

## APPLICATION NOTE

# Flow-pressure data at bioprocess scale

The largest difference in functionality between small, lab-scale columns and bioprocess columns is the impact of so-called wall effects; the support to the resin given by the column walls that results in better flow properties in smaller columns. Therefore, it is important to obtain flow-pressure data for the resins in bioprocess columns where the wall effects are minimized. Flow velocity vs. pressure curves for a resin are obtained by increasing the column diameter in several consecutive flow-pressure runs. In this curve, critical flow, packing flow and running flow are measured at increasing column diameters. Resins with higher degrees of cross-linking, such as derivatized resins, and resins with smaller pore sizes will tolerate higher flow rates before the bed collapses. The data presented here should be helpful in creating a packing protocol for Bio-Works' resins in different types of columns.

## Introduction

Chromatography is a crucial step in the purification of biopharmaceuticals and must be thoroughly optimized in commercial manufacturing. There is always a need to increase the scale of the chromatography purification steps which are typically developed and optimized in small-scale laboratory experiments, where parameters such as dynamic binding capacity and purity assessments are studied. Chromatography operating conditions (e.g., bed heights, flow rates, and buffer solutions) identified at small scale may not produce comparable product quality or process performance if packing quality or wall effects change during the scale-up. One extremely important parameter to study, that cannot be determined in small scale, is the flow-pressure relationship of the resin. Due to the column's wall-effects, performance with regard to flow and pressure properties will increase at smaller scales, and this must be accounted for when scaling-up. The maximal flow rate, packing flow rate and maximal running flow rate can be determined by studying these properties at increasing column diameters using increasing flow rates until the bed starts to collapse.

## Wall effect

The largest difference in functionality between small lab scale columns and bioprocess columns is the impact of the wall effect. The wall effect is the support that the column walls give to the resin which results in better flow properties in smaller columns. This effect decreases with an increasing diameter of the column. This is due to the fact that, when columns are scaled-up and the column diameter ratios increase, the wall support to the packed bed decreases. Flow induces increased compression of the resin, causing increased pressure drops across the packed bed and decreased bed stability. A further complication is that different chromatography resins withstand varying degrees of compression. Resins with a significant lower level of compressibility may be particularly sensitive to the operating scale and packing conditions. In a correctly packed column, there is a linear relationship between flow and pressure up to the packing flow rate and the angle to the x axis is related to mean particle size.

A priori knowledge of the maximal flow rates and pressure limits for a specific resin at different column dimensions are therefore essential for setting up a robust bioprocess purification method.

- **Critical flow**  
Maximal flow rate before resin bed collapses.
- **Packing flow**  
Flow rate at 70% of the critical flow pressure.
- **Maximal run flow**  
Flow rate at 70% of packing flow rate.

## Flow-pressure method

The flow-pressure method is performed to determine the relation between the pressure over column ( $\Delta P$ ) and the flow rate for a specific column and resin setup.

An open bed is used, which means that the adaptor is not adjusted to contact the resin surface. The method is carried out by pumping water through the column at increasing pressure steps (the pressure is stabilized for  $\geq 1$  column volume (CV) before the next pressure step is initialized) until the bed collapses and the collapse point in terms of critical flow-critical pressure is reached. The interval that includes the collapse point can be determined as the place from which the linearity of the pressure ends until the place where the pressure increases unabated. The pressure increment steps around the critical point where the plateau is reached must be very small. The flow-pressure data obtained will later be used to determine the maximal flow rate, packing flow rate and maximal running flow rate for the resin. The corresponding pressure data will also be obtained, see Figure 1.

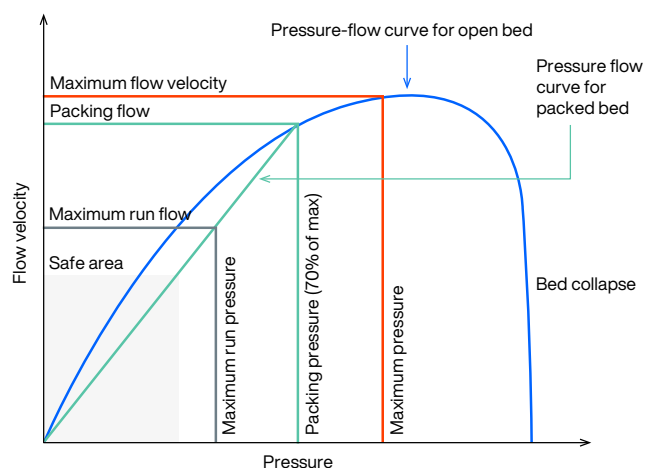


Figure 1. Theory: flow-pressure curve.

## WorkBeads resins

WorkBeads™ are agarose-based chromatographic resins manufactured using a proprietary method that results in porous beads with a tight size distribution and superior mechanical stability. WorkBeads 40 have an average bead size of 45  $\mu\text{m}$ . The particle size has been selected to enable high resolution separations at moderate backpressure. The base matrices in the WorkBeads 40 resin family consist of 40/100, 40/1000, 40/10 000, and 40/30 000, where the last digits specify the approximately exclusion limit for molecules in kDa. Most of Bio-Works derivatized resins are based on the 40/1000 base matrix. The functional groups are coupled to the resin via chemically stable linkages, *i.e.* the derivatized resins are more robust compared to their base matrices.

## Model used

Pressure-flow modeling based on the Blake-Kozeny<sup>1,2,3</sup> equation can be implemented to understand bed compressibility, wall effects, and determine the minimum diameter for a representative scale-up or scale-down model that can accurately predict bed integrity and performance at commercial manufacturing scale. Using this model, a non-linear regression analysis was performed to calculate maximal flow-pressure parameters (critical flow, maximal packing flow, maximal run flow, critical  $\Delta P$ , maximal packing  $\Delta P$  and maximal run  $\Delta P$ ) for each resin and setup.

## Flow-pressure data for different WorkBeads 40 matrices

### WorkBeads 40/1000

To generate the graph in Figure 2 the flow-pressure data from the system with the column bypassed (system contribution) was first subtracted from all data obtained using the flow-pressure method on a column with WorkBeads 40/1000. Thereby only the pressure over the column is measured. Columns with increasing diameter, BPG 100 mm, BPG 200 mm and BPG 300 mm, were used at two bed heights (BPG 200), 150 mm and 200 mm.

From this graph, all parameters such as maximal flow rate (critical flow), packing flow rate and maximal running flow rate could be determined, see Table 1, as well as maximal critical  $\Delta P$ , maximal packing  $\Delta P$  and maximal run  $\Delta P$ , see Table 2.

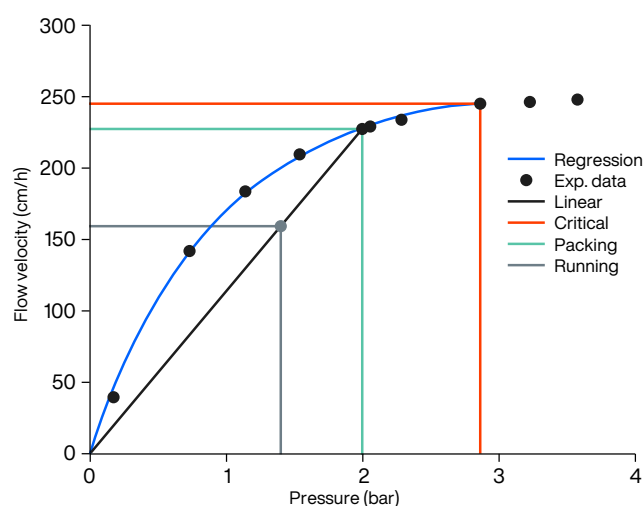


Figure 2. Flow-pressure curve for WorkBeads 40/1000 in BPG 300 x 200 mm (diameter x bed height).

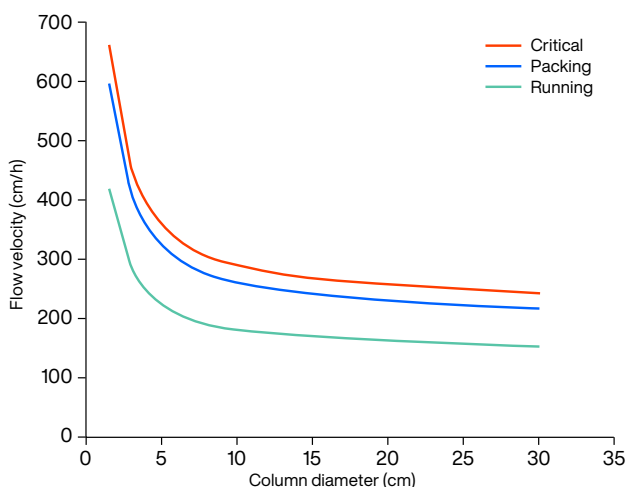
Table 1. Maximal flow rates obtained from flow-pressure curve for WorkBeads 40/1000 in BPG 300 x 200 mm.

Critical flow (cm/h)	Max. packing flow (cm/h)	Max. run flow (cm/h)
245	228	159

**Table 2.** Maximal pressures over the column obtained from flow-pressure curve for WorkBeads 40/1000 in BPG 300 x 200 mm.

Critical $\Delta P$ (bar)	Max. packing $\Delta P$ (bar)	Max. run $\Delta P$ (bar)
2.9	2.0	1.4

Additionally, another graph can be obtained: flow-pressure vs column diameters. By varying the size in both dimensions, reliable data could be obtained. Figure 3 shows how the flow rate correlates with the column diameter and this is useful in scale-up or scale-down phases. Scale-dependent wall effects became negligible for columns with larger diameters than 150 mm.



**Figure 3.** Flow velocity vs column diameter curve for WorkBeads 40/1000.

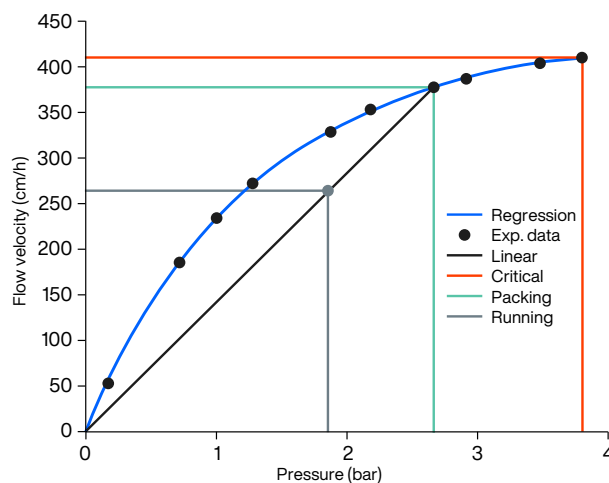
## WorkBeads 40S

WorkBeads 40S is an agarose-based strong cation exchange chromatography resin. It is completely ionized over a broad pH range (pH 2-12) and derivatized with sulfonate ligands. This resin is used both as a capture step and as a polishing step depending on the application. Many of these applications involve peptide purifications. Figure 4 shows a flow-pressure curve obtained for WorkBeads 40S in a BPG column with a diameter of 300 mm and a bed height of 240 mm.

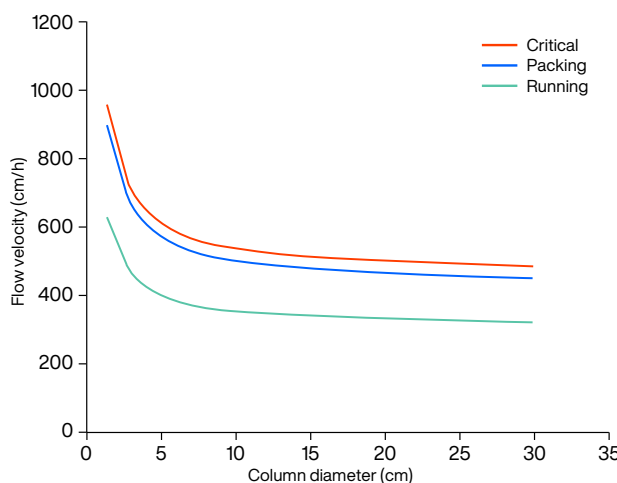
In Figure 5 the flow velocity vs column diameter is shown. The flow-pressure data, such as maximal flow rates, packing flow rates, maximal running flow rates and max pressures, are shown in Table 3.

WorkBeads 40S is based on the base matrix 40/1000, in which the sulfonate ligands are coupled. In Figure 5 we can see the increased maximal flow rates obtained compared to the values seen in Figure 3, i.e. the derivatised resin is much more rigid compared to its base matrix. Figure 6 shows this relationship.

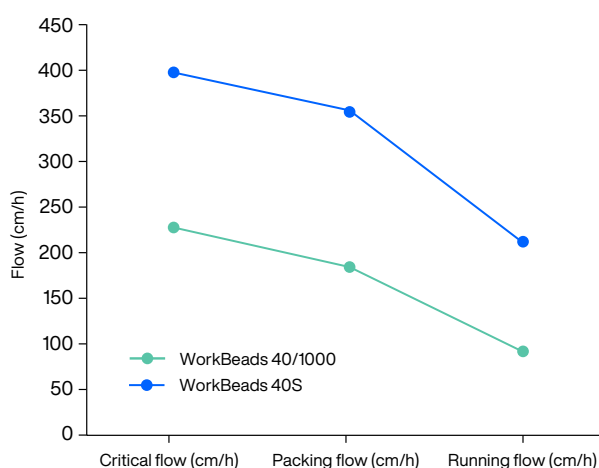
This means that it will be possible to use higher flow rates for the derivatised resins. Figure 6 shows a 1.5-fold higher rigidity for WorkBeads 40S compared to the base matrix itself. This value is also expected to be valid for e.g. WorkBeads 40Q.



**Figure 4.** Flow-pressure curve for WorkBeads 40S in BPG 300 x 240 mm.



**Figure 5.** Flow velocity vs column diameter curve for WorkBeads 40S.



**Figure 6.** Base matrix compared to its derivatised resin.

## Maximal flow rates depend on the porosity

To investigate the effect of the porosity of the WorkBeads 40 base matrix on pressure-flow rates, experiments were also conducted using the base matrices WorkBeads 40/100, WorkBeads 40/10 000 and WorkBeads 40/30 000. BPG 200x500 mm were used. These experiments are summarized in Figure 7, where there is an inverse correlation between pore sizes and tolerated flow rates/pressures. A trendline (red) is drawn in the plot to illustrate this relationship.

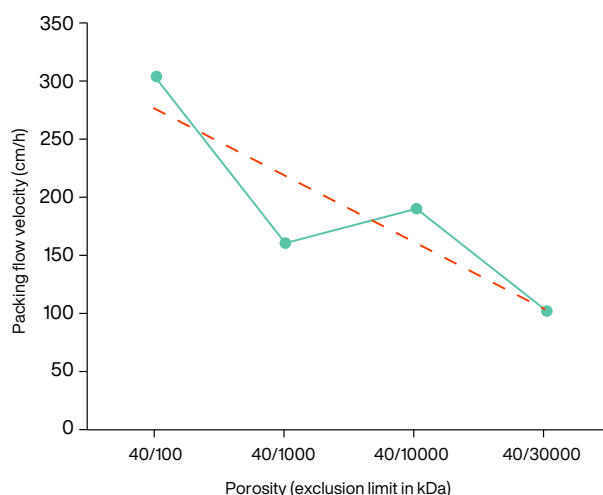


Figure 7. Packing flow velocity vs porosity.

WorkBeads 40/100 which has the lowest cut-off value can tolerate much higher flow rates than the larger pore-sized WorkBeads 40/30 000. Here we show the maximal packing flow rate, but the same correlation is seen for the other maximal flow rates (maximal running flow rate and maximal critical flow rate  $\Delta P$ , maximal packing  $\Delta P$  and maximal run  $\Delta P$ ).

## Conclusion

Flow-pressure data obtained at bioprocess scale (here at column diameters 100 mm to 300 mm) are important for

performing robust bioprocess scale purifications. Here we can see that so-called wall effect (when the solid wall support the resin) are negligible for column diameters larger than 100 mm to 150 mm using WorkBeads 40 resins. We further see an increase of 1.5-fold in tolerance against flow rates and pressures for derivatised resins compared to the base matrices and an increase in rigidity in smaller pore-sized resins. There is e.g. a 1.6 fold difference in maximal flow rate allowed between WorkBeads 40/100 and WorkBeads 40/30 000. A rough estimation of tolerated maximal flow rates for derivatised resins not tested in this study (based on WorkBeads 40 base matrices) can be calculated by multiplying the obtained value for the base matrix by 1.5 (WorkBeads IMAC resins and WorkBeads 40Q are based on WorkBeads 40/10000 base matrix; WorkBeads affimAb is based on WorkBeads 40/30 000 base matrix, etc.).

The derived data from all flow-pressure experiments are shown in Table 3.

## References

1. F. C. Blake, Transactions of American Institute of Chemical Engineers., 14 (1922), pp. 415-421
2. J. Kozeny, Sitzungsber. Akad. Wiss. Wien. Math. Naturwiss. Kl. Abt. IIa, 136 (1927), pp. 271-306
3. J. Prentice, et al., Journal of Chromatography A, Vol 1625, 16 August 2020

## Ordering information

Visit [www.bio-works.com](http://www.bio-works.com) for information regarding all WorkBeads resins.

Orders: [sales@bio-works.com](mailto:sales@bio-works.com) or contact your local distributor.

For more information about local distributor and products visit [www.bio-works.com](http://www.bio-works.com) or contact us at [info@bio-works.com](mailto:info@bio-works.com)

Table 3. Flow-pressure values obtained for resins at specified setup.

WorkBeads resin	Diameter (cm)	Height (cm)	Critical flow <sup>1</sup> (cm/h)	Max. packing flow <sup>2</sup> (cm/h)	Max. run flow <sup>3</sup> (cm/h)	Critical pressure <sup>4</sup> (bar)	Packing pressure <sup>5</sup> (bar)	Running pressure <sup>6</sup> (bar)
40/1000	30	20	245	228	159	2.9	2.0	1.4
40/1000	20	20	277	243	170	2.5	1.7	1.2
40/1000	10	20	283	259	181	2.4	1.7	1.2
40/1000	20	15	295	259	181	3.0	2.1	1.5
40/100	20	20	359	325	228	4.7	3.3	2.3
40/10 000	20	20	272	259	181	4.3	3.0	2.1
40/30 000	20	20	222	209	146	3.0	2.0	1.4
40S	30	24	410	377	264	3.8	2.7	1.9

<sup>1</sup> Max flow velocity (critical flow) is maximal flow velocity before resin bed collapses.

<sup>2</sup> Packing flow is flow rate at 70% of maximal flow pressure.

<sup>3</sup> Maximal run flow is 70% of packing flow rate.

<sup>4</sup> Maximal pressure (critical pressure) is maximal pressure before resin bed collapses.

<sup>5</sup> Packing pressure is pressure at 70% of maximal pressure.

<sup>6</sup> Maximal run pressure is 70% of packing pressure.

## **bio-works.com**

Bio-Works, WorkBeads and GoBio are trademarks of Bio-Works Technologies.  
All third-party trademarks are the property of their respective owners.

© Bio-Works.

All goods and services are sold subject to Bio-Works terms and conditions of sale.  
Contact your local Bio-Works representative for the most current information.

Bio-Works, Virdings allé 18, 754 50 Uppsala, Sweden. For local office contact  
information, visit [bio-works.com/contact](http://bio-works.com/contact).

AN 40 300 010 BA

