Scale-up of Anti-TSH IgG Purification from ToyoScreen™ Columns to Semi-Preparative Columns

TOYOSCREEN APPLICATION NOTE

Author: T.J. Higley, Tosoh Bioscience LLC

Abstract:

Prepacked 1mL ToyoScreen columns provide a more convenient way to screen Toyopearl resins without sacrificing separation quality.

Introduction:

Resin screening is a vital part of the methodology involved in developing large scale purification schemes. Typically, small volumes of bulk resins are used to evaluate the optimal resin chemistry by hand-packing various resins into semi-preparative LC column housings. To cut down on time and labor expense, Tosoh Bioscience recently introduced 1mL and 5mL ToyoScreen pre-packed scouting columns that provide convenient and affordable alternatives to self packing. The usefulness of ToyoScreen columns will be highlighted in the purification of a monoclonal antibody. Specifically, the packing quality and resin scalability will be compared for anti-TSH IgG using a 1mL ToyoScreen column and a self packed 10mL semi-preparative column, both columns are packed with Toyopearl Phenyl-650M.

Results:

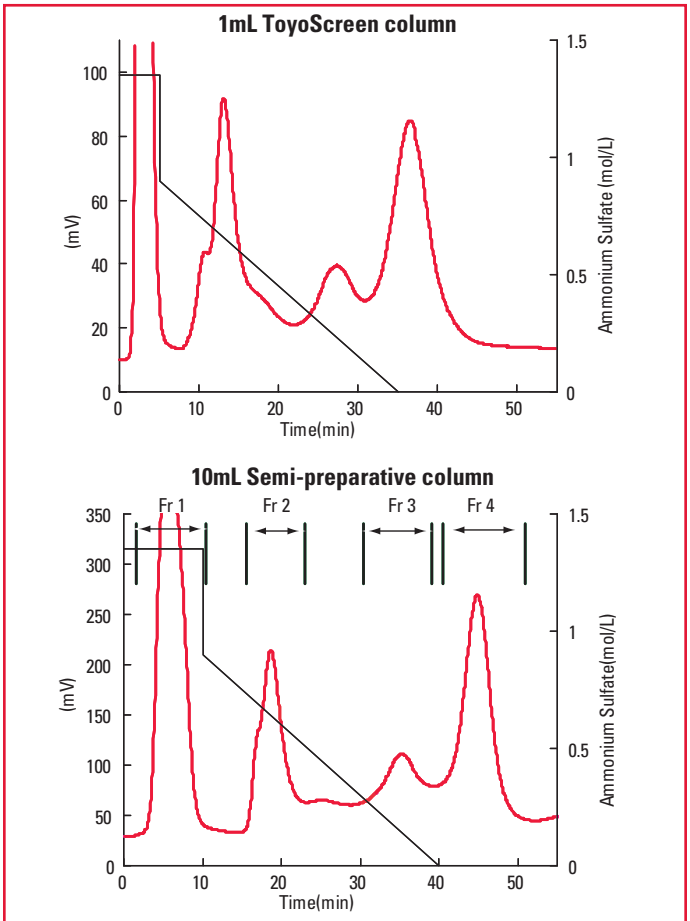
Several methodologies exist to judge the scale-up from a small column volume to larger scale preparative or even pilot plant columns. One practical methodology, developed by Yamamoto *et al.*, is shown in the equation below. According to Yamamoto's theory, the resolution obtained on one column will be the same as that obtained on a column of different dimensions if this equation is kept constant. To demonstrate the value of Yamamoto's theory we developed the conditions for the purification of Anti-TSH on a 1mL (6.4mm x 30mm) ToyoScreen column, filled with Toyopearl Phenyl-650M. The separation was scaled up to a 10mL column (14.6mm x 60mm) filled with the same packing material. In the experimental design we maintained the same particle size and linear velocity, increased column volume and void volume 10-fold, increased column length 2-fold and decreased the gradient slope 5-fold on the 10mL column. The net effect of these changes, according to Yamamoto's equation, is that resolution is kept constant.

$$Rs \propto \frac{1}{dp} \frac{z^{1/2}}{u^{1/2}\,(g(V_t-V_o))^{1/2}}$$

Rs: Resolution
u: Linear velocity
g: Gradient slope
dp: Particle size
V_o: Void volume
z: Column length
V_t: Column volume

As shown in *Figure 1*, similar resolution is obtained on both columns, indicating that results obtained on 1mL ToyoScreen columns can be successfully scaled up to larger column dimensions. Fraction 4, (data not shown) contains the IgG antibody as verified by SEC and gel electrophoresis.

Figure 1. Similar resolution is obtained on both 1mL ToyoScreen and 10mL Semi-preparative columns.



Packing: Toyopearl Phenyl-650M
Eluent: (A) 0.1M phosphate buffer containing 1.8M $(NH_4^+)_2SO_4$, pH7.0
(B) 0.1M phosphate buffer, pH7.0
Sample: Anti-TSH from cell culture supernatant (x4 diluted)

	1mL ToyoScreen	10mL Semi-preparative
Column Dimensions:	6.4mmID x 3cmL	14.6mmID x 6cmL
Injection Volume:	500µL	5000µL
Flow Rate:	0.5mL/min; 0.5CV/min; 93cm/hr	2.5mL/min; 0.25CV/min; 90cm/hr
Gradient Profile:	25% B; 0-5min (isocratic) 50% B: 5min (step) 50% to 100% B; 5-35min (linear)	25% B; 0-10min (isocratic) 50% B: 10min (step) 50% to 100% B; 10-40min (linear)
Gradient Slope*:	0.06M/mL	0.012M/mL

**The gradient slope is the change in ionic strength per unit volume. Gradient volume is the product of flow rate and gradient time.*

Conclusions:

The ToyoScreen columns provide similar separation efficiencies to larger (semi) preparative columns allowing for accurate screening assessments in more convenient and less costly format.

References:

S. Yamamoto, M. Nomura and Y.Sano, *J. of Chromatography*, 409 (1987) 101

TOSOH

TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.separations.us.tosohbioscience.com
email: info.tbl@tosoh.com

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