Use of Heparin Affinity Chromatography for the Purification of Proteins

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The selectivity intrinsic to affinity chromatography can simplify protein purification trains by eliminating one or more unit operations. Affinity chromatography has a demonstrated capability to reduce production costs and improve process throughput.

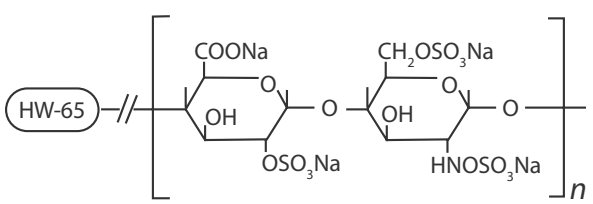
Much is written about the use of Protein A affinity in the production of monoclonal antibodies (MAbs). When Protein A is used in a capture step, its selectivity enables the resin's capacity to be used primarily for the binding of the MAb while letting other feedstock components such as host cell proteins flow through unretained. Eluted MAb fractions are significantly enriched in purity and further processed downstream by intermediate steps using ion exchange chromatography. A polishing step such as hydrophobic interaction chromatography can be used as well.

Another Affinity Adsorbant

Heparin, like Protein A, is another naturally occurring molecule with affinity properties. It is a linear and highly sulfated glycosaminoglycan (Figure 1) composed of alternating subunits of uronic acid and D-glucosamine. Heparin has an innate recognition for thrombin, factor Xa, factor IXa, and other plasma proteins. The noncovalent interaction of heparin with such proteins disrupts the blot-clotting cascade, an action that manifests itself clinically as an anticoagulant.

The covalent attachment of heparin to chromatographic supports is well documented. Recently, Tosoh Bioscience introduced two new and improved affinity resin product lines, Toyopearl AF-Heparin HC-650M ("HC" indicating high capacity) bulk media and prepacked TSKgel Heparin-5PW columns for the purification of positively charged proteins in addition to the above-mentioned blood coagulation factors.

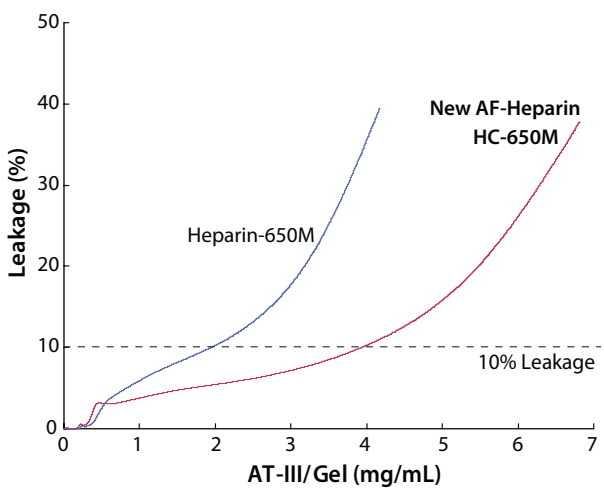
Figure 1: Structure of NEW Toyopearl AF-Heparin HC-650M (5 mg/mL approximate ligand density)



A Heparin-Based Resin

Toyopearl AF-Heparin HC-650M was produced using the Tosoh Resin Innovation Program (TRIP) using an improved attachment chemistry for ligand immobilization, which greatly enhances the heparin conformation for affinity chromatography. As seen in Figure 2, this modification doubled the dynamic capacity of Toyopearl AF-Heparin HC-650M for antithrombin III (AT-III at 300 cm/hr). This increased dynamic binding capacity allows almost twice as much feedstock to be loaded onto a process

Figure 2: Comparison of dynamic AT-III binding capacity at 300 cm/h



	AT-III Binding Capacity (10% leakage in mg/mL gel)
New AF-Heparin HC-650M	3.9
Heparin-650M	1.9

Column Size	6.0 mm ID × 4 cm (1.13 mL)
Mobile phase	0.4 mg/mL AT-III in 0.01 mol/L Tris-HCl containing 0.15 mol/L NaCl at pH 7.5
Linear Velocity	300 cm/h
Detection	UV@280 nm

column. If an existing process is being replaced, this reduces the need to expand the manufacturing footprint for increased production demand.

Another throughput advantage for the new Toyopearl AF-Heparin HC-650M is the resin's average particle size of 65 µm (40–90 µm range). The combination of smaller particle size and a narrower particle size distribution typically produces sharper elution band widths and higher target concentration (Figure 3) when compared with other commercially offered products. This results in a smaller feedstock volume for further downstream unit operations and it could add incremental target purity depending upon the impact of the increased chromatographic resolution.

A Polymeric Product

The polymeric backbone of Toyopearl sustains higher linear velocities, and Toyopearl AF-Heparin HC-650M is no exception when compared with similarly sized softer gels (Figure 4). The acknowledged rigidity of this backbone creates additional opportunities for improved process throughput by running at higher linear velocities.

One concern for all purification specialists is the source of animal-derived products. The heparin used by Tosoh Bioscience is obtained from the intestinal mucosa of healthy pigs grown in countries that are officially free of common and exotic animal diseases. Donor pigs are fed nonruminant feedstock and the heparin is extracted under permanent supervision of the appropriate veterinary department in each country of origin. The crude heparin is then purified in the United States using a process that is validated for the removal of TSE infectious agents and other viral particles.

At Tosoh Bioscience, the heparin (Heparin Sodium, *USP, EP, JP*) is covalently attached to Toyopearl or TSK-GEL base beads to create Toyopearl AF-Heparin HC-650M or TSKgel Heparin-5PW. Then heparin-modified Toyopearl media or TSKgel Heparin-5PW prepacked columns are reimported into the United States under a permit issued by the United States Department of Agriculture's Animal Plant Health Inspection Service (USDA-APHIS). Tosoh Bioscience LLC is pleased to provide appropriate documentation upon request to meet your regulatory needs.

In conclusion, Tosoh Bioscience now offers two new lines of high performance products: Toyopearl AF-Heparin HC-650M bulk media and TSKgel Heparin-5PW prepacked columns for your resin toolbox. For established manufacturing processes — or for those now in development — the new Toyopearl AF-Heparin HC-650M creates unique opportunities to reduce process costs and increase throughput, whereas the TSKgel Heparin-5PW prepacked columns are ideal for scale-down studies.

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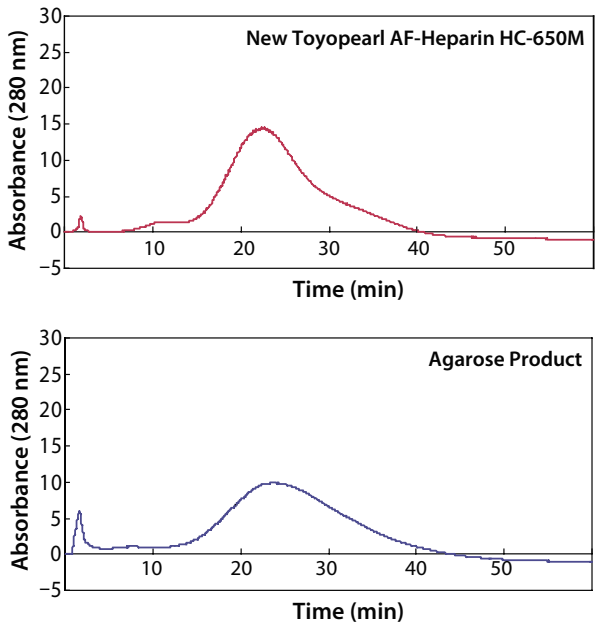
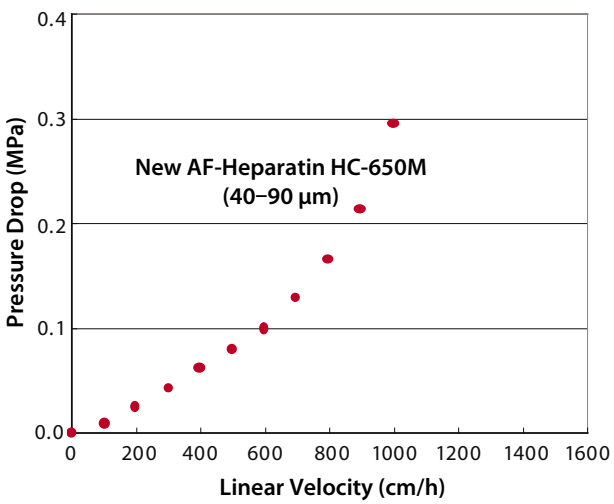
Figure 3: Affinity chromatography of AT-III on heparin adsorbents



Column Size	6 mm ID × 4 cm
Flow Rate	1.0 mL/min
Sample	Antithrombin III, 1 mg
Buffer A	0.1mol/L Tris, 0.01 mol/L Citrate (pH 7.5)
Buffer B	0.1mol/L Tris, 0.01 mol/L Citrate, 2.0 mol/L NaCl (pH 7.5)
Elution	60-min linear gradient from buffer A to buffer B
Detection	UV@280 nm
Temperature	25 °C

Figure 4: Pressure flow curve of NEW Toyopearl AF-Heparin HC-650M



Column Size	22 mm I.D. × 20 cm
Detection	UV @ 280 nm
Mobile Phase	Water
Temperature	Room Temperature