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Comparison of Binding Capacity and Affinity of Monoclonal Antibody towards Different Affinity Resins using High-throughput Chromatography Method

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Abstract: Protein-A Affinity chromatography is the widely used key method for purification of monoclonal antibodies. Selection of a most suitable affinity resin based on binding capacity and affinity is typically performed prior to optimization. Development of high-throughput chromatography method in 96-well filter plate significantly reduced consumption of antibody sample and shortens the experimental time as compared to a typical column chromatography approach. In this study, five different affinity resins were evaluated, rProtein-A FF, MabSelect Sure, ProSep-vA Ultra and two novel synthetically derived affinity ligands immobilized on agarose media, the GF1 and GF2 resins. Resins were dispensed on a 96-well filter plate and antibody sample with different protein concentration was loaded to evaluate resins affinity and static binding capacity. MabSelect Sure, an agarose based matrix with alkaline resistance Protein-A ligand and ProSep-vA Ultra that is a rigid pore glass resin exhibit the highest static binding capacity at ~60-63 mg IgG mL^{-1} of resin. The two novel resins, GF1 and GF2 show moderate binding capacity at ~28-34 mg IgG mL^{-1} of resin. By addition of salts during binding, the capacity of the novel resins was enhanced to ~33-42 mg IgG mL^{-1} resin. Affinity of all evaluated resins was quite comparable. Few other factors for resin selection such as dynamic binding capacity, ligand stability and resistance including resin cost will be briefly discussed.

Key words: Affinity resin, monoclonal antibody, high-throughput chromatography, adsorption isotherm

INTRODUCTION

Affinity chromatography, employing Protein-A molecule as a biospecific ligand, is the most widely used method for capture purification of monoclonal antibodies. Protein-A binds selectively to the F_c part of an antibody where the interaction appears to be characterized by hydrophobic interaction and stabilized by some hydrogen bonds and salt bridges (Vunnum *et al*., 2009). Due to the high selectivity and stability of the ligand, it is the most preferred method incorporated into a standard antibody purification platform. Already in 2007, among the 17 approved monoclonal antibodies in the market for therapeutic application, 16 products were utilizing Protein-A affinity chromatography as the first capture step in their purification process (Shukla *et al*., 2007).

Protein-A affinity resin had been regarded as one of the most expensive raw materials used in production of monoclonal antibody (Shukla *et al*., 2007; Hober *et al*., 2007). Due to the high cost, it is very crucial to maximize the use of this expensive resin. Selection of the suitable Protein-A resins is typically performed prior to further development of the step with a focus to maximize the binding capacity of the antibody to the resins in order to improve productivity and economics of the process. Also due to its high cost, researchers are currently looking at alternatives to this specific resin. Some reported alternative purification methods include cation exchange chromatography, mixed mode chromatography and chromatography using other affinity or mimetic resins (Hober *et al*., 2007; Arunakumari and Wang, 2009; Arnold *et al*., 2011). Other types of affinity ligands which could be synthetically derived such as peptide based ligand will be of great interest if one could demonstrate their purification performance, selectivity and binding capacity is comparable to Protein-A ligand. The great advantage of this type of ligand could be in the reduction of raw material cost contributed from replacing the Protein-A resin with a cheaper ligand immobilized on a standard chromatography matrix.

Investigation and development of affinity chromatography involve several steps, starting with the selection of suitable affinity ligand or resin, binding condition optimization as well as optimization on specific

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removal of impurities due to non-specific binding and antibody elution (Shukla *et al*., 2007). As binding of antibody to affinity resin is driven by hydrophobic interaction between the molecules, the mechanism can be described by an adsorption isotherm to evaluate effect of antibody concentration to the equilibrium binding capacity. From the isotherm, maximum binding capacity under a static condition can be predicted together with information on the resin affinity constant (GE Healthcare, 2009).

A typical approach of performing a chromatography process investigation in a column format is time consuming and requires a significant amount of materials including the monoclonal antibody product. These lead to high development cost contributed from the time spent and the consumption of materials. Introduction of 'high-throughput chromatography operation' in a 96-well filter plate format had provided several advantages. The approach had managed to shorten the investigation timeline as large number of chromatographic experiments can be performed in parallel in the same 96-well filter plate (Coffman *et al*., 2008; Lacki and Brekkan, 2011).

This study was focus on investigating the adsorption isotherm for five different affinity resins that includes commercially available Protein-A resins and novel chemically synthesized affinity ligand immobilized on conventional agarose matrix. The investigation was aimed to provide useful information to select suitable affinity resins based on binding capacity and affinity of the antibody to the specific resin. The high-throughput chromatography operation in 96-well filter plate is to be explored. A review on other factors for affinity resin selection such as dynamic binding capacity, ligand resistance and stability including resin cost and overall production cost was also be discussed.

MATERIALS AND METHODS

Materials: Purified monoclonal antibody, of IgG2 subclass was used throughout the study. The antibody was produced from a CHO cell culture and purified using series of Protein-A, Anion Exchange (AEX) and Cation Exchange (CEX) chromatography. The overall study was performed using a 96-well filter plate (Pall Lifesciences, USA). Commercial Protein-A resins, Protein-A Sepharose FF, MabSelect Sure (GE Healthcare, Sweden), ProSep vA Ultra (Millipore, USA) and two novel resins, GF1 and GF2 (Graffinity GmbH, Germany) were being evaluated. Table 1 provides some properties of the evaluated affinity resins.

Operation of the 96-well filter plate was performed using a vacuum manifold unit (Pall Lifesciences, USA) and a vacuum pump (Millipore, USA).

96-well filter plate preparation: The five resins evaluated were prepared as 20% (v/v) slurry. The 100 μL resin slurries were dispensed manually into the filter plate. Eighteen wells were dedicated per type of resins. The resins were equilibrated three times with binding buffer (20 mM phosphate buffer, pH 7.5) followed by elution buffer (10 mM Sodium Citrate pH 3.3). Finally, resins were re-equilibrated for another three times with binding buffer at 5 min incubation for each step. Plate was incubated on orbital shaker at 900 rpm.

The second filter plate for the binding improvement study was prepared at the same resin slurry concentration. Three different resins were studied and 100 μL resin slurries were dispensed manually into the filter plate, with 28 wells dedicated per type of resin. The plate was equilibrated using dedicated binding buffer.

Adsorption isotherm study: Adsorption isotherm study was performed for three commercially available resins, rProtein-A FF, MabSelect Sure, ProSep-vA Ultra and two novel chemically synthesized affinity ligands immobilized on agarose media, the GF1 and GF2 resins under static conditions. Antibody samples at six different concentrations (0.2, 0.6, 1.2, 1.8, 2.4 and 3.0 mg mL^{-1}) were used to plot the isotherm. The antibody sample was buffer exchanged into 20 mM phosphate buffer, 180 mM NaCl, pH 7.5. The 500 μL of each different concentration samples were loaded to a respective well and incubated for 45 min on the orbital shaker at 900 rpm. Unbound samples were recovered in a UV-transparent collection plate (Corning, No. 3635) by vacuum suction to determined unbound concentration by UV absorbance at 280 nm (EC: 1.35). The filter plate was then

Table 1: Properties of evaluated affinity resins

Resin	Manufacture	Ligand	Matrix	Mean particle diameter (μm)
rProtein-A FF	GE Healthcare	Recombinant protein-A	4% cross linked agarose	90
MabSelect Sure	GE Healthcare	Engineered/ alkaline resistant protein-A	Rigid cross linked agarose	85
ProSep-vA Ultra	Millipore	Recombinant protein-A	Controlled pore glass	75[a]
GF1	Graffinity/GE	Chemical/ small molecule	4% cross linked agarose	90
GF2	Graffinity/GE	Chemical/ small molecule	4% cross linked agarose	90

[a]Irregular size/particle diameter

washed with binding buffer, followed by elution cycle with 10 mM Glycine-HCl, pH 3.3. All filtrate samples were collected for further analysis.

Binding improvement for GF resins: As derived from the adsorption isotherm study, both Graffinity resins showed a lower static binding capacity as compared to the commercially available Protein-A resins. To improve binding of antibody to these resins, addition of salt in the binding buffer and sample was introduced. Five different concentration of sodium chloride (150, 250, 350, 450 and 550 mM NaCl) and five different concentration of sodium sulphate (100, 150, 200, 250 and 300 mM Na_2SO_4) were added to both binding buffer and sample to evaluate the antibody binding. The resins were equilibrated with three times of binding buffer (20 mM phosphate buffer, pH 7.5) containing the respective salts concentration as above, followed by addition of elution buffer, 10 mM Sodium Citrate pH 3.3. Finally, resins were re-equilibrated for another three times with binding buffer at 5 min incubation for each step. Total 500 μL of samples were loaded to each well and incubated at room temperature on an orbital shaker at 900 rpm. After 45 min, unbound was collected and protein concentration was determined by UV absorbance at 280 nm.

RESULTS AND DISCUSSION

Adsorption isotherm: The adsorption isotherm study was performed to evaluate the equilibrium capacity of the antibody adsorb to the affinity resins at a specific antibody concentration. Following incubation of the antibody to the resins, the equilibrium concentration was measured to determine the equilibrium binding capacity of the resins. The equilibrium concentration which is determined from this batch experiments in 96-well plate chromatography method provides a good estimation on the overall adsorption isotherm with very minimal antibody consumption is required throughout the study.

To determine the capacity of the resins, a simple mass balance can be performed according to the following equation:

$$Mass_{bound} = \frac{Mass_{added} - Mass_{unbound}}{V_{sample}\ x\ (C_0 - C_{unbound})} \quad (1)$$

where, mass bound = Capacity (q) × V_{resin}, $C_{unbound}$ = Equilibrium concentration (C_{eq}).

Therefore, capacity (q) can be determined from:

$$Capacity\ (q) = \frac{V_{sample}\ x\ (C_0 - C_{eq})}{V_{resin}} \quad (2)$$

Table 2: Estimated maximum capacity and dissociation constant for evaluated affinity resins

Resin	q_{max} (mg mL^{-1})	K_d (mg mL^{-1})
rProtein-A FF	56.23	0.055
MabSelect Sure	59.61	0.017
ProSep-vA Ultra	63.91	0.042
GF1	34.02	0.172
GF2	28.03	0.340

Having the capacity at the specific equilibrium concentration, a plot of q against C_{eq} was plotted and the data was fitted into Langmuir adsorption isotherm which is the most representative isotherm used to describe protein adsorption on a chromatography media (Hahn *et al*., 2003). The isotherm is as per following equation:

$$q = \frac{q_{max}\ ,\ xC_{eq}}{K_d + C_{eq}} \quad (3)$$

where, q_{max} is the maximum capacity and K_d is the dissociation constant. Figure 1 provides the adsorption isotherm plots for the five different affinity resins and Table 2 lists the Langmuir isotherm parameter estimates based on the plots.

The three commercially available Protein-A resins are having a high equilibrium capacity, with the highest capacity is achieved from ProSep-vA Ultra resin at ~63 mg IgG mL^{-1} of resin. The MabSelect Sure resin with the engineered alkaline-resistance Protein-A ligand, is having about 5% lower equilibrium capacity as compared to ProSep-vA Ultra followed by the widely used rProtein-A FF resin with an equilibrium capacity of ~56 mg IgG mL^{-1} of resin. The two novel resins, each using a different chemically synthesized affinity ligand, GF1 and GF2 showed a lower binding capacity as compared to other evaluated resins, in a range between ~28-34 mg IgG mL^{-1} of resin. As immobilization of both Graffinity ligands to agarose matrix is not yet optimized, this could be one of the causes of the lower capacity.

Affinity of the resins to bind to antibody was assessed from the dissociation constant parameter estimates. As binding in affinity resins is always very specific, the values of K_d can also predict how tight the antibody will bind to the affinity ligand. In general, the smaller the K_d the higher the affinity and binding of antibody to the ligand will be. From the plots, highest affinity was observed from MabSelect Sure resin with ProSep-vA and rProtein-A values of K_d being quite comparable. However, the affinity for both Graffinity resins is also lower with a K_d value of around 0.17-0.34 mg mL^{-1}.

In general, the early screening of affinity chromatography resins by evaluating the adsorption

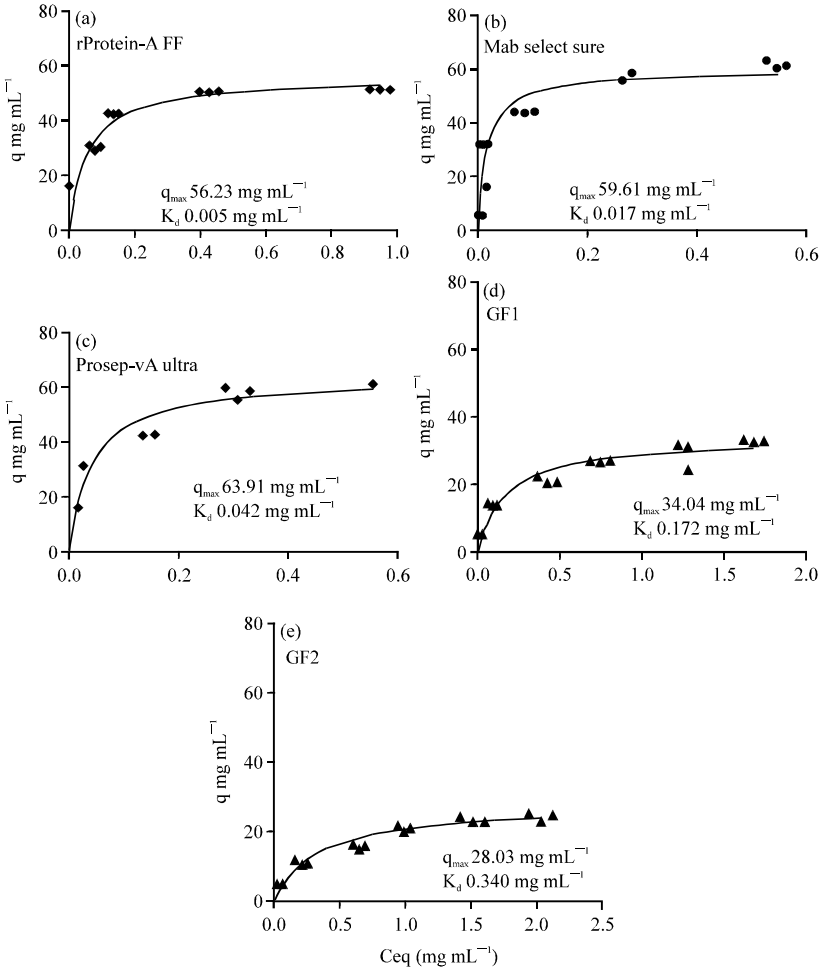

Fig. 1(a-e): The adsorption isotherm plots of monoclonal antibody to different affinity resins, (a) rProtein-A FF, (b) MabSelect Sure, (c) ProSep-vA Ultra, (d) GF1 and (e) GF2 resin

isotherm plots provides useful information on the maximum equilibrium capacity that can be achieved under a static condition. This information helps in selecting the most suitable resin if one is looking at maximizing the productivity rate and economics of the chromatography step. In general, higher binding capacity will help in reducing the amount of required resins to be packed in a column. It will also help in reducing cycle number when a higher amount of antibody can be purified at one time. The information on dissociation constant helps in evaluating the affinity of the monoclonal antibody to bind onto the specific resin. The values predict the minimum concentration of antibody feed solution when half of the maximum binding capacity can be achieved. Also with both parameter estimates from the isotherm, the capacity of a specific affinity resin with effect to feed antibody concentration can be predicted, thus providing an indication on the robustness of specific chromatographic step with variation in feed concentration (GE Healthcare, 2009).

Binding Improvement for GF resins: From the previous adsorption isotherm study, lower equilibrium capacity had been observed for the two novel resins, GF1 and GF2. As binding is characterized by hydrophobic interaction, addition of a certain salts at a certain concentration during binding typically will help in promoting the binding of antibody to the ligand. In this study, the maximum equilibrium capacity of the two resins in comparison with rProtein-A FF resin, was evaluated by overloading the resins (>60 mg IgG mL^{-1} resin) with antibody at high concentration, (Co = 3.0 mg mL^{-1}). The equilibrium capacity is calculated as per previous Eq. 2. The effect of different type of salts, sodium chloride and sodium sulfate at a range of concentration, for the two GF resins and rProtein-A FF resin is shown in Fig. 2.

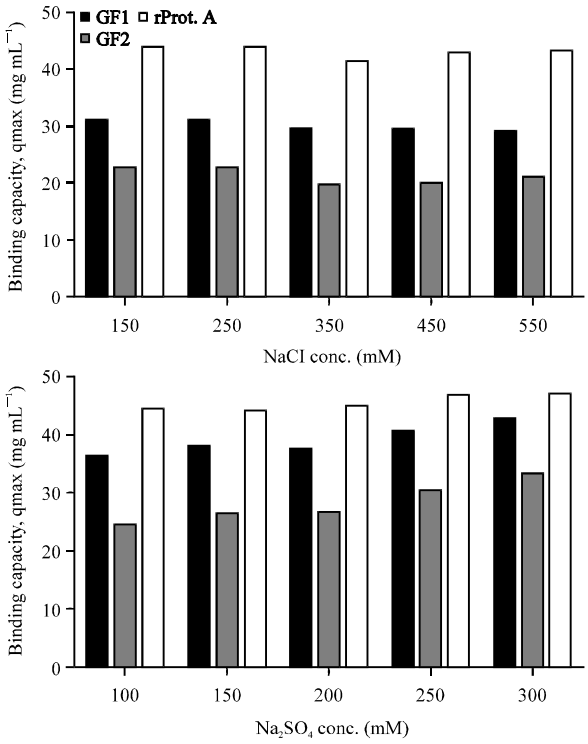

Fig. 2(a-b): Binding capacity of GF1, GF2 and rProtein-A FF resins (a) with addition of NaCl and (b) $NaSO_4$ at different concentration

By addition of sodium chloride (NaCl) salts up to 550 mM, no further increase in binding capacity was observed for all three resins evaluated, where the maximum capacity is observed at 44 mg IgG mL^{-1} resin for rProtein-A FF, 32 mg IgG mL^{-1} resin for GF1 resin and 23 mg IgG mL^{-1} resin for GF2 resin at a lower NaCl concentration. For addition of sodium sulfate (Na_2SO_4) salts at a concentration from 100-300 mM, significant improvement on the binding capacity of both GF resins was observed at high Na_2SO_4, as compared to rProtein-A FF resin. Binding capacity for GF1 resin, at 42 mg IgG mL^{-1} resin is quite comparable to rProtein-A FF resin at 44 mg IgG mL^{-1} resin. The GF2 resin binding capacity had increased to 33 mg IgG mL^{-1} resin, however, still lower than the other two evaluated resins.

In general, the binding condition for a specific affinity resin need to be further investigated if one is targeting to improve the binding capacity. Addition of promoting salts during binding will enhance the hydrophobic interaction between the affinity ligand and the antibody thus will increase the binding capacity. However, in real production sample derived from a cell culture, the non-specific binding of other impurities to the resins will also be affected. Thus it is also very crucial to balance this non-specific binding to a lower level while targeting to maximize the binding capacity.

Other factors for resin selection: Apart from evaluation on adsorption isotherm, several other factors shall be additionally considered when screening for the most suitable affinity resins for purification of a specific monoclonal antibody. Extensive evaluation and review on several affinity resins had been elaborated in many literatures (Vunnum *et al*., 2009; Hober *et al*., 2007; Swinnen *et al*., 2007). Another key factor that has to be considered during resin selection is the dynamic binding capacity beyond the static equilibrium capacity. Studies had showed that dynamic binding capacity is related to the residence time of antibody in a column and is also related to the mass transfer properties of a specific resin, i.e., being basically dependent on particle size and porosity of a resin (Hahn *et al*., 2003; Hahn *et al*., 2005). However, excessive residence time may not be favorable as it will increase the overall process time. Therefore, a more rigid resin that can be operated at shorter residence time with a higher porosity is highly desirable in order to improve dynamic binding capacity.

Factors related to the affinity ligand such as ligand stability and resistance to extreme condition during antibody elution and sanitization is a decisive factor that should be considered for large scale commercial manufacturing. One should also evaluate if a possible leaching of the ligand is observed during the low pH antibody elution as this will add a specific process related

impurities to the product (Hahn *et al*., 2005). Purification performance of a resin over a number of purification cycles, including highly alkaline cleaning and sanitization cycles should also be evaluated to determine the stability or lifetime of a resin (Hahn *et al*., 2006). In general, information on binding capacity, ligand leakage and clearance of specific impurities over purification cycles (e.g., >50 cycles) will provide better information on the resin stability.

Cost of a resin should also be considered during selection as this will have a direct impact on the overall production cost. For rProtein-A FF, the price is approximated at ~USD 10,000 L^{-1} while the improved MabSelect Sure with the engineered ligand is at 50% higher price. For other synthetically derived ligand on standard matrices, the price is at a magnitude lower than other protein-A resins at a range between USD 7,000 L^{-1} to USD 8,000 L^{-1}. For a very large monoclonal antibody production, significant savings could be obtained from the reduction in this raw material price. However in general, the overall economic of the affinity chromatography process will still rely on other factors during the process such as buffer consumption, number of cycles, including cost on the specific chemicals required for ligand regeneration.

CONCLUSION

Useful information on maximum binding capacity and affinity of a specific affinity resins can be determined by evaluating the adsorption isotherm plots. This information that are obtained under a static condition from high-throughput chromatography method in 96 well filter plates, provides a representative information during early screening of several resins prior to selection of potential resin for further development. Also by optimizing binding condition by addition of salts to promote hydrophobic interaction, one should be able to improve the binding capacity of a certain affinity resin. However, other factors should also be considered when selecting the most suitable resin. Further evaluation on dynamic binding capacity in relation to specific resin properties such as rigidity and porosity, resin resistance and stability throughout a number of purification cycles including a review on resin cost will provide a better guide for resin selection.

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