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TOYOPEARL® SuperButyl-550C

#427

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.

The newly developed Toyopearl SuperButyl-550C HIC resin incorporates higher binding capacity for low molecular weight proteins (MW< 30 KDa), high recovery and low pressure drop.

Usage of Toyopearl SuperButyl-550C

The molecular weight of a wide variety of proteins is in the range of 12-36 KDa. Very small molecules like insulin and larger proteins such as antibodies are less common.

Insulin can be purified by RP chromatography. Nevertheless, this mode is less appropriate for proteins, due to the risk of denaturation. In such cases milder hydrophobic interaction chromatography (HIC) is preferable, with which insulin can also be successfully purified.

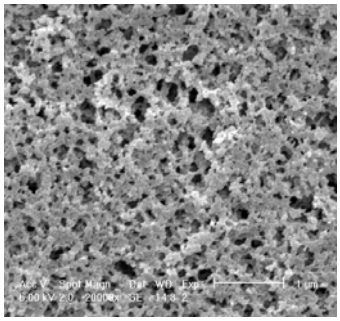
Dynamic Binding Capacity as a Function of Pore Size:

Resin	Mean Pore Size [nm]	Mean Dynamic Binding Capacity for Lysozyme [mg/ml]
Toyopearl Butyl-650C	100	40
Toyopearl SuperButyl-550C	50	61

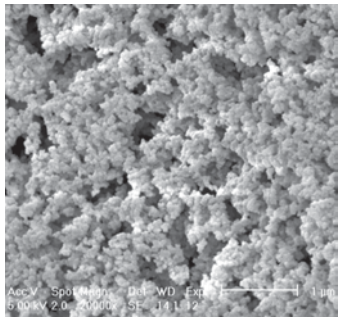
Table 1

See below 50 and 100 nm pore sizes under the TEM with 20.000 fold magnification

Toyopearl with 50 nm pore size



Toyopearl with 100 nm pore size



For smaller proteins the mass transfer is fast, and resins with smaller pores (e.g. 50 nm) can be used. Such resins provide a large internal surface area and this results in a high protein binding capacity.

The recovery for different proteins is comparable to competitive resins. Examination of BSA recovery shows that Toyopearl SuperButyl-550C performs better than all other resins (Table 2).

Mass Recovery (% of proteins)

Protein	Toyopearl SuperButyl 550C	Agarose Based Butyl	Agarose Based Phenyl (highly subst.)
Ribonuclease A	98	98	106
Lysozyme	93	94	90
Chymotrypsinogen	92	86	96
Bovine Serum Albumin	86	82	51

Table 2

Loading: 0.1 M phosphate buffer / 1.8 M ammonium sulfate (pH 7)
Sample: 1 mg/ml protein in loading buffer
After column saturation the unbound protein was removed with the loading buffer without salt.
The protein elutes in 0.1 M phosphate (pH 7)

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Toyopearl SuperButyl-550C is a coarse grade resin with a particle size range between 50-150 µm, and is well suited to capture steps. The pressure drop of a packed bed remains low over a wide flow-rate range. Pressure is lower than competitive HIC resins in aqueous mobile phase (Figure 1).

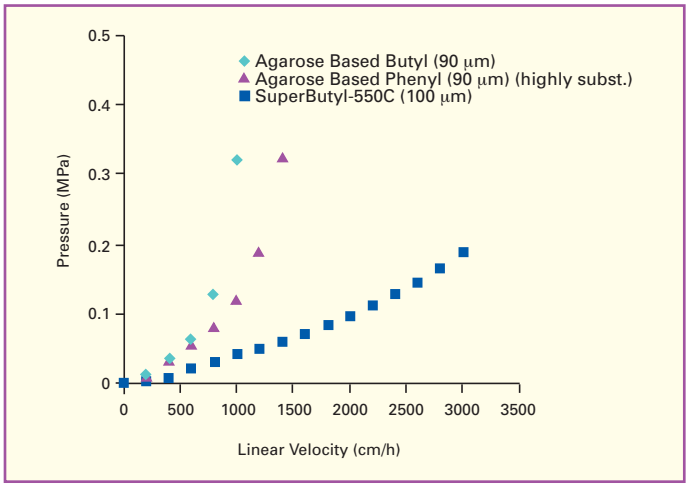
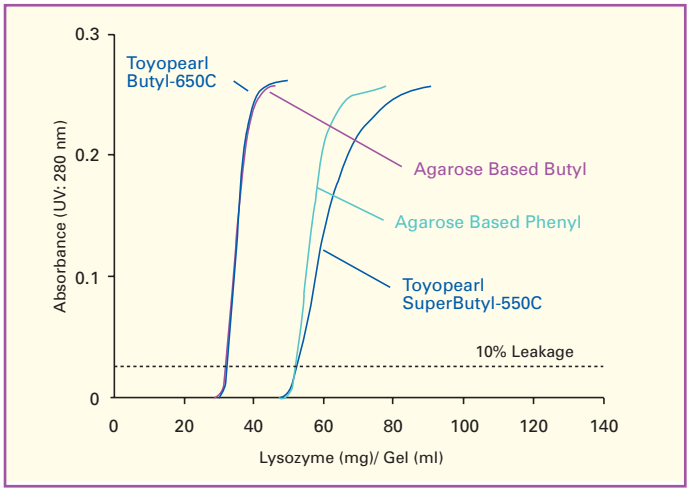


Figure 1

Fast mass transfer action is desired during the capture step. Dynamic binding capacity of four different resins is plotted in Figure 2. Toyopearl Butyl-650C and the agarose based Butyl behave very similarly, whereas Toyopearl SuperButyl-550C compares with the agarose based Phenyl offering high dynamic capacity. Toyopearl SuperButyl-550C however shows higher overall capacity. The resolving power of the new Toyopearl SuperButyl-550C resin was compared to other Toyopearl HIC resins with 100 µm particle size (Figure 3).



Conditions for binding capacity tests:

Loading:	1 mg/ml lysozyme in 0.1 M phosphate buffer/1.8 M ammonium sulfate (pH 7)
Flow rate:	300 cm/hour

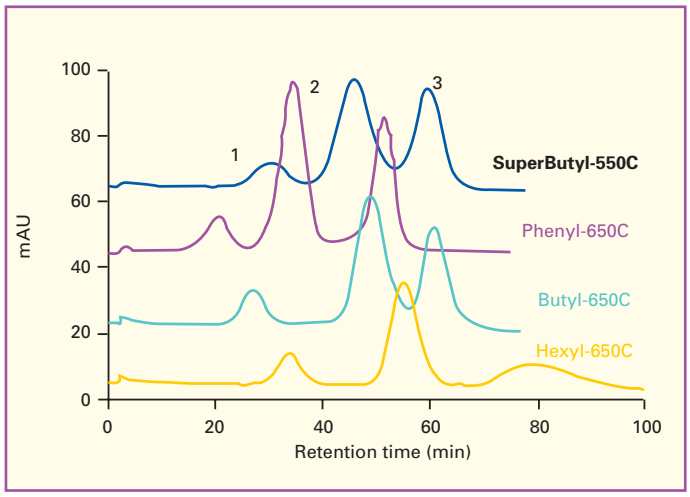


Figure 3

Conditions:

Column:	7.5 mm ID X 7.5 cm length
Eluent A:	0.1 M phosphate buffer / 1.8 M ammonium sulfate, pH 7
Eluent B:	0.1 M phosphate buffer, pH 7
	Linear gradient from A to B in 60 min
Flow rate:	1 ml/min (136 cm/hour)
UV-Monitor:	280 nm
Sample:	1. Ribonuclease
	2. ysozyme
	3. a-Chymotrypsinogen
	(1mg/ml each)
Injection vol.:	100 µl

Summary:

- Toyopearl SuperButyl-550C offers a high dynamic binding capacity for smaller proteins.
- The coarse grade resin generates low pressures even at high linear flow rates.

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

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