Packing a 10 mm ID column with BioPro IEX SmartSep S30

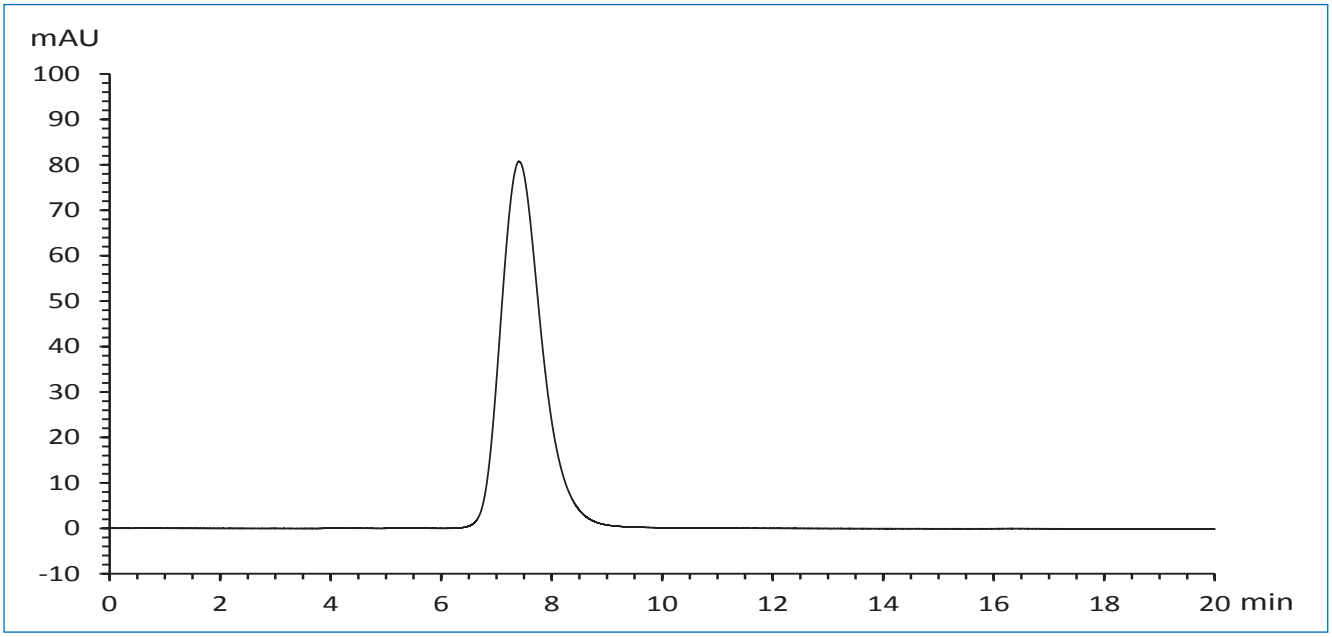
A method has been developed for packing BioPro SmartSep S30 into 10 mm ID glass columns. The influence of parameters such as flow velocity, packing factor and packing buffer was investigated.
The result is a two-step method using water as the packing buffer. This method was successfully tested by packing columns with different bed lengths. Method parameters are given in Table 1.

Table 1: Packing Method Parameters

Bed Length	10 cm ± 20 %	20 cm ± 10 %
Slurry- / Packing Buffer	Water	
Slurry Concentration	30 %	
Number of Steps	2	
Step 1	Consolidation at 76 cm/h (1 mL/min) for 25 minutes	Consolidation at 76 cm/h (1 mL/min) for 45 minutes
Step 2	Mechanical compression, packing factor 1.05	Mechanical compression, packing factor 1.05

Typical results for this method using water as packing buffer are:
Asymmetry = 1.1–1.4
Plate count per meter = 9,000–15,000

These results confirm the general target specifications for BioPro IEX SmartSep S30:
Asymmetry = 0.7–1.4
Plate count per meter ≥ 7,000



Pressure-Flow-Relation

The pressure drop of a consolidated column at 10.5 cm bed length was measured for a range of flow velocities from 0 cm/h to 3,300 cm/h to investigate packing conditions. In addition, the degree of compression (packing factor) was determined by measuring bed lengths at the different flow velocities. The results are shown in Figure 1.

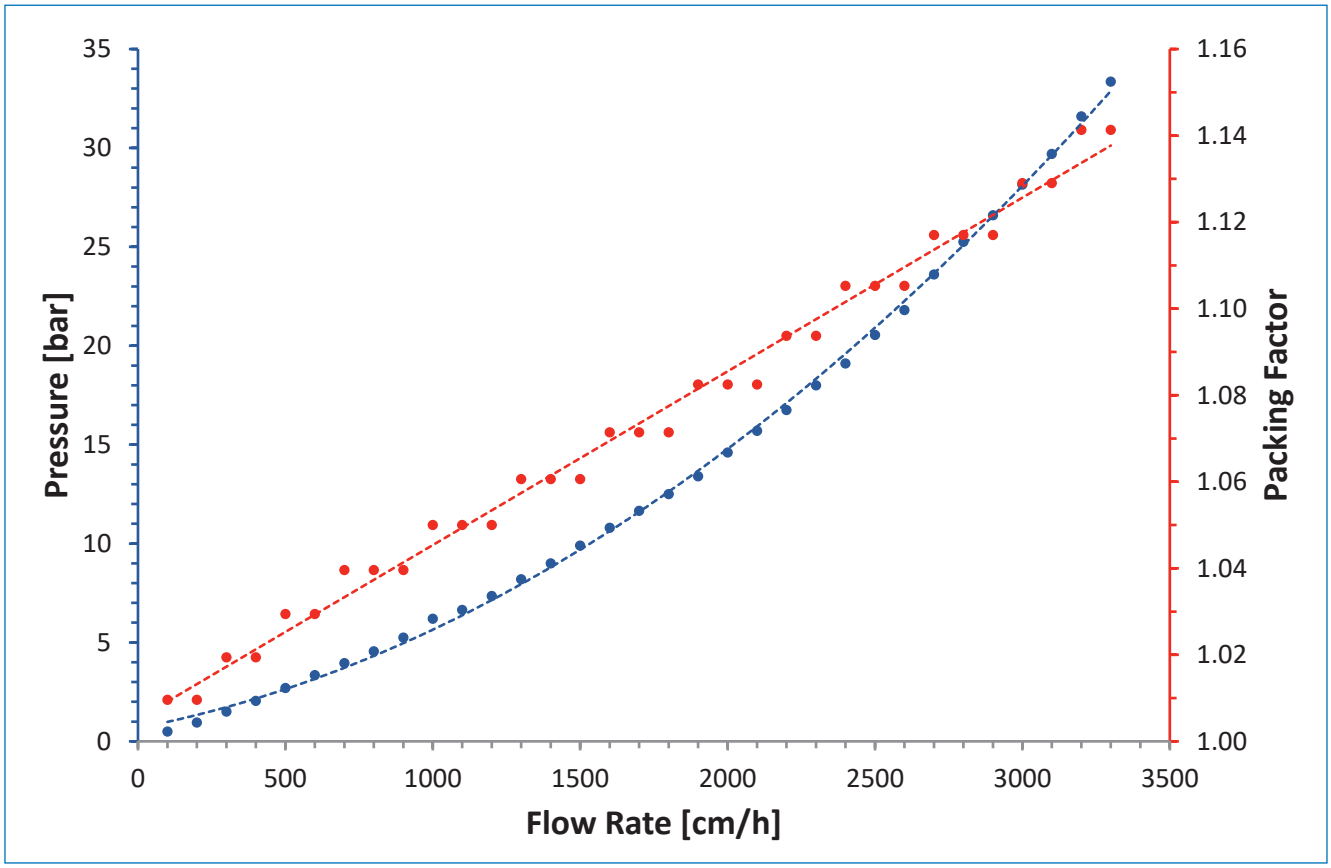


Figure 1: Pressure Flow Relation for BioPro IEX SmartSep S30 in a 10mm ID column.

General Description of Packing Methods Used

The slurry was prepared with a predetermined concentration. To be able to use slurries with low concentrations, a packing adapter was used to allow for higher slurry volumes. Subsequently, the column was packed under flow (target bed height 10 cm ± 20%), applying both one-step and two-step methods:

- One-step method: only one flow rate was used for resin compression. The compression flow rate was varied to evaluate its influence.
- Two-step method: A low flow rate was used to consolidate the resin bed. Then, the bed was mechanically compressed by the piston. Packing factors were varied.

The packed columns were inspected using a pulse test (see table 7 for method parameters). Parameters used for packing quality were asymmetry and plate count per meter (N/m). In the following, packing quality trends for selected variations of packing parameters are described.

A) Variation of Flow Rate – One-Step Method

Constant Conditions: slurry concentration = 30%
slurry and packing medium = 1 M NaCl

Table 2: Variation of Flow Rate.

Flow rate [cm/h]	N/m	Asymmetry
153	± 0 %	± 0 %
688	- 9 %	+ 4 %
1,528	+ 79 %	- 17 %

Conclusion: By using a higher flow rate, higher compression was achieved, which results in higher plate count and lower asymmetry.

B) Variation of Packing Factor – Two-Step Method, Packing Medium 1 M NaCl

Constant Conditions: slurry concentration = 30%
slurry and packing medium = 1 M NaCl
consolidation flow rate = 76 cm/h

Table 3: Variation of Packing Factors, Packing Medium 1 M NaCl.

Packing Factor	N/m	Asymmetry
1.00	± 0 %	± 0 %
1.05	+ 6 %	- 9 %
1.10	- 6 %	± 0 %
1.15	- 15 %	± 0 %
1.20	- 40 %	+22 %

Conclusion: If compression is too strong, the column bed becomes inhomogeneous. This has a detrimental influence on plate count and asymmetry. The best results were obtained with a packing factor of 1.05.

C) Variation of Salt Concentration – Two-Step Method

Constant Conditions: slurry concentration = 30%
consolidation flow rate = 76 cm/h
packing factor = 1.05

Table 4: Variation of Salt Concentration.

Packing Medium	N/m	Asymmetry
1 M NaCl	± 0 %	± 0 %
2 M NaCl	- 53 %	+ 55 %
Water	-10 %	- 35 %

Conclusion: Increasing salt concentration leads to higher asymmetry and also to lower plate count. The best symmetry values were obtained using pure water.

D) Variation of Packing Factor – Two-Step Method, Packing Medium Water

Constant Conditions: slurry concentration = 30%
slurry and packing medium = water
consolidation flow rate = 76 cm/h

Table 5: Variation of Packing Factor, Packing Medium Water.

Packing faktor	N/m	Asymmetry
1 M NaCl	± 0 %	± 0 %
2 M NaCl	± 10 %	+ 13 %
Water	-11 %	- 19 %

Conclusion: As in B), decreased plate count and increased asymmetry is observed when compression is too strong. The best results were obtained with a packing factor of 1.05.

E) Variation of Slurry Concentration – Two-Step Method

Constant Conditions: slurry and packing medium = water
consolidation flow rate = 76 cm/h
packing factor = 1.075

Table 6: Variation of Slurry Concentration.

Slurry Concentration	N/m	Asymmetry
50 %	± 0 %	± 0 %
30 %	+12 %	- 5 %

Conclusion: A lower slurry concentration results in a more homogeneous column bed which significantly results in higher plate count.

Table 7: Chromatographic Conditions for Column Qualification.

Mobile Phase	Sodium Phosphate Buffer, 20 mM, pH 7
Flow Rate	1 mL/min
Detection	UV, 220 nm
Sample	Formamide, 2µL/mL, dissolved in mobile phase
Injection	80 µL