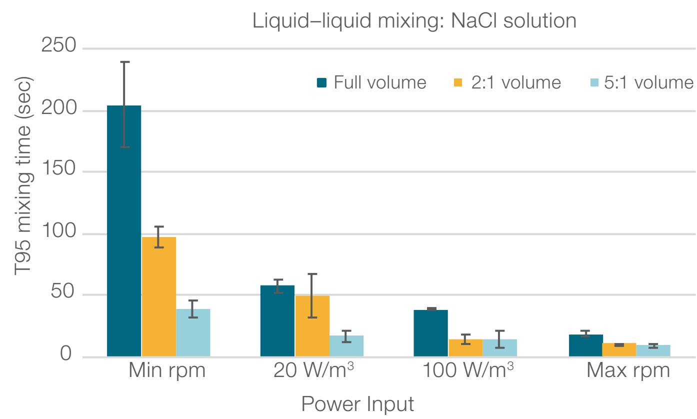


Figure 2. The average T95 mixing times (± 1 standard deviation) calculated for varying working volumes at minimum rpm (20 rpm), 20 W/m^3, 100 W/m^3, and maximum rpm (356 rpm).

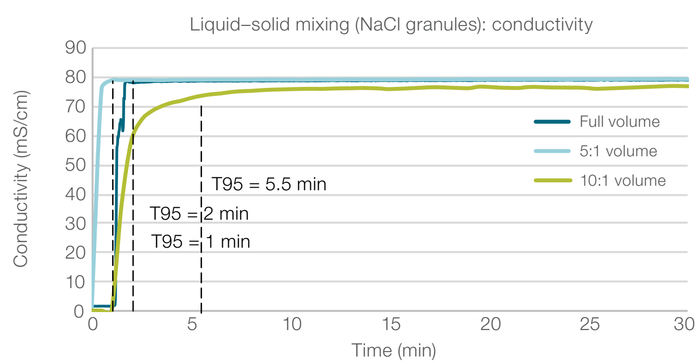


Figure 3. Conductivity data recorded at 10:1, 5:1, and full volume.

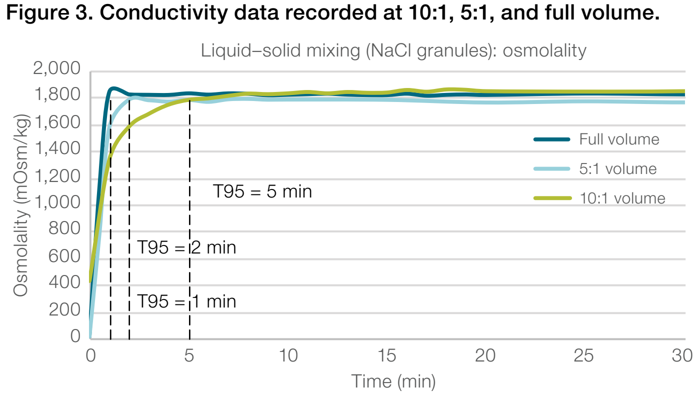


Figure 4. Osmolality recorded at 10:1, 5:1, and full volume.

Thermo Scientific™ imPULSE™ S.U.M. at low working volumes. Figure 4 shows the osmolality recorded for the three working volumes. The T95 mixing times were reached in 5 minutes for the 10:1 working volume, 2 minutes for the 5:1 working volume, and 1 minute for the full volume, similar to mixing times observed for conductivity.

Liquid–solid mixing: AGT medium

For liquid–solid mixing of representative AGT medium granules, the glucose and osmolality were measured offline after samples were collected. The results of the glucose concentration and osmolality measurements are shown in Figure 5. At all volumes tested, the glucose levels and osmolality had a T95 mixing time of 2 minutes.

The T95 mixing times calculated from the conductivity data were 1 minute at 5:1 working volume and 2 minutes at full volume (Figure 6). The conductivity was not measured at 10:1 volume due to a sensor error. At low working volumes there was a distinct upward shift in pH over time (Figure 7). It is likely that this shift is a result of CO_2 degassing due to the low working volume and rapid mixing. This shift is not ideal, because cell metabolism can be altered by pH changes [2]. The pH shift can be corrected by the addition of acid or base; however, studies have shown that base or acid addition can be correlated with process variability and osmolality increase [3]. Generally, it is considered a best practice to avoid the addition of base or acid to cell culture media. During this study, the purpose was to show how rapidly a medium could be incorporated, and ideal conditions for cell culture medium preparation were not followed. More ideal conditions for mixing cell culture media are discussed in the conclusions and recommendations section.

The powder in this liquid–solid mixing study floated on top of the fluid, and the settling that was observed in the liquid–solid mixing with NaCl granules did not occur. Therefore, at lower working volumes, rapid mixing times were observed.

Figure 8 shows photos taken at various times during the mixing study at full volume. After 2 minutes of mixing, the powder was completely in suspension and foaming was diminished. This corresponds with the data in Figures 5 and 6 that show osmolality, conductivity, and glucose stabilizing in a similar time frame.

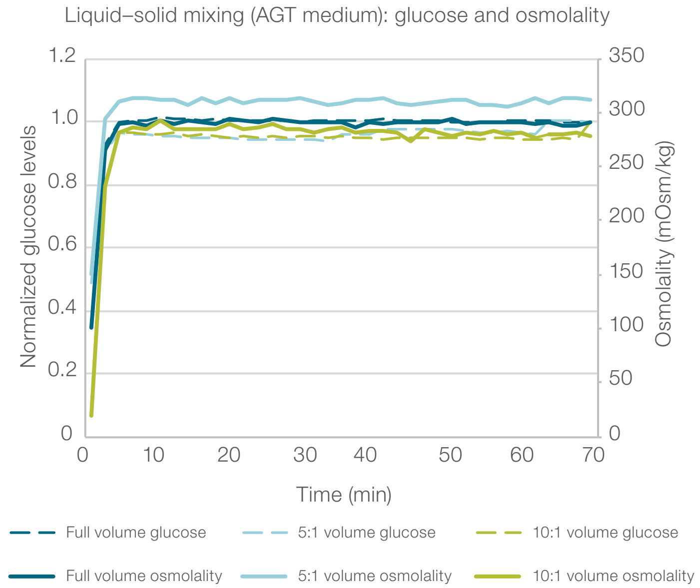


Figure 5. Glucose concentration and osmolality recorded at 10:1, 5:1, and full volume.

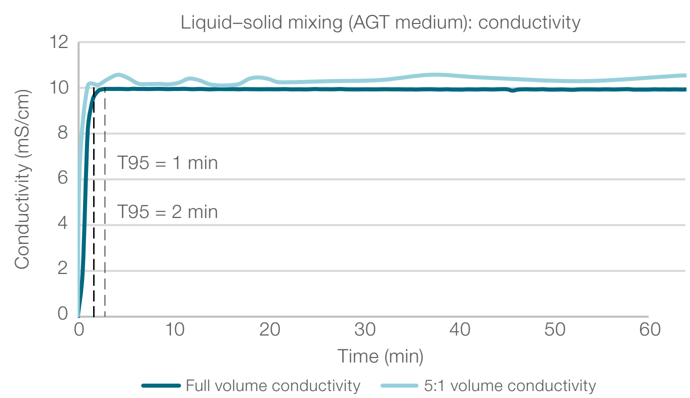


Figure 6. Conductivity data of the AGT medium recorded at the different volumes tested.

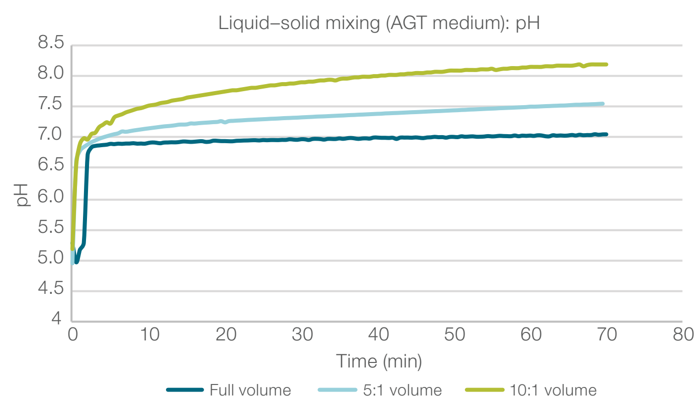


Figure 7. pH of the AGT medium recorded at the different volumes tested.

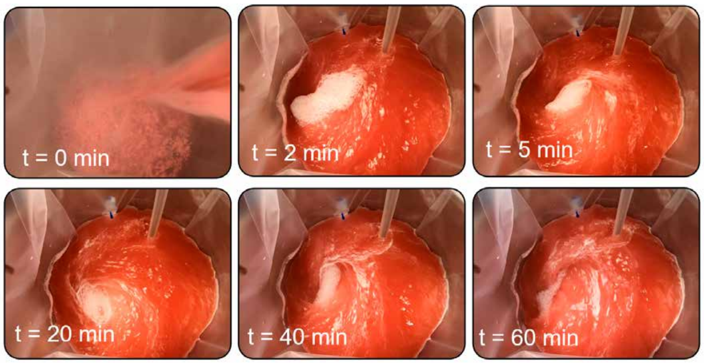


Figure 8. Photos of the fluid at the top of the mixer at various time points during the full-volume AGT medium mixing study.

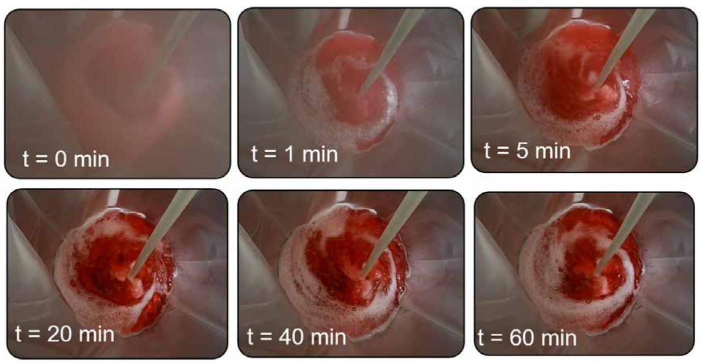


Figure 9. Photos of the fluid at the top of the mixer at various time points during the 5:1 volume AGT medium mixing study.

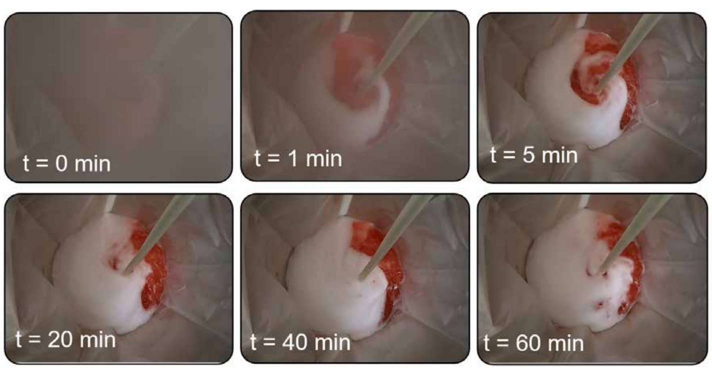


Figure 10. Photos of the fluid at the top of the mixer at various time points during the 10:1 volume AGT medium mixing study.

Figure 9 shows photos taken at various times during the mixing study at 5:1 working volume. After 1 minute of mixing, it was observed that the powder was completely in suspension and foaming was diminished. This corresponds with the data shown in Figures 5 and 6.

Figure 10 shows photos taken at various times during the mixing study at 10:1 volume. After 1 minute of mixing, it was observed that the powder was completely in suspension and foaming was diminished. This corresponds with the data shown in Figures 5 and 6 for the 10:1 volume. Homogeneity in conductivity, osmolality, pH, and glucose was achieved in a similar time frame.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

The measured viscosity of the corn syrup mixtures containing 80–88% corn syrup by approximate volume ranged from 246 cP to 861 cP, which is within the range of medium- to high-viscosity fluids seen in some applications. The conductivity data recorded are shown in Figure 11. The average T95 mixing times were determined to be 31 seconds for 80% corn syrup (266 cP), 49 seconds for 84% corn syrup (464 cP), and 146 seconds for 88% corn syrup (820 cP).

Viscous liquid–solid mixing: corn syrup with NaCl granules

The measured viscosity resulting from corn syrup mixtures containing 80–89% corn syrup by volume ranged from 328 cP to 1,216 cP, which is within the range of medium- to high-viscosity fluids seen in some applications. High-viscosity fluids (700–1,000 cP) were completely mixed in under 10 minutes, and medium-viscosity fluids (200–400 cP) were completely mixed in under 6 minutes (Figure 12).

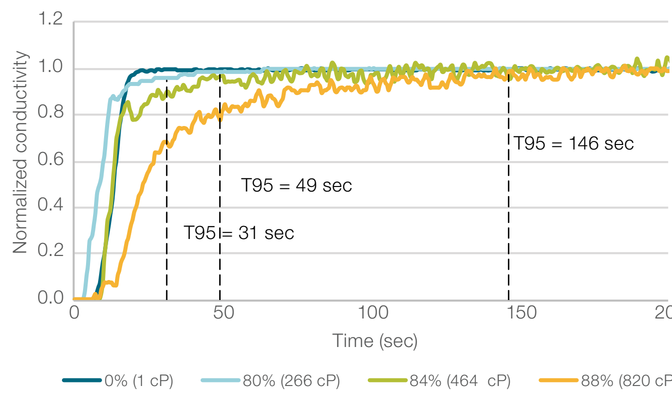


Figure 11. Conductivity data recorded during viscous liquid–liquid mixing with solutions of 0–88% corn syrup.

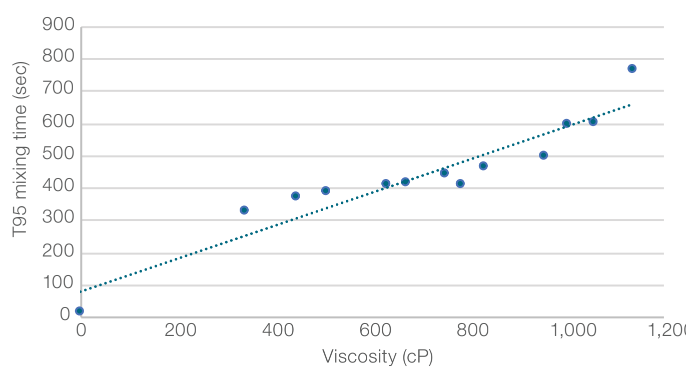


Figure 12. Average T95 mixing times of viscous liquid–solid mixing with corn syrup solutions of 0–89%.

Conclusions and recommendations

It was found that the 200 L HyPerforma S.U.M. exhibits robust and efficient mixing for volumes ranging from 10:1 to full volume. It is capable of mixing liquids, sinking solids, and floating powder in under 6 minutes (Table 3). It is also capable of mixing medium-viscosity fluids in under 6 minutes and high-viscosity fluids in under 10 minutes during liquid–solid testing. During viscous liquid–liquid testing, T95 mixing times of 0.8 minutes were observed for medium-viscosity fluids and 2.4 minutes for high-viscosity fluids. In general, at lower working volumes, faster mixing was observed. However, mixing sinking solids was found to produce longer mixing times at low working volumes due to settling. Table 3 shows when the solution reached a T95 mixing time based on the analytic used.

For optimal mixing, we suggest filling the mixer, setting a desired agitation, and adding product incrementally. If complex medium is being prepared at 5:1 working volume or lower, we recommend that the mixer not be set to the manufacturer-recommended stirring speed at that working volume. Lowering the agitation, decreasing overall processing time, and paying attention to pH drift will help ensure that the pH stays within range. Low working volumes with sinking solids can be problematic, and longer mixing times may be observed.

The 200 L HyPerforma S.U.M. performs complete and rapid mixing that can be used in a variety of upstream and downstream processes that contain a wide variety of materials, ranging from liquids, floating solids, sinking solids, and viscous liquids, at different working volumes. With the addition of the Touchscreen Console, the 200 L HyPerforma S.U.M. is a user-friendly, rapid, and robust mixer that meets the mixing requirements that are essential in today's competitive mixing industry.

Table 3. Average times for mixing of various materials in the 200 L HyPerforma S.U.M.

Mixing study	Mixing material	Working volume	Agitation	Analytic	T95 mixing time (min)
Liquid–liquid	NaCl solution	5:1	356 rpm (3,509 W/m^3)	Conductivity	0.2
		2:1	356 rpm (1,404 W/m^3)	Conductivity	0.2
		Full volume	356 rpm (702 W/m^3)	Conductivity	0.3
Liquid–solid	NaCl granules	10:1	140 rpm (427 W/m^3)	Conductivity	5.5
				Osmolality	5.0
		5:1	170 rpm (382 W/m^3)	Conductivity	1.0
				Osmolality	2.0
		Full volume	356 rpm (702 W/m^3)	Conductivity	2.0
				Osmolality	1.0
Liquid–solid	AGT medium	10:1	140 rpm (427 W/m^3)	Osmolality	2.0
				Glucose	2.0
				Visual inspection	1.0
		5:1	170 rpm (382 W/m^3)	Conductivity	1.0
				Osmolality	2.0
				Glucose	2.0
				Visual inspection	1.0
		Full volume	356 rpm (702 W/m^3)	Conductivity	2.0
				Osmolality	2.0
				Glucose	2.0
				Visual inspection	2.0
Viscous liquid–liquid	High-viscosity liquid (700–1,000 cP)	Full volume	356 rpm (~926–938 W/m^3)	Conductivity	2.4
	Medium-viscosity liquid (200–400 cP)	Full volume	356 rpm (~913–926 W/m^3)	Conductivity	0.8
Viscous liquid–solid	High-viscosity liquid (700–1,000 cP)	Full volume	356 rpm (~926–938 W/m^3)	Conductivity	10.0
	Medium-viscosity liquid (200–400 cP)	Full volume	356 rpm (~913–926 W/m^3)	Conductivity	6.0

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References

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3. Hoshan L, Jiang R, Moroney J et al. (2019) Effective bioreactor pH control using only sparging gases. *Biotechnol Prog* 35(1):e2743.

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