

A Disposable Mixing System for Hydrating Powdered Media and Reagents

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The technology of disposable or single-use systems has grown dramatically in recent years. The advantages have been identified in previous articles (1). Manufacturers continually focus on improvements to this new and emerging technology.

Several significant improvements to mixing technology have recently been developed by HyNetics, a joint and equally owned venture of HyClone and Alfa Laval Biokinetics, Inc. The mixing system developed in this partnership is a modular unit: a completely disposable and integral bag assembly called the “One-Touch” system. The system has a capacity range of 30–10,000 L, is fully enclosed, and can be used in unclassified or “gray” spaces. The disposable product contact surfaces prevent cross contamination and require no sterilization or cleaning, greatly reducing equipment turnover times. The

system can be used to prepare media, buffers, intermediate product solutions, IV solutions, and homogeneous suspensions such as adjuvanted vaccines.

The heart of this system is a proprietary mixing disc designed to improve agitation over other disposable systems. It allows granulated powders to enter the liquid phase quickly and efficiently, reducing mixing and process times.

DESIGN FEATURES

The HyNetics system is designed for use in biopharmaceutical production facilities, R&D facilities, and other installations that require a system for hydrating powdered media and reagents. The fully integrated unit is designed to meet cGMP guidelines and other regulatory requirements. Additional options allow configuration to meet individual needs. Photo 1 shows a One-Touch system with an operator access platform.

In combination with the mixing disc, the other important design feature is a hexagonal holding vessel with a specially engineered angled bottom. This configuration of tank design and mixing disc provides turbulence at the corners for enhanced mixing. The mixer shaft is connected to a drive that converts rotational output from the motor into linear motion. The stroke varies from 10 to 20 cm depending on vessel size. The speed can be varied from 0 to 2 Hz, providing slow or mild agitation up to aggressive mixing. The contact surfaces of the disposable system components (bags, ports, tubing, valves, and filter sets) are made of animal-derived–component-free (ADCF) materials.

Temperature is measured and controlled using an optional heat transfer surface integrated into the vessel with a proprietary temperature-sensing element. A peristaltic pump (the pump type preferred for this application) transfers solutions through filtration for final processing. **Liquid level** is monitored using load cells. An automated system for inflating the bag and controlling the operating pressure is also standard. The entire One-Touch system is controlled from a central panel with an analog interface or optional PLC. The system has interlocks to ensure operator safety.

The mixing technology used for the 25–10,000-L vessels is identical, and the system can operate down to 30% of the maximum volume. The mixing disc is made of rigid, engineered plastic with multiple slots that extend through the disc. The underside of these slots comprises film sections or “flaps” attached only to one edge down the given radius of the disc. They open as the disc moves up from the bottom of the mixing bag on the drive’s upstroke and the fluid flows through the slots of the disc. On the down stroke, the flaps close, directing energy toward the bottom of the vessel and up the walls to produce a very effective turbulent mixing pattern. The mixing disc, flaps, plastic shaft, and a shaft cover that attaches to the bag film are all disposable materials that pass Class VI testing. The entire bag assembly is easily placed inside the holding vessel, which is equipped with a side access panel (Photo 2) for visual monitoring during processing.

PRODUCT FOCUS: MEDIA, BUFFERS, INTERMEDIATE PRODUCT SOLUTIONS, IV SOLUTIONS, AND HOMOGENEOUS SUSPENSIONS

PROCESS FOCUS: PRODUCTION

WHO SHOULD READ: CELL CULTURE AND FERMENTATION, PURIFICATION, FORMULATION, PROCESS DEVELOPMENT, AND MANUFACTURING

KEYWORDS: SINGLE-USE (DISPOSABLE) SYSTEMS, HYDRATION, DESIGN OF EXPERIMENTS

LEVEL: INTERMEDIATE

Mixing trials have shown that the HyNetics agitation scheme is efficient and that powders go into solution quickly. High turbulence created by the interaction between the vessel shape and the disposable mixing unit geometry helps pull materials into the fluid stream as they are metered into the bioprocess container (BPC) mixer vessel. That provides a marked difference from traditional mixing tank designs in which powders are usually “dumped” and stirred. The mixing bag contains a powder port on the top, to which a powder feed bag (containing either medium or buffer powdered material) mates using a tri-clamp. Powder is then delivered through that port dust free.

At the top of the powder feedbag is a port with tubing and end treatment through which high-purity water can be fed if the powder bag needs to be rinsed. A water/liquid port is also located on top of the mixing bag for connecting equipment such as a water-for-injection source. An integral part of this water/liquid port, fitted inside the bag, is a spray device. As water or fluid is added to the mixing bag, it sprays out to wash down any powder either in the headspace or on the bag walls, and it falls on any powder still floating on the liquid surface. These measures help ensure that the powder goes into solution quickly. A vent port is also located on top of the mixing bag. There are two ports on the bottom of the bag: One is to evacuate the bag; the other is a recirculation port so that if sampling or supplementing is required, either can be accomplished through a recirculation loop. The preferred

filtration scheme is located downstream of the peristaltic pump, followed by a customer’s choice of either a filling system or some other specific use of the content mix.

The mixer shaft is mechanically crimped to a cover that is heat welded to the bag. The shaft is held by the mechanical drive system and connects on the other end to the mixer disc. Process fluid is contained within the cover by a heat weld to the bag at the top and the mechanical crimp in the shaft.

Figure 1 shows a cross-sectional view of the mixing system. The mixer head (A) is machined from an HDPE sheet stock plastic. The film is heat welded to the bottom face of the mixer head to act as the valve (B). The drive shaft (F), machined from an extrusion grade HDPE, is press-fit into the head. The shaft has geometric features at both ends that retain the mixer head and secure the drive shaft into the skid. An injection-molded elastomer cover (C) is heat welded to the liner and crimped to the drive shaft with an aluminum crimp ring (D).

MIXING DYNAMICS

The mixing head of the HyNetics system creates high turbulence, benefiting from the shape of the holding vessel and the design of the mixing unit. Such interaction draws dry materials into the fluid stream as they are metered into the vessel. This differs greatly from the “dump and stir” methodology normally used.

Using a series of initial evaluations, a design of experiments (DOE) analysis of the mixing process indicated that the system was optimized to a maximum frequency and stroke with the mixing disc size and hardware design. The final determination of tank geometry was a hexagonal shape, which optimized the mixing

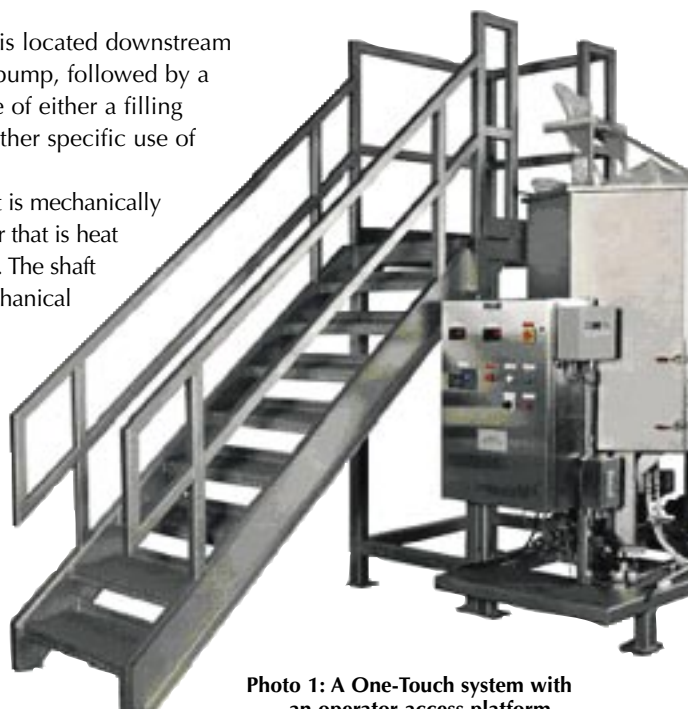


Photo 1: A One-Touch system with an operator access platform

and also minimized cost. In most cases, the tank diameter to mixer head ratio was designed to 2:1. The target 2:1 ratio varied slightly to maintain the slot and rib widths of the mixing disc.

The mixing range is defined at 100% nominal volume for the upper limit. The lower limit is based on two factors:

- The minimum static pressure required for the top portion of the solution to effectively transfer to the bottom layer during mixing
- The minimum fluid volume height required to maintain a liquid level above the mixer disc.

Data from the DOE studies show that all solutions can be mixed at a 3:1 turndown or 33% volume. Actual mixing volumes are at approximately 26% of nominal tank volume.

Fluid velocities were measured at six locations throughout the tank. The sensors were mounted vertically to a metal harness that was positioned at the outside diameter of the tank. Those tank positions are known to provide the highest fluid velocities. The motion of liquid in the tank was best described as a series of pulses created by the pumping action of the mixer head check valve. Those fluid motion pulses radiated outward from the head as fluid was compressed and forced outward during the down stroke of the mixer cycle. As pulses move upward along the outer wall, interactions between them



Photo 2: A holding vessel equipped with a side access panel

Table 1: Results of hydration tests using RPMI and salt

Linear Size	Head Size	Time to Hydration (minutes)			
		RPMI 26%	RPMI 80%	NaCl 26%	NaCl 80%
250	12.75	NT	5	NT	1
750	16.5	3	6.5	3	3
1500	21	3	6	1	1
3000	27	3	12	1	3
5000	30	NT	10	NT	1
10,000	30	3	11	2	2
100,000	27	NT	12	NT	3

NT = not tested

create a random motion. Fluid is then pulled down through the center of the tank and back to the mixer disc, where particles and other agglomerates are broken up when they reach the mixer. This technology mixes powders in a recirculating motion. Figure 2 is a computer model of a typical mixing profile using a 10,000-L One Touch system with its mixing disc and hexagonal holding vessel with angled bottom.

Sample tests were performed with two types of powders known to be either difficult or easy to hydrate (RPMI and salt). Salt sinks to the bottom of tanks, and RPMI clumps and floats on a liquid surface. During mixing, osmolality samples were taken from the tank center at the bottom, middle, and top of the tank. The time to hydrate salt ranged between one and three minutes depending on tank and head size. The time to hydrate RPMI was between five and 12 minutes depending on tank and head size while the clumps were pulled down into the mixer disc and dispersed.

Concentration profiles were approximately equal for samples taken from the bottom, middle, and top of the tank. Table one summarizes results of this study.

MIXING EVALUATION OF THE 250-L SYSTEM

A mixing study was performed by HyClone using the 250-L HyNetics mixing system and the standard mixing validation protocol. The disposable components were placed into a holding vessel, which was filled with water up to 90% by volume. The mixer was turned on (2.5 Hz), and media components were added one at a time. The solution was sampled at one-minute intervals for pH and osmolality as well as for sodium and calcium chemical profiles. Two different batches were evaluated for these mixing studies.

Data from each batch showed that hydration was successful after about 20 minutes to perform all of the addition steps including pH adjustment and Q.S. to final volume (150 L). Observations

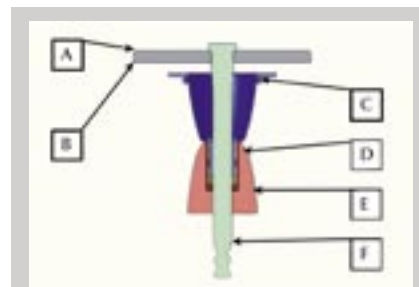


Figure 1: Cross-sectional model view of the HyNetics mixing system (labels are defined in the text).

made during the mixing process showed complete mixing for each ingredient after only a few minutes. Samples taken at the end of the mixing time (42 minutes for batch 1 and 38 minutes for batch 2) show consistent pH and osmolality readings from top, middle, and bottom of the tank. Detailed results of mixing studies for batches 1 and 2 are available from HyNetics upon request.

AN EFFICIENT TECHNOLOGY

The One-Touch system mixes powders and solutions efficiently and with less expenditure of time and money than other mixing technologies. Time and labor are saved by efficient use of single-use, disposable technology and a mixing method using the disc and hexagonal-shaped tank with unique angled bottom. Cost is reduced by eliminating the need for typical processes such as cleaning and sterilization validation and by reducing turnover time between batches.

Mixing studies demonstrate that mixing patterns generated by the tank system and disc allow for quick hydration of powders into solution.

REFERENCE

- 1 Aranha H, et al. The Disposables Option. *BioProcess International 2* (supplement 4) October 2004.

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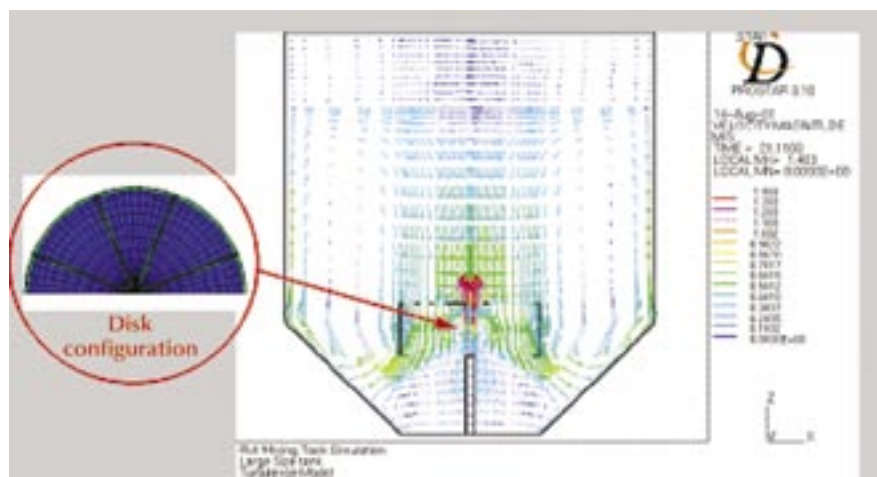


Figure 2: Computer model of a typical mixing profile using a One Touch system; velocity vectors for the 10,000-L tank: disk speed at 2Hz, time = 21.11 sec