A study of mixing behavior in disposable 3D bags used in cross-flow filtration

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The time it takes to mix fluids in disposable bags under different operating conditions is a key issue for many process developers. We have investigated this phenomenon using a tubing loop to recirculate solutions via convective forces in disposable 100 L and 200 L 3D rectangular bags. This method is applicable in cross-flow filtration. Results show that good mixing in a relatively short time can be achieved for all conditions tested if sufficiently high recirculation flow rates are used. In many situations, this can be 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.

This Application note describes studies on the mixing of homogeneous solutions spiked with a high salt concentration solution in 100 L and 200 L 3D rectangular bags. It examines the impact of parameters such as liquid density and viscosity, vessel design and power input (i.e. recirculation flow rate). A similar study using disposable 50 L 2D pillow bags has also been described (see Related literature).

Experimental details

Bag descriptions and arrangements

All bags and assemblies used are from the GE Healthcare ReadyCircuit™ range. This includes sterile, quick to configure and assemble circuits for a variety of fluid processing application needs in filtration systems. Bag sizes range from 250 mL to 50 L for the 2D (pillow) format and from 100 L to 200 L for the 3D (rectangular) format. Figure 1 shows disposable 3D bags from this range.

Fig 1. Flexible 3D plastic bags (empty) from the ReadyCircuit range similar to those used in this mixing study.

In the configuration used for this study, a recirculation loop was assembled by connecting a 5 ft jumper to the bag outlet, from which the following components were connected: pump tubing, an injection port to introduce sample, a SciCon™ conductivity sensor, and a jumper back-directed to the bag inlet. Recirculation loop inner diameter was ¾". The conductivity sensor was connected to a SciPress™ pressure monitor and a computer for logging data. Liquid flow was driven by a Watson Marlow™ 720 peristaltic pump. During recirculation, the outlet was at the bottom of the bag (slightly off-center) and the inlet at the top (center). Figure 2 shows this set up.

To ensure proper mixing, the inlet was directed into the bulk liquid and not towards the bag inner wall in all working volumes investigated.

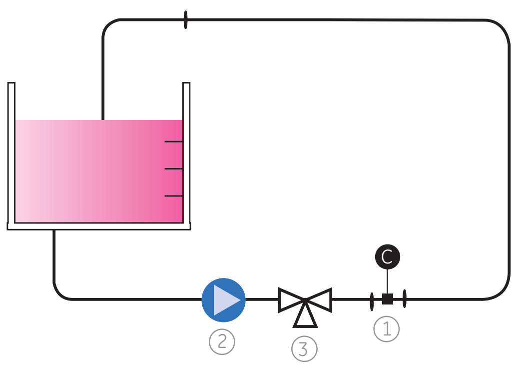

Fig 2. Experimental set-up showing the recurculation assembly and component locations: 1) conductivity sensor, 2) pump tubing and 3) injection port.

Testing of mixing behavior

Each bag was filled to a specified working volume with a test solution: room temperature water (viscosity 1 cP) or aqueous glycerol (5 cP). The recirculation flow rate was set and a high conductivity spike of 50 to 400 ml 3 M NaCl (sufficient to raise vessel conductivity by at least 200 µS/cm) was introduced.

Conductivity was monitored before the spike was applied, as it was applied (t = 0), and every second thereafter until it fully stabilized. Mixing behavior was observed by noting recirculation stream conductivity until a stable level was reached.

Time to reach T95 (95% of a step from the starting point to the end point) was defined as the mixing time, i.e. the time to render the recirculating mixture well-mixed.

Table 1 lists the parameters and ranges investigated. These were combined and evaluated by creating multiple linear regression (MLR) models in a Design of Experiment (DoE) approach. The definition of good mixing (T95) was set to 6 min or less.

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)
Recirculation flow rate (L/min)	3	3.75	15
Working volume (L)			
100 L bag	4	25	100
200 L bag	4	75	200

Results

Data evaluation

Data from all mixing combinations were evaluated using Modde 9.2 software. Four MLR models (two viscosities and two bag sizes) where created.

To evaluate the models' normal probability plot, R^2 and Q^2 were assessed.

All models evaluated were considered statistically valid. No data point was considered as outliers at 95% confidence interval from the normal probability plot. The models generated from the experiments at 1 cP viscosity were better than those at 5 cP.

Table 2 summarizes the model validation data. The models were considered as acceptable for the intended purpose, i.e. to recommend flow rates that give satisfactory mixing. They were thus used to generate contour plots that visualize mixing behavior.

Table 2. Summary of model validation

Bag volume (L)	Solution viscosity (cP)	R^{2*}	$Q^{2\dagger}$
100	1	0.80	0.53
100	5	0.88	0.37
200	1	0.81	0.60
200	5	0.73	0.35

* R^2 is the percentage of the variation of the responses explained by the model.
† Q^2 is the percentage of the variation of the response by the model according to cross validation.

Mixing behavior

Figure 3 shows contour plots of mixing time for the 100 L and 200 L 3D bags. Sufficient mixing (mixing time < 6 min) can be achieved for all working volumes at both viscosities in the 100 L bag if the recirculation flow rate is not too low (>5 L/min). The same situation is seen for the 200 L bag at low viscosity. At high viscosity, however, the required recirculation flow rate has to be at least 9 L/min to ensure sufficient mixing (Fig 3C).

Other findings of a practical nature were that the return of the recirculation loop in a 3D bag has to be positioned directly into the liquid and not towards the bag inner wall. This will ensure proper mixing. In addition, air inside a bag should be avoided. Otherwise, mixing times will increase and foam will be formed. When folding the 100 L and 200 L bags to reduce unused space, avoid sharp folds that in any way interfere with the recirculation flow as it enters the bag.

In general, high viscosity gives longer mixing times than low viscosity. This result is expected and is due to free-space diffusion being slower at higher viscosity. Creating the same motion in a higher viscosity liquid requires more power input. Nevertheless, good mixing can be achieved at the investigated working volumes if the flow rate is high enough.

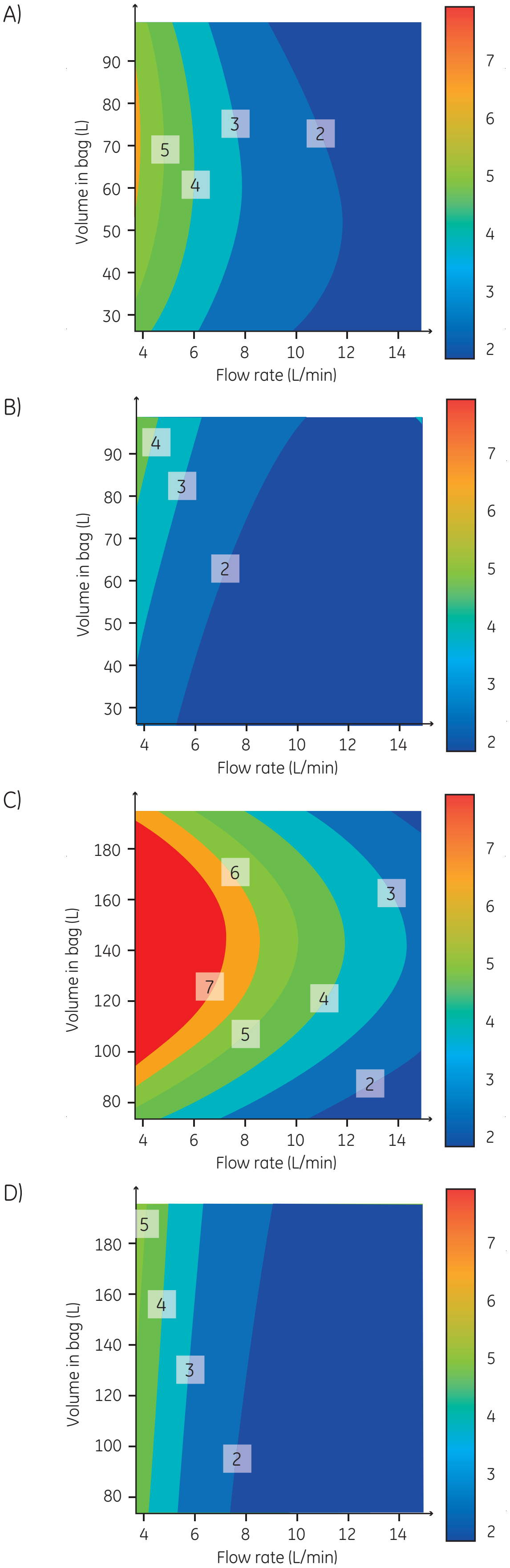

Fig 3. Contour plots of mixing time for 100 L and 200 L 3D bags. A) 100 L bag, high viscosity, B) 100 L bag, low viscosity, C) 200 L bag, high viscosity, and D) 200 L bag, low viscosity. The numbers (white boxes/black font) indicate the time in minutes to reach T95. The shortest mixing times are shown with a blue background.

At low viscosities the results show that at a constant bag volume, mixing time will decrease with increasing recirculation flow rate. This is expected and is due to the increased power input/volume ratio used. Keeping recirculation flow constant and changing the volume inside the bag will result in the same effect on mixing time; the increased power input/ volume ratio again gives better mixing.

At high viscosities, however, the mixing behaves differently. The effect of a change in vessel geometry when the liquid volume in the bag alters becomes apparent. As working volume decreases from 200 L to 100 L and below at constant recirculation flow rate in the high viscosity case, fig. 3C show a maximum in mixing time at a working volume of 140 L.

For a cross-flow filtration unit operation, the flow rate is not optimized for mixing but rather for the filtration process. In general, hollow fibers are operated from 2000 up to 16,000 s^{-1} in shear. Table 3 shows how shear is related to the hollow fiber size and flow rate. A general guide is that size 8 and 9 are used for the 100 L bag and size 35 & 55 for the 200 L bag. If the process can be operated according to this recommendation, the mixing should be adequate (<6 min) at all filter sizes and process viscosities if run above 2500 s^{-1} in shear.

Table 3. Hollow fiber filter shear rates (s^{-1})

Recirculation flow rate (L/m)	Size 8 and 9 hollow fiber filters		Size 35 and 55 hollow fiber filters	
	C-Lumen	E-Lumen	C-Lumen	E-Lumen
3.75 (low)	2800	1250	1150	500
7.5 (medium)	5600	2500	2300	1000
15 (high)	11200	5000	4600	2000

Conclusions

Good mixing in a relatively short time span (T95 < 6 min) can be achieved for all conditions tested for typical flow rates used in hollow fiber processes. In many situations, this can be attained in three minutes. Mixing in disposable bags during cross-flow filtration operations can therefore be achieved using recirculation flow without having to resort to bags with an incorporated impeller.

For both 3D bag sizes, the recirculation return port has to be directed into the bulk liquid in order to enable mixing.

The contour plots from the Design of Experiment studies generally reveal large operating ranges with a wide window of conditions. Process developers can therefore choose the correct conditions that will give them good control over the mixing. When extreme conditions leading to poor results are avoided, good mixing can be achieved in most situations.

Related literature

A study of mixing behavior in disposable 2D bags used in cross-flow filtration. Application note 29-0187-31. GE Healthcare Life Sciences.

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