PlasmaCap EBA™, an innovative method of isolating plasma proteins from human plasma

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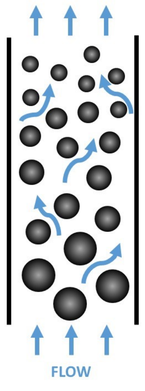
Introduction

Expanded bed adsorption (EBA) chromatography technology has the potential to be the first major advancement in plasma protein extraction in over 75 years. It allows for isolation and purification of Immunoglobulin G (IgG) and other plasma derived proteins at improved levels of quality, consistency, and purity compared to conventional methods, such as cold ethanol fractionation. It also provides a potential cost and time-saving manufacturing process. This technology offers greater efficiency in isolating plasma proteins and will allow us the potential to deliver improved therapies to patients with rare diseases.

Direct chromatographic capture of native IgG from plasma is made possible by creating a stable fluidized bed that is not affected by the variability in lipid, lipoproteins, micelles, and soluble aggregates that have made previous attempts to directly capture IgG by chromatographic methods unsuccessful.

EBA Operation



EBA columns operate in upward flow, facilitating increased void volume between resin beads. This allows highly turbid liquids, such as plasma, to be processed without aggressive cleaning, sanitizing, and conditioning agents and little risk of plugging or fouling.

Figure 1: Upward flow of liquid through an EBA column

Figure 2: Clinical scale EBA column (d=30 cm) running in up flow

Resin structure

The EBA resin used to capture IgG contains high density tungsten carbide imbedded in an microporous agarose bead. Binding specificity for IgG is achieved by covalent attachment of 4-aminobenzoic acid to the porous agarose support. Other chemical names for 4-aminobenzoic acid include PABA and Vitamin B10.

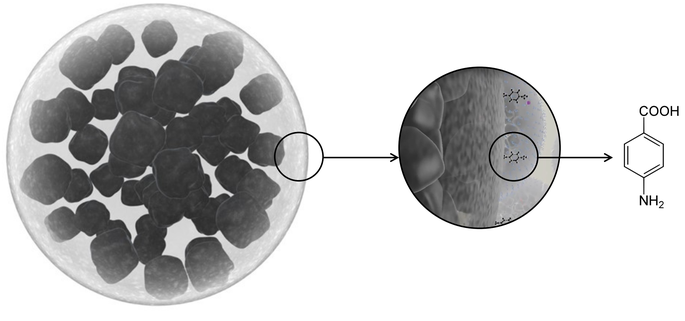


Figure 3: Tungsten-carbide agarose beads with 4-aminobenzoic acid ligands

The multimode ligand has carboxyl and amine moieties and carries a net negative charge at pH >5.5. Selective binding of the Fc portion of IgG occurs by isoelectric attraction, hydrogen bonding, van der waals forces, π-π stacking, π-cation, aromatic ring, and hydrophobic interaction.

Protein Capture

The EBA column is fluidized and equilibrated by upward flow. Plasma that was conditioned to a specific pH and conductivity to promote capture of native IgG is loaded. After the maximum amount of IgG is bound to the resin, the column is washed, then IgG disassociated and collected in an elution step.

Equilibration

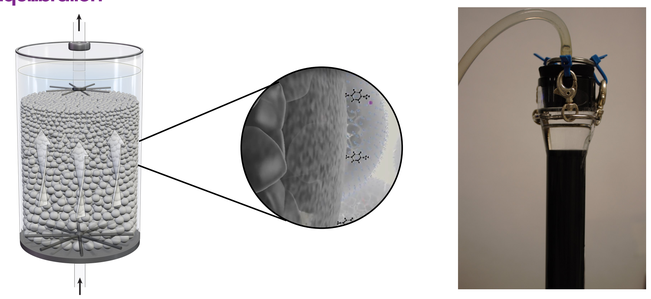


Figure 4: Lab scale EBA column (d=2 cm) in equilibration

EBA columns are expanded by flowing liquid upward through the column and equilibrated to condition the ligand for optimal binding of IgG (Figure 4).

Load

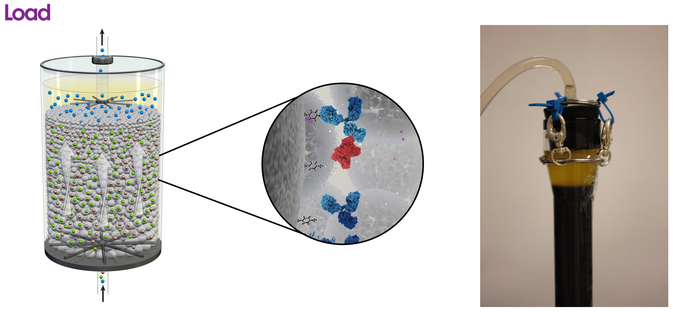


Figure 5: Lab scale EBA column (d=2 cm) in load

Columns are loaded by flowing plasma upward, exposing the ligand to all plasma proteins (Figure 5). The ligand preferentially binds the targeted IgG molecules while allowing other proteins that can potentially bind to subsequent EBA columns to flow through the column such as AAT, C1-inh, and albumin.

Elution

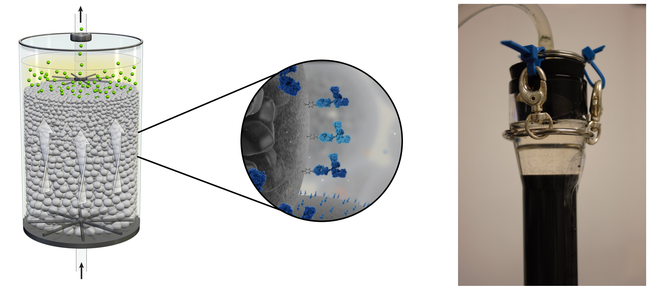


Figure 6: Lab scale EBA column (d=2 cm) in elution

The IgG molecules are unbound from the column, flowing upwards from the flow of elution buffer (Figure 6). The EBA column recovers >90% of the native IgG present in the column load. The IgG is eluted in a buffer which is suitable for subsequent virus reduction and clarification steps.

PlasmaCap™ IG

Purification of IgG using PlasmaCap EBA allows for production of highly pure IgG with little to no aggregation and the high recovery of IgG throughout the process means it produces the most possible product for patients. Increasing IgG purity through the PlasmaCap EBA process is demonstrated in Figure 7.

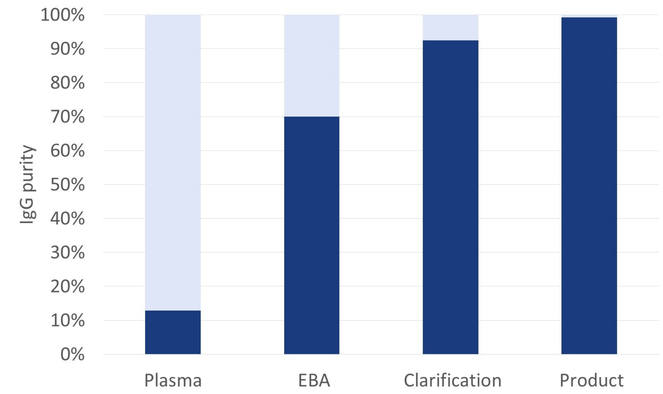


Figure 7: Increasing IgG purity throughout PlasmaCap EBA™ process

The PlasmaCap EBA™ process also puts the patient first by being able to formulate a well tolerated IVIG product, demonstrated in Table 1.

Table 1: PlasmaCap IG attributes

Attribute	Average	n
Recovery	68.7 ± 2.5	21
IgG monomer plus dimer	99.5 ± 0.3%	34
Fc function	30 mg: 111 ± 14%	10
Sodium	21 ± 3 mM	10
Osmolality	279 ± 3 mOsm/kg	34
pH	4.82 ± 0.07	34
Glycine	257 ± 3 mM	34

Other proteins that do not bind and flow through the column, such as AAT, C1-inh, and albumin, can potentially be captured by subsequent EBA columns to produce other desirable plasma products.

Thanks to the flexibility, selectivity and accuracy of EBA, plasma-derived products manufactured using this technology will have competitive product profiles compared to existing products in the market.

Conclusion

The PlasmaCap EBA process was used to manufacture clinical material for a clinical trial that started in 2017. Today, the clinical trial is nearing completion. Preliminary results are presented in the poster : *"A prospective, open-label, multicenter study of the efficacy, safety, tolerability, and pharmacokinetics of PlasmaCap™ IG (Immunoglobulin) in adults and children with primary immunodeficiency".*

Disclosures

All authors of this presentation are current employees of Evolve Biologics, a Division of Therapure Biopharma Inc., the commercial manufacturer using the PlasmaCap EBA™ process.