Affinity chromatography media efficient in purification of coagulation factors

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Introduction

Here, we present affinity chromatography media (resins), VIIISelect, IXSelect, and VIISelect, for use in efficient large-scale purification of the coagulation factors factor VIII (FVIII), factor IX (FIX), and factor VII (FVII), respectively, to achieve high purity and yield on one step.

Hemophilia is a group of inherited bleeding disorders caused by a deficiency in one or several coagulation factors. Haemophilia type A is the most common form and is caused by a deficiency or defect in FVIII. Hemophilia type B is the second most common form and is known as FIX deficiency, or Christmas disease. Both FVIII and FIX are used in replacement therapy for the respective disorder. FVII is used for hemophilia patients with FVIII or FIX deficiencies who have developed inhibitors against the replacement coagulation factor. Coagulation factors can be obtained from plasma or expressed as a recombinant proteins in various cell types.

The medium development projects were a collaboration between former BAC BV (now part of Life Technologies, owned by Thermo Fisher Scientific) and the Custom Design Media (CDM) group at GE Healthcare.

Chromatography medium development

The final medium products were developed by GE Healthcare's CDM group and are commercially available under the product names VIIISelect, IXSelect, and VIISelect, respectively. The CDM group of chromatography medium specialists works together with bioprocessing customers worldwide to develop media suitable for commercial manufacturing of biopharmaceuticals in a GMP environment.

The affinity ligands were developed using technology from BAC BV and were specifically selected for binding and elution of FVIII, FIX, or FVII, respectively. The ligands are based on single-domain heavy chain antibody fragments from Camelidae. The antigen-binding domain constitute single-domain fragments of approx. M_r 13 000 to 14 000 in size. The ligands were recombinantly produced in Saccharomyces cerevisiae and showed no toxic reactions in animal studies.

VIIISelect, IXSelect, and VIISelect media are designed with the ligand attached to the base matrix through a hydrophilic spacer arm to facilitate binding of the target molecule (Fig 1). The ligand is immobilized to the agarose base matrix via stable amide bonds to ensure high chemical stability and low leakage.

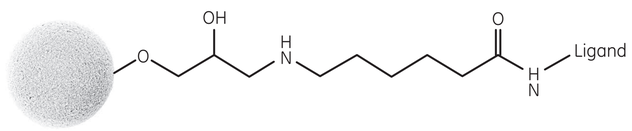

Fig 1. Structure of VIIISelect, IXSelect, and VIISelect media.

Results and discussion

Medium characteristic

Medium characteristics are shown in Table 1.

Performance

The use of VIIISelect in purification of β-domain-depleated recombinant FVIII has been described in publications (1,2). Purification of FIX from Chinese hamster ovary (CHO) cell supernatant using IXSelect medium is shown in Figure 2. Figure 3 shows FVII purification from human plasma. As shown, high purity was obtained for both coagulation factors in one single chromatography step.

Table 1. Medium characteristics

	VIIISelect	IXSelect	VIISelect
Target molecule	β-domain-depleated recombinant FVIII (dissociated from von Willebrand factor)	Recombinant or plasma FIX	Recombinant or plasma FVII
Matrix	Highly cross-linked high-flow agarose		
Particle size	75 µm (mean)		
Ligand size	M_r 13 000	M_r 13 000	M_r 14 000
Ligand density	Approx. 0.7 mg ligand/mL of medium (specification ≤ 1.0 mg ligand/mL medium)	Approx. 8 mg ligand/mL of medium (specification > 6.5 mg ligand/mL medium)	Approx. 6 mg ligand/mL of medium (specification 4.2–7.2 mg ligand/mL medium)
Binding capacity	Approx. 20 000 IU FVIII/mL medium	Approx. 6 mg FIX/mL medium	Approx. 8 mg FVII/mL medium
Long term pH stability (operational)	3–10		
Short term pH stability (CIP)	2–12		
Flow velocity	Up to 300 cm/h at 30 cm bed height	Min. 600 cm/h in a 1 m diameter column, with 20 cm bed height at 20°C using buffers with the same viscosity as water at < 3 bar (0.3 Mpa)	

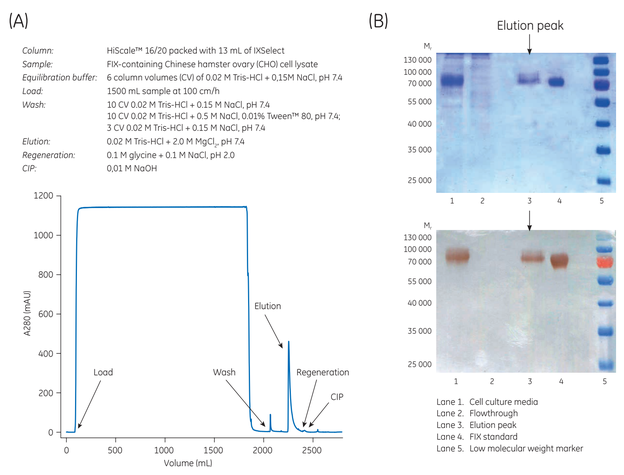

Fig 2. (A) Chromatogram and (B) SDS-PAGE analysis show FIX capture from CHO cell supernatant to high purity using IXSelect medium.

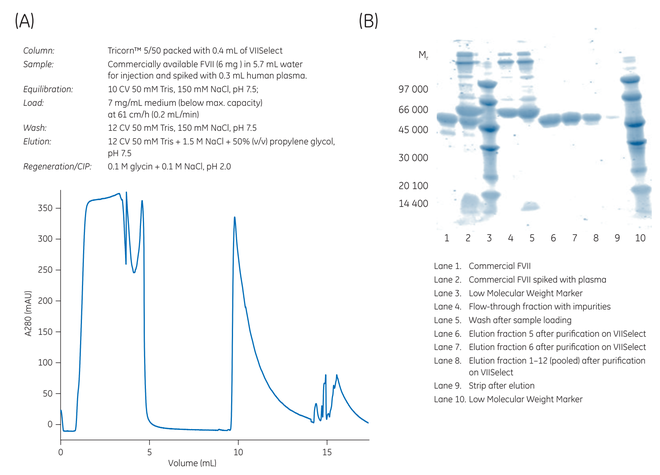

Fig 3. (A) Chromatogram and (B) SDS-PAGE analysis show FVII capture from human plasma to high purity using VIISelect medium.

Stability

The chemical stability of VIIISelect, IXSelect, and VIISelect has been investigated over the wide pH range used in industrial processes and in some cases also recommended for cleaning and sanitizing. A forced stability study was performed with static incubation of the media at pH 2 to 14 for one week at 20°C and 40°C. The degradation was studied by loss of carbon and nitrogen. Results from this study supports the recommendations of pH ranges for short term and long term use.

Figure 4 shows typical stability results after medium storage in different solutions of various pH at 20°C and 40°C during one week. Results were obtained for VIISelect but are similar for VIIISelect and IXSelect.

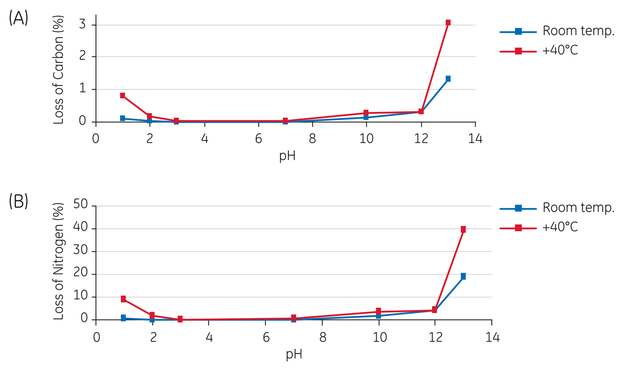

Fig 4. Relative (A) carbon loss and (B) nitrogen loss of VIISelect medium.

Stability after exposure to different alkaline and acidic CIP solutions was studied for VIIISelect and results are shown in Figure 5. After exposure to the different CIP solutions for various periods of time, binding capacity of VIIISelect for FVIII was studied in multiwell plates filled with VIIISelect medium. FVIII activity was determined using COAMATIC™ FVIII activity test (CHROMOGENIX). The most promising CIP solution in this study was shown to be acidic. However, a CIP protocol should be designed for the specific application.

Ligand leakage can be determined by an assay, for all three products, available through the following websites:

VIISelect: www.lifetechnologies.com/order/catalog/product/810299001

VIIISelect: www.lifetechnologies.com/order/catalog/product/810286001

IXSelect: www.lifetechnologies.com/order/catalog/product/810300001

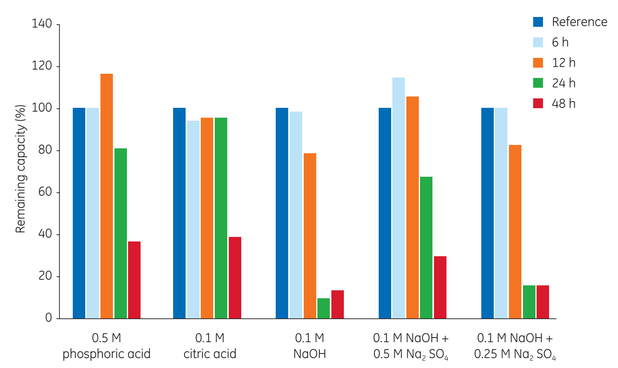

Fig 5. Effect on binding capacity of VIIISelect after exposure to CIP solutions for various length of time.

References

1. McCue, J. *et al. J Chromatogr A*, **1216**, 7824–7830 (2009).

2. Casademunt *et al. Eur J Haematol* **89**, 165–176 (2012).

Conclusions

VIIISelect, IXSelect, and VIISelect affinity chromatography media offer the following benefits:

- Efficient purification of the coagulation factors FVIII, FIX, and FVII can be performed to high purity and yield.
- Rapid processing of large sample volumes.
- Animal-component free to reduces regulatory concerns in the production of respective blood coagulation factor for therapeutic applications.

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