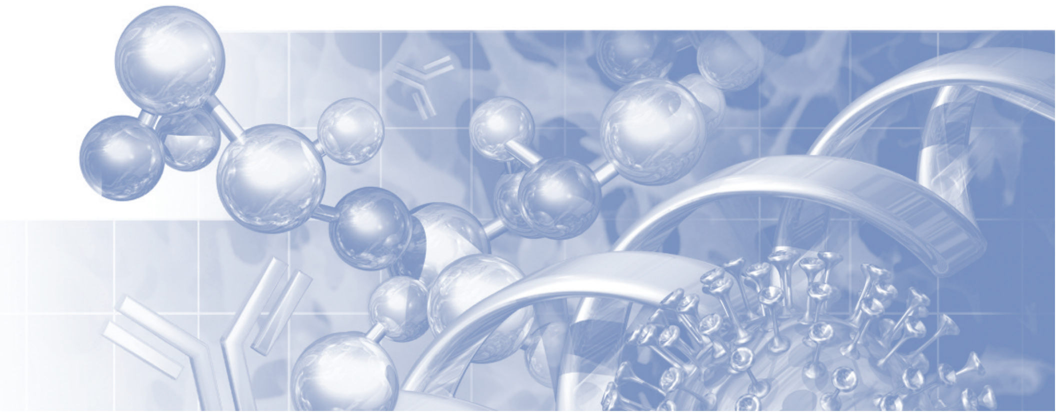
Life Sciences

Application Note

USD 2747

Allegro™ 2D Powder Port Biocontainer – Recirculation Mixing at 20 and 50 L

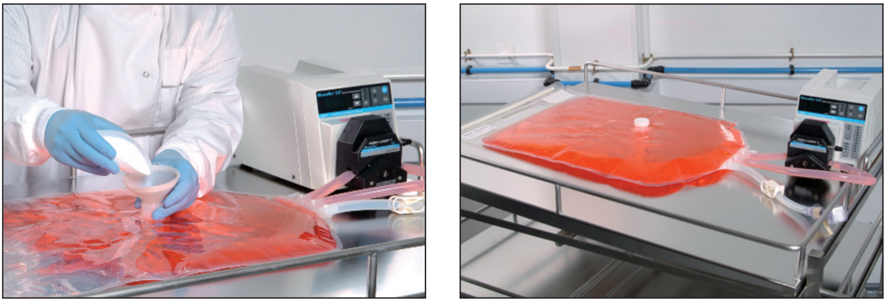


*Filtration. Separation. Solution.*SM

1. Introduction

Mixing systems are often used to prepare buffer and media solutions while controlling important fluid parameters such as pH and conductivity. Such systems can vary for a wide range of applications and volume sizes. Pall Life Sciences has introduced a user-friendly, single-use mixing system for small volume batches. This system, as shown in Figure 1, is comprised of an Allegro 2D biocontainer that contains a powder port intended for solids introduction and associated tubing. Added liquids and solids are mixed using a peristaltic pump to transfer the fluid from the outlet port of the biocontainer to the inlet port through a recirculation loop. The peristaltic pump creates a jet stream from the recirculation loop into the biocontainer resulting in turbulent mixing. The Allegro biocontainer mixing systems are available in 20 L and 50 L sizes each containing ½ in. inlet and outlet fittings, with targeted application volumes from 10 to 50 liters.

Figure 1:
Picture of the Pall Allegro 2D 20 L mixing system.



2. Summary

The Allegro 2D biocontainer/mixer was evaluated for optimum mixing conditions for solids and liquids. Studies showed the most rapid and complete mixing of solids with liquids is achieved by tilting the biocontainer toward the inlet, tilting the inlet ports upward, and adding solids after partial filling with liquid, then completing the liquid addition. Test methods and results are described below.

3. Description of Test Methods

Liquid-solid recirculation and mixing experiments were conducted for buffer and media preparation applications in the Allegro 2D mixer biocontainer. A series of experiments were designed to determine the optimal operating conditions to achieve efficient mixing. The mixing efficiency was determined by mixing time, defined as the time required to completely mix the solution. Complete mixing is determined by monitoring the solution conductivity within the recirculation loop until the value is at a steady state and there are no visible solids left in the biocontainer.

In addition to determining whether this mixing system is suitable for a certain solution or application, other critical parameters must be considered. For example, the proper positioning of the Allegro biocontainer and of the inlet fluid jet into the biocontainer needs to be determined. Therefore, experiments were conducted evaluating the effects of the orientation of the biocontainer with respect to how it lays on the tray surface. In these experiments, mixing tests were conducted with the biocontainers lying flat on the tray as well as having the tray tilted. For the addition of fluids and solids, addition was tested with 100% fluid addition, then 100% solid addition and by 25% fluid addition, 100% solid addition, then 75% fluid addition.

The recirculation rate throughout all of the experiments was set at 13 L/min (LPM). This speed is the highest flow rate achievable with ½ in. peristaltic tubing and a peristaltic pump. If a different pump is used, pump slippage may occur at higher or lower flow rates.

4. Results

The Allegro 2D mixer biocontainer is capable of mixing buffer and media solutions as shown in Table 1.

Table 1:
Summary of mixing results for solutions tested within the Allegro 2D mixer biocontainer.*

	Mixing Achieved in <1 hour?	
Solution	**20 L**	**50 L**
1 M NaCl	Yes	Yes
0.75 mM NaOH	Yes	Yes
Phosphate Buffered Saline (Dulbecco's formulation)	Yes	Yes
Tris Buffer	Yes	Yes
Sodium Citrate Buffer	Yes	No
1M Ammonium Sulfate	No	No
13.4 g/L DMEM (Dulbecco's Modified Eagle's Medium)	Yes	Yes
47.6 g/L Terrific Broth	Yes	Yes
30 g/L Tryptic Soy Broth	Yes	No

**All tests were conducted under the best conditions, tray tilted and nozzle pointed downwards*

Figure 2 and Figure 3 show the performance of the Allegro 2D mixing system when mixing 10 g/L of sodium chloride powder into DI water. Figure 2 shows the mixing times for the 50% and 100% fill volume cases as well as tilted versus flat mixing systems. The tilted system is able to reach a steady state quicker and thus has a faster mixing time than when the biocontainer is positioned flat. This is due to more of the fluid and solid settling closer to the inlet port because the biocontainer is tilted downwards towards the inlet. The closer solids are to the inlet port, the more turbulent the mixing is, and thus more solid is in a turbulent mixing area than when the biocontainer is flat. Figure 3 shows mixing for the inlet nozzle flat versus nozzle pointed downwards. The nozzle pointing downwards produces a quick mixing; however with the nozzle pointed flat the solid could not be mixed into solution within 1 hour. When the inlet nozzle is pointed downwards, the jet stream, produced by the recirculation loop, produces a more turbulent mixing in the same area as where the powder port is and thus where the majority of the solid lies. With high density solids, the solid will settle on the bottom of the biocontainer and thus when the inlet nozzle is pointed downwards, the jet stream directly hits the solid, producing the most efficient mixing.

Figure 2:
High Density Salt - Biocontainer tilted vs. Flat

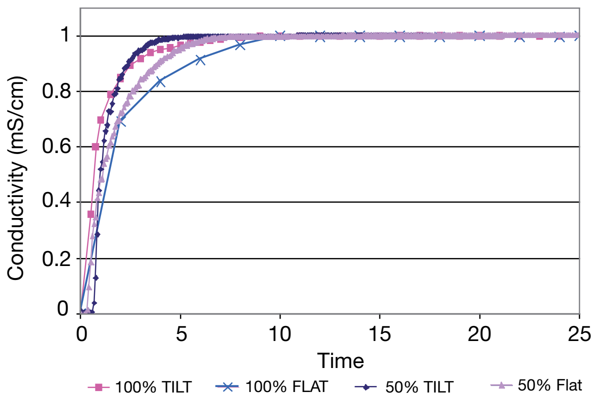


Figure 2 illustrates mixing NaCl within 50 L biocontainer using the maximum recirculation speed of 13 L/min. The solution mixed quicker when the tray and biocontainer were tilted. In all of these trials, the nozzle was pointed downwards.

Figure 3:
High Density Salt - Nozzle Downwards vs. Flat

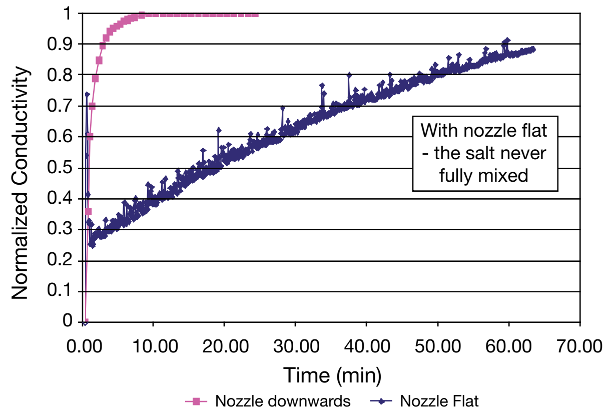


Figure 3 illustrates mixing NaCl within 50 L biocontainer using 13 L/min recirculation speed. With the inlet nozzle flat, the salt never fully mixed, seen by the curve never reaching the fully mixed conductivity. With the inlet nozzle pointed downwards, the solution mixed quickly and fully, due to the nozzle pointing directly at the solid and aiding its mixing. The tray was tilted in both of these trials.

Figure 4 presents different methods of fluid/solid addition. The most efficient addition process is by adding 25% of the fluid, then 100% of the solid, and then adding the rest of the fluid (75%). This two-step liquid addition allows for more space in the biocontainer to add the solid because the biocontainer is not yet full. The one-step liquid addition (all liquid added initially) makes it very difficult for solid addition of low density solids that tend to float on top of the liquid. Moreover, when adding the second 75% of the fluid, the solid begins to mix even before all the fluid is in the biocontainer, and is thus a more efficient use of the operator's time.

Figure 4:
Low Density TSB - 1 vs. 2 Liquid Additions

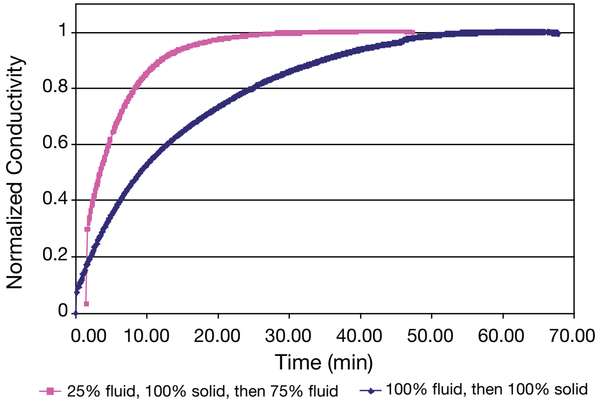
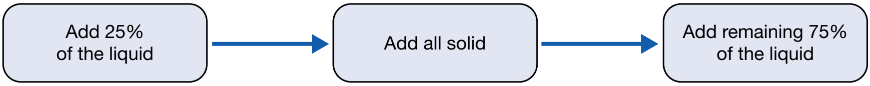


Figure 4 illustrates mixing Tryptic Soy Broth within 20 L biocontainer using 13 L/min recirculation speed. When the addition of fluid is conducted in 2 additions with the solid addition in between the solution mixes much quicker. The tray was tilted in both of these trials.

5. Recommended Procedures

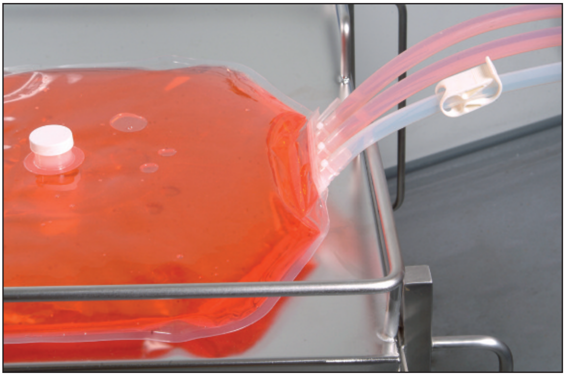
- The powder addition should be completed in 3 stages. The operator should first add about 25% of the desired liquid volume into the biocontainers followed by the addition of the solid. Then the remaining of the liquid (75%), should be added. This method allows for easier addition because the operator is not required to add the solid when the biocontainer is completely full of fluid. This results in more efficient mixing because the solid will already have been mixing during the liquid fill stage.

Figure 5:
Flow chart of how to add liquid and solid to the mixing system.



- It is recommended to tilt the biocontainer on the Allegro 2D cart so that a larger portion of the solid and fluid material is closer to the inlet port.
- It is also recommended to tilt the inlet port so that it points downwards (as shown in Figure 6 below) which will allow the fluid jet stream from the inlet port to come in contact with the powdered components more directly. This is especially important when mixing high density solids.

Figure 6:
The tubing is attached to the inlet and outlet and is angled so the ports are pointed downwards.



- This mixing system is capable of mixing easy solutions, however it is unable to mix some more “difficult to mix” solutions (ie: 1 M ammonium sulfate).
  All applications should be tested thoroughly to determine if mixing is possible with any given system configuration.

6. Conclusions

The modified design of the Allegro 2D biocontainer, in combination with a recirculation loop in the system design, has been successfully shown to efficiently mix many of the commonly used buffers and media listed in Table 1. The mixing in the 20 L Allegro biocontainer system was shown to be faster than in the 50 L size due to the smaller amount of solids required. The powder port biocontainer with recirculation mixing can efficiently mix up to 1M solid NaCl in both the 20 L and 50 L mixer biocontainers. However, this investigation found that some “difficult to mix” powders, such as Tryptic Soy Broth, could not be fully mixed within the 50 L biocontainer using a recirculation rate of 13 L/min.

The minimum working volume for the 20 L biocontainers is 10 L, and for the 50 L biocontainer it is 25 L.

Pall Life Sciences can provide additional support through our Scientific and Laboratory Services to support any potential mixing applications. Please contact Pall for further details.

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