



TOYOPEARL® Toyoscreen

Toyoscreen - Fast Screening and speedy Setup of Separation Conditions!
Find the right resin for your intended application by testing Toyopearl® process resins!

Toyopearl is a methacrylic polymer incorporating high mechanical stability. Resins are available as non-functionalized "HW-" series resins for SEC or derivatised with surface chemistries for alternative modes of chromatography such as IEC, HIC and AFC.

In the production of therapeutic, recombinant proteins, downstream processing is one of the most cost intensive procedures. The essential part of recovery is the chromatographic purification, where usually 2-4 chromatographic column steps are employed.

In production, where large columns with dimensions of more than 2 meter diameter are used, the development of a purification process is a scale-up procedure. The very first step of developing this purification process is a thorough examination of the resin to be used.

Separation media for the purification of proteins are hydrophilic, mechanical and chemical stable polymeric materials. By applying subsequent chemical modifications they can be functionalized for the intended purpose (e.g. ion exchange, hydrophobic interaction chromatography, affinity chromatography). The application of a particular resin is mainly determined by the kind of ligand attached to it. The base materials can be synthetic or natural polymers (e.g. Agarose, Methacrylates, Styrol/Divinylbenzole).

Beyond the general decision for a particular base material company, it is not an easy job to find the right resin, even when screening materials just from one resin supplier.

As physical and chemical properties of proteins are so complex, the separation efficacy of a resin is not only a simple function of the ligand. Other determining parameters are: particle size distribution, pore size distribution, pore structure, ligand density and surface hydrophobicity. So it is wise for a resin supplier to provide different grades of resins to the customer.

Toyopearl, for example, is produced with four different particle sizes and three different pore sizes. So selection of a particular resin requires testing of, in this case, 12 columns for one ligand. This resin evaluation process is a time consuming optimisation process in a multidimensional parameter space (see example in Figure 2).

The screening of different resins can be easily performed by using pre-packed small columns with the same chromatographic performance.



Figure 1: ToyoScreen Ion Exchanger Kit with Holder and six small 1 ml Columns

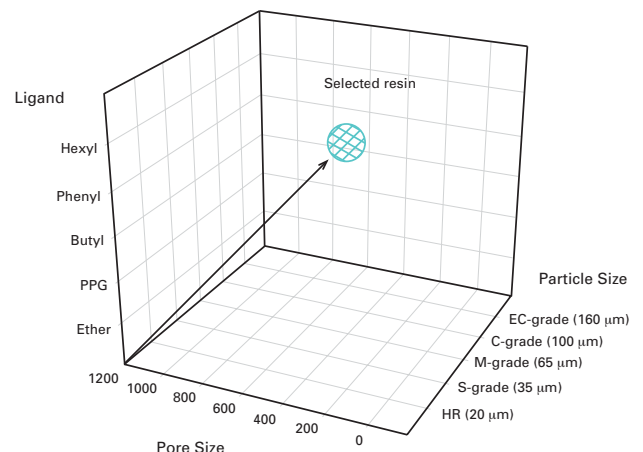


Figure 2: Resin Property Parameter Space for the Evaluation of Toyopearl HIC Resins

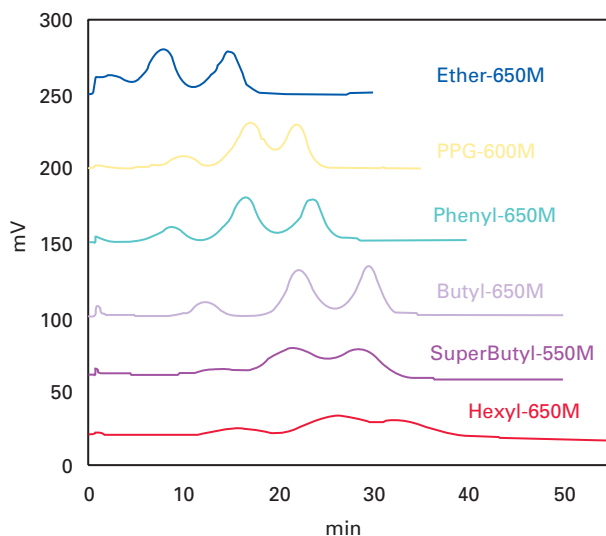


Figure 3: Ligand Screening of Toyopearl HIC Resins on a Standard Protein

Column: Toyoscreen (1 ml)
 Eluent A: 0.1 M Phosphate buffer + 1.8 M Sodium Sulfate (pH 7.0)
 Eluent B: 0.1 M Phosphate buffer (pH 7.0)
 Flow Rate: 1 ml/min
 Gradient: 30 min linear
 Injection Vol.: 50 µl
 Samples: Ribonuclease A, Lysozyme, alfa-Chymotrypsinogen 1 mg/ml

The theoretical plate heights of this columns are almost the same as in larger columns.

A ligand screening for Toyopearl HIC resins for the separation of standard proteins is shown in Figure 3. A ToyoScreen kit consists of small 1 or 5 ml columns and holder (Figure 1). The columns can be reused several times and can be connected to all HPLC and Bio-HPLC systems.

Screening of operational Parameters:

In process development a fast change in elution-, equilibration- and purification conditions is required. Parameters like pH, salt content, gradient volume, flow rate and loading has to be changed many times to find optimal conditions. Common factorial designs are used to minimise the experimental efforts. A prerequisite is the reproducibility of the experiments which is strongly dependent from a good quality of the testing columns which can not be always assured by self packed columns. For this ToyoScreen is an ideal tool.

The effect of gradient volume and flow rate for the resolution for a ToyoScreen SuperQ-650M column is shown in Figure 4.

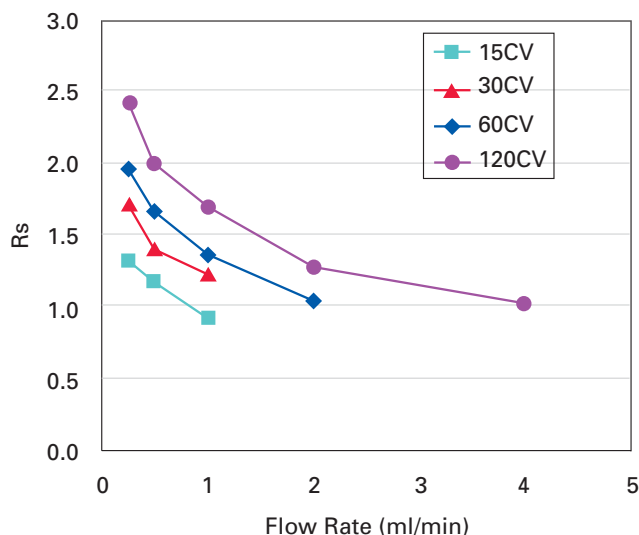


Figure 4: Change in Resolution in Correlation of Gradient Volume and Flow Rate

Column: Toyoscreen SuperQ-650M (1 ml)
 Eluent A: 20 mM Tris-HCl (pH 8.0)
 Eluent B: 20 mM Tris-HCl + 0.5 NaCl (pH 8.0)
 Flow Rate: 0.25, 0.5, 1, 2, 4 ml/min
 Gradient Vol.: 15, 30, 60, 120 CV
 Injection Vol.: 100 µl
 Samples: Transferrin, Ovalbumin 1 mg/ml

Summary:

- fast screening tool to find the best Toyopearl resin for your application
- easy connection to all HPLC and FPLC systems
- small columns for short cycle times simulating your production in downscale with the real process particles prepacked in 1ml and 5 ml ready to use columns

**For further details
 of choice and selection of
 the Toyopearl® resin
 that best
 suits your particular
 process purification needs,
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