Mixing efficiencies for the 50 L HyPerforma Single-Use Mixer

Introduction

The 50 L Thermo Scientific™ HyPerforma™ Single-Use Mixer (S.U.M.) provides rapid mixing for essential upstream and downstream processes. The addition of the Touchscreen Console to the 50 L HyPerforma S.U.M. provides 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, four different mixing studies (Table 1) were performed with the 50 L HyPerforma S.U.M. at various working volumes and mixing speeds. The materials used in the 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 50 L HyPerforma S.U.M.

Materials and methods

A standard 50 L HyPerforma S.U.M. was used along with a standard 50 L Thermo Scientific™ HyPerforma™ BioProcess Container (BPC) modified with sampling ports and probe ports at the top, middle, and bottom of the BPC.

Table 1. Mixing studies performed with the 50 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, glucose concentration, visual, pH
Viscous liquid–liquid	Corn syrup with NaCl solution	80–88% corn syrup	Conductivity

To test the wide range of applications of the 50 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.

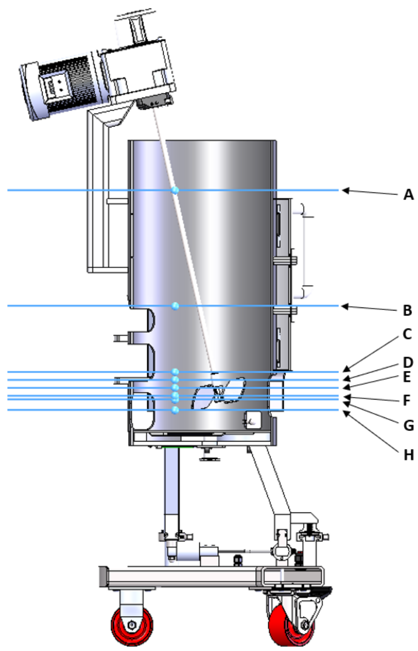
$$P = N_p \rho N_i^3 D_i^5 \qquad \text{(Equation 1)}$$

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 (5 L)	130 rpm (283 W/m^3)
5:1 (10 L)	144 rpm (193 W/m^3)
2:1 (25 L)	356 rpm (1,164 W/m^3)
Full volume (50 L)	356 rpm (582 W/m^3)

The impeller coverage at various locations on the 50 L HyPerforma S.U.M. was determined through visual inspection, and the results are shown in Figure 1.



	Description	Height	Volume (L)
A	100% working volume	55.88 cm (22 in.)	50
B	50% working volume	27.94 cm (11 in.)	25
C	20% working volume	12.07 cm (4.75 in.)	10
D	Top of impeller	10.16 cm (4 in.)	8.25
E	Probe belt covered	8.26 cm (3.25 in.)	6.5
F	10% working volume	6.35 cm (2.5 in.)	5
G	Middle of impeller	5.40 cm (2.13 in.)	4
H	Bottom of Impeller	2.86 cm (1.13 in.)	2

Figure 1. The impeller coverage at various locations on the 50 L HyPerforma S.U.M., and the corresponding height and volume. Height was measured as the distance from the bottom center of the vessel to the top of the fluid at a specific volume or location.

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). Although 356 rpm is above the manufacturer-recommended maximum stirring speed for 5:1 working volume, it was tested for comparative purposes.

The 50 L HyPerforma S.U.M. was set to the predetermined agitation and allowed to mix for 2 minutes, until mixing flow patterns stabilized. 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. A T95 mixing time—when the conductivity 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 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 twenty 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 and pH probe were inserted into the bottom and top ports of the BPC. A camera was attached to the top of the mixer to observe the time when foam and powder from the medium were completely incorporated. 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 set to the predetermined agitation, and the manufacturer-recommended amount of AGT medium was 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 osmolality and glucose levels of the samples were tested and recorded, and the conductivity and pH were measured online with the Touchscreen Console.

Viscous liquid–liquid mixing: corn syrup with NaCl solution

Conductivity probes were inserted into the BPC in the bottom, middle, and top ports. The BPC was initially filled with 50 L of 88% corn syrup/12% DI water (v/v). A solution of 350 g/L NaCl was made, and a 500 mL mixture containing 12% of the salt solution and 88% corn syrup (v/v) was prepared. The agitation was set to 356 rpm, and 50 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 same method described in the prior liquid–liquid mixing section.

Results

Liquid–liquid mixing: NaCl solution

For liquid–liquid mixing of a salt solution, T95 mixing times were less than 2.5 minutes regardless of agitation or working volume, as shown in Figure 2. As working volumes were lowered, mixing times also decreased. An increase in power corresponded to a decrease in T95 mixing times.

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 4.5 minutes for 10:1 working volume, 1.5 minutes for 5:1 working volume, and 0.5 minutes for full volume. This mixing trend is different from the liquid–liquid mixing results observed previously where T95 mixing times decreased 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 200 and 2,000 L HyPerforma S.U.M.s as well as the 2,000 L 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, and 2 minutes for both the 5:1 and full volume, similar to mixing times observed for conductivity.

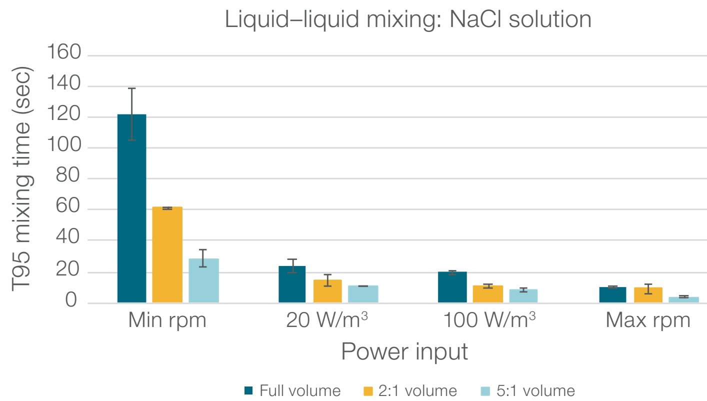


Figure 2. The average T95 mixing times (± 1 standard deviation) calculated for three working volumes at minimum rpm (20 rpm), 20 W/m³, 100 W/m³, and maximum rpm (356 rpm).

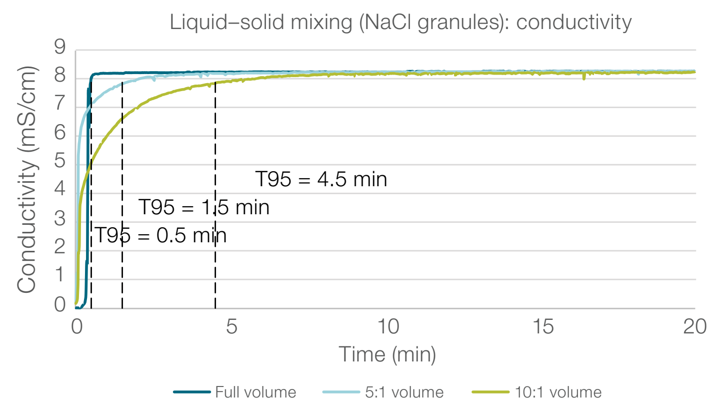


Figure 3. Conductivity recorded at 10:1, 5:1, and full volume.

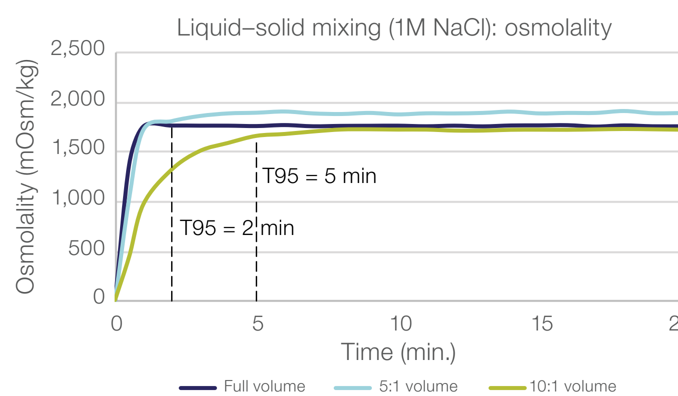


Figure 4. Osmolality recorded at 10:1, 5:1, and full volume.

Liquid–solid mixing: AGT medium

For liquid–solid mixing of representative AGT medium granules, the glucose concentration and osmolality were measured offline after samples were collected. The results of the glucose and osmolality measurements are shown in Figure 5. The T95 mixing times for glucose and osmolality were 2 minutes at the 10:1 working volume and 1 minute at the 5:1 working volume. At full volume, the T95 mixing times for glucose and osmolality were 3 minutes.

The T95 mixing times calculated from the conductivity data were 1.5 minutes at the 5:1 and 10:1 volumes and 1 minute at full volume (Figure 6). 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 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 10:1 volume. After 2 minutes of mixing, 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 working volume. Homogeneity in glucose, osmolality, and conductivity was achieved in 2 minutes.

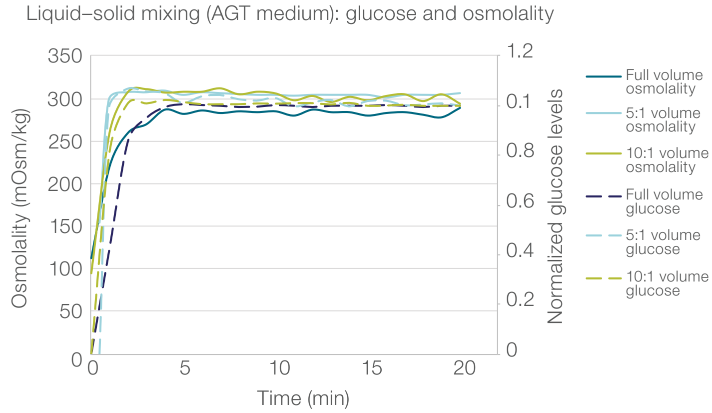


Figure 5. Glucose concentration and osmolality recorded at 10:1, 5:1, and full volume.

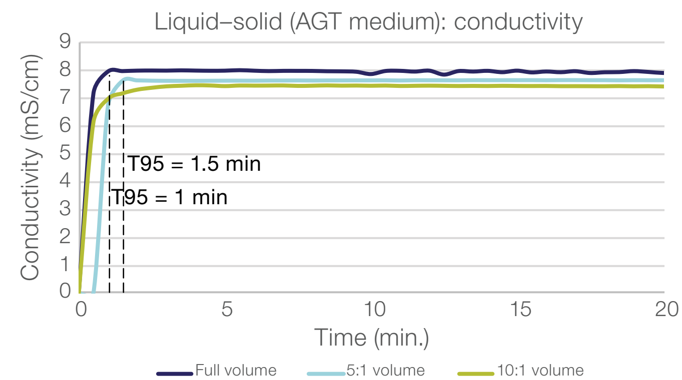


Figure 6. Conductivity recorded at the different volumes tested.

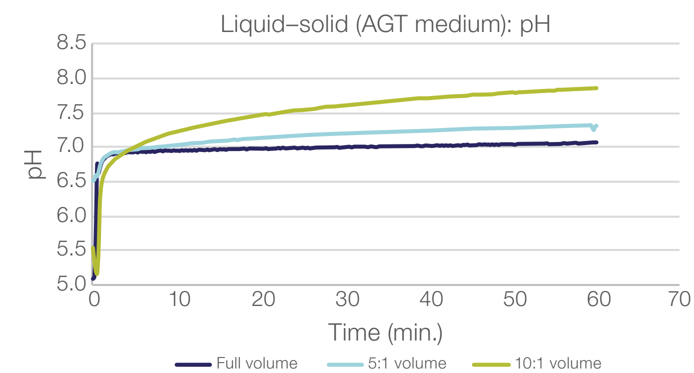


Figure 7. pH recorded at the different volumes tested.

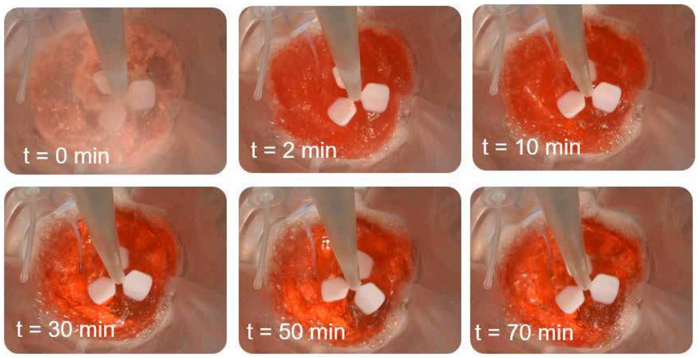


Figure 8. Photos of the fluid at the top of the mixer at various time points during the 10:1 volume AGT medium mixing study.

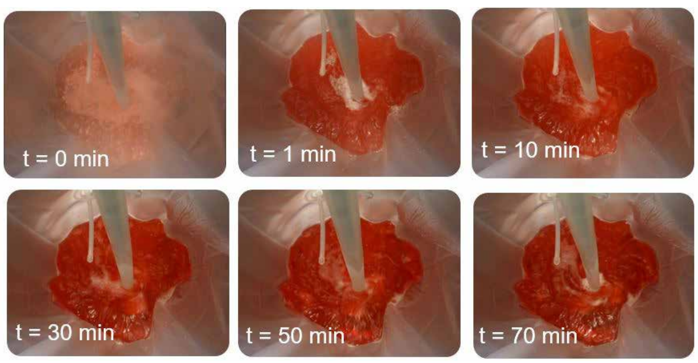


Figure 9. Photos of the fluid at the top of the mixer at various time points during the 5:1 volume AGT medium mixing study.

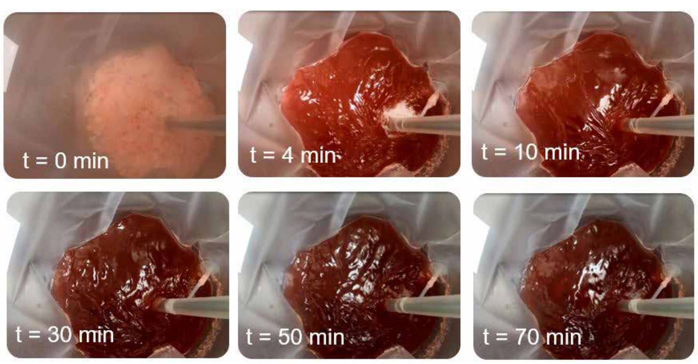


Figure 10. Photos of the fluid at the top of the mixer at various time points during the full-volume AGT medium mixing study.

Figure 9 shows images captured at various times during the mixing of AGT medium at 5:1 working volume. After 1 minute of mixing, the powder was completely in suspension and foaming was diminished. This corresponds with data displayed in Figures 5 and 6 at 5:1 working volume.

Figure 10 shows images captured at various times during the mixing of AGT medium at full working volume. After 4 minutes of mixing, the powder was completely in suspension and foaming was diminished. This corresponds with the data in Figure 5 at full volume.

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 242 cP to 836 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 T95 mixing times were determined to be 0.5 minutes for 80% corn syrup (243 cP), 0.6 minutes for 84% corn syrup (399 cP), and 3.2 minutes for 88% corn syrup (835 cP).

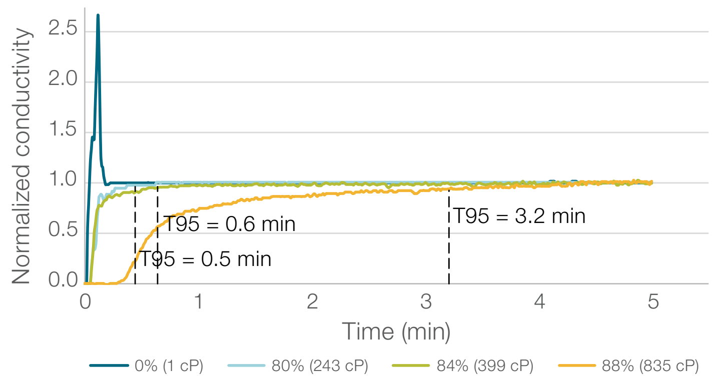


Figure 11. Conductivity data recorded during viscous liquid–liquid mixing with solutions of 0–88% corn syrup.

Conclusions and recommendations

It was found that the 50 L HyPerforma S.U.M. exhibits robust and efficient mixing for working volumes ranging from 10:1 to full volume. It is capable of mixing liquids, sinking solids, and floating powder in under 5 minutes. It is also capable of mixing medium-viscosity liquids in under 1 minute and high-viscosity liquids in under 4 minutes. In general, faster mixing at lower working volumes was observed. However, mixing sinking solids was found to produce longer mixing times at lower 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 a complex medium is being prepared at 5:1 working volume or lower, we recommend that the mixer is not set to the manufacturer-recommended maximum stirring speed at that working volume. Lowering the agitation, lowering overall mixing time, and paying attention to pH drift helps ensure that the pH remains in range. The solution is then mixed until homogeneity occurs. Low working volumes with sinking solids can be problematic, and longer mixing times may be observed.

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The 50 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 50 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.

References

1. Doran PM (2013) Bioprocess Engineering Principles, Second Edition. Elsevier: Waltham, MA.
2. Hagrot E (2011) Development of a culture system for modeling of pH effects in CHO cells. Student thesis. KTH School of Biotechnology, Department of Process Technology, Stockholm, Sweden.
3. Hoshan L, Jiang R, Moroney J et al. (2019) Effective bioreactor pH control using only sparging gases. *Biotechnol Prog* 35(1):e2743.

Table 3. Average times for mixing of various materials in the 50 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 (2,910 W/m^3)	Conductivity	0.1
		2:1	356 rpm (1,164 W/m^3)	Conductivity	0.2
		Full volume	356 rpm (582 W/m^3)	Conductivity	0.2
Liquid–solid	NaCl granules	10:1	130 rpm (283 W/m^3)	Conductivity	4.5
				Osmolality	5.0
		5:1	144 rpm (193 W/m^3)	Conductivity	1.5
				Osmolality	2.0
		Full volume	356 rpm (582 W/m^3)	Conductivity	0.5
				Osmolality	2.0
Liquid–solid	AGT medium	10:1	130 rpm (283 W/m^3)	Conductivity	1.5
				Osmolality	2.0
				Glucose	2.0
				Visual inspection	2.0
		5:1	144 rpm (193 W/m^3)	Conductivity	1.5
				Osmolality	1.0
				Glucose	1.0
				Visual inspection	1.0
		Full volume	356 rpm (582 W/m^3)	Conductivity	1.0
				Osmolality	3.0
				Glucose	3.0
				Visual inspection	4.0
Viscous liquid–liquid	High-viscosity liquid (700–1,000 cP)	Full volume	356 rpm (~768–778 W/m^3)	Conductivity	3.2
	Medium-viscosity liquid (200–400 cP)	Full volume	356 rpm (~758–768 W/m^3)	Conductivity	0.6

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