A study of mixing behavior in disposable 2D bags used in cross-flow filtration

The time it takes to mix fluids in disposable bags is a key issue for many process developers. This study demonstrates recirculation of 1 cP and 5 cP viscous solutions in 50 L, 2-dimensional (2D) pillow bags using a tubing loop and peristaltic pump. This method is particularly applicable to cross flow filtration. The results show that good mixing in relatively short time can be achieved when sufficiently high recirculation flow rates are used. Many times well-mixed solutions are attained in three minutes or less.

Introduction

The growing interest in using disposables in biopharmaceutical processing can be seen in the increasing use of single-use bags instead of stainless steel vessels. The driving force behind this switch is the desire to minimize cleaning and cleaning validation for product and buffer containers. Disposable systems also afford increased design flexibility. GE Healthcare Life Sciences ReadyToProcess product platform brings a plug-and-play alternative to multiple bioprocessing unit operations including cross flow filtration.

The change from fixed-geometry stainless steel vessels to disposable bags generates new mixing considerations. Apart from magnetically-driven impellers, which often are the option when employing disposable bags, an alternative method of mixing is to simply recirculate the solution within the reservoir bag. Because cross flow filtration generally requires that the bulk suspension (retentate), processed by the filter, is actively circulated, in-bag recirculation is suitable for this mixing application.

Cross flow retentates are sometimes higher in density and/or viscosity than their respective feed streams. Thus, mixing behavior in disposable cross flow filtration reservoir bags is of particular interest. In this application note the mixing of homogeneous solutions in 50 L, 2D pillow bags is demonstrated. The effects of liquid density and viscosity differences, vessel working volume, and power input per unit volume (i.e. recirculation flow rate) are presented. Of special interest is determining the minimum working volume of the reservoir bags. Hence, a related study on 100 L and 200 L 3-dimensional bags also deserves the reader's attention (1).

Experimental details

Bag descriptions and arrangements

All bags and assemblies used in this study were from GE Healthcare ReadyCircuit™ product portfolio including sterile, quick-to-configure and assemble circuits for a variety of fluid processing applications for filtration systems. Available bag sizes range from 250 mL to 50 L in the 2D format. Figure 1 shows the 50 L, 2D (pillow) disposable bag type used in this study.

Fig 1. A ReadyCircuit disposable 50 L, 2D bag, with inlet and outlet ports, similar to the ones used in this mixing study. The tray is tilted at a 3 degree angle to the bag outlet, which ensures that the solution is directed towards the outlet.

The 2D format used was a 50 L bag with four ports placed in a tray at a 3 degree angle to the bag outlet, which ensures that the solution is directed towards the outlet. This bag design has three ports on its lower side that can be used as either inlets or outlets, one sample port, and a fourth inlet port located on the upper part of the bag. Set-ups, with both upper and lower inlets, were therefore studied (Fig 2).

In both set-ups, a recirculation loop was assembled by connecting a 5′ jumper to the bag outlet, from which the following components were connected: a SciCon™ conductivity sensor, pump tubing, an injection port for sample introduction, and a jumper tube directed back to the bag inlet used. Recirculation loop inner diameter was ¾”. The conductivity sensor was connected to a SciCon conductivity monitor and a computer for data logging. Liquid flow was driven by a Watson-Marlow™ 720 peristaltic pump.

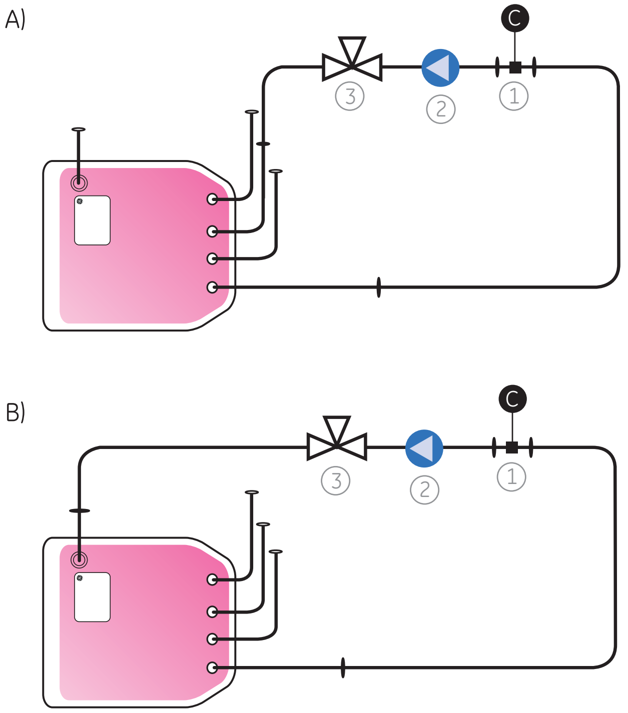


Fig 2. Experimental set-up showing recirculation assemblies and component locations for: A) 50 L, 2D bag equipped with a low inlet adjacent to the bag’s outlet, and B) 50 L, 2D bag equipped with a high inlet located at the opposite end of the bag from its outlet. In both cases the recirculation loop was assembled by connecting a 5 ft section of tubing to the bag’s outlet. Also included in the equipment set-up: 1) a Scilog conductivity sensor, 2) pump tubing, 3) an injection port to introduce sample (concentrated NaCL) solution spike. Recirculation loop inner diameter was ¾ in. The conductivity sensor was connected to a SciCon conductivity monitor and a PC equipped for data logging using LabVIEW™ software. Liquid flow was driven by a Watson Marlow Bredel 720DU Peristaltic pump equipped with 3/4” ID Pumpsil™ tubing.

Testing of mixing behavior

Each bag was filled to a specified working volume with either room temperature water (viscosity = 1 cP) or 47% (v:v) glycerol (viscosity = 5 cP). After recirculation flow rate was set, a high conductivity spike of test liquid saturated with NaCl (0.1% of the working volume) was introduced at the injection port (t=0). Conductivity was monitored prior to injection and at each second after injection until it was fully stabilized. The time to reach T95 (95% of the concentration step change from starting point to end point concentration) was defined as the mixing time, that is, the time it took to render the recirculating mixture well-mixed. Table 1 lists the parameters and levels investigated. Data from all testing combinations were evaluated using Modde™ 9.2 software and four multiple linear regression (MLR) models were created in a design of experiment (DoE) approach. All tests were conducted in duplicates.

Table 1. Factors and levels investigated in the bag mixing experiments

Parameter	Levels	Low Range	High Range
Viscosity (cP)	2	1 (water)	5 (47% glycerol)
Inlet configuration	2	Upper	Lower
Recirculation flow rate (L/min)	5	1.6	16
Working volume (L)	5	5	50

Determination of minimum working volume during recirculation

Figure 3 shows the assembly used for studying the minimum working volume during recirculation. Each recirculation loop tested was equipped with a pressure sensor, an inlet line, an outlet line, and a recirculation pump. Recirculation loops with both upper and lower inlet types were studied. Prior to minimum working volume determination, each type of recirculation loop was filled with water and its dead volume was determined by weighing. A fresh 50 L bag was filled with 10 L of test solution - either water or 47% glycerol. The 50 L bag was mounted in its tray and filled up to about 10 L with test solution. Excess air was removed from the bag. The recirculation pump was started, and bag outlet pressure was noted. Liquid was then slowly removed from the bag by a second peristaltic pump. The point at which the bag’s outlet pressure was observed to significantly decrease, as a consequence of low volume in the bag, was determined and defined as the minimum working volume during recirculation. All tests were conducted in duplicates.

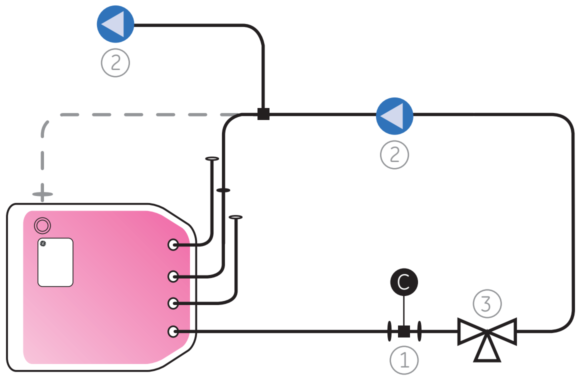


Fig 3. Experimental set-up showing the recirculation assembly and component locations: 1) conductivity sensor, 2) pump tubing and 3) injection port, for determining the minimum working volume of a 50 L, 2D bag. All testing was performed at room temperature (20°C).

Results

Mixing data evaluation

Four MLR models were created - one for each combination of inlet position and viscosity. Tests, in which it took longer than 20 minutes to reach T95, were excluded. To validate the model's normal probability plot, R^2, Q^2, ANOVA and reproducibility were assessed. The models for low viscosity were considered to be statistically valid. However, high viscosity restricts mixing to a greater extent than low viscosity, leading to more variable mixing behaviors (large variations in mixing times when performing replicates). This limits the high viscosity operating range. When the input ranges for high viscosity models were restricted, valid models could be obtained. When using the upper bag inlet during recirculation, flow rate levels were restricted to ranging from 8 to 16 L/min at four levels, and bag working volume levels were restricted to a range from 12.5 to 50 L at three levels. When using the lower bag inlet during recirculation, flow rate levels were restricted to a range from 4 to 16 L/min at four levels, and bag working volume levels were restricted to ranging from 25 to 50 L at three levels. The model validation data are summarized in Table 2. These models were determined to be acceptable for the intended purpose, that is, recommendation of flow rates that lead to satisfactory mixing. Hence, these models were used to generate contour plots that visualize mixing behavior. Settings outside these ranges, were excluded from the model.

Recirculation flow rate levels were restricted in the models to a range from 4 to 16 L/min at four levels and bag operating volumes were restricted to ranging from 25 to 50 L at three levels. Settings outside these ranges were exclued from the model.

Table 2. Summary of model validation including R^2, Q^2 and reproducibility

Solution viscosity (cP)	Inlet configuration	R^{2*}	$Q^{2\dagger}$	Reproducibility‡
1	Upper	0.86	0.80	0.91
1	Lower	0.82	0.77	0.96
5	Upper	0.82	0.60	0.92
5	Lower	0.82	0.72	0.83

* R^2 is the percent of variation of responses explained by the model.

† Q^2 is the percent of variation of responses explained by the model according to cross-validation.

‡ Reproducibility is the variation of the response under the same conditions as compared to total variation of the response.

Mixing behavior

Figure 4 shows the mixing behavior at low viscosity and Figure 5 shows mixing behavior at high viscosity. As expected, when all other parameters were constant, a high recirculation flow rate led to shorter mixing times for all bag configurations and viscosities since higher flow rates impart higher power input per volume inside the bag. At high recirculation flow rate, mixing times at low viscosity were shorter than the mixing times at high viscosity (as expected). At low viscosity, mixing was efficient (< 6 minutes) if the recirculation flow rate was held at or above 5 L/min for all bag working volumes tested. These results are not greatly affected by the choice of inlet configuration, but the use of the lower port appears to be slightly more efficient from a mixing standpoint than the use of the upper port. At low viscosity the upper inlet port supports a broad working volume range for the bag.

Choice of working volume affects the mixing behavior, and changes in working volume affect the power input to volume ratio (the primary determinant of mixing behavior). Therefore, mixing at constant recirculation flow rate is generally more favorable at low working volumes than at high working volumes. However, at very low working volume in 2D bags, the close proximity of the upper and lower bag walls begins to resist the convective flow generated by recirculation. This combination of factors gives rise to a maximum mixing time within the 2D bag as working volume at constant flow rate is decreased.

At low working volumes there is also a critical working volume below which channeling occurs. This is when a large fraction of the bag's volume is completely excluded from convective flow and controlled mixing. At the upper inlet configuration, this threshold is observed at working volumes between 8 and 12.5 L. For the low inlet configuration, the equivalent range is 12.5 to 25 L. Working volumes should therefore be kept above these levels to ensure proper mixing, especially at high viscosity. If operations at volumes below these critical thresholds are needed, the bag should occasionally be manipulated or flexed to overcome this natural phenomenon.

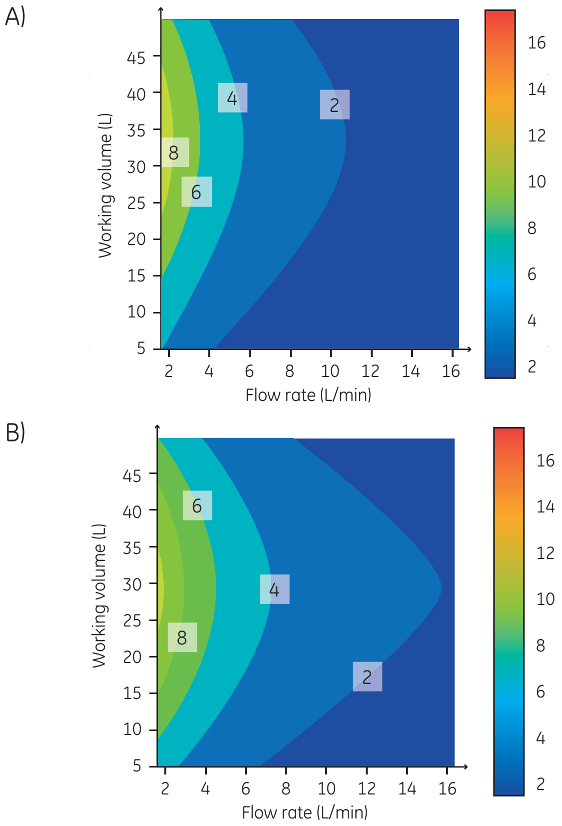


Fig 4. Contour plots showing mixing time (minutes) at **low** viscosity in the 50 L, 2D bag: A) low inlet configuration, and B) high inlet configuration. The numbers (white box, black font) indicate the time in minutes required to achieve T95. Shortest mixing times are denoted with a dark blue background.

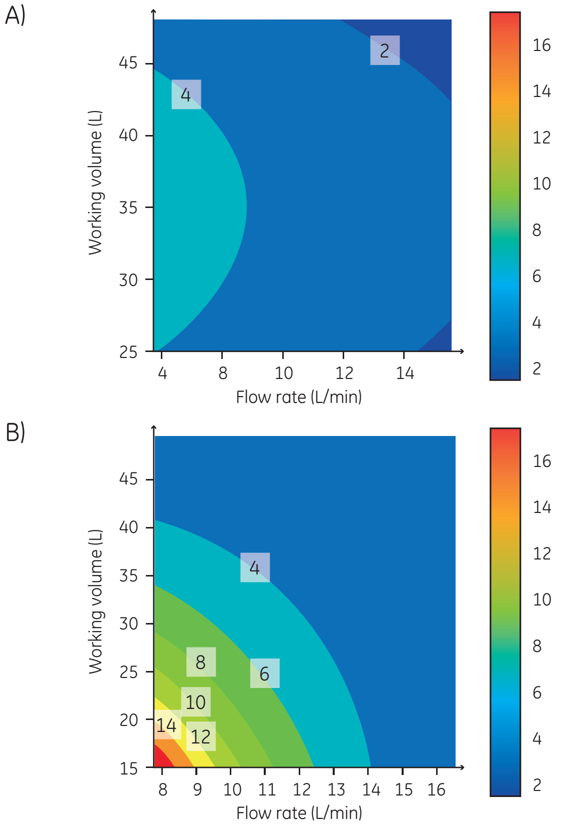
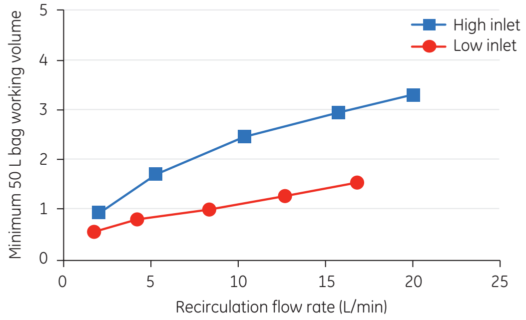


Fig 5. Contour plots showing mixing time (minutes) at **high** viscosity in the 50 L, 2D bag: A) low inlet configuration, and B) high inlet configuration. The numbers (white box, black font) indicate the time in minutes required to achieve T95. Shortest mixing times are denoted with a dark blue background. Note the difference in the X- and Y-axes. In the high viscosity case the positive effect of high recirculation flow rate is very apparent.

Minimum working volume volume

The minimum working volume for a given equipment set-up inevitably depends upon the minimum working volume of the selected bag and the liquid hold up volume of the recirculation loop. The minimum working volumes presented here only represent the minimum working volumes of the 50L bag itself. When using this information you should calculate the holdup volume of your specific recirculation flow path and add that volume to those shown here, in determining the system minimum working volume.

A) Low viscosity



A) High viscosity

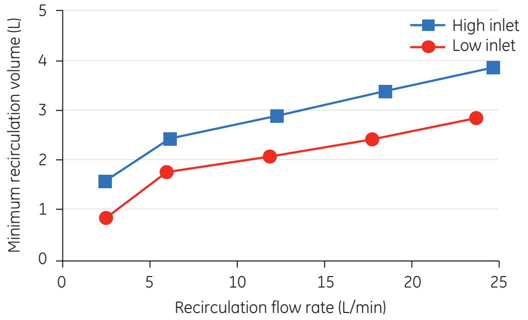


Fig 6. Minimum working volume as a function of recirculation flow rate, viscosity, and inlet types for the 50 L, 2D bag: A) low viscosity, and B) high viscosity. Relative standard deviation was below 5.5% for low viscosity and 2.8% for high viscosity.

As expected, a lower minimum working volume is obtained at low viscosity as compared to high viscosity at the same flow rate (Fig 6). This difference is magnified by the choice of the lower inlet. A working volume of at least 4 L is required to ensure that the pump can be used at its maximum flow rate of 25 L/min.

Note that the volume in the recirculation flow path depends upon the recirculation set-up (upper or lower inlet). Choosing the lower inlet set-up, affords the selection of 3 L as the minimum working volume at high viscosity and 2 L at low viscosity.

This volume is added to the minimum working volume from this study to achieve the required volume for the intended system. If the viscosity is not known, the minimum working volume data for the high viscosity condition should be used.

Applying this data to other types of mixing or filtration operations, which use recirculation, requires determination of the holdup volume in the recirculation flow path.

Conclusions

During cross flow filtration, good mixing in relatively short time spans (T95 < 6 min) is generally achieved at low as well as high viscosity, with both upper and lower inlet configurations, and over a broad range of recirculation flow rates. In some cases, T95 is attained in three minutes or less. Therefore, the necessity of the use of an exogenous mixer (impeller) can be avoided with one single reservation: to assure proper mixing at high viscosity, low working volumes should be avoided or external mixing (bag manipulation) applied. For 50 L, 2D bags, decreased convective flow and high viscosity lead to increased mixing times. Very low working volumes affect mixing and are due to changes in bag geometry. Contour plots from the Design of Experiments (DoE) studies generally revealed large operating ranges within a wide window of operating conditions. Process developers can therefore choose the correct conditions from which they will obtain good mixing control. If extreme conditions, which might lead to long mixing times, are avoided, good mixing should be easily achievable.

Related literature

1. Application note: A study of mixing behavior in disposable 3D bags used in cross-flow filtration, GE Healthcare Life Sciences, 29-0187-32 (2012).

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