A Novel Single-Use Mixing System for Buffer Preparation

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The global biotechnology manufacturing industry recorded industry revenues of $39 billion in 2003 and is estimated to be growing at 15% per year (1). Manufacturing operations constitute about 20–25% of a biopharmaceutical company's operating costs (2). The most significant costs involved are in facility time, which can account for up to 55% of total manufacturing costs, and validation, which can account for 10–20% (2). One cost-reduction option that is increasingly adopted by biopharmaceutical manufacturers is the move to disposable manufacturing. "Use-once, throw-away" technology is helping many to improve facility throughput by reducing downtime between campaigns, allowing the development of multipurpose plant designs because of the inherent flexibility of disposable systems. Key disposable components for biopharmaceutical production include filtration, tubing, clamps, connectors, storage vessels, small-volume bioreactors (<500-L), and now mixing systems.

Traditional mixing systems for biopharmaceutical processing require considerable time for cleaning, sterilization, and validation after each mixing run. Disposable systems can eliminate many or all of those costly steps. However there are several different types of closed disposable mixing systems to select from, and

Newmix Pad-Drive 1000 mixing system
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they are based on a number of mixing technologies: levitated magnetic impellers, magnetically driven stir bars, motor-driven impellors, small motor-driven wands, and bellows systems that force perforated plates through solutions to be mixed. One thing that most of those systems have in common is that they are all relatively efficient for liquid–liquid mixing at small to medium scales, but most are significantly less efficient for powder mixing applications, which usually require high torque to overcome flow resistance.

Innogenetics has two divisions. The therapeutics group develops and manufactures recombinant vaccines against chronic hepatitis B and C and human papilloma virus, as well as treatments for pulmonary edema, sepsis ands chromic venous leg ulcers. That division also operates a contract development and manufacturing program under CGMP and biosafety level 2 conditions. The second division is a specialty diagnostics group that develops test systems against infectious diseases and neurodegradation as well as genetic testing for a range of disorders.

At its manufacturing facility, the company sought to implement disposable systems for buffer preparation both to reduce costs and increase flexibility. Choosing the correct system is important for implementing new projects and especially for contract manufacturing activities. The decision process involved first identifying those systems that could undertake both liquid–liquid and liquid–powder mixing in a scalable format (from R&D laboratory to full production volumes), with cost-effective mixing and a system that could be easily integrated into existing processes.

We evaluated a new noninvasive disposable liquid–liquid and liquid–powder mixing system that potentially circumvents the time-consuming and costly cleaning, sterilization, and validation steps identified above. Initially, this mixing system was tested to determine its suitability and effectiveness for use in the scalable and homogeneous mixing of sodium chloride (NaCl), which was used to

Figure 1: Scalability of mixing results from 50-L to 200-L, shown by measuring conductivity of 5-M NaCl mixtures

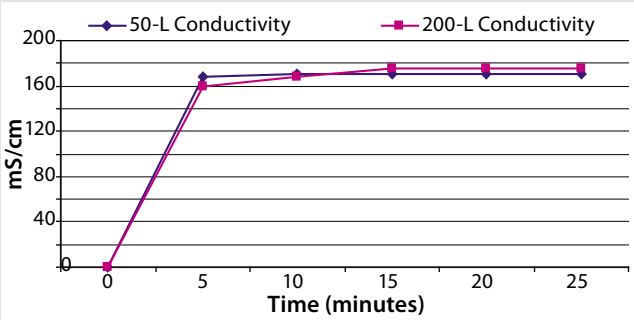
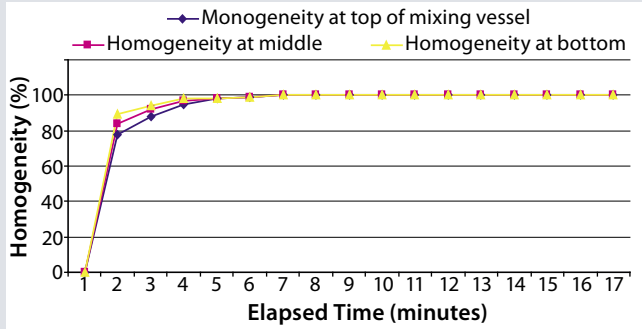


Figure 2: Homogeneity of salt mixing efficiency through the liquid column of a C-Mix 200-L bag



simulate salt-based buffers. We describe our test protocols and results obtained below.

Following completion of those first experiments, we reviewed the design of the system and its disposable elements for efficiency and ease of use, then made suggestions for optimizing both design and functionality. We evaluated the redesigned disposable element to determine the efficiency of changes made and its compatibility for use in a CGMP environment.

Mixing Study

We use the Newmix Pad-Drive 1000 mixing system from ATMI Life Sciences (www.atmi-lifesciences.com) in combination with a 200-L circular holding tank fitted with a standard 200-L Newmix-C disposable mixing bag, also from ATMI. The C-mix bag contains an integrated mixing paddle sheathed in the same film used in construction of the bag to maintain a continuous and homogeneous fluid-contact layer.

The mixing system operates by stirring its paddle within the liquid column in a similar action to that demonstrated when a coffee spoon is used to stir a cup of coffee. Although the paddle circulates within the liquid column, neither it nor its associated shaft rotate. The direction of circulation, speed, and frequency of change in direction of the paddle can all be set and saved by an operator in the system's 99-program memory. This design provides a reproducible, highly efficient, low-shear mixing system without integrated bearings, which has the added benefit of placing no rotational torque on the bag film, thus minimizing shear-generated failure of the film–paddle interface. Also, coupling of the bag to the system is very easy to perform, with no tools, by one operator after minimal training.

The bags used in these scalability and homogeneity trials were standard 200-L bags supplied by ATMI with integral filling lines, a 6-in. diameter powder transfer port, air supply line, and bottom drain line.

Scalability: NaCl was weighted out to give a final concentration (once dissolved and mixed) of 5 M: 292 g/L NaCl was dissolved and mixed at 50-L (14.6kg NaCl) and 200-L (58.4 kg NaCl) volumes by adding the appropriate amount through the integral powder transfer port to an appropriate volume of water for injection (WFI) in the disposable mixing bag. We then started the mixing operation. Mixing efficiency was determined by measuring conductivity at the top of the bag until it stabilized. Measurements were taken opposite the point of powder addition during mixing.

Homogeneity: The bag was prefilled with 170 L water for injection, to which was added 60 kg NaCl (353 g/L) for a final concentration of 6.55-M NaCl. We determined the homogeneity of mixing by measuring the conductivity of the solution at the top, middle, and bottom of the bag until those measurements stabilized. A stable measurement was determined to represent 100% homogeneity.

Results: Tables 1 and 2 and Figures 1–3 describe the efficiency of mixing for 50-L and 200-L volumes of 5-M NaCl and water.

Table 1: Mix data results at 50-L and 200-L mix volumes

Time (min)	Mixing of 50 L 5-M NaCl		Mixing of 200 L 5-M NaCL	
	Speed (rpm)	Conductivity (mS/cm)	Speed (rpm)	Conductivity (mS/cm)
5	50	168	80	159
10	50	170	80	168
15	50	171	80	176
20	50	171	80	176
25	50	171	80	176

Table 2: Homogeneity results from top, middle, and bottom of bag during mixing

Time (min)	Homogeneity (%)		
	Top	Middle	Bottom
0.0	0.0	0.0	0.0
0.5	78.0	84.0	89.0
1.0	88.0	92.0	94.0
2.0	95.0	97.0	98.0
3.0	98.0	98.0	98.5
4.0	98.5	99.0	99.5
5.0	100.0	100.0	100.0
10.0	100.0	100.0	100.0
15.0	100.0	100.0	100.0

Initial Summary

We found that the Pad-Drive 1000 unit with the simple and easy-to-use 200-L Newmix-C bag gave excellent mixing characteristics and very quickly produced a homogeneous solution (determined by measuring conductivity) of high-molarity NaCl. The tested system fulfilled all parameters identified as important for Innogenetics before the start of the experimental trials.

During mixing operations, however, it became apparent that there was a significant accumulation of undissolved salt particles in the drain port at the bottom of the mixing bag, between the top of the drain connector and the pinch clamp used to seal the discharge tubing (Figure 3). No matter how vigorous the mixing cycle was, salt particles remained in the drain port. We did not measure the amount of accumulated salt, although it was visible to the naked eye. When mixing buffers for tangential-flow filtration and chromatography applications, a small amount of accumulated undissolved salt poses blockage issues based on the flow geometries of TFF cassettes and chromatography resin particles.

As part of our company's contract manufacturing operations, the selected mixing system also could be used in preparation of finished product formulations, where the presence of undissolved product could lead to inaccurate final doses being prepared. Therefore, we entered into a second series of experiments with the equipment manufacturer, seeking to identify the root cause of the problem and to develop suitable solutions.

Drain-Port Testing

Several suppliers have used offset positioning of mixing systems within disposable bags to create turbulent or nonlinear flow in circular mixing systems for better mixing efficiency. So we looked at offset positioning of the drain-ports as a possible, simple, and inexpensive solution to the problem of particle accumulation in the drain port. To quickly determine whether this was actually a viable option to pursue, Innogenetics worked with ATMI to produce a multiple-drain-port bag that might allow quick determination of salt accumulation. A standard 200-L container was modified to facilitate this round of experiments.

For this purpose, a 200-L C-Mix bag was manufactured with 13 different drain connectors in various positions (Figure 4). We visually inspected the drain connectors and tubing during and after mixing to see whether salt had completely washed out of the drain connector inlets. If salt had accumulated in the drain connector, the amount was recorded and scored between zero and six, with zero being no salt observed and six being salt filled to the top of the drain port. The same operator scored all experiments to eliminate personal variabilities in determination of particulate accumulation. The same design of drain port was fitted to each test location (Figure 4).

Figure 3: Design of drain port with collection of undissolved salt particles in the drain

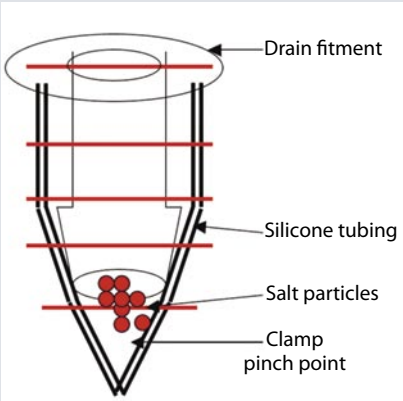
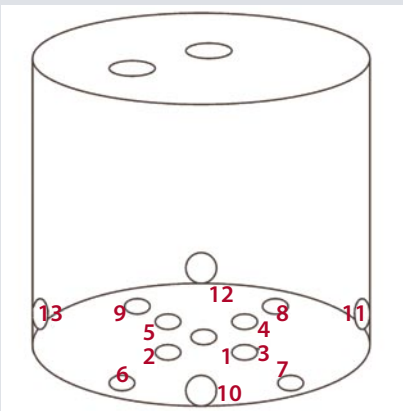


Figure 4: Locations of test drains on the 200-L C-mix bag in drain-port testing



Results: As Table 3 shows, considerable differences were observed in the amounts of undissolved salt depending on the position of the drain fitments. On average, salt accumulation at drains placed on the side of the bag were minimal compared with those located at the bottom center (drain 1), bottom middle (drains 2–5), and bottom side (drains 6–9) of the bag.

If the average salt accumulation per region is reviewed from the data in Table 3, a higher salt accumulation is also discovered in ports located at the bottom side of the vessel than in those located at the bottom middle (Table 4).

Drain-Port Design: From the results in Tables 3 and 4, it would appear that incorporating a drain into the side wall of this type of mixing bag would be a simple solution to eliminate most particulate deposition and accumulation during mixing. However, locating the drain port on the side wall would significantly increase the volume of liquid retained in (and unrecoverable from) the mixing vessel. For buffer mixing, that may not represent a significant cost addition to the final process; for mixing of either intermediates or final products, such additional loss is unacceptable.

Two other concerns relating to side-wall positioning of the drain valve are also worth noting. A side-wall position would require significant reengineering of the mixing tank, specifically the jacketed ones for

Table 3: Results of visual inspection of salt accumulation in each drain

Drain	Test 1	Test 2
1	6	6
2	2	1
3	3	3
4	5	2
5	2	4
6	2	4
7	2	4
8	5	5
9	2	2
10	1	0–1
11	0	0
12	0	0
13	0	1

Table 4: Averaging the salt accumulation scores

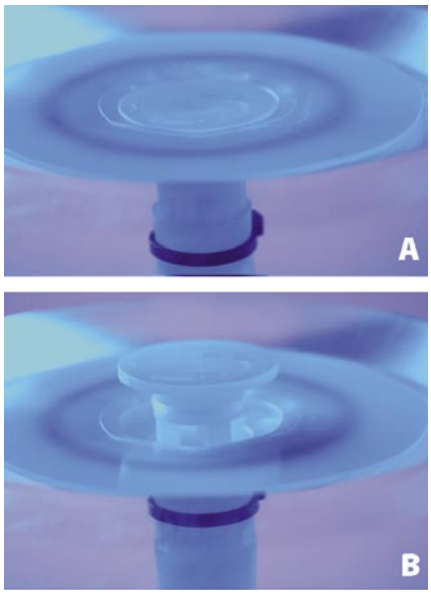
Location	Results	Number of Data Points	Average Value
Bottom center	6, 6	2	6
Bottom middle	2, 3, 5, 2, 1, 3, 2, 4	8	2.75
Bottom side	2, 2, 5, 2, 4, 4, 5, 2	8	3.25
Vertical side	1, 0, 0, 0, 1, 0, 0, 1	8	0.375

temperature control. It would also allow the bag to be positioned only in one orientation within the mixing tank, therefore eliminating the flexibility of location for the powder-addition port that operators enjoy with the current design.

While these experiments were underway, ATMI was developing a new drain port that could potentially solve the problems of particulate accumulation in the old-style ports as well as be easy to use, cost-effective to manufacture and install, and maintain the inherent flexibility of the original design. The newly designed drain port is made of linear low-density polyethylene (LLDPE) and works by closing the port entrance during the filling and mixing cycles (Photo 1A), thus preventing the accumulation of undissolved particles in the port before the dissolution step of the mixing cycle is completed. A smooth surface is created at the bottom of the bag during mixing so that efficiency is not compromised. Following mixing, the center-sealing plug in the drain is easily raised by a simple lever on the side of the mixing station, which allows mixed liquid to drain thoroughly from the bag (Photo 1B).

Creative Solutions

The Newmix Pad-Drive 1000 is a single-use mixing system that provides a practical and cost-effective solution for homogeneous, scalable, high-efficiency mixing. Its disposable nature eliminates the need for cleaning, sterilization, and validation following a mixing run. We determined the mixing process to be efficient and easy to use. It seemed appropriate for preparation of chromatography and TFF buffers in a CGMP biopharmaceutical manufacturing environment. Optimization of the drain-valve geometry has eliminated the major defect we found in the original bag design. Our adoption of a disposable mixing step significantly reduced the turn-around time required to transition between operating configurations, therefore significantly improving our company's use of facility time and reducing operating costs per run.



Photos 1A and 1B: New drain-port design in the closed (A) and open (B) positions.
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This also provides a much greater degree of confidence in the aseptic integrity of the entire system.

Providing Creative Solutions

As the trend toward disposables continues, the use of thermoplastic tubing welders and sealers will no doubt gain momentum as well. When implemented alongside single-use process components, these welders and sealers offer biopharmaceutical manufacturers greater flexibility and increased efficiency. With a tubing welder, an end-user can make quick and reliable tubing connections without absolute dependence on Class A clean zones. Consequently, end-users can conduct onsite customization, which reduces time-to-market as well as the costs associated with validation and cleaning. Additionally, with tubing sealers, end-users can conduct small-volume sampling with decreased risk of process variation due to lapses in operator technique and/or training. That translates to a much higher level of assurance for aseptic integrity.

In the years to come, disposable technologies — and the systems used to implement them, such as thermoplastic tubing welders and sealers — will continue to transform the biopharmaceutical industry and the way in which manufacturers meet its changing needs.

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