Using Xcellerex mixing system as a slurry tank when packing chromatography columns

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Using Xcellerex™ mixing system as a slurry tank when packing chromatography columns

This application note describes the performance of single-use Xcellerex XDM Quad Mixing System used as a slurry tank when packing chromatography columns. The capability of the mixing system to keep the chromatography resin in suspension and the possibility to integrate the system in column packing were studied. Further, an evaluation of the effect of the integrated, disposable mixer bag impeller on chromatography resin particles was performed. The results show that XDM Quad Mixing System can be used as a slurry tank for chromatography resin in 50 or 100 L bags, when packing columns. For larger volume, our recommendation would be to use dedicated, stainless steel slurry tanks.

Introduction

Xcellerex XDM Quad Mixing System is designed for convenient and efficient mixing of buffer, resin, product, and other process fluids (Fig 1). The single-use system eliminates the hassle of time-consuming and costly steam-in-place, clean-in-place, and cleaning validation procedures. The result is an efficient processing and rapid batch-to-batch turnover time.

The XDM Quad Mixing System is a rigid containment system complete with motor and controls, and an irradiated USP Class VI, single-use, low-density polyethylene plastic bag equipped with a disposable bottom-mounted impeller. The connection between motor and disposable impeller is via a robust magnetic coupling. The agitation is driven through stainless steel bearings within the impeller, providing high torque and rapid mixing to the system.

Fig 1. XDM Quad Mixing System, with a powerful motor and magnetically locked impeller, effectively mixes even highly viscous materials.

DEAE Sepharose™ Fast Flow chromatography resin was used to investigate the ability of XDM Quad Mixing System to keep the resin in suspension. The resin was mixed for 1 h before the bag was drained and resin sedimentation was monitored. In a related study, the XDM Quad Mixing System was evaluated for use with an AxiChrom™ 300 chromatography column. The mixer was used with the Intelligent Packing concept of AxiChrom columns for packing Capto™ adhere ImpRes.

The effect of the mixer bag impellers on Sephacryl™ S-200 HR and Capto DEAE particles was also evaluated in this study.

Materials and methods

Resin mixing

Xcellerex XDM Quad Mixing System was used to mix DEAE Sepharose Fast Flow chromatography resin. A 100-L XDM-100 basic Quad mixing bag was filled to 50% with 50 L of resin at 42% slurry concentration by pumping. The slurry was mixed for 1 h with the impeller set to 100 rpm. After 1 h, the slurry was drained through the bottom hose and resin sedimentation was monitored in the mixing bag.

Column packing

Xcellerex XDM Quad Mixing System was used for packing of Capto adhere ImpRes chromatography resin in AxiChrom 300 column. A 100-L XDM-100 basic Quad mixing bag was filled with resin to give a 20 cm bed height in the column. The impeller speed was set to 50 rpm for mixing and was reduced gradually during packing.

The column was packed with Intelligent Packing controlled by the AxiChrom Master and the resin was automatically transferred from the mixer tank to the column. When the filling was finished, the resin valve and column tubes were rinsed.

The packed column was tested for reduced plate height and asymmetry factor (A_s).

Effect of impeller on resin particles

A mixer test was performed to evaluate the impact of the bag impeller on the chromatography resin particles. Sephacryl S-200 HR and Capto DEAE were tested separately in a mixer bag. Samples for particle analysis were taken both before the resins were added to the bag and after 1, 2, and 3 h of agitation at 100 rpm. The samples were measured using a Multisizer™ 3 Coulter Counter™.

Results and discussion

Resin mixing

To study the performance of XDM Quad Mixing System when used in slurry preparation of chromatography resins, DEAE Sepharose Fast Flow resin was mixed for 1 h before the bag was drained and monitored for sedimentation.

It is important that the mixing bag is filled with buffer or water covering the impeller before adding slurry and that the resin is in slurry when filling the mixing bag.

No sedimentation of resin was observed in the mixing bag.

Column packing

AxiChrom 300 column and Capto adhere ImpRes resin were used for studying the possibility of integrating the XDM Quad Mixing System into the Intelligent Packing concept of the AxiChrom columns.

The initial filling of the column with an excess of resin in the bag proceeded smoothly. When the resin level in the bag was lowered, turbulence in the center could be observed. The turbulence did not affect the resin outlet, that is, no air was introduced into the column. However, to minimize the risk of allowing air into the slurry, and subsequently the column, the running speed of the agitator did not exceed 50 rpm. Further, when the resin level was below the impeller, the resin was not mixed properly and instead started to sediment. This was overcome by buffer addition to keep the slurry level above the impeller, with a dilution of the resin as a result.

To get most of the resin out of the bag, the bag can be lifted and tilted towards the resin outlet. After the packing, a minor volume of resin slurry was left in the bag and hose (including rinse volumes). The volume will depend on optimization and how the user develops their standard operating procedures.

The efficiency of the packed column was tested and the results are summarized in Table 1.

Table 1. Column efficiency data for AxiChrom 300 column packed with Capto adhere ImpRes resin

Bed height (cm)	20
Packing factor	1.15
Flow velocity (cm/h)	30
Upflow	
A_s factor	1.25
Reduced plate height	1.77
Downflow	
A_s factor	1.19
Reduced plate height	1.60

The results show that the XDM Quad Mixing System is suitable for mixing of DEAE Sepharose Fast Flow resin slurry. It is also possible to integrate the system in the Intelligent Packing concept of the AxiChrom 300 for packing of Capto adhere ImpRes resin into the column.

Effect of impeller on resin particles

The effect of the impeller on Sephacryl S-200 HR and Capto DEAE particles after mixing was evaluated. Samples taken before (0 h) and after 1, 2, and 3 h were compared to the initial sample. No significant effect on the particle size distribution or mean particle size ($d_{50,volume}$) of the chromatography resins from the bag impeller was observed.

Conclusion

In most scales, a dedicated stainless steel tank will be the most versatile chromatography resin handling vessel. An XDM Quad Mixing System, equipped with a 50 or 100 L bag, can be integrated in automated packing of chromatography columns and be used as an alternative to stainless steel tanks. When using an XDM Quad Mixing System as a slurry tank, procedures must be developed to resuspend sedimented resin in the bag and avoid sediment blocking the outlet tubing as well as determination of the slurry concentration in the bag, where needed. Measures should also be taken to minimize the residue volume of slurry in the bag at completion of the packing process.

Ordering information

Product		Product code
XDM Quad	Mixing system	29048373
XDM-100	Basic Quad mixing bag, 100 L	On request

Related literature	
Packing Capto ImpRes media with verified packing methods, Application note	29030698

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