Fast Resin-Screening and Method Development with Pre-Packed Process Development Columns

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Introduction

In the large-scale purification of proteins relevant for therapeutic or diagnostic use, liquid chromatography plays the most important role. In general, LC performance parameters such as selectivity, binding capacity, recovery etc. are mainly influenced by the properties of the chromatographic medium. Therefore, selection of the most suitable medium is the significant key point to succeed in purification. This resin screening historically was accomplished by packing various bulk resins into small columns by hand, which required significant investments in time and cost.

In order to improve the efficiency of these resin-screening experiments, recently new cartridge type pre-packed scouting columns were introduced by Tosoh Bioscience. The 1ml and 5ml ToyoScreen® columns are packed with various TOYOPEARL® process resins and are a convenient and affordable alternative to self-packing.

In this work, the utilisability of the ToyoScreen columns was evaluated on the purification of a monoclonal antibody, Anti-Thyroid Stimulating Hormone (Anti-TSH) IgG from a cell culture supernatant.

Experimental Conditions

LC system

HPLC equipment (autosampler, pump, UV-detector) was supplied from Tosoh Corporation.

Columns

A ToyoScreen HIC Kit, containing 6 columns packed with Ether-650M, PPG-600M, Phenyl-650M, Hexyl-650C and SuperButyl-550C
(see Table 1).

The 1 ml ToyoScreen columns with the dimension of 6.4 mm ID x 3 cm L were mounted in an acrylic holder allowing the columns to be connected to a standard HPLC/FPLCTM instrument (see fig.1).
The Glass column with dimensions of 14.6 mm ID x 6 cm L, was packed with Toyopearl Phenyl-650M

Sample

Anti-Thyroid Stimulating Hormone (Anti-TSH) IgG, and cell culture supernatant are provided from internal source. The sample was diluted 4 times in a mixture of elution buffer (buffer A:buffer B 1:2) prior to injection onto the columns.

Conditions

Screening conditions were the same for all resins as shown in Fig. 2. Conditions for the Scale up experiment are shown in Table 2.

Experimental Conditions

Figure 1: 1 ml ToyoScreen column mounted in a holder

List of TOYOPEARL® HIC grade

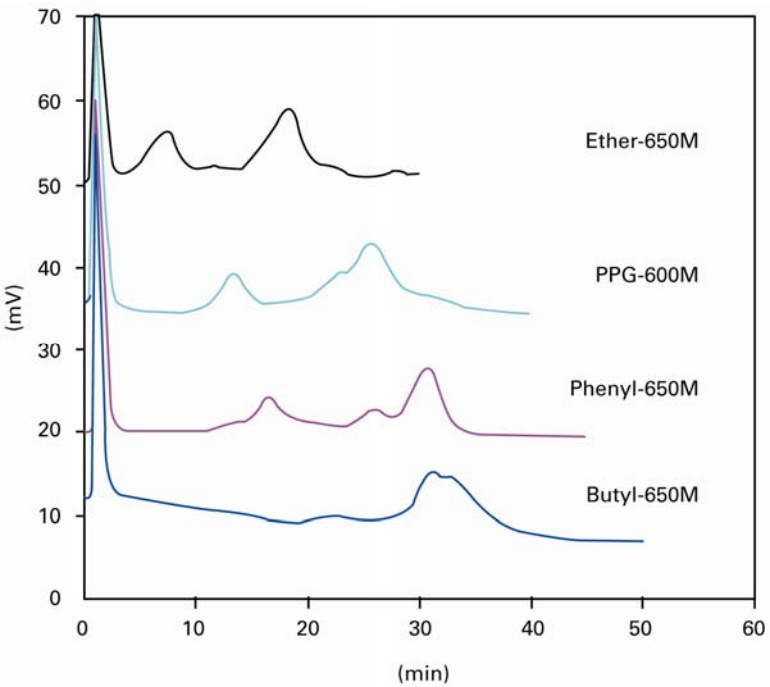
	Hydrophobicity	Particle Size
Ether-650M	Low	40 - 90 mm
PPG-600M	↕	40 - 90 mm
Phenyl-650M		40 - 90 mm
Butyl-650M		40 - 90 mm
SuperButyl-550C		50 - 150 mm
Hexyl-650C	High	50 - 150 mm

Table 1: List of Toyopearl HIC grade

Resin Screening Experiment

For the purification step by Hydrophobic Interaction Chromatography, ToyoScreen HIC columns packed with Toyopearl Ether-650M, PPG-600M, Phenyl-650M and Butyl-650M were used for selecting the HIC resin with the best selectivity.

As demonstrated in Fig. 2, the best separation was obtained by Toyopearl Phenyl-650M. It is assumed that not only hydrophobicity but also the phenyl ligand selectivity works effectively for the separation of this sample. Therefore, Phenyl-650M was selected as a separation resin, and used for the following study.



Chromatographic Conditions

Column:	ToyoScreen 1mL
Eluent:	(A) 0.1 mol/L phosphate buffer containing 1.8 mol/L ammonium sulfate (pH 7.0)
	(B) 0.1 mol/L phosphate buffer (pH 7.0)
Flow Rate:	1 mL/min (1 CV/min)
Gradient:	(A) to (B) linear
Gradient:	Volume : 30CV
Sample:	Cell culture supernatant (x4 diluted) (Antibody: Anti-TSH)

Injection Volume:50uL

Figure 2: Screening for the best Toyopearl HIC resin for the purification of Anti-TSH anitbody

Resin Screening Experiment

Subsequently, several method parameters as type of salt, salt concentration and pH of the elution buffer as well as the flow rate and gradient volume were examined. As a result, 1.35M ammonium sulfate at pH 7.0 was selected as the elution buffer, the optimal elution flow rate was determined to be 0.5 mL/min with a gradient volume of 30CV (gradient slop: 0.06 (mol/L)/min).

As an example, Fig. 3 shows the influence of the starting concentration of ammoinium sulfate on the resolution of the Anti-TSH sample.

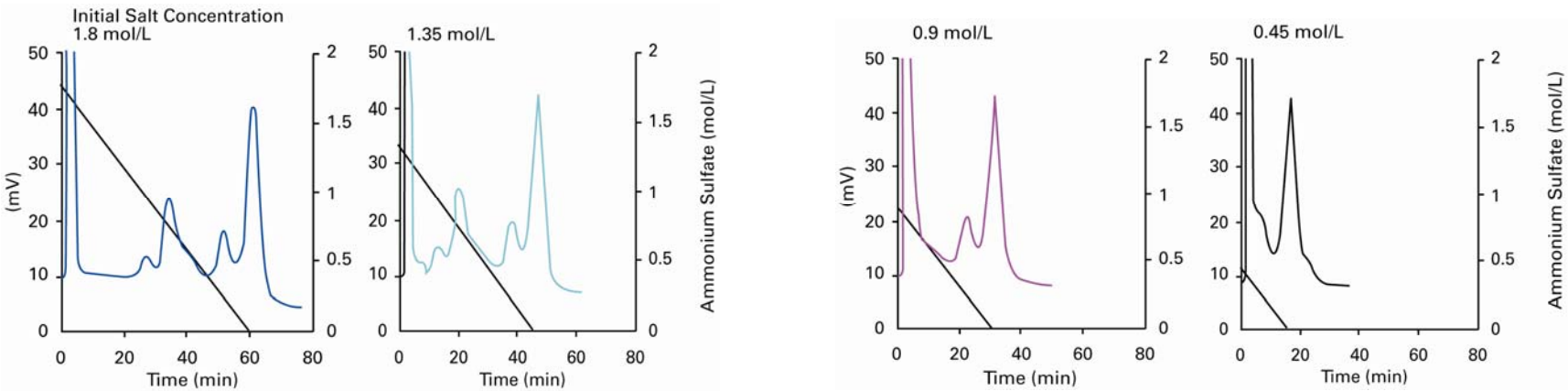


Figure 3: Effect of initial salt concentration on chromatogram
(Separation of cell culture supernatant (anti TSH))

Chromatographic Conditions

Column:	ToyoScreen Phenyl-650M (1 mL)
Eluent:	(A) 0.1 mol/L phosphate buffer containing 0.45, 0.9, 1.35 or 1.8 mol/L ammonium sulfate (pH7.0)
	(B) 0.1 mol/L phosphate buffer (pH 7.0)
Flow Rate:	0.5 mL/min (0.5 CV/min)
Gradient:	(A) to (B) linear
Gradient Slope:	0.06 (mol/L)/mL
Sample:	Cell culture supernatant (x4 diluted) (Antibody: Anti-TSH)
Injection Volume:	200 µL

Resin Screening Experiment

Also, maximal sample loading capacity could be determined on the ToyoScreen column. Fig. 4 shows the effect of increesing injection volume on the resolution of Anti-TSH antibody from its main by products.

Deterioration of resolution was seen at sample loads higher than about 5mL. However, regarding to this sample, resolution was acceptable at injection volumes of around 1000 µL.

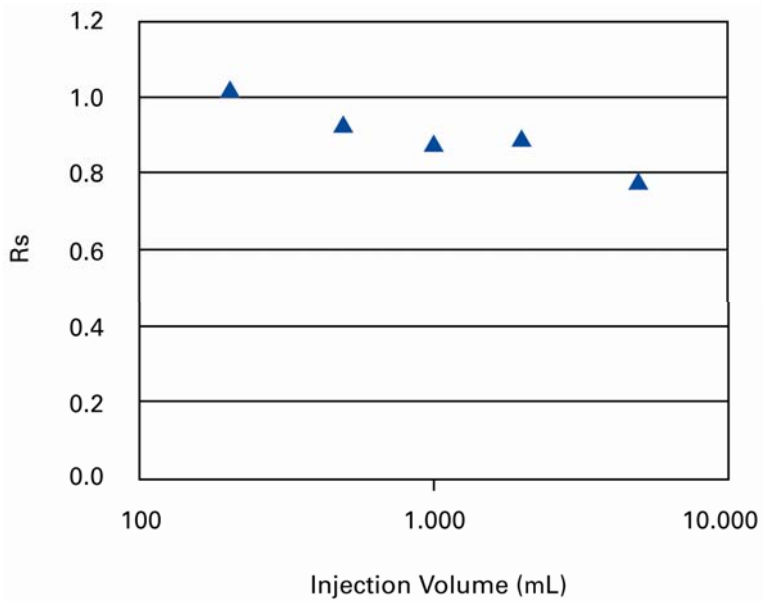


Figure 4: Influence on injection volume on resolution

Conditions

Column:	ToyoScreen Phenyl-650M (1 mL)
Eluent:	(A) 0.1 mol/L phosphate buffer containing 1.8 mol/L ammonium sulfate (pH 7.0)
	(B) 0.1 mol/L phosphate buffer (pH 7.0)
Flow Rate:	0.5 mL/min
Gradient Profile:	(B) 25% (0 min) - 25% (5 min) - 50% (5 min,step) - 100% (35 min,linear, slope:0.06 (mol/L)/mL)
Sample:	Cell culture supernatant (x 4 diluted) (Antibody: Anti-TSH)
Injection Volume:	200 µL

Rs : Resolution between IgG and impurity eluted prior to IgG

Scale-up Experiment

In a third step, the scalability of the method was investigated. The purification method, developed on the 1ml ToyoScreen Phenyl-650M column was scaled up to a self packed 10ml semi-preparative Toyopearl column, filled with the same packing material.

The chromatographic conditions were settled according to the following theoretical equation.

$$Rs \propto (1/dp * z1/2 / u1/2 (g(Vt-V0))1/2$$

Rs :	Resolution	dp :	Particle size
z :	Column length	u :	Linear velocity
g :	Gradient slope	Vt :	Column Volume
V0 :	Void Volume		

According to Yamamoto et al., 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. This means in the experimental design the particle size and linear velocity was maintained, column volume and void volume were increased 10-fold, column length increased 2-fold and the gradient slope decreased 5-fold on the 10ml column. The net effect of these changes, is that resolution is kept constant.

As shown in Figure 5, 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 contains the IgG antibody as verified by SEC and gel electrophoresis (see fig. 6).

Scale-up Experiment

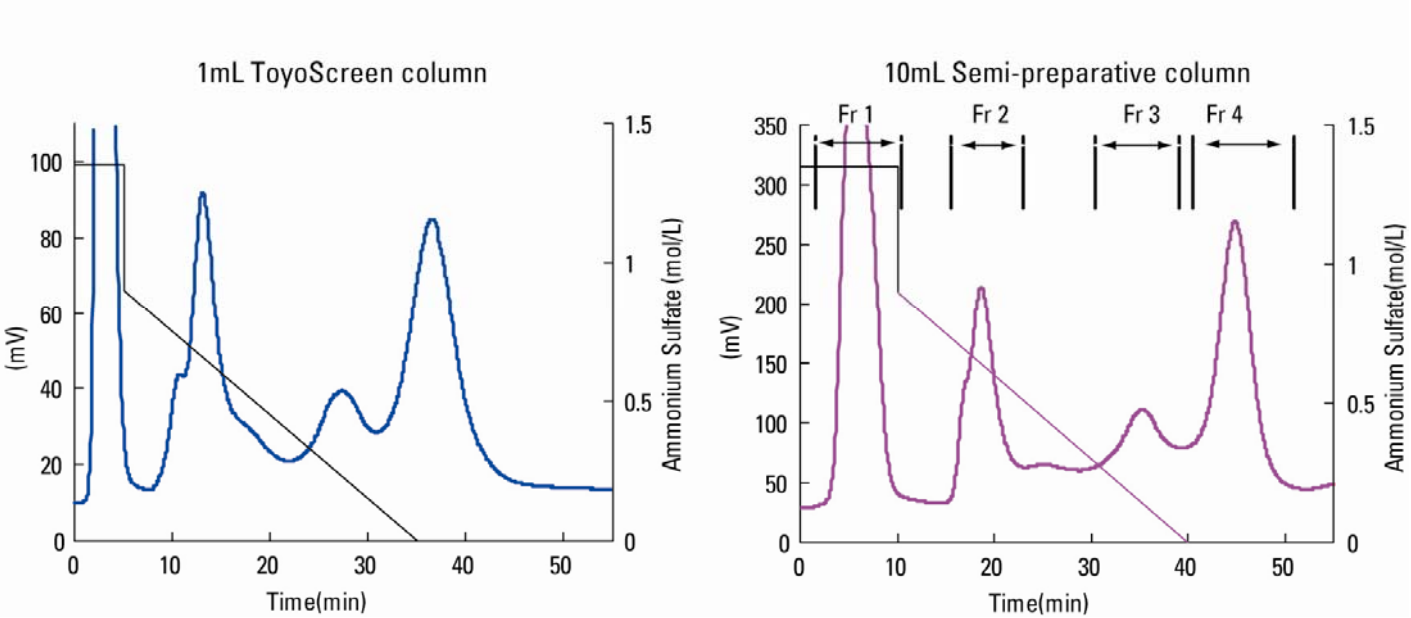


Figure 5: Separation of anti-TSH from cell culture supernatant on a 1 ml ToyoScreen column and a 10 ml Semi-preparative column.

Scale-up Experiment

Packing : Toyopearl Phenyl-650M
Eluent: (A) 0.1 M phosphate bffer containing 1.8 M $(NH_4)_2SO_4$, pH 7.0
(B) 0.1 M phosphate buffer, pH 7.0
Detection: UV@280nm
Sample: Anti-TSH from cell culture super natant (x4 diluted)

	1ml ToyoScreen	10ml ToyoScreen
Col. Dimension	6.4mmID x 3cmL	14.6mmID x 6cmL
Inj. 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.

Table 2: Separating conditions for anti-TSH from cell culture supernatant on a 1ml ToyoScreen column and a 10ml Semi-preparative column.

Figure 6: Purity of antibody separated on Toyopearl Phenyl-650M SDS gel of collected fractions.
Fraction 4 shows very good purity of the Anti-TSH antibody

Conclusion

By developing a separation method and scale up for the purification of Anti-TSH antibody, it could be demonstrated that ToyoScreen columns are a very powerful tool for resin screening, method development and optimization. The versatility of the ToyoScreen format also allows the columns to be applied to process optimization experiments and scale up to semi-preparative purification columns.

ToyoScreen columns are

- easy to use
- a good tool for fast resin screening
- suitable for method development
- and optimization
- useful for scale up

Reference: S. Yamamoto, M. Nomura and Y. Sano, Journal of Chromatography, 409 (1987) 101