BIOTECH

Application Note

USD 3465

Evaluation of the Wand Mixer Single-Use Mixing System for Sorbent Slurry Homogenization Before and After Packing of a 400 mm Auto-Packing Chromatography Column

Contents

1 Introduction

Conventional reusable mixers are commonly used to create a homogeneous sorbent slurry prior to packing of chromatography columns. As the popularity of single-use mixers has grown, so has interest in using them for such sorbent slurry mixing applications. In this study, a 50 L Wand Mixer single-use mixing system was evaluated for its ability to produce a homogeneous sorbent slurry.

Figure 1

Wand Mixer system in floor-based model with various compatible mixing containers and bench-top model with various compatible mixing containers

2 Experimental

2.1 Mixing Before Automated Packing

Before use, the 50 L Wand Mixer wand was measured and photographed to establish baseline dimensions.

A 50 L Wand Mixer biocontainer was installed in a 50 L Wand Mixer round MDPE tank, inflated with air through a top inlet, then connected to a floor-based Wand Mixer drive unit. A T-piece with sampling port was then installed on the mixing biocontainer's bottom drain outlet tubing to allow representative samples to be taken.

The sorbent slurry used in this study consisted of the Cytiva♦ 64% Q Sepharose XL resin in water. The Wand Mixer drive head was lowered until the sweeper was 5 cm from the bottom of the biocontainer, then mixing was initiated at a speed of 100 rpm. Slurry was then transferred to the mixing biocontainer using a diaphragm pump and tubing routed through the biocontainer's powder port. After ~20 L of slurry had been transferred, the mixing speed was increased to 150 rpm, then pumping continued until ~40 L of slurry had been transferred – in total, this transfer took ~5 minutes. Mixing was continued at 150 rpm for 5 minutes after transfer was complete. Slurry was then sampled from the top (through the powder port) and bottom (at the T-piece) of the mixing biocontainer to confirm homogeneity.

With the powder port open to the atmosphere and the impeller continuing to run at 150 rpm, the slurry was transferred from the mixing biocontainer to a 400 mm automatically packing chromatogaphy column via a diaphragm pump. At the conclusion of the draw, the mixing wand was found to be no longer in contact with the slurry. Lastly, the slurry was sampled from the bottom (at the T-piece) of the mixing biocontainer to confirm homogeneity.

For all samples taken, 10 mL aliquots were centrifuged at 1000 G for 2 x 5 minutes then quantified by measurement of the solid bed height.

2.2 Mixing After Automated Unpacking and Settling

Mixing in the Wand Mixer was resumed at 150 rpm. The slurry was resuspended inside the column through an introduction of air from the bottom of the column, then the column was drained back into the mixing biocontainer via a top inlet port. An automated rinse of the column with a further 7 L of water was performed to ensure all the sorbent had been returned to the Wand Mixer, resulting in a dilution of the sorbent to a concentration of ~58%.

Once transfer to the Wand Mixer was complete, mixing was stopped, and the sorbent was allowed to settle overnight (16-18 hours – deemed to be a worst-case scenario). Resuspension was then assessed by resuming mixing at incrementally increasing impeller speeds; 30 rpm for 1 minute, then 50 rpm for 1 minute, then 100 rpm for 1.5 minutes, and finally 150 rpm for 5 minutes. Slurry was then sampled from the top (through the powder port) and bottom (at the T-piece) of the mixing biocontainer to confirm homogeneity.

For all samples taken, 10 mL aliquots were centrifuged at 1000 G for 2 x 5 minutes then quantified by measurement of the solid bed height. After use, the 50 L Wand Mixer wand was measured and photographed to assess whether deformation had occurred.

3 Results

Pre-packing mixing efficiency results are given in Table 1. Figure 1 shows typical sample appearance in centrifuge tubes after centrifugation. Post-unpacking/post-settling mixing efficiency results are given in Table 2.

Table 1

Slurry concentration before column packing and after 5 minutes mixing at 150 rpm

	Reference*	After 5 Minutes Mixing at 150 rpm		At the End of the Slurry Draw
Sampling Level		Samples from the Top	Samples from the Bottom	Samples from the Bottom
Average Slurry Concentration (%)	64.1	65.3	65.6	63.9

*The reference is based on collected data from the slurry after manual mixing

During pre-packing mixing, the sorbent slurry was found to be homogeneous after 10 minutes (5 minutes transfer followed by 5 minutes of post-transfer mixing at 150 rpm) and remained homogeneous until the end of the slurry draw, even though the wand was no longer in contact with the slurry.

Figure 2

Samples from the top (left two tubes) and the bottom (right two tubes) of the mixing bag after 5 minutes mixing and 2 x 5 minutes centrifugation

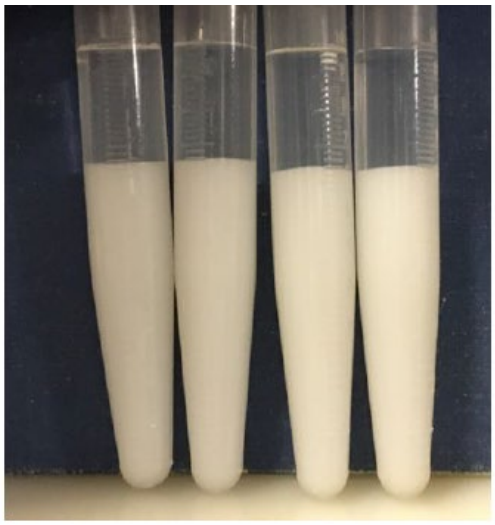


Table 2

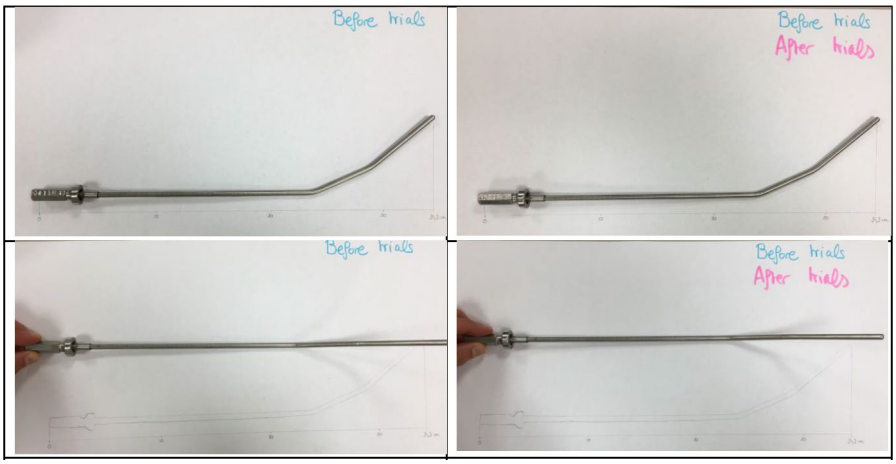
Sorbent concentrations after one night settling and 8.5 minutes mixing from 30 to 150 rpm

	Reference*	After 8.5 Minutes Mixing from 30 to 150 rpm	
Sampling Level		Samples from the Top	Samples from the Bottom
Average Sorbent Concentration (%)	58.0	57.1	56.4

During post-unpacking/post-settling mixing, the sorbent slurry was found to be homogeneous after 8.5 minutes mixing in increments from 30 to 150 rpm. The shape and dimensions of the mixing wand were evaluated (Figure 3). No changes in dimensions or appearance were noted, confirming that the forces experienced during the mixing study did not over-stress the wand, even when mixing was initiated while its lower section was fully immersed in the sorbent bed.

Figure 3

Mixing wand before and after study



4 Recommendations

The tubing leading in and out of the biocontainer should be checked to ensure it is free of kinks before initiating the automated packing and unpacking sequences.

Not more than 0.4 bar static pressure should be applied to the bottom drain line during the slurry vessel air agitation before packing.

The mixing biocontainer should be properly vented to avoid over-pressurization during air agitation and column unpacking. The maximum operating pressure of a mixer biocontainer is 0.4 bar. We recommend using a Pall Inflation box for a controlled pressure environment of the biocontainer. More information can be obtained by contacting your local Pall contact person or at contact form at ww.pall.com

5 Conclusions

The 50 L Wand Mixer system is able to create a homogenous 64% Q Sepharose XL sorbent slurry in less than 10 minutes before column packing, and again after column unpacking with an overnight settling period.

The mixing procedure of incrementally increasing mixing speed from 30 to 150 rpm over 8.5 minutes provided safe and efficient mixing with no damage to the mixing wand. Pall recommends using this procedure to create a homogenous slurry with the Q Sepharose XL and similar sorbents.

PALL CORPORATION

Visit us on the Web at www.pall.com/industry
Contact us at www.pall.com/contact

Corporate Headquarters
Port Washington, NY, USA
+1-800-717-7255 toll free (USA)
+1-516-484-5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

Pall Corporation has offices and plants throughout the world. To locate the Pall office or distributor nearest you, visit www.pall.com/contact.

The information provided in this literature was reviewed for accuracy at the time of publication. Product data may be subject to change without notice. For current information consult your local Pall distributor or contact Pall directly.

IF APPLICABLE Please contact Pall Corporation to verify that the product conforms to your national legislation and/or regional regulatory requirements for water and food contact use.

© Copyright 2021, Pall Corporation. Pall, PALL , are trademarks of Pall Corporation. ® Indicates a trademark registered in the USA.
•Cytiva is a trademark of Global Life Sciences Solutions USA LLC

USD 3465 21.07799
03/2021