Cellufine™ MAX DexS-HbP

A new chromatography resin incorporating a dextran sulfate polymeric ligand, acting as a Heparin mimetic for isolation and purification of plasma proteins

Dextran sulfate is a synthetic derivative of the natural polysaccharide dextran and is reported to have similar bioactivity as heparin. For example, dextran sulfate selectively inhibits HIV-1 replication *in vivo* or rapidly binds heparin cofactor II. Dextran sulfate is also used to prepare cation exchange chromatography resins. JNC has developed a new chromatography resin, Cellufine MAX DexS-HbP incorporating dextran sulfate to act as a pseudo affinity heparin mimetic ligand. This new resin is built on a crystalline highly cross-linked stable cellulose base bead ideally suited for large scale bio-pharma manufacturing processes. The new bead structure is resistant to base CIP and can be operated under high flow modes with minimal back pressure. Table 1 summarizes the properties of this new heparin mimetic affinity resin.

Table 1. Properties of Cellufine MAX DexS-HbP.

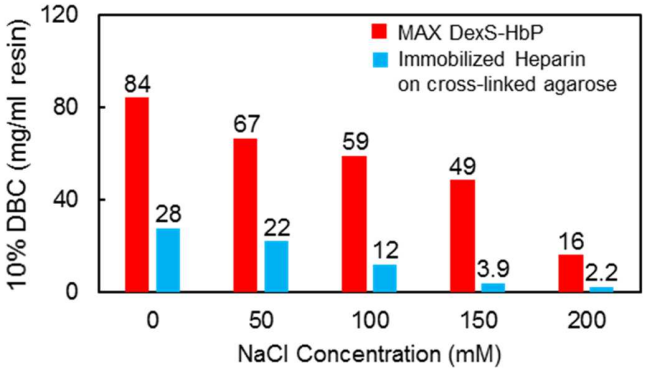
Properties	
Base bead matrix	Highly cross-linked cellulose
Particle size	90 µm average (40 – 130 µm)
Ligand	Dextran sulfate
CIP	0.5 M NaOH
Lysozyme adsorption capacity (mg/ml)	≥ 50 mg/ml
Operating pressure	< 0.3 MPa
Operating flow rate	Up to 1200 cm/h

Model Protein adsorption to Cellufine MAX DexS-HbP resin

A series of model protein binding to this Heparin mimetic resin were investigated to characterize the resin comparing to Heparin on agarose beads.

Lysozyme binding has been used to characterize the Cellufine Heparin mimetic resin and compared to Heparin on agarose beads. Dynamic binding capacity (DBC) data over a range of salt concentrations is summarized in Figure 1, below.

Figure 1. DBC of lysozyme with Cellufine MAX DexS-HbP and Heparin on agarose beads.

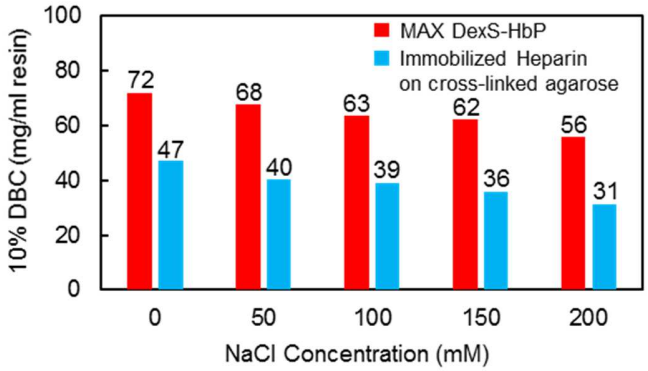


DBC was measured in a 5 mmID x 2.5 cmL (volume 0.49 ml) flow packed column at a flow rate of 0.125 ml/min for a residence time of 4 min. Lysozyme at 2 mg/ml was loaded in a 50 mM Tris

HCl buffer pH 9.5 + 0, 50, 100, 150 and 200 mM NaCl.

Lactoferrin binding has also been used to characterize the Cellufine Heparin mimetic resin and compared to Heparin on agarose beads. Dynamic binding capacity (DBC) data over a range of salt concentrations is summarized in Figure 2, below.

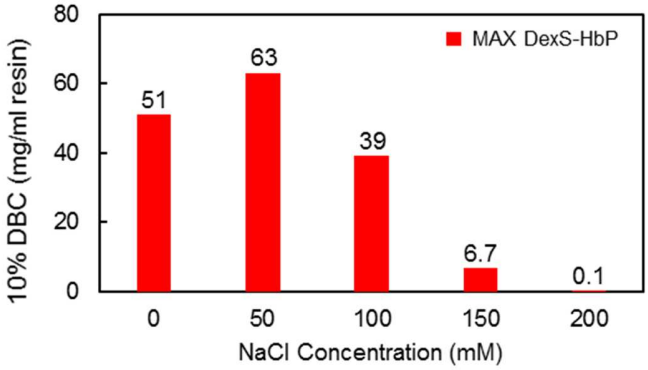
Figure 2. DBC of Lactoferrin with Cellufine MAX DexS-HbP and Heparin on agarose beads.



DBC was measured in a 5 mmID x 2.5 cmL (volume 0.49 ml) flow packed column at a flow rate of 0.125 ml/min for a residence time of 4 min. Lactoferrin at 2 mg/ml was loaded in a 10 mM Na Phosphate buffer pH 7.5 + 0, 50, 100, 150 and 200 mM NaCl.

Polyclonal immunoglobulin (IgG) binding was investigated over a range of salt concentrations at pH 5.0 in Acetate buffer. DBC data is summarized in Figure 3, below.

Figure 3. DBC of Polyclonal IgG binding to Cellufine DexS-HbP.



DBC was measured in a 5 mmID x 2.5 cmL (volume 0.49 ml) flow packed column at a flow rate of 0.125 ml/min for a residence time of 4 min. Polyclonal IgG at 2 mg/ml was loaded in a 10 mM Na Acetate buffer pH 5.0 + 0, 50, 100, 150 and 200 mM NaCl. A more extensive study looking at both salt and pH variables is summarized in Table 2, below.

Table 2. DBC of Polyclonal IgG between pH 5 – 8 over a range of NaCl concentrations.

10% DBC Polyclonal IgG (mg/ml)			
NaCl Concentration (mM)	150	200	250
pH 5.0 *a	6.7	0.1	< 0.1
pH 7.0 *b	< 1	0	0
pH 8.0 *c	0	0	0

DBC was measured as described in Figure 3 with the polyclonal IgG (2 mg/ml) in the following buffers; a) 10 mM Acetate pH 5.0, b) 10 mM Na Phosphate buffer pH 7.0 and c) 50 mM Tris HCl buffer pH 8.0.

Discussion

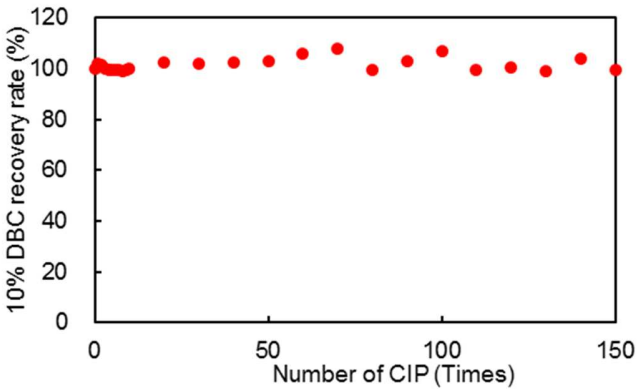
Lysozyme and Lactoferrin showed significantly higher binding compared to Heparin on agarose beads. Binding > 45 mg/ml was seen for both proteins at up to 150 mM NaCl. Heparin affinity has been used (see Ref. 1) as a final polishing step in the production of recombinant therapeutic human Lactoferrin. In contrast, polyclonal IgG was not adsorbed to Cellufine MAX DxS-HbP at > 150 mM NaCl concentration between pH 7 to pH 8.

Ref. 1: Choi et al Glycoconjugate J. 2008 August; 25(6): 581–593

Base Clean in Place (CIP) for multiple cycles of re-use

The new Cellufine DexS-HbP resin showed stable 10% DBC Lysozyme binding performance at up to 150 cycles of base CIP with 0.5 M NaOH. The recovery of protein binding performance is summarized in Figure 4, below.

Figure 4. CIP by 0.5 M NaOH (10 CV) with Cellufine MAX DexS-HbP during 150 cycles of re-use.



Protein recovery after multiple cycles of base CIP was measured in a 5 mmID x 2.5 cmL (volume 0.49 ml) flow packed column at a flow rate of 0.125 ml/min for a residence time of 4 min. Lysozyme at 2 mg/ml was loaded in a Buffer A; 50 mM Tris HCl pH 9.5 + 150 mM NaCl. Retained protein was recovered by elution with Buffer B; 50 mM Tris HCl pH 9.5 + 1.0 M NaCl.

The column was then subjected to a CIP cycle as outlined in Table 3 below.

Discussion

Cellufine MAX DexS-HbP shows excellent retention of lysozyme binding activity in a simulation of 150 cycles of CIP in 0.5 M NaOH.

Table 3. Base CIP Process Workflow.

Process Step	Flow Velocity (ml/min)	CV
Equilibrate with Buffer A (50 mM Tris HCl pH 9.5 + 150 mM NaCl	0.5	10
Load 2 mg/ml Lysozyme in Buffer A	0.125	70
Wash with Buffer A	0.5	10
Elution with Buffer B (Buffer A + 1M NaCl)	0.5	30
Wash with buffer A	0.5	10
Base (0.5M NaOH) CIP	0.5	10
Re-equilibrate with Buffer A	0.5	10

Purification of Antithrombin III (AT III) with Cellufine MAX DexS-HbP

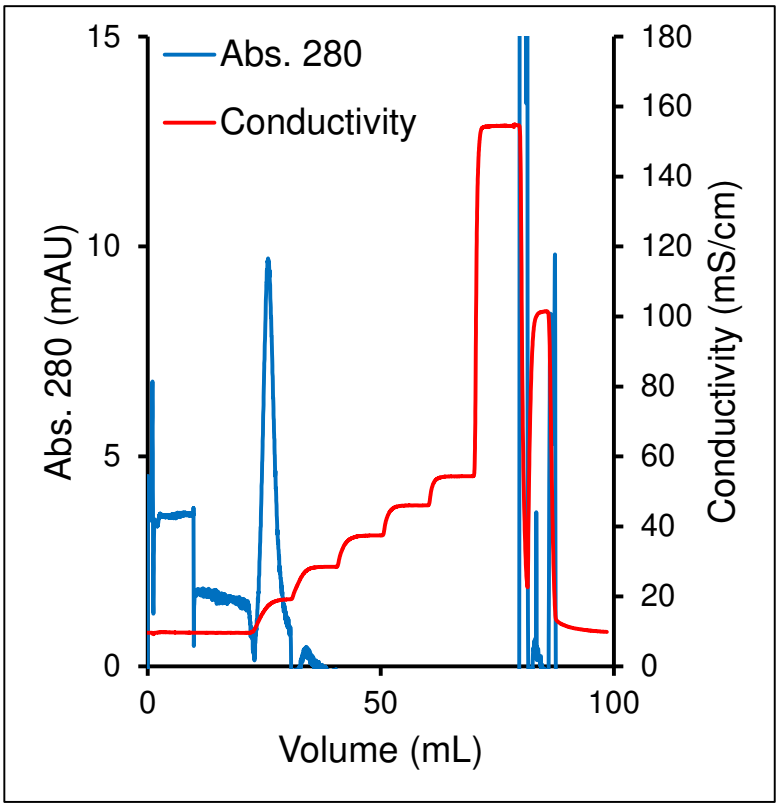
Heparin affinity on Agarose has been used to purify Antithrombin III directly from bovine whole plasma (see Ref. 2) with a high degree of purity.

Ref. 2, Data file 18-1134-77 AE HiTrap Heparin HP Figure 5. GE Healthcare Biosciences AB

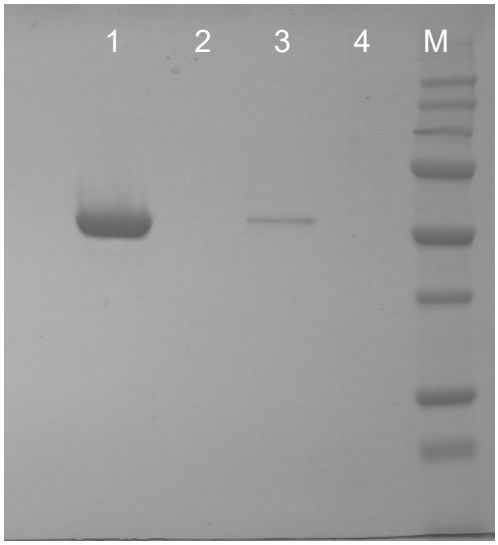
An Antithrombin III adsorption and elution purification workflow is summarized in Figure 5 below. The study was carried out with a 5 mmID x 2.5 cmL (0.49 ml volume) column in 100 mM Tris HCl, 10 mM Trisodium citrate buffer pH 7.4 for loading of 0.15 ml of Antithrombin III (Neuart) at 2.0 mg/ml. Material retained by Cellufine MAX DexS-HbP was eluted with a step gradient of 0.1, 0.2, 0.3, 0.4, 0.5 and 2 M NaCl in loading buffer. Elution fractions were then analyzed by SDS-PAGE under reducing conditions.

Figure 5. Chromatography of Antithrombin III on Cellufine MAX DexS-HbP.

Panel A. Chromatogram.



Panel B. SDS-PAGE Analysis.



1 Antithrombin III (Neuart)
2 Flow through (FT)
3 0.1 M NaCl elution step
4 2.0 M wash step
M MWt. markers

Discussion

Lactoferrin showed 10% DBC binding to MAX DexS-HbP > 56 mg/ml at up to 200 mM NaCl while loading at up to pH 7.5. Under the same conditions immobilized heparin showed only 31 mg/ml capacity. In both case their capacities declined as the [NaCl] increased. Polyclonal IgG showed little or no binding to Immobilized heparin. In contrast, MAX DexS-HbP showed > 63 mg/ml capacity at up to 50 mM NaCl but declined rapidly up to 200 mM NaCl.

In summary, the Dextran sulfate modified Cellufine MAX DexS-HbP showed significantly higher binding capacity for Lactoferrin and Lysozyme with minimal retention of polyclonal antibody at physiological ionic strength. This resin will be very useful for fractionation of plasma proteins, such as Antithrombin III.

Additional Cellufine Products used during Plasma Protein Purification

Cellufine ET clean (poly(ε-lysine)) - can remove endotoxin from a cellular product solution at physiological pH, ionic strength of μ = 0.02 – 1.0, and 0 – 25 °C.

ET Clean S (2,000 MWt. cut-off pore size)
ET Clean L (> 2 x 10^6 MWt. cut-off pore size)

Cellufine GH-25 desalting media - based on porous, spherical, highly crosslinked cellulose particles. The sharp 3 kDa exclusion limit allows proteins to pass through the column in the void volume while retarding smaller molecular weight solutes in the internal pores.

Description	Quantity	Catalogue No.
Cellufine MAX DexS-HbP	5 x 1 ml mini column	21 700-51
	1 x 5 ml mini column	21 700-15
	10 ml	21 700
	50 ml	21 701
	500 ml	21 702
	5 lt	21 703
	10 lt	21 704

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