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Affinity Chromatography for Purification of IgG from Human Plasma

Lucia Hofbauer, Leopold Bruckschwaiger,
Harald Arno Butterweck and Wolfgang Teschner
Baxter Innovations GmbH, Vienna,
Austria

1. Introduction

Edwin J. Cohn et al. developed cold ethanol fractionation for isolating different blood plasma fractions on an industrial scale during the first half of the last century. The fractionation process uses different solubility of plasma proteins by varying the pH, ethanol concentration, temperature, ionic strength and protein concentration (Cohn et al., 1946). Initially, the main reason for developing plasma fractionation on a large scale was for purification of albumin. Albumin is used for treating shock, hypoproteinemia (Janeway et al., 1944), acute or chronic nephritis (Thorn & Armstrong, 1945) and hepatic cirrhosis (Thorn et al., 1946) as well as other disorders.

Colonel Bruton was the first to use polyclonal immunoglobulin G (IgG) as a treatment (Bruton, 1952). During his work at the Walter Reed Army Hospital in 1952, he used IgG to successfully cure a young boy who had recurrent sepsis. Bruton discovered that the γ-globulin content in blood plasma can be enhanced by monthly subcutaneous (SC) injection of immune human serum globulin. Subsequently, immunoglobulin administration became the standard treatment for patients with hypogammaglobulinemia.

In the late 1970s, intravenous (IV) administration of IgG became the method of choice because the large volumes of the immunoglobulin product that are necessary for providing the physiologic levels of IgG for the effective treatment of various diseases such as primary immune deficiencies (PID), immune (idiopathic) thrombocytopenic purpura (ITP) or Kawasaki syndrome can be applied by this route (Weiler, 2004). New purification techniques were established to produce IVIG (intravenously administrable immunoglobulin G) preparations which would not give rise to the adverse effects such as fever, headache, arthralgia, serum sickness, aseptic meningitis, myocardial infarction and thromboembolic events typically seen after IV administration of immunoglobulin products intended for the intramuscular or subcutaneous route of administration.

Chromatography for protein purification on an industrial scale was developed supplementary to the use of different precipitating agents (e.g. polyethylene glycol (PEG) (Polson et al., 1964)) or batch-adsorption on DEAE Sephadex (Hoppe et al., 1967), starting from plasma itself or intermediates derived from Cohn's, Oncley's or Kistler & Nitschmann's processes (Falksveden & Lundblad, 1980, Hoppe et al., 1967, Kistler &

Nitschmann, 1962, Oncley et al., 1949, Suomela, 1980). Later, caprylic acid precipitation (Audran & Pejaudier, 1975, Steinbruch & Audran, 1969) was used for the purification of IgG. Further processes were developed which combined some of these methods. The resulting products were of high quality containing functionally intact IgG that showed a similar subclass distribution to that of plasma (Ballow, 2002). Suomela as well as Falksveden and Lundblad presented ion exchange chromatographic processes for the isolation of IgG in the book "The methods of plasma protein fractionation" edited by Curling. While Falksveden used PEG as a precipitation agent and in addition a combination of cation and anion exchange chromatography, Suomela added a Lysine Sepharose affinity chromatographic step after ion exchange chromatography to remove proteolytic activities, thus enhancing the quality of the final preparation (Falksveden & Lundblad, 1980, Suomela, 1980). Travis et al. described the advantages of removing albumin by mimetic dye affinity chromatography prior to fractionation in different fractionation schemes (Travis et al., 1976). Later Gianazza and Arnaud developed a method for purifying plasma proteins, which included the use of Cibacron Blue Sepharose affinity chromatography resin. They studied the behavior of 27 different plasma proteins and suggested affinity chromatography as a useful initial step in plasma fractionation (Gianazza & Arnaud, 1982).

Since the early nineteen seventies, affinity chromatography has been investigated for its use in the purification of many biomolecules. The hurdles which need to be overcome were already identified in the nineteen seventies: Ligand leaching, low capacity and harsh elution conditions (Travis et al., 1976). On the other hand, this method had and still has the advantages of a several thousand-fold enrichment of the target protein out of large volumes of crude starting materials, combined with high recoveries as well as the targeted separation of active and inactive material of denatured or functionally different forms. Roque et al. (Roque et al., 2007) and Low et al. (Low et al., 2007) have written excellent reviews of the development of affinity ligands used for antibody purification from biological to bioengineered or fully synthetic ligands.

Affinity chromatography has become the method of choice for the purification of monoclonal antibodies (Kelley, 2007, Kelley et al., 2009, Low et al., 2007), while the purification procedures for polyclonal plasma-derived antibodies traditionally incorporate precipitation steps combined with ion exchange chromatography.

In this chapter we describe the potential advantages and draw-backs of affinity chromatography for capturing IgG from clarified crude polyclonal IgG fractions of human plasma. IgSelect affinity media and Protein G Sepharose 4 Fast Flow (FF), both from GE Healthcare, were investigated for this purpose. Cohn fraction II+III paste, which mainly consists of α-, β- and γ-globulin (Cohn et al., 1944), was used as the starting material. Ideally the process involves the clarification of dissolved II+III paste by filtration and a one-step affinity chromatography process leading to an intravenously administrable IgG. A reduction in the complexity of the manufacturing should also lead to an improved IgG yield, which would increase the market supply needed for new indications like neurological disorders (e.g. Alzheimer's disease (Relkin et al., 2009)).

2. Affinity chromatography resins for polyclonal human IgG capture

Most commercially available affinity chromatography resins are protein A based. Hahn and coworkers (Hahn et al., 2003, Hahn et al., 2005, Hahn et al., 2006) did a comprehensive study

of 15 currently available protein A affinity media, including investigating their mass transfer characteristics and selectivity. The 3 resins that came out with the top dynamic binding capacities (DBC) at low residence times were MabSelect Xtra ™, MabSelect SuRe™, both from GE Healthcare, and ProSep®-vA Ultra from Millipore, all with a DBC at 10% of about 40 mg/mL IgG at a residence time of 4 minutes. The residence time (e.g. in minutes) is the bed height divided by the linear flow rate (e.g. expressed in cm bed height per minute). It is preferable that the target protein binds at lower residence times, because manufacturing productivity is increased. Although the residence times and capacities of these protein A resins are better than those of first generation protein A resins like Protein A Sepharose 4 Fast Flow (GE Healthcare), the capacities of cation exchange resins used in large scale IgG capturing of about 150 mg/mL at a DBC of 10% (data not shown) are still superior. Protein A, a bacterial surface protein isolated from *Staphylococcus aureus,* interacts with the Fc (Fragment, crystallizable) part of the antibody. The Fc region is the tail of the antibody interacting with cell surface receptors and complement proteins to modulate the immune system. Even though the Fc part is regarded as constant across IgGs, not all IgG subclasses have identical Fc parts. This is the reason why protein A ligands have only low affinity to IgG_3. Protein-A-based media are not suitable for polyclonal IgG purification because the European Pharmacopoeia requires IgG preparations to have a subclass distribution similar to that found in human plasma (Morell et al., 1972, Schauer et al., 2003).

The remaining candidates suitable for large scale manufacturing were found to be the bacterial surface protein G and a novel camelid-antibody-based ligand from BAC company.

A 17-kDa recombinant protein G fragment manufactured in an *E. coli* where the albumin binding region of native protein G has been genetically deleted is used as a ligand for protein G affinity resins. Compared with protein A, which has only weak affinity to IgG_3, the protein G fragment has a high binding affinity to all human IgG subclasses, including IgG_3 (GE Healthcare, 2007b). Therefore the experiments described below were performed with Protein G Sepharose 4 Fast Flow, which has a capacity of 17 mg/mL at 7 min residence time according to the claim of the manufacturer. This resin is an example of a protein G resin belonging to the BioProcess Media which GE Healthcare (Björkgatan 30, 75109 Uppsala, Sweden) offers for industrial scale purification processes.

IgSelect is a new affinity resin, also manufactured by GE Healthcare, which uses technology from BAC (BAC B.V. Huizerstraatweg 28, 1411 GP Naarden, The Netherlands). According to BAC their CaptureSelect® ligands offer a unique affinity purification solution based on camelid-derived single domain antibody fragments. These small 14-kDa affinity ligands, which are produced in *Saccharomyces cerevisiae*, can be used as a platform solution for any biopharmaceutical purification challenge and, also according to BAC, have been proven in many applications to result in a high yield and purity of the biopharmaceutical as well as fewer purification steps than needed in conventional chromatography methods. All these factors affect the cost of the biopharmaceutical products. In addition, CaptureSelect ligands can be tailored to guarantee mild elution conditions, thereby maintaining the native state of the biopharmaceutical molecule of interest. The ligand is coupled to the matrix (cross-linked high flow agarose) by a long hydrophilic spacer (GE Healthcare, 2007a, GE Healthcare, 2007b).

According to BAC and GE Healthcare the resin has the following benefits:

- Binds to all subclasses of human IgG
- Rigid base matrix to allow high flow rates
- Animal-free production, generally recognized by authorities as safe (GRAS status)
- Mild elution conditions
- Capacity: 17 mg/mL at 2.4 min residence time

Low et al. (Low et al., 2007) emphasized in their review that CaptureSelect was the only ligand which showed a selectivity comparable to protein A and that it has the advantage of a higher elution pH than other resins that had been investigated such as protein-A-based resin (MabSelect®, GE Healthcare) and the synthetic ligands based on mimetic dye resin (Mabsorbent® A1P and A2P, Prometic Biosciences). Because of the advantages reported, we also selected this resin for capturing IgG from clarified dissolved Cohn fraction II+III paste.

3. Description of the IgG purification process

The Cohn separation methods result in five main precipitates. Fraction I, which mainly consists of fibrinogen, is obtained from either plasma or cryosupernatant after separation of cryoprecipitate or after additional adsorption of blood coagulation factors and inhibitors (as, for example, described for the Baxter product KIOVIG/Gammagard Liquid), adding 8% alcohol and adjusting the temperature to approximately –2°C. Fraction II, which mainly consists of IgG, is purified from fractionation II+III paste generated by separating raw immunoglobulin and raw albumin. Fraction III is a waste fraction containing, for example, lipid-bearing β-globulins and IgA. Fraction IV, which consists of α-globulins, can also be obtained in two steps: Fraction IV-1 enriched with α-1 antitrypsin, and fraction IV-4, which is used for further purification of transferrin or, most recently, butyrylcholinesterase (Weber et al., 2011). Fraction V is mainly composed of albumin.

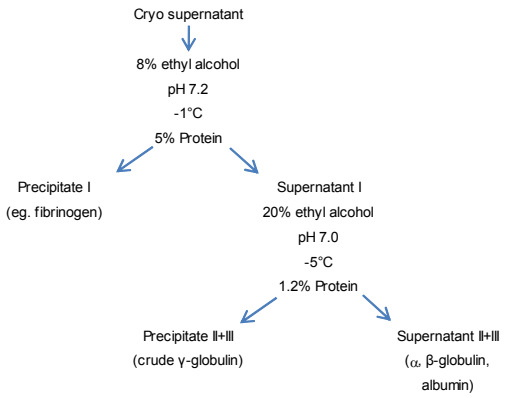

Fig. 1. Cold ethanol fractionation scheme from cryo-supernatant to fraction II+III

As the production of fraction III by the Cohn method leads to a considerable yield loss in the range of 20% (Buchacher & Iberer, 2006), most modern immunoglobulin purification methods that use plasma start with II+III paste and apply chromatographic purification methods after resuspending the paste and clarifying the suspension by filtration or centrifugation.

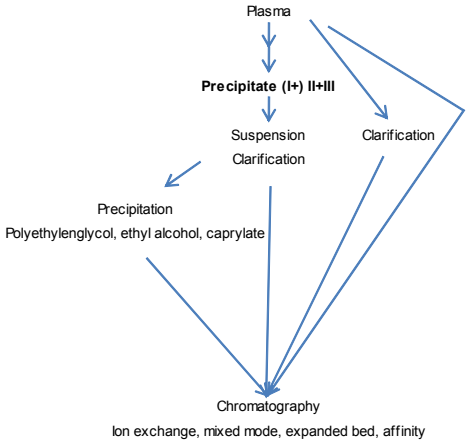

Fig. 2. Overview of modern IgG purification processes from plasma (see also Buchacher & Iberer, 2006)

We selected the II+III paste for IgG purification with affinity resins to compare yield and purity with final containers manufactured with conventional ion exchange chromatography, and also to avoid influencing the albumin and α-1 antitrypsin purification. This is of particular importance as regulatory authorities can require expensive and time-consuming clinical studies before they accept changes in manufacturing schemes.

Prevention of virus transmission has been a key aspect in the development of intravenous products as a large number of blood donations are required to produce a single immunoglobulin lot out of human plasma. In addition to donor selection and plasma testing, virus removal or inactivation steps during the manufacturing process are critical for assuring the safety of the product. Solvent-detergent (S/D) treatment, which works by irreversible destruction of the lipid envelope of viruses and is otherwise very gentle on sensitive proteins, is the most reliable and acceptable method for inactivating lipid-enveloped viruses. The concentrations of S/D reagents are low, typically 0.3% Tri-n-Butyl Phosphat (TnBP) and 1% detergent. Non-ionic detergents such as Tween 80 or Triton X-100 are preferred as they are easier to remove in subsequent chromatography steps. Because of these advantages of the S/D treatment, we subjected dissolved and filtered II+III paste to this virus inactivation step before affinity chromatography. The conditions used for the affinity chromatography are summarized below.

IgSelect affinity resin:

- Equilibration and washing: 20 mM NaH_2PO_4 + 150 mM NaCl, pH 7.4
- Loading: 17.4 mS/cm, ~ 10 g IgG/mL resin, pH 7.4
- Elution: 250 mM glycine, pH 4.0

Protein G Sepharose FF:

- Equilibration and washing: 20 mM NaH_2PO_4, pH 7.0
- Loading: 5.15 mS/cm, ~ 10 g IgG/mL resin, pH 7.0
- Elution: 100 mM glycine, pH 3.5

After affinity chromatography the eluate was concentrated and diafiltered against 250 mM glycine leading to a final bulk with 100 mg/mL protein at pH 4.4 to 4.9, similar to the commercially available IGIV product Gammagard Liquid/KIOVIG. An in-depth final container characterization was performed after sterile filtration and the results compared with Gammagard Liquid / KIOVIG specifications (see Table 1).

Measure	**Method** / **Unit**	**IgSelect**	**Protein G**	**Specifications Gammagard Liquid**
IgG recovery in eluate	% of Cohn pool	78	85	-
CAE	% γ-globulin	98.6	99	≥ 98
Molecular size distribution	Aggregates (>450 kDa) [%]	1.0	1.9	≤ 2
	Monomer/Dimers (160 – 320 kDa) [%]	99.0	98.0	≥ 95
IgG subclass	% IgG1/2/3/4	58.4/37.1/**0.6**/3.9	65.8/31.0/8.7/3.5	-
Amidolytic Activity	nmol/mL min	< 10	< 10	< 10
PKA	IU/mL	< 4	8.2	< 10
ACA	%	48.2	fixed all complement	< 50
IgA	mg/mL (at 10% protein)	0.7	1.0	≤ 0.14
Ligand	µg/mL (at 10% protein)	1.45	1.76	-

Table 1. Final container comparison of IgSelect affinity resin and protein G affinity resin versus the Gammagard Liquid product specifications (red = unfavorable results)

Both resins showed promising IgG recoveries of greater than 75% in the eluate. Purity (γ-globulin content), as measured by cellulose acetate electrophoresis (CAE) which has for decades commonly been used to determine the protein composition in plasma fractions (Kawai, 1973, Putnam, 1975), met Gammagard Liquid specifications. The IgG subclass distribution of the final container produced with IgSelect resin had an unusually low IgG_3 content, notwithstanding GE Healthcare's claim that IgSelect will bind all human IgG subclasses. Zandian & Jungbauer also used polyclonal IgG for their IgSelect evaluation but did not scrutinize the IgG subclass selectivity (Zandian & Jungbauer, 2009). In contrast to IgSelect, we found that final containers produced with Protein G Sepharose FF showed an IgG subclass distribution similar to the normal IgG subclass distribution in human blood plasma, as expected (Morell et al., 1972, Schauer et al., 2003).

Amidolytic activity was below the detection limit for both final containers. Amidolytic activity is a sum criterion, where the consumption of the chromogenic substrate PL-1 (D-norleucyl-L-lysin-p-nitroanilide-dihydochloride) is photometrically determined.

The Protein G Sepharose FF process resulted in a higher prekallikrein activator activity (PKA) than IgSelect (8.2 IU/mL compared with < 4 IU/mL), but it was still within the specifications for Gammagard Liquid (≤ 10 IU/mL) and the European Pharmacopoeia's specifications for IGIV (≤ 35 IU/mL). The prekallikrein activator activity in the sample tested forms kallikrein out of purified prekallikrein. The kallikrein activity converts the chromogenic substrate Pk-1 (D-α-aminobutanoic acid-L-cyclohexyl alanyl-L-arginin-p-nitroanilin-diacetate) into p-nitroanilin (pNA), which is measured photometrically.

The IgSelect final container complied with the European Pharmacopoeia specifications for anticomplementary activity (ACA), which is a measure of the non-specific consumption of complement by the immunoglobulin preparation (≤ 50%). By contrast, the final container obtained with protein G consumed all complement. All 3 activities measured are criteria to assess the tolerability of these potential immunoglobulin products.

Neither of the chromatography affinity methods were able to reach the high purity standards of Gammagard Liquid with respect to IgA content (≤ 0.14 mg/mL IgA). Additional chromatographic purification steps would be required to reach Gammagard Liquid specifications.

One of the main disadvantages of both affinity resins was the ligand leaching. As much as 1.45 µg/mL and 1.76 µg/mL ligand were detected in the final container concentrated to a 10% protein solution for IgSelect and protein G, respectively. Considering a dose of the polyclonal plasma IgG of 1.0 g/kg (10 mL) body weight, a patient (75 kg) would receive 1.45 µg/mL * 75 * 10 mL = 1087.5 µg. Compared with monoclonal antibodies which are given at a much lower dose, the high ligand content of the polyclonal preparation might lead to unpredictable long-term side effects.

4. Reusability of IgSelect and protein G Sepharose FF

We performed a reusability study to test the stability of the resins. A fresh IgSelect resin was packed into the column with a final bed height of 15 cm. The flow-through was collected in fractions after equilibration and loading with a protein solution to reach a final IgG load of approximately 25 mg IgG/mL resin. The resin was then washed with 30 column volumes of washing buffer, eluted with three column volumes of elution buffer and cleaned with three column volumes of phosphoric acid, acetic acid and butyl alcohol (PAB) (Millipore, 2011) solution. Finally, after a hold time of 24 min, another three column volumes of PAB solution and five column volumes of 1 M NaCl were applied. After the first break-through curve (BTC), consecutive serial runs and cleaning cycles were performed without any fractions being collected. S/D-treated loading material was used throughout this study (for break-through and serial runs). Furthermore, a filter with a pore size of 15 µm was used as a column guard.

Fig. 3 compares the break-through curves (BTC 1 to 3) performed with fresh resin and with resins after 90 and 180 runs and cleaning cycles. The dynamic binding capacity (DBC) at 1% IgG loss decreased after 90 runs and cleaning steps from 16.5 mg to 12.5 mg IgG/mL resin. During these cycles a pressure rise was observed even though only S/D treated loading solutions were used. The pressure rise indicated fouling of the chromatographic media and showed that the recommended PAB cleaning was an insufficient cleaning step for the

loading material used. A change of cleaning reagents or longer hold times may increase the cleaning efficiency.

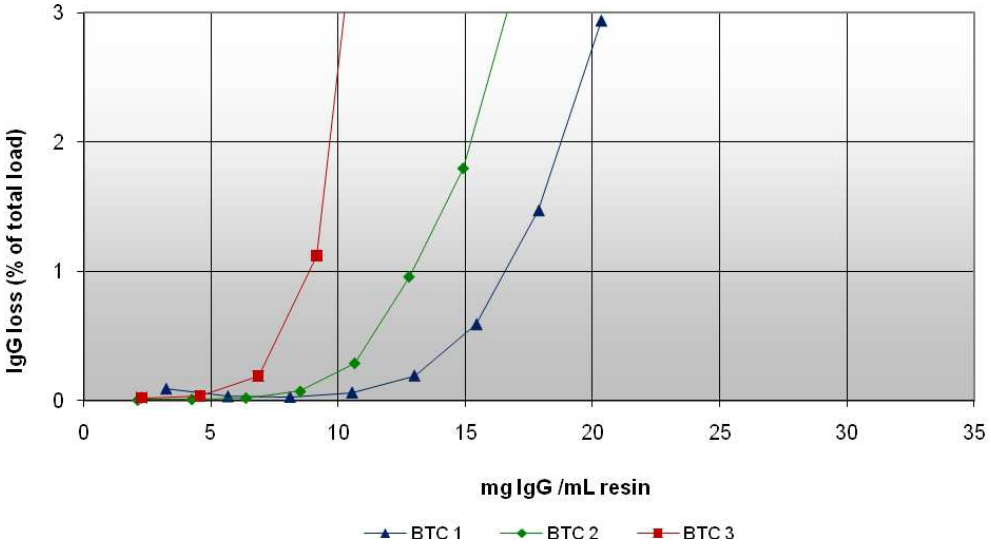

Fig. 3. IgSelect affinity media: break-through curves (BTC). BTC 1 = first run before PAB cleaning, BTC 2 = after 90 runs and PAB cleaning cycles, and BTC 3 = after 180 runs and PAB cleaning cycles

The protein yields in the eluates decreased concomitantly with the decline in resin capacity, which can be explained by the considerable ligand leaching or fouling of the chromatographic media, as already mentioned. 3 µg IgSelect ligand/mL 10% protein solution were found in the eluate after the first run, diminishing to 2.3 µg IgSelect ligand/mL 10% protein solution after 90 cycles. After 180 cycles the eluate contained 5 µg IgSelect ligand/mL 10% protein solution. The details are given in Table 2.

	IgG yield		*IgSelect Ligand*	
	[%]	*[g/L Plasma]*	*[ng/mL]*	*[µg/mL 10% protein solution]*
BTC 1 - Eluate	101.1	4.72	142	3.0
BTC 2 - Eluate	88.7	3.93	134	2.3
BTC 3 - Eluate	67.7	2.92	120	5.0

Table 2. IgG yield and IgSelect ligand concentration in eluate fraction

Our reusability study with Protein G Sepharose FF showed similar characteristics. The loading capacity decreased from a DBC at 1% of 28 mg per mL with fresh resin (see Fig. 4, BTC 1) to a DBC at 1% of approximately 20 mg per mL of resin after 57 runs and cleaning cycles (see Fig. 4, BTC 3). The pH of the loading material seemed to play only a minor, if any, role as shown by the results after 3 runs and cleaning cycles (see Fig. 4, BTC 2).

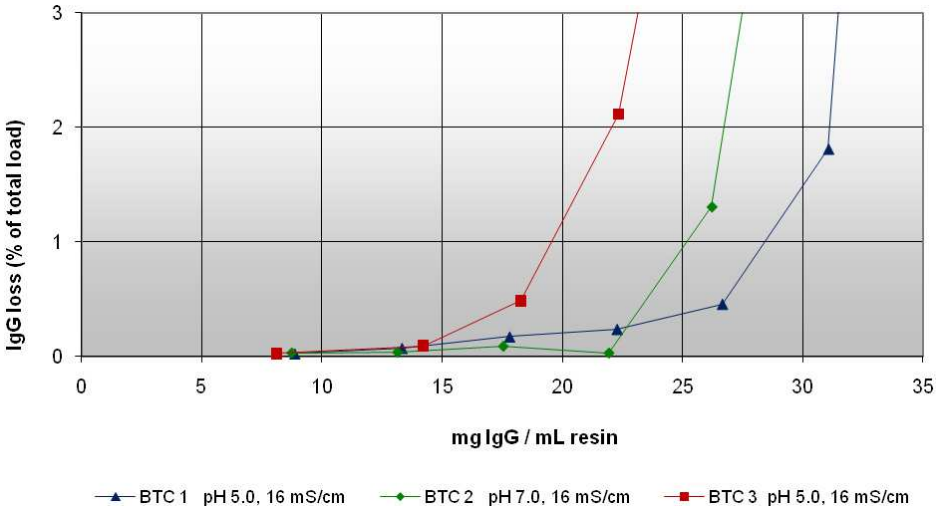

Fig. 4. Protein G Sepharose FF break-through curves (BTC): BTC 1 = without cleaning, BTC 2 = after 3 runs and cleaning cycles, and BTC 3 = after 57 runs and cleaning cycles (different loading pH)

5. Cost-effectiveness of affinity chromatography for the purification of polyclonal IgG from plasma

Reusability and loading capacity are the main criteria for calculating the cost-effectiveness of chromatography steps for IgG purification from human plasma. In the following example cost-effectiveness is estimated based on the results gathered with IgSelect affinity resin and Protein G Sepharose FF. The example assumes a manufacturing through-put of one million liters of human plasma per year. This through-put is the same as initially planned for the new fractionation plant in Barcelona by the Spanish company Grifols, one of the world's leading producers of plasma products (reported by PRNewswire on February 18th, 2011).

As shown in Section 4, with a loading capacity of 10 g IgG/L resin the IgSelect affinity resin can be used for a maximum of 120 runs (with 120 PAB cleaning cycles) without a major loss of IgG during the loading and washing procedure. Assuming an IgG yield of 5.2 g/L plasma (US source plasma) in the IgSelect starting material, 5.2 million grams IgG have to be bound to the resin. The calculation further assumes a maximum column size for large-scale manufacturing of 1,200 L resin, which can be delivered as a radial flow column by Proxcys (Proxcys BV, Bedrijvenweg 4, NL-7833 JH Nieuw-Amsterdam, The Netherlands). 12,000 g IgG can be bound in one purification run on such a column. 434 runs would be needed to process the equivalent of 1 million liters of plasma (5.2 million g IgG/12,000 g IgG per run = 434 runs).

As the column can be used for a maximum of 120 times, repacking the column with fresh resin (4,800 L of resin) needs to be done four (3.62) times for 434 runs. The resin costs approximately 8,500 €/L. A total expenditure of approximately 36.9 million € would be required solely for the resin to process the equivalent of one million liters of plasma. Assuming a final container yield of 4.5 g/L plasma and a revenue of 50 €/g IgG, a total

revenue of 225 million € would be expected. Therefore approximately 16.5% of the product revenue would be spent on the affinity media.

On the other hand one 1,200 L column of a conventional ion exchange column as described for Gammagard Liquid purification (Teschner et al., 2007) with a loading capacity of the resin of 100 mg IgG/mL can bind 120,000 g IgG. Only 43 runs would be necessary to process the equivalent of 1 million liters of plasma. This one column can be used for 23 years assuming a more than 1,000 times reuse of the resin. This means less than 0.05 column changes per year. Assuming a resin price of 2,000 €/L, the resin price per year would be 120,000 € or 300-fold less than the variant based on affinity chromatography. This calculation is summarized in Table 3.

	IgSelect	**Protein G Sepharose FF**	**Cation exchange media**	**Unit**
Starting material	1,000,000			L plasma
IgG concentration	5.2			g IgG / L plasma
IgG amount	5,200,000			g IgG / 1 million L plasma
Column size	1200			L resin
Load	10	20	100	mg IgG / mL resin
IgG load/run	12,000	24,000	120,000	g IgG / column
Reusability of one column	120	60	>1000	runs / column and resin
Total runs	434	217	43	runs / 1 million L plasma
New resin	3.62	3.62	<0.05	refilled columns / year
Resin costs	8500	8500	2000	€ / L resin
Resin costs per year	36.9	36.9	0.12	price (million €) / column
Water needed for SD removal (30CV)	15.6	7.8	1.55	(million L) /year
Water costs for SD removal (0.37€/L)	5.78	2.9	0.57	million € / year

Table 3. Calculation of cost-effectiveness: Data for IgSelect affinity resin and Protein G Sepharose FF compared with cation exchange resin

Additionally, the greater column resin and run requirements of the affinity column variant implies more buffer consumption, more labor and a higher environmental burden. This is illustrated in the following calculation. Usually 20 to 30 column volumes are needed to remove solvent and detergent reagents from the IgG bound to the column. In the above-mentioned example for IgSelect affinity resin, 434 runs with a 1,200 L column require 15.6 million L of water, alone for the washing process. The same removal process for S/D reagents will only consume 43 runs*1,200 L*30 = 1,548 million L of water in the ion exchange variant—10 times less. Assuming a water price of 0.37 € per L, just for S/D removal, additional water cost of 5 million € have to be added for the IgSelect process. Considering water is also used for cleaning, regeneration and equilibration, the additional water cost for the IgSelect process compared with ion exchange chromatography would be much higher than those estimated for S/D removal.

6. State of the art IgG preparations and their use

Traditionally human plasma immunoglobulin is mainly used for primary immunodeficiency and severe combined immunodeficiency (Haeney, 1994, Toubi & Etzioni, 2005, Weiler, 2004), but also patients with autoimmune diseases are treated with IgG (Kaveri et al., 1991, Schwartz, 1990). More recently, neurological disorders come into focus (Blaes et al., 1999, Elovaara et al., 2008, Wiles et al., 2002). Currently more than 20 million liters of plasma are fractionated worldwide per year and more than 50 million grams of IgG are manufactured thereof (Buchacher & Iberer, 2006, Burnouf, 2011). These numbers are only one reason to continuously improve productivity and keeping an eye on cost effectiveness.

The safety of the final product is another key aspect which is monitored with increasing attention. During the last decades the control of the donors, the test of the plasma donations and the incorporation of dedicated virus reduction steps into the manufacturing schemes was regularly improved to a very high standard (Furuya et al., 2006, Kreil et al., 2004, Poelsler et al., 2008, Stucki et al., 2008, Trejo et al., 2003) in order to reduce the risk of virus transmission with blood products as often reported in the early days (Bresee et al., 1996, Farrugia & Poulis, 2001).

Very recently, a growing number of reported adverse events for one IGIV product on the market led to the withdrawal of the product and prompted the manufacturer, the authorities and the competitors to investigate the root cause (Roemisch et al., 2011). The investigation resulted in a further requirement for normal human immunoglobulin to show that the manufacturing process has steps incorporated removing the thromboembolic potential from the IgG (as announced by EMEA).

Another actual trend in the immunoglobulin market is the transition from the intravenous (IV) to the subcutaneous (SC) route of administration which allows more flexibility for the patient. This trend is reflected by new IGSC products on the market or in the clinical stage (Jolles et al., 2011, Teschner et al., 2009).

Most of the immunoglobulin products from human plasma are manufactured conventionally combining precipitation and ion exchange chromatography (Ballow et al., 2003, Stein et al., 2009, Teschner et al., 2007), but there are also attempts to introduce new techniques like expanded bed chromatography (Anspach et al., 1999, Barnfield Frej et al., 1997, Hubbuch et al., 2001) or affinity chromatography in the manufacturing of plasma derived therapeutic proteins (Suomela, 1980). In view of the limitations of affinity chromatography the successful implementation is reported for low aboundant plasma proteins (Weber et al., 2011) and hyperimmune IgG preparations (Bryant et al., 2005) which are produced in lower amounts and given at lower doses intramuscularly. Some plasma derived hyperimmune products soon may even be replaced by recombinant products (Frandsen et al., 2011).

Another more general trend is the replacement of IGIV by monoclonal antibodies tailored for specific autoimmune diseases or cancer (Waldmann, 2006) and more recent developments of chimeric molecules and biosimilars (Goldsmith et al., 2007, Kaneko & Niwa, 2011, Wozniak-Knopp et al., 2010). As the number of monoclonal antibodies on the market and their demand is continuously growing, the manufacturers already took the first hurdle and dramatically improved the cell culture yield. The next bottleneck is the

downstream processing of the cell culture supernatant and interestingly with higher volumes and IgG concentrations similar restrictions are encountered as for the plasma fractionation processes shown above (Birch & Racher, 2006).

These developments are supported by efforts undertaken by the producers of chromatography resins to offer new resins with ligands depicting affinity properties combined with a higher stability (Baines et al., 2009, ProMetic Bioscience, 2005, Shi et al., 2009). In a certain respect alkali stable mixed mode resins might be the answer to the challenge as there is a trend to more complex ligand structures resulting in the combination of ionic, hydrophobic hydrogen bounding and thiophilic interaction properties resembling at the end affinity like interactions.

7. Conclusion

Affinity chromatography is known to be a highly efficient purification step for trace proteins and monoclonal IgG. We did a detailed evaluation of the feasibility of this technique for purifying polyclonal human IgG using IgSelect affinity media and Protein G Sepharose FF.

Starting with II+III paste and including solvent-detergent treatment, the main advantage of the two affinity chromatography media investigated was found to be the potential of a single step purification process to reach a high γ-globulin purity (almost 100%) as well as an excellent process efficiency of ≥ 75%. However, we encountered drawbacks with this purification technique during the optimization experiments.

Reusability experiments showed considerable ligand leaching, which resulted in a deterioration of the binding capacity of the resin from run to run. This disadvantage was even more severe as the initial binding capacity was already low at 10 mg IgG/mL affinity resin for the use of IgSelect affinity resin and 20 mg IgG/mL affinity resin for the use of Protein G Sepharose FF. This is five to ten times lower than what is usually seen for ion exchange resins. Even with a 2-fold improvement in the binding capacity or reusability, the affinity chromatography option is more expensive and labor intensive than the classic ion exchange purification technique.

Affinity resins cannot be cleaned with sodium hydroxide without a significant reduction in binding capacities. Cleaning steps with either 0.1%Triton X-100 or PAB were not sufficient to exclude fouling of the resin, as indicated by a pressure rise in our experiments. This emphasizes the importance of developing a potent cleaning step or a more resistant resin. Such a cleaning step is mandatory for resins used in the blood plasma fractionation industry to prevent cross contamination from batch to batch.

Both affinity resins investigated were 300-fold more expensive in terms of resin cost, reusability, and binding capacity and at least 5- to 10-fold more expensive in terms of water consumption than common ion exchange resins, e.g. CM Sepharose FF.

From a large scale perspective, intravenously administrable polyclonal IgG produced from human plasma by one-step affinity chromatography using IgSelect or Protein G Sepharose FF as a single purification step was not found to be feasible in terms of IVIG specifications, ligand leaching and because of the significantly higher cost. However, it is an option for the purification of considerable lower amounts of hyperimmunes from human plasma, suitable for subcutaneous or intramuscular administration.

The key step for the introduction of affinity chromatography into large scale plasma fractionation remains the development of a more stable affinity or pseudo-affinity resin without the loss of specificity.

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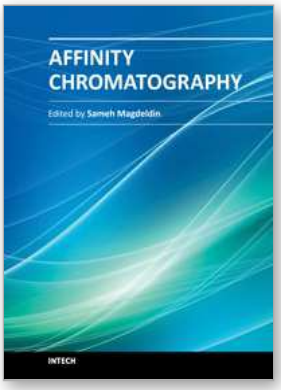

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Most will agree that one major achievement in the bio-separation techniques is affinity chromatography. This coined terminology covers a myriad of separation approaches that relies mainly on reversible adsorption of biomolecules through biospecific interactions on the ligand. Within this book, the authors tried to deliver for you simplified fundamentals of affinity chromatography together with exemplarily applications of this versatile technique. We have always been endeavor to keep the contents of the book crisp and easily comprehensive, hoping that this book will receive an overwhelming interest, deliver benefits and valuable information to the readers.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821