# Optimizing Pore Size to Improve Throughput in Downstream Purification of Biological Therapeutic Products

Process engineers strive continuously to improve the throughput of their downstream chromatographic purifications. A simple way of doing this for a specific therapeutic target is to select a resin with the highest available dynamic binding capacity. The higher binding capacity results in the ability to purify more product per unit time while maintaining a constant plant footprint and buffer tankage. A number of resin manufacturers increase binding capacity by varying ligand type and density.

Other factors influencing the binding capacity for a particular resin are the target molecule's size, the total surface area of the bead, and the accessibility to that surface area for the target. A molecule's accessibility to a resin's total surface area is a function of the pore diameter of the resin bead.

With the broad range of pore diameters available on TOSOH Corporation's methacrylic Toyopearl® and TSKgel® PW polymer beads *(Figure 1)*, developers can select the one with optimum accessible surface area and binding capacity for their target.

The sizes of these therapeutic molecules can range from small therapeutics such as insulin to larger molecules such as IgG and IgM.

## Pore Size Screening and Selection

In order to select an optimum resin bead one first needs available a complete range of pore sizes on one polymer backbone. *(Figure 1)* Having the same bead chemistry throughout a wide range of products ensures that the interactions of the target molecule with the ligand and with the resin backbone does not vary during the screening experiment.

*Figure 2* shows a simple pore size screening for the insulin molecule. The resins being evaluated are the same chemistry as the TSKgel SP-5PW cation exchanger (sulfopropyl) with only the pore size being varied for the experiment. For the purpose of this article we will identify those resins as a generic TSKgel SP-PW type material. The X-axis of the chart is a measure of increasing pore size using the exclusion limit of the bead pores. As the pore size increases the dynamic binding capacity increases because the insulin molecule has more access into the larger pores.

By itself, though, the increasing pore size is also decreasing the absolute surface area of the bead. This means that once a resin pore size becomes large enough for a given target, increasing the pore size beyond that value decreases the binding capacity because of the decreasing surface area

An experimental SP-PW cation exchanger was prepared using the optimum pore size for insulin as determined in *Figure 2*. In *Figure 3* the experimental resin has a dynamic binding capacity of 230 mg/mL-gel while the commercial TSKgel SP-5PW generates only 170 mg/mL-gel. This is a dynamic capacity increase of 35%.

**Figure 1.** Tosoh Bioscience methacrylic base beads

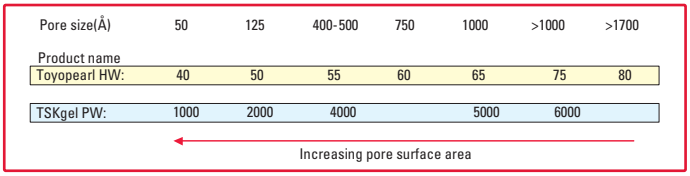



**Figure 2.** Screen of insulin binding capacity and pore size for TSKgel SP-PW type resins.

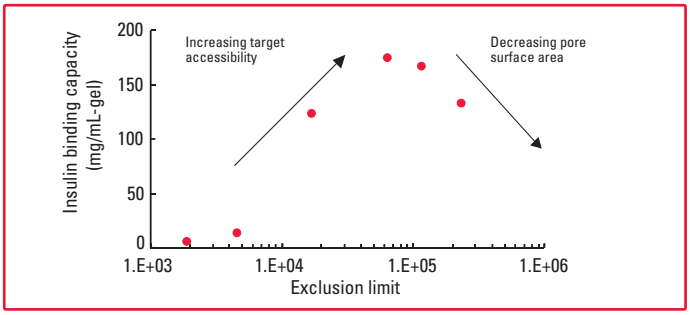



**Figure 3.** Improvement of binding capacity for insulin (5.7kD) on TSKgel SP-5PW.

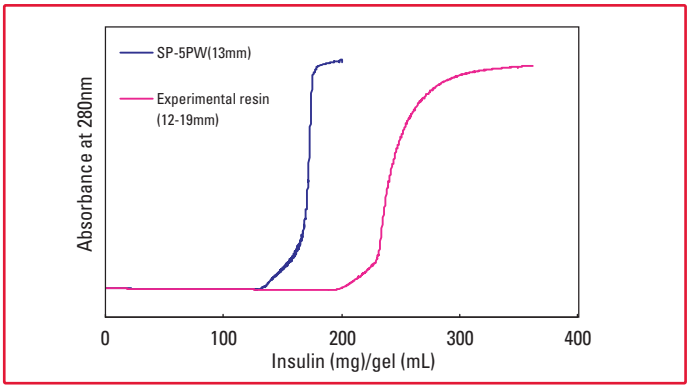
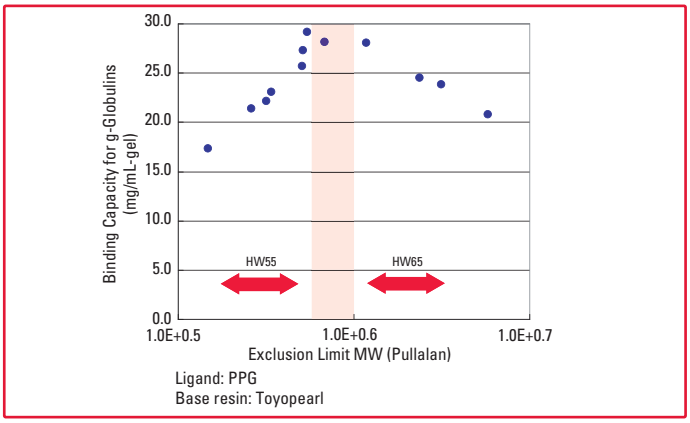



**Figure 4.** Relationship between the exclusion limit of base resin and the static binding capacities for γ-globulin.




**Monoclonal antibody-resin optimization**

## Monoclonal antibody-resin optimization

Pore size screening also works for large molecules such as γ-globulin. The X-axis in *Figure 4* is a measure of the resin's increasing pore diameter. In this case the optimum pore size is between that of the commercially available Toyopearl HW-55 and HW-65 size exclusion products. So a new pore size was created for the Toyopearl line, a product called Toyopearl HW-60.

## Monoclonal antibody-ligand optimization

Unlike the singular insulin molecule, monoclonal antibodies are very diversified in their degree of hydrophobicity. *Figure 5* shows the elution times of 51 different mouse monoclonals on a Phenyl-5PW hydrophobic interaction chromatography column.

Because of this molecular diversity it is very useful to have a broad ligand library available to chemically modify the HW-60 bead and its optimized pore size. *Figure 6* shows four of the ligand types used for hydrophobic interaction purifications.

## Dynamic Binding Capacity Optimization

*Figure 7* shows a dynamic binding capacity comparison of Toyopearl Phenyl-650 (HW-65 base bead), Toyopearl PPG-600 (HW-60 base bead), and Toyopearl Butyl-600 using γ-globulin. The optimized pore for γ-globulin of Toyopearl HW-60 provides a clear advantage in dynamic binding capacity over the other resins screened and shows 50 mg/mL of resin capacity.

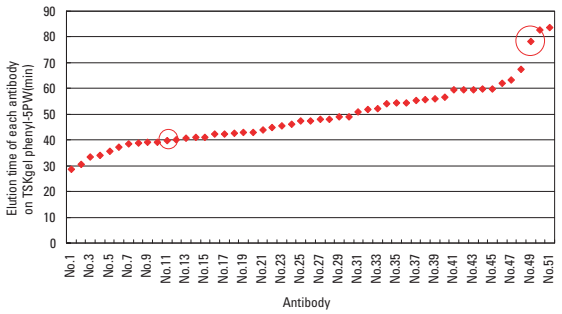
## Aggregate Removal

*Figure 1* includes the large pore product Toyopearl HW-75. This wider pore resin particle could be useful for purifying large monoclonal antibody aggregates or IgM. For these applications we have developed an experimental product Toyopearl Phenyl-750. With capacities likely to be in the 30-50 mg/mL range, this material may have some significant advantages over more traditional aggregate removal materials like hydroxyapatite. Please contact me if you are interested in discussing this topic or to arrange for sample.

## Summary

Using pore size selection to improve the dynamic binding capacity of a therapeutic target can greatly improve the process economics of a downstream purification step. The range of product pore sizes with the same polymer backbone available from Tosoh Bioscience LLC is unique in the industry and ideal for these experiments.Having the same polymer chemistry means scaling up from the lab bench analytical method or scaling down from process purification back to a needed analytical method, is transparent with our products.

**Figure 5.** Hydrophobic diversity of mouse monoclonal antibodies.




Plot of chromatographic elution time against 51 kinds of mouse monoclonal Abs

| | |
|---|---|
| Column : | TSKgel Phenyl-5PW |
| 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 |
| Gradient : | (B)0%(0min)--0%(5min)--100%(65min) linear |
| Samples : | 51 kinds of mouse monoclonal antibodies |

**Figure 6.** HIC Ligand Candidates

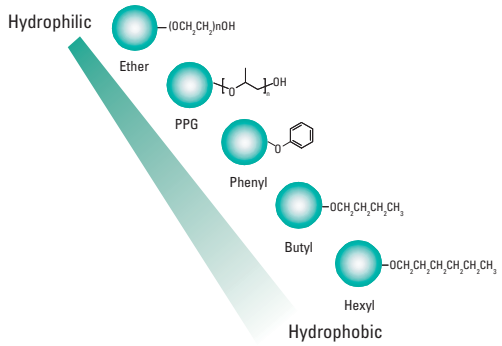
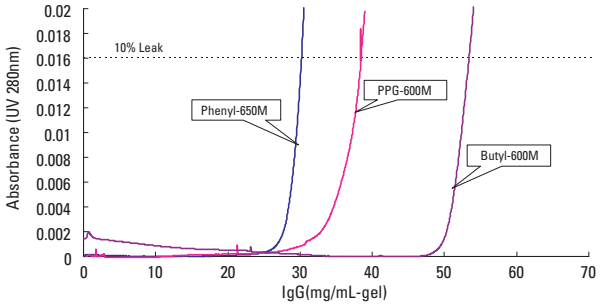



**Figure 7.** Improvement of binding capacity for γ-globulin on HIC bonded phases.

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