#425

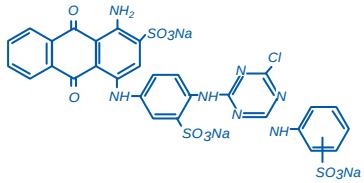
TOYOPEARL® AF-BlueHC-650M

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.

Toyopearl AF-Blue is functionalized with Cibacron Blue F3GA, useful in the purification of NAD/NADP dependant enzymes and blood components, like Interferon, Coagulation factors and Albumin (Table 1).

Advantages: Toyopearl AF-BlueHC provides enhanced protein binding capacity and reduc ed dye leakage for pre-parative applications. The original Toyopearl AF-Blue product remains available in support of existing processes.

Formula of Cibacron Blue F3GA:



Applications for Affinity Blue Resin:

The ligand attached is Cibacron Blue F3GA. This is a mono-chlorinated triazine dye, which is immobilised by an elimi-nation reaction involving the chlorine atom attached to the triazine ring and hydrogen atoms at the resin surface.

Some proteins bind biospecifically with this dye ligand due to its structural similarity to cofactors such as NAD/NADP, while others, such as albumin and interferon bind in a less spe-cific manner through ionic and/or hydrophobic interactions.

Biospecifically adsorbed proteins may be eluted by low con-centrations of free cofactor (1-20mM). Less specifically adsorbed molecules require the use of higher concentra-tions of cofactor (> 50 mM), salt (Table 2) or a pH-step.

Regeneration of the gel is achieved using alternate low and high pH (e.g. pH 5 followed by pH 11) or by using high salt (2M KCl or 3M NaCl). Tightly bound lipids can be removed with 4M Urea.

Table 2

Solvents recommended for elution and regenera-tion of Toyopearl® AF-Blue HC-650M

Solvent	Concentration
Potassium chloride	*2 M*
Sodium cloride	*3 M*
Urea	*4 M*
Ammonium sulfate (saturated)	*4,2 M*
Sodium thiocyanate	*1 M*
Sodium hydroxide	*0,1 N*
Triton X-100	*1 %*
Ethylene Glycol	*75 %*
Chloroform / Methanol	*50/50%*

Table 1

Commercial Processes using the Blue ligand

Protein	*Lactoferrin*
Source	*Human Whey*
Binding buffer	*5 mM Na-barbital/HCl, pH 7.4; 50 mM NaCl*
Eluent	*0.05 - 1M NaCl /linear gradient)*
Protein	*Albumin*
Source	*Human Serum*
Binding buffer	*50 mM Tris-HCl, pH 8.0, 50 mM NaCl*
Eluent	*0.2M NaSCN*
Protein	*Interferon*
Source	*Human Fibroblasts or Leucocytes*
Binding buffer	*20mM Sodium phosphate, pH 7.4*
Eluent	*0-50 % (v/) ethylene glycol or 0,15-1,0 m NaCl*
Protein	*Lactate dehydrogenase*
Source	*Rabbit muscle*
Binding buffer	*50mM Phosphate, pH 7.0*
Eluent	*1mM NAD^+ and 2mM Na_2SO_3*

(Protein Liquid Chromatography. Kastner, 1999 Elsevier)

Caustic Stability:

The caustic stability of AF-Blue HC-650M was tested in 0.1 N and 0.5 N NaOH solutions at 25°C.

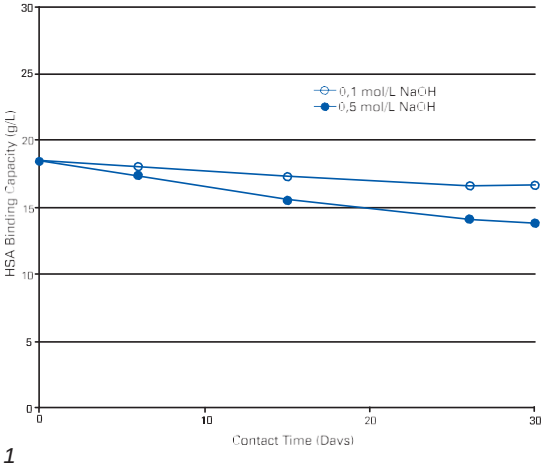


Figure 1

TOSOH

1 ml of adsorbent was suspended with 5 ml of each solution. Stability was determined by measuring HSA equilibrium binding capacity.

Toyopearl AF-BlueHC-650M withstands exposure to 0.1N NaOH for 30 days. When contamination is severe, 0.5 N NaOH may be used for short exposure times. Longer contact times decrease binding capacity for HSA.

Ligand Leakage:
The dye can be measured spectrophotometrically at 620 nm. It also strongly adsorbs at UV280. Toxicity investigations for the dye in vitro with eukaryotic and prokaryotic cells demonstrated zero or slight toxicity only [Mohammad, J. (1995), J. Chrom. 510, 155-164]

Competitive leakage study

Conditions:
200 mg of each resin was suspended in 4 ml of solvent (Table 3) and incubated at RT on a shaker for 24h. Absorption of the supernatant at 620 nm was measured after adjustment to neutrality. Dye concentration was estimated assuming a molar extinction coefficient of 12.750 (L/M cm^{-1}).

Thermal Stability:

The resin can be autoclaved at 121°C for 20 min.

Binding Capacity:

The binding capacity for HSA is > 18 mg/ml swollen gel and is dependant from pH (Figure 2) and from linear flowrate (Figure 3).

Ligand Leakage

Packing

Table 3

Solvent	Concentration of Cibacron Blue F3GA (µmol/l)	
	Toyopearl AF-Blue HC-650M (TOSOH)	Affinity Blue Resin Agarose based resin
0,1 N NaOH	1,1	1,9
0,5 N NaOH	3,1	11,7
0,1 M HCl	4,8	6,7
0,5 M HCl	4,1	11,5

Please refer to the brochure "Toyopearl Packing Instructions and Use".

HSA Capacity vs. pH

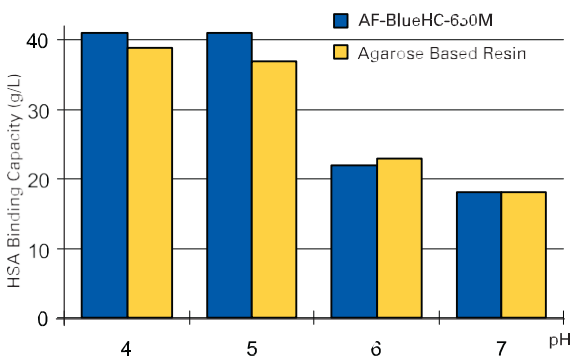


Figure 2

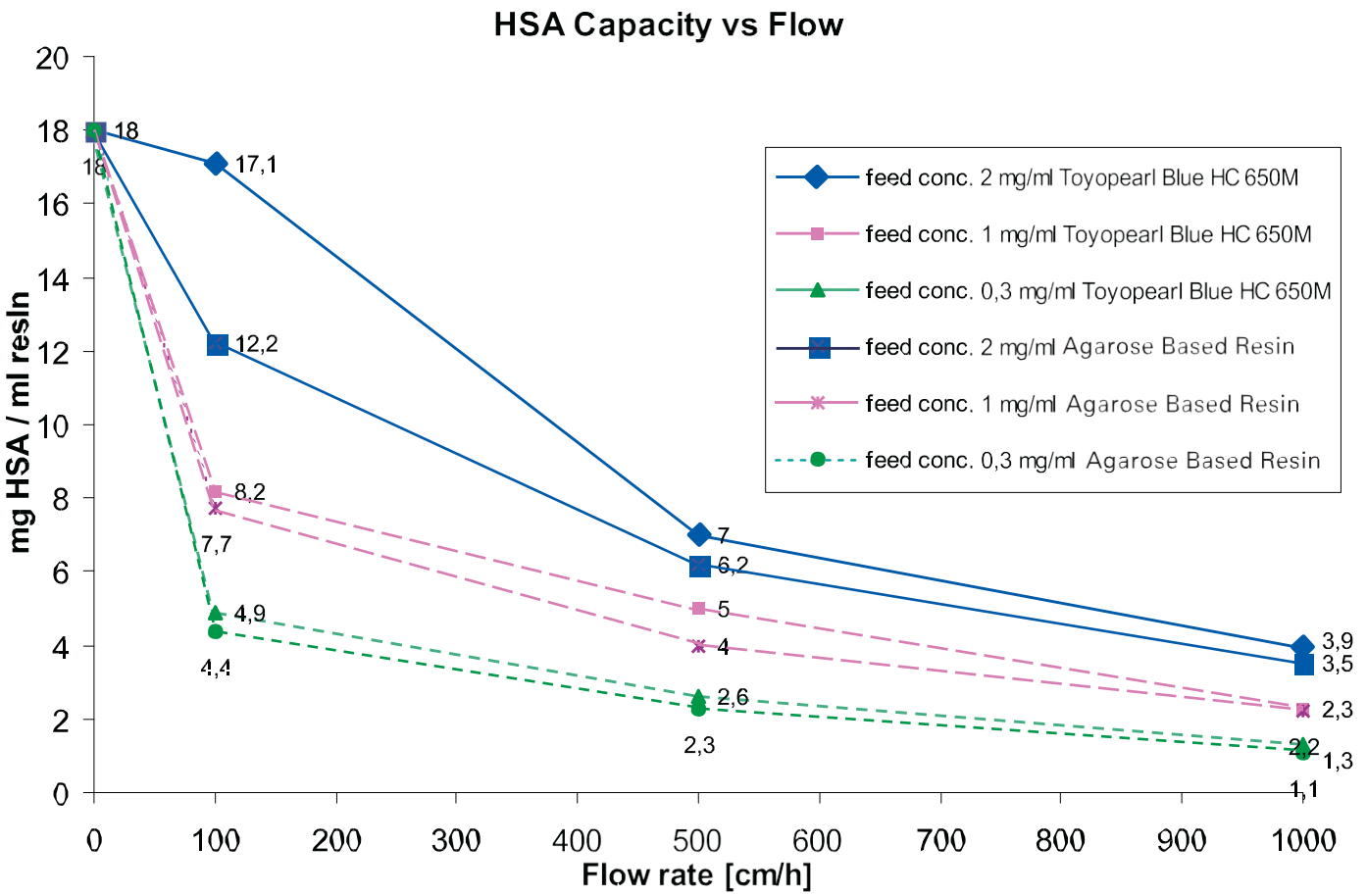


Figure 3

F03P07A

With courtesy of TOSOH

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