The Next Wave in Disposable Mixing
Technology Review and Application Summary

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**Introduction**

When the first disposable bioreactors were developed in 1996, mixing to ensure gas transfer and cell viability were key operational requirements. However, mixing and doing so in a single-use system became a unique design challenge.

There were a number of classic mixing models known, but most did not lend themselves well to the concept of a single-use system. Impellers were an unlikely option, due to the challenges of introducing the mixing element in a sterile manner. Magnetic stir bars could address this problem, but their size limited the top end working volumes due to their mixing mechanics.

To address these challenges a novel mixing mechanism was developed for the bioreactor application. A polyethylene film was used to create a “pillow” to contain the cell culture medium to be inoculated. This pillow was secured to a rocking table which would then induce a wave motion to promote mixing and gas transfer.

To address the needs of this new bioreactor, materials were improved, and soon 2-D and 3-D disposable vessels were made of layered films—typically low-density polyethylene blends (LDPE) for biocompatibility and layered with ethylene-vinyl alcohol (EVOH) film for gas barrier protection.

These films are lightweight, durable and relatively easy to construct into various sized “bags” by heat or ultrasonic sealing operations. Tubing and fittings were also added to the disposable bioreactor to allow for sampling and material transfer. All materials used in the bag construction could be gamma irradiated to ensure sterilization before use.

For the first time, a disposable, single-use system for growing cell culture was available which could eliminate the risk of cross contamination and reduce cleaning requirements, validation needs and set-up time. Soon, cost analysis proved that the disposable bioreactor route was an economical way to reduce materials, labor and cleaning costs. These advantages paved the way for disposable mixing systems for media and buffer preparation or downstream processes.

**The First Wave in Disposable Mixing**

The biopharma industry embraced the disposable vessel concept. Most of these early systems relied on the impeller mixing concept or a pump around loop. Although the mixing mechanism or container varied from vendor to vendor, the central theme of an inexpensive, easy to operate, single-use system remained the same. These initial systems were sufficient for basic liquid-liquid applications but were often challenged by high viscosity, suspensions or powder-liquid mixing particularly in the +100L regime. These early systems were also limited from an analytical standpoint, since sensors were not commercially available that could be easily integrated into a disposable mixing system.
Survey of Disposable Mixing Systems

1. Lined Stirred Tank

The stirred tank is the classic mixer; lining a tank with a disposable plastic film was the next logical step in its evolution. A rotating impeller is used in a vessel to mix. While there are many impeller designs, the basic principle is the same. Energy is imparted by the rotating impeller which pumps fluid throughout the tank. The major problem with the stirred tank is the localization of shear. The area near the impeller is highly agitated, but the fluid velocity decays rapidly away from the impeller. A typical velocity pattern is shown in Figure 1. In order to provide good mixing, especially on scale-up, a lot of energy needs to be imparted in order to get the fluid to circulate to distant parts of the tank. Unfortunately this gives rises to very large shear gradients – very high shear in the impeller zone and almost stagnant fluid away from the impeller. These shear gradients cause damage, especially to biological systems.

![Figure 1. Velocity Profiles in Stirred Tank](image)

**Figure 1. Velocity Profiles in Stirred Tank**

Another difficulty with a stirred tank is that in order to be efficient, the impeller must be fairly large compared to the tank diameter. Contrast the flow fields produced by the different size impellers in Figure 2.

![Figure 2. Circulation Patterns with Large and Small Impellers](image)
Empirically, it has been determined that the impeller diameter must be between $\frac{1}{3}$ and $\frac{1}{2}$ of the tank diameter. This makes the construction of a disposable impeller or magnetically drive stir bar-type device impractical for volumes of over 10 liters. For example, a 100 liter tank with a diameter of 24 inches requires a throw away impeller of at least 8 inches in diameter. So the only solution is to use a smaller impeller and increase the RPM. As a result the shear force and probability of foaming due to aeration is increased. This can be better exemplified by a series of simple calculations:

The mixing time can be correlated to the number of times the fluid is turned over ($T$).

$$T = \frac{Q}{V}$$  \hspace{1cm} (1)

$Q =$ Fluid Pumped (liter/minute), $V =$ the fill volume. Typically, 5-10 complete fluid turnovers per minute are required for homogeneity

The fluid pumped ($Q$) is also a function of the impeller speed, impeller diameter and coefficient:

$$Q = N_0 ND^3$$  \hspace{1cm} (2)

$N_0 = .5$ (axial impeller)
$N =$ mixer speed (RPM)
$D =$ impeller diameter

So, to mix 100L with a turnover ($T$) of 10/minute we need:

- Impeller diameter 1": $N$ (rpm) required = 122,000
- Impeller diameter 8": $N$ (rpm) required = 240

Here the challenge is apparent, with a disposable impeller based system one may select a small impeller and risk the effects caused by high shear. The alternative option is for one to select the larger impeller and associated drive, but this is impractical in a disposable system.

2. **Pump Around Loop**

The pump around loop is one of those ideas that, at first glance, appears to be an elegant and simple solution to the problem of mixing in a closed system. In this system, a tube is used to externally circulate fluid in order to mix the contents of a disposable vessel.

However, the system is not an efficient mixing device. First of all, in order to achieve a closed system, it is necessary to recirculate using a peristaltic pump. This kind of pump and the tubing available pose an upper limit of the fluid flow in the recirculation loop.

Typically, peristaltic pumps used in these applications can only provide a flow of around 10 liters/minute. Again considering $T = \frac{Q}{V}$ this leads to a circulation time about 100-fold less than a comparable stirred tank with 8” impeller. Furthermore, the velocity is limited due to collapse of the tubing or the inlet port. High linear fluid velocity in the recirculation tube can cause significant shear damage to labile substances. Finally, as the recirculation flow enters the mixing vessel, the fluid velocity slows dramatically as the cross-sectional area increases.
This low fluid velocity is often too little to suspend and disperse particles in the mixing bag. All these effects are depicted graphically in Figure 3.

![Diagram showing problems with practical pumparound mixer]

**Figure 3. Problems with Practical Pumparound Mixer**

In summary, the pump-around loop is essentially useless as a mixing device. Its turnover rate is significantly less than other technologies. It is volume limited due to tubing limitations and in no way can it handle mixing powders into viscous solutions.

3. **Oscillating disks**

Oscillating disks attempt a different approach to mixing. They consist of a mixing bag with an internal mixing disk mounted on the bottom. The disk is moved up and down, and this imparts fluid motion. Examining the fluid velocity profiles of such system shows that the fluid velocity decays rapidly as you move away from the disk generating a shear gradient – with a high shear zone near the disk and potential stagnant zones further away. The disk must also be designed correctly so that there are no stagnant regions under the disk. Mixing trials show that, while the oscillating disk provides efficient mixing with low viscosity fluids and salt solutions, it is not clear how well the device performs with viscous or non-Newtonian fluids. While the oscillating disk mixer is an innovative step to overcome the problems associated with a stirred tank and also provides a disposable format, it may not be suitable for all applications.

4. **Wave Mixer**

The wave mixing concept started the disposable movement but it is mostly applied to liquid-liquid mixing or powders that easily dissolve. As described earlier a “pillow” shaped flexible vessel is filled with liquid and secured to a rocking bed which induces a wave motion (see figure 4).
Figure 4. Wave Mixing System

This system does not create local high energy zones. However, viscous materials (+1,000cP) can be a challenge. Powder addition is possible using a screw cap, but, since the bag is two-dimensional in design, processing large volumes may be a challenge. Finally, the footprint of the wave style mixer can grow quickly once above the 200L range as the pillow design of the bag and its rocking base are rectangular in shape.

Disposable Mixer Summary

To summarize thus far, there are now several mixing technologies that have been adapted to serve as a disposable system. Each of these options offers the end user a reduced risk of cross contamination, a reduction in start up and operating time, and virtually no cleaning requirements. But to date there has yet to be a “one size fits all” solution that can address the full range of application challenges encountered while utilizing disposable components.

The impeller concept has been the age-old solution, and it is relatively easy to design a lined tank but as outlined earlier there are several weaknesses to this system. In order to provide a system with a low recurring cost on the disposable components, the impeller must be small in diameter regardless of whether it is a stir bar in nature or an overhead drive.

If the stir bar or levitating impeller is employed, an external magnetic drive has to provide the motive force. This is acceptable for operations in small glass beakers or carboys but it is not practical in pilot or production scale operations. In the large scale operation the external drive is not capable of imparting the necessary energy to the impeller and since the impeller has to be small a very high RPM has to be used which again is not desirable.

Recently there have been disposable systems introduced where an overhead impeller is introduced into a 3-D disposable bag through a tri-clamp port molded into the bag. Even with a fold up impeller approach this concept limits the overall diameter and once again the issue of high RPM to induce mixing is faced in the large volume scale. Additionally, it is a challenge to introduce an efficient impeller in a sterile manner so these systems are limited to operations where the mixed solutions can either be filter sterilized or where sterility is not an issue at all.

As discussed the pump around loop with its low turn over rate is not very practical in the large scale. It should also be noted that this approach does little to address suspensions that may need to be homogenized such as in filling line applications. Also, there are a number of applications where powders are to be mixed into solutions at higher viscosities.
For these applications there needs to be intimate contact between the mixing element and the components in the system and this is not possible in the pump around format.

The oscillating disk is another spin on the impeller concept and although it can be integrated into a disposable platform it offers little advantage over the traditional stirred tank approach. As mentioned earlier the wave mixing concept is novel and ideal for the bioreactor applications but has it’s limitations in the mixing arena.

Jet Mixing: The Next Wave in Disposable Mixing

In order to address the mixing challenges encountered by manufacturers on a daily basis a new concept had to be developed. A cost effective, disposable system was needed that could mix simple liquid-liquid recipes, high viscosity solutions, suspensions, and high powder to liquid solutions. The system also had to be able to do so across the full range of volumes from pilot to production and needed to scale up.

This challenge led to the development of the jet mixing concept which was then transformed into the recently introduced FlexMixer® system. The FlexMixer operates differently than any other system to date. The mixer consists of a flexible vessel called the FlexBag™ with a perforated septum. The septum can be moved up and down by an external actuator (See Figure 5). Each hole in the mixing septum creates a jet mixing element. A jet of fluid going through each hole entrains and mixes the surrounding fluid. The mechanical energy for the jet is supplied by the forced movement of the septum within the vessel. The septum motive power is supplied by an external non-invasive mechanical actuator which moves the septum up and down (See Figure 6).

Figure 5. FlexBag and FlexMixer System
Like most disposable mixing technologies the FlexBag is constructed of a LDPE with all the required tubing & sample ports for operation and the entire unit can be gamma sterilized. By varying the film thickness in key places the FlexBag has flexibility where required and rigidity such as at the septum.

**Advantages of the FlexMixer**

With this unique mixing concept, the *FlexMixer* has many advantages over conventional mixing devices:

1. There are no circulation zones carrying material to and from the mixing zone like those associated with conventional stirred tank mixers. The mixing jets themselves are spread across the horizontal area of the mixing septum and travel vertically so that they travel throughout the entire vessel volume on every stroke.

2. Volumetric fluid turnover is essentially equal to one stroke, the *FlexMixer* volumetric turnover rates are inherently higher than those associated with conventional stirred tank mixers since the fluid is not in a circulation zone going into and out of the “mixing zone.”

3. Scale-up is very simple because for a given fluid the maximum fluid velocity of the jets is a function of the vertical speed of the mixing septum and the % open area (total area of the mixing holes) of the septum. Linear average speed is kept constant on scale-up. Units up to 10,000 liters in volume are possible.

4. Rapid dispersion of additions - The conventional stirred tank mixer typically utilizes a single fixed injection port for ingredient addition. In the *FlexMixer*, addition ports can be incorporated into the mixing septum allowing direct-metered injection of ingredients throughout the vessel volume, minimizing or eliminating zones of high ingredient concentration.
5. Power requirements – most of the power applied to a conventional stirred tank mixing system is used to a) develop the pumping head in the vicinity of the impeller, and b) develop the circulation flow to and from the impeller. In general, the FlexMixer requires significantly less power since the circulation flow is very minimal as the velocity heads generated from each septum hole cycle throughout the vessel volume.

6. Shear control is maximized in the FlexMixer. High FlexMixer shear rates are achieved by a combination of higher stroke speeds and/or a reduction in the percent open area in the mixing septum. Conversely, low shear rates are achieved by lower stroke speeds and/or an increase in the percent open area of the mixing septum. To generalize, it is clear that the FlexMixer imparts less localized, high shear than a stirred tank system since the work is spread across each mixing element of the septum.

7. High Viscosity Mixing – the FlexMixer mixes high viscosity fluids by convective mixing. All of the viscous fluid is forced through the mixing septum on every stroke. Each return stroke is in the opposite direction, which provides the frequent blending/folding action necessary for efficient high viscosity mixing.

8. Powder Hydration – the FlexMixer is very efficient at hydrating powders since the shear zones are carried throughout the vessel ensuring that the mixing jets come in contact with the powder (top or bottom of the vessel). For floating powders, the mixing septum can be programmed to rise above the liquid level and physically pull the powder into the liquid.

9. Suspensions – the mixing jets lift particles off the bottom and easily achieve uniform suspensions since shear zones move throughout the fluid volume. Typical time to reach homogeneity is less than a minute.

10. Foaming – By removing the gas head prior to mixing, non-coalescing fluids can be aggressively mixed without foaming (no gas to create bubbles). Conversely, by providing a head space, it is possible to provide efficient gas-liquid dispersion.

11. Low cost – The FlexMixer is constructed of blow molded plastic. The mixing septum is simply a piece of perforated plastic film.

12. Single-use design – It is designed to be a disposable system eliminating the cost of cleaning, validation, and reuse. This translates to lower operating cost and less chance of contamination.

13. Validatable – The FlexMixer contact components are made of USP Class VI resins and validation data are provided for pharmaceutical applications. Units are made in a clean room environment and can be provided sterilized by gamma radiation.

14. High fluid velocities make it possible to even provide heating and cooling options.
15. Footprint – the FlexMixer is totally unique in that the system can be designed tall and narrow and still have excellent mixing efficiencies. This is a function of using the septum which can traverse the entire fluid zone.

**FlexMixer Dissolution Studies**

Dissolution studies were conducted to demonstrate how rapidly the FlexMixer can achieve homogeneity. Figure 7 shows the dissolution of salt in a low-viscosity (water-like) fluid. Conductivity measurements were used to follow the dissolution process.

![Figure 7. Dissolution of NaCl in low viscosity fluid](chart)

Unlike most other mixing devices, the FlexMixer is also effective in highly viscous fluids. Figure 8 shows the dissolution of NaCl in 8000 cP, carboxymethylcellulose solution (CMC). Time to achieve homogeneity was less than 8 minutes. Experiments have been performed up to 400,000 cP.
FlexMixer Fluid Circulation Studies

A number of experimental studies were conducted using laser anemometry to determine the velocity profile, strain rate, and vorticity. These studies were used to determine the basic performance of the FlexMixer at different operating conditions and geometry. The data were also used to validate subsequent computational fluid dynamic (CFD) modeling. Figure 9 below shows the typical correlation between the experimental data and computer simulation. Computer simulation was then extensively used to optimize the system.

Figure 8. Dissolution in Viscous Fluid

Figure 9  Velocity profile at 15cm/s septum speed (LEFT=laser data, RIGHT=computer simulation

Computer simulations were used to determine velocity distributions. An example is shown in Figure 10. Hundreds of such simulations were used to determine the optimal septum design, open area, septum speed and motion profile. The septum design and operation are patented.
More and more users are looking for ways to determine the quality of their product through the use of in-situ monitors and instrumentation. As mentioned earlier the Wave Bioreactor was faced with new challenges during its development, one of which was the need for probes that could be used with a disposable system. Typically it is desirable to monitor dissolved oxygen (DO), pH, and temperature for cell culture work and at that time there was nothing off the shelf that could be inserted into a disposable system in a sterile manner. Today smaller temperature probes are available that are no more than a few millimeters in diameter which can be inserted into a sleeve built into the disposable bag. By using a semi-permeable sheath and fiber optic based systems, dissolved oxygen and conductivity can be measured with miniscule probes inserted into the disposable system.

Most biologic processes require media and buffer preparations which contain various conductive salts. These solutions are then added to the bioreactor or in some cases for downstream processes such as cell lysis and chromatography. As shown in some of the data presented earlier, it is quite easy to use conductivity as a trending parameter of homogeneity for such salt solutions. For large scale mixing systems Wave Biotech, LLC does provide options for pH and conductivity measurement and feedback control to alert operators when homogeneity has been reached.

Figure 10. Fluid Velocity Streamline CFD Simulation – FlexMixer
The Disposable Bioprocessing Operation

From the advent of the disposable bioreactor came a whole market of ancillary components. Disposable tubing is now the norm in any bio-pharmaceutical facility and there are countless options for quick connect fittings, slide clamps, transitions, and adapters. There are even plastic components for steam-in-place (SIP) when connecting disposable systems to rigid stainless steel components. Most tubing is constructed of thermoplastic materials which can be fused closed for terminations and there are unique tube welding devices used to fuse two tubes in a sterile manner. It is now possible to connect tubing from a disposable mixer to tubing to feed a bioreactor by the means of a sterile tube fuser without the need of a hood. Through the integration of these components the bioprocessing manufacturer can now prepare their media and buffer solutions in advance using a disposable mixing vessel. With the aid of a sterile tube fuser these mixing systems can be linked to large scale disposable bioreactors and then harvested cell lines can be pumped to downstream processes for purification – Figure 11.

Figure 11. Disposable Bioprocessing System Layout

Conclusions

With better disposable mixing technologies, the use of in-situ sensors and instrumentation and the ancillary components to integrated various unit operations, the next wave in disposable mixing is upon us. With the advent of the jet mixing mechanism it is now possible to address nearly all challenges common to the bio-pharmaceutical manufacturer.
In addition to basic liquid-liquid mixing, it is no possible to mix high viscosity solutions, suspensions and powder-liquid recipes in a completely disposable system. With the use of micro sensors for dissolved oxygen, pH, and conductivity, process control and automation can be used to ensure product quality. All the ancillary components are now in place to transport fluids and suspensions to and from various unit processes many of which are now disposable in nature. Valuable time and money can be saved through quicker set up time, elimination of cleaning, and less process validation. Also, through the use of disposable mixers and ancillary technologies money can be saved in facility development as well. The disposable bioreactors, mixing systems, and flexible interconnecting tubing create their own contained environment reducing the need for higher clean room classification.

For the start up facility, disposables offer a clear economic advantage on several levels. The capital costs for most disposables are 50% less than their equivalent volume of stainless steel systems. Lately, manufacturers are seeing first hand the effect of rising steel costs as equipment prices are increasing due to short materials supply. Ongoing operating costs are nearly 70% less than that with traditional stainless steel systems. This savings is largely due to the significant reduction of man hours associated with the cleaning, verification, and set up time in traditional systems. This savings is also in the form of factors commonly overlooked when one is selecting a traditional stainless steel plant process. Disposable mixers are generally much easier to operate and require less ongoing maintenance as there are no seals, bearings and motor assemblies to upkeep, particularly with the FlexMixer system which does not use an impeller. This means that operators and plant maintenance technicians require less training on the system. Finally there is the hidden killer to the bottom line operating cost and efficiency of the manufacturing process – the enormous volumes of cleaning agents, and water for CIP/ SIP used. As a rule of thumb for every liter of water used in various stages of the manufacturing process, roughly 5-10 times that volume is used in the form of cleaning agents, alcohols, and CIP/SIP water. The use of disposables can significantly reduce this waste.

Disposable mixers for basic liquid applications were the first step in allowing biopharmaceutical manufacturers to reduce manufacturing costs. With the advent of jet mixing technology, a variety of applications can now use disposable mixing technology, and in-situ sensors can be used to streamline the manufacturing process and improve the bottom line.

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