

Characterization of a new albumin binding adsorbent

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Introduction

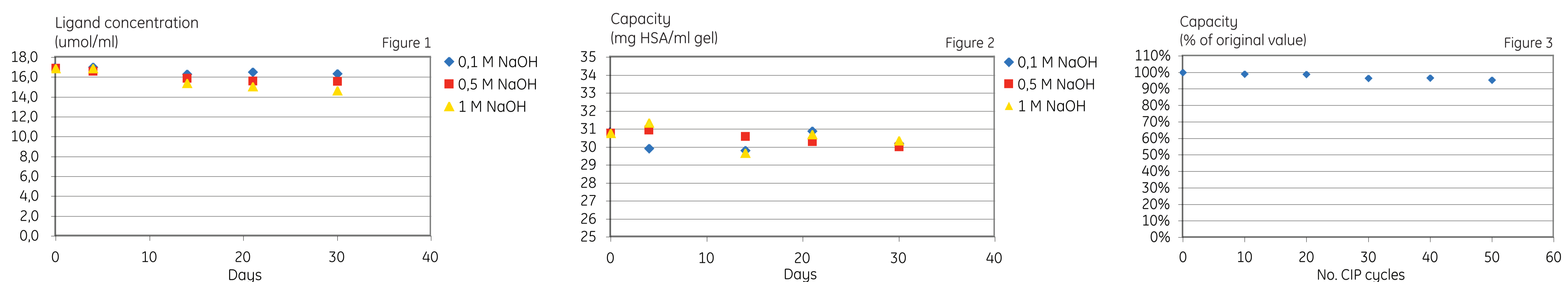
A new affinity chromatography prototype resin for purification or removal of HSA was investigated. The prototype resin, Cpto™ Blue, is made by immobilizing Cibacron™ Blue to a new High Flow Agarose matrix with superior robustness and flow properties compared to existing agarose matrices. A new immobilization chemistry has greatly improved chemical stability and reduced leakage. The combination of high throughput and CIP stability makes this an excellent resin for large scale chromatography.

Description

Composition	highly cross-linked agarose
Particle size	75 µm average (d50, vol)
Pressure / flow spec.	3 bar at 600 cm/h, 1 m diameter column, 20 cm bed height in water
pH stability operational	4 – 12
CIP stability	3 – 14
Ligand	Cibacron blue 3G

Stability study

The stability of the resin has been tested for storage in three different concentrations of sodium hydroxide at ambient temperature. Samples have been withdrawn at several occasions and the ligand concentration and HSA capacity have been determined according to standard quality control methods. As can be seen in figure 1 and 2 the HSA capacity is retained after 30 days of storage even though the ligand concentration has decreased 3 to 13 %. It can be noted that no change at all is observed after the contact time of 96 h.



Cpto Blue has been challenged in a repetitive way by exposing it to a number of cycles of binding buffer, human serum, wash, elution of HSA and cleaning with 0,5 M NaOH. After every tenth cycle the column was tested for HSA capacity according to standard quality control method. A small decrease in capacity can be observed after 50 cycles. Figure 3.

Application

The selectivity of this new adsorbent was investigated by a one step purification of HSA from human serum figure 4. Total load of HSA corresponds to 50 % of the maximum capacity. Load, wash and eluate were analyzed with electrophoresis, figure 5. The selectivity is as expected with purity comparable to the reference sample used.

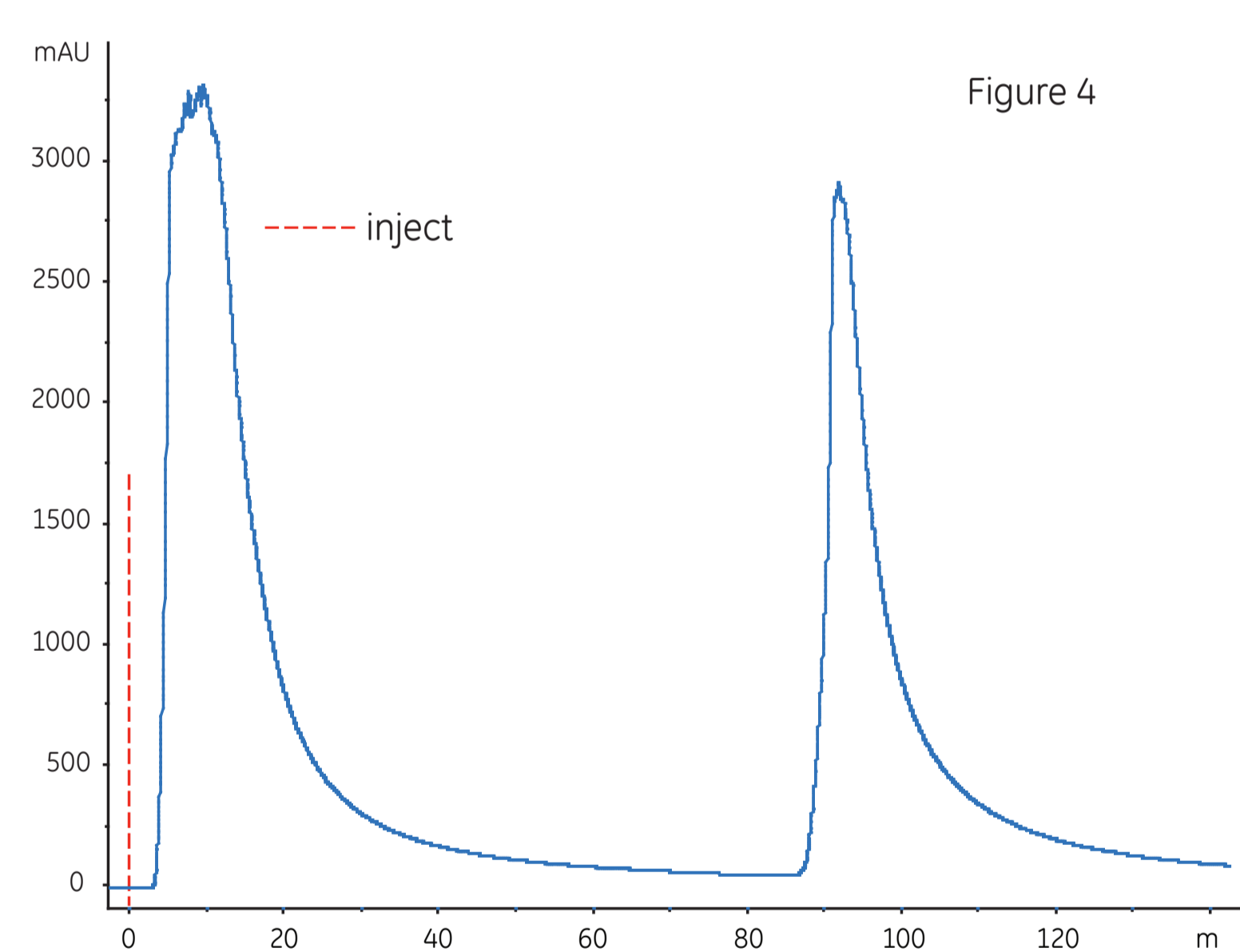
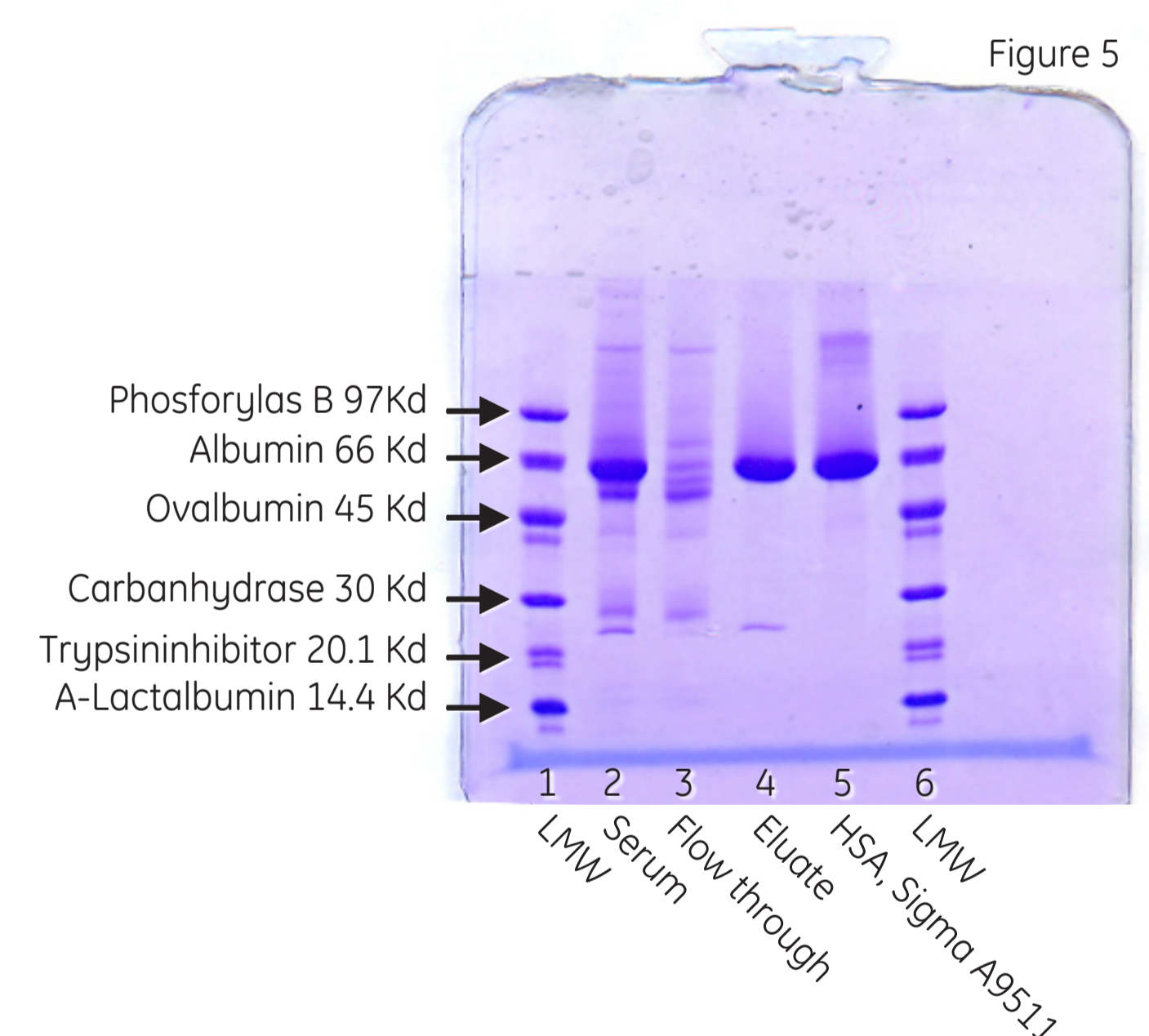


Figure 4. CHROMATOGRAM

Column: Tricorn™ 10/100, 10 cm bed height
Equilibration: 10 column volumes, 0,05M Tris, 0,5 M NaCl, pH 8,0, 535 cm/h
Load material: 2,5 ml human serum, filtered 0,45µm, 300 cm/h
Wash: 10 column volumes, 0,05M Tris, 0,5 M NaCl, pH 8,0, 535 cm/h
Elution: 7,5 column volumes, 0,05M Tris, 0,2 M NaSCN, pH 8,0, 300 cm/h

Figure 5. ELECTROPHORESE
SDS-PAGE, gradient gel 10 - 15 % under reducing conditions



Conclusion

The binding capacity is in the range 30 mg HSA/ml gel. The stability studies show that the resin has a good stability to alkaline conditions which extends functional lifetime and reduces overall production costs. As expected the selectivity for HSA is good. The electrophoreses profiles show that the purity of the eluate is comparable to the commercial reference.

Custom Design Media (CDM)

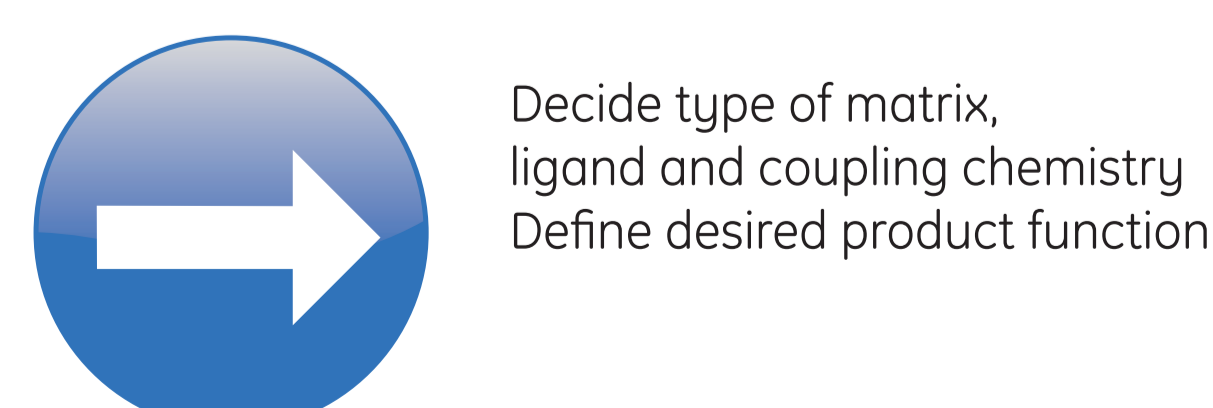
Custom Design Media (CDM) has over 20 years experience in the preparation of chromatography media.

New chromatography media will be prepared in close collaboration with the customer to fulfill the requirements of their designated process.

Development is fast and CDM can manufacture in process scale within four months under normal circumstances.

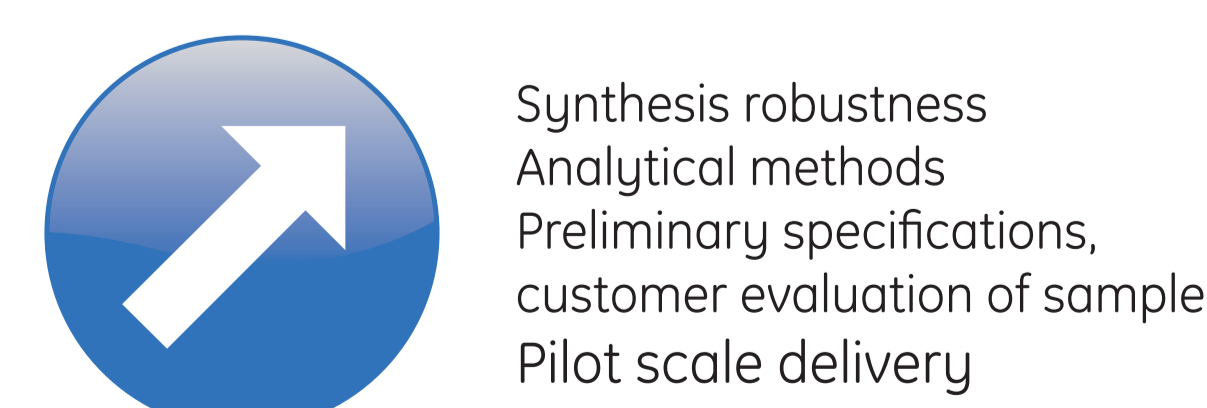
Several CDM chromatography media have become standard products and are fully supported with regulatory documentation. All projects run according to ISO 9001.

1 Media definition



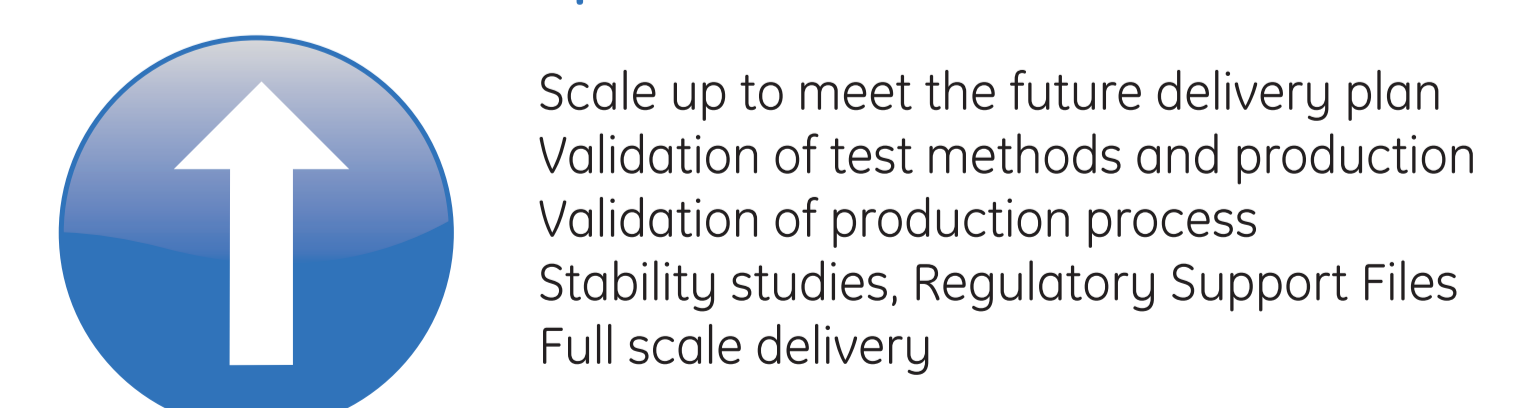
Decide type of matrix, ligand and coupling chemistry
Define desired product function

2 Media assurance



Synthesis robustness
Analytical methods
Preliminary specifications, customer evaluation of sample
Pilot scale delivery

3 Full scale production and validation



Scale up to meet the future delivery plan
Validation of test methods and production
Validation of production process
Stability studies, Regulatory Support Files
Full scale delivery