Protein A Affinity Column for Monoclonal Antibody (MAb) Titer Analysis

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Overview

Purpose: Demonstrate the capability of a prototype Protein A column for titer analysis of monoclonal antibodies (MAbs).

Methods: The prototype Protein A column was analyzed on a HPLC hybrid system. Chromatographic conditions such as flow rate, column temperature, and sample loadings were tested to evaluate the column performance.

Results: The prototype Protein A column can accurately determine the MAb titer in the range of 0.025 mg/mL to 5 mg/mL. The total analysis time is less than two min.

Introduction

Early in the development of recombinant MAbs, a large number of harvest cell culture (HCC) samples must be screened for IgG titer. Affinity chromatography employing a Protein A ligand is often used to determine the MAb concentration as well as to purify it for downstream aggregate and charge variant analysis. The challenge facing the analytical laboratories in the pharmaceutical industry is to develop a high-throughput and robust titer assay.

In the current study, a prototype Protein A column for fast MAb titer analysis is presented. This prototype Protein A column was developed based on a novel polymeric resin. The hydrophilic surface has been designed to accommodate protein conjugation. A recombinant Protein A ligand has been covalently attached onto the hydrophilic resin surface. The functionalized resin with recombinant Protein A is then packed into a 4 × 35 mm PEEK™ column body.

The hydrophilic nature of the backbone minimizes nonspecific binding, and therefore enables accurate quantification of the MAb titer. In addition, the small particle size of the resin produces a highly efficient column. When injecting 20 µg of rabbit IgG, the IgG peak width at half height is about 0.01 min. The sharp peak shape also provides great sensitivity. As little as 0.25 µg of MAb can be easily detected. The prototype Protein A column has very low back pressure to allow a high flow rate, and therefore fast analysis. At 2.5 mL/min, the entire analysis, including equilibration, takes only 1.6 min. Ruggedness testing shows that this column can go through more than 2,000 cycles with very little loss of performance. The HPLC compatibility of this column allows automation while providing accurate and high throughput analysis.

Methods

Sample Preparation

MAb harvest cell culture was a gift from a local biotech company. The HCC was filtered through a 0.22 µm membrane prior to sample injection.

Columns

Prototype Protein A column, 12 µm, 4 × 35 mm

Buffers

Eluent A: 50mM Sodium Phosphate, 150 mM NaCl, 5% acetonitrile, pH 7.5
Eluent B: 50mM Sodium Phosphate, 150 mM NaCl, 5% acetonitrile, pH 2.5

Liquid Chromatography

HPLC experiments were carried out using a hybrid system equipped with:

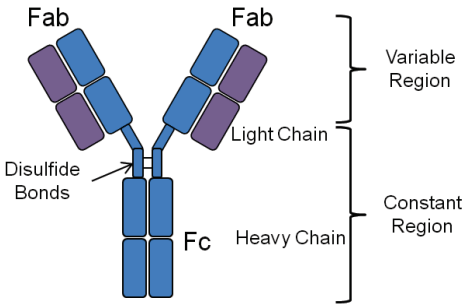
- Thermo Scientific Dionex ICS-3000 Dual Gradient Pump System
- Thermo Scientific Dionex TCC-100 Thermostatted Column Compartment
- Thermo Scientific WPS-3000 Pull-Loop AutoSampler
- Thermo Scientific Dionex VWD-3400RS UV Detector equipped with a 2.5 µl Micro Flow Cell

Results

Staphylococcal protein A (SPA) plays an important role in immunology and biochemistry, owing to its specific interaction with the Fc part of immunoglobulin G (IgG) from many mammals. SPA is a cell-wall-associated protein domain exposed on the surface of the gram-positive bacterium, Staphylococcus aureus. SPA consists of three different regions: S is the signal sequence that is processed during secretion; the five homologous IgG binding domains are E, D, A, B, and C; and a cell wall anchoring region is XM. SPA and the smaller ligands derived from SPA have been used widely for the affinity purification of antibodies.[1]

Recombinant Protein A (rSPA) is expressed in *E. coli* as opposed to the native protein extracted from Staphylococcus aureus. rSPA is a 45 kDa protein containing the same amino acid sequence and molecular mass as the native Protein A sourced from S. aureus. rSPA is used as the affinity ligand for the prototype Protein A column.

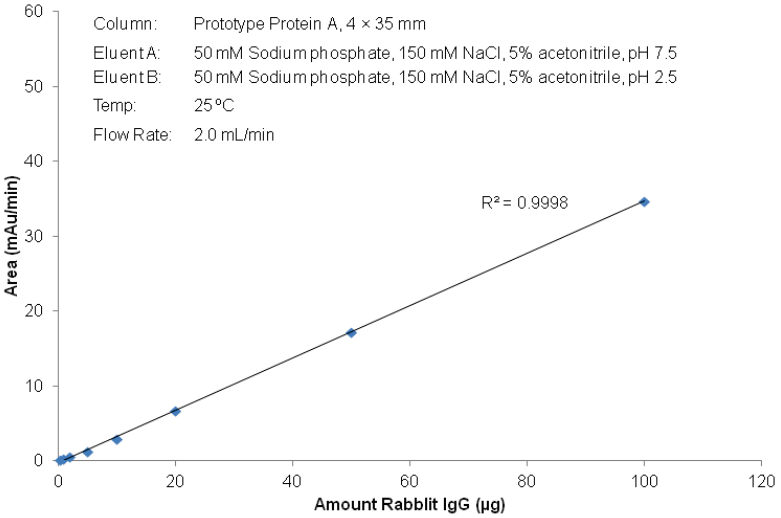
FIGURE 1. Schematic of an antibody showing the fragment crystallizable region (Fc) and the fragment antigen binding region.



Dynamic Loading Capacity

The dynamic loading capacity of rabbit IgG, isolated from pooled normal serum, is no less than 100 µg on the prototype Protein A column, analyzed at a flow rate of 2 mL/min. Figure 2 shows the linearity of area to sample load for rabbit IgG when loaded onto the prototype Protein A column. This correlation allows the prototype Protein A column to be used for quantitation of MAb in harvest cell culture over a wide range of concentrations.

FIGURE 2. Area dependence on rabbit IgG Loading. Gradient: 0% B for 0.2 min, 100% B for 0.6 min, 0% B for 1.2 min.



Influence of Flow Rate on Column Pressure, Cycle Time, and Peak Area

The prototype Protein A column can be used at a flow rate up to 2.5 mL/min. The recommended flow rate is 2 mL/min. Figure 3 shows the effect on increasing flow rate on column backpressure. Figure 4 shows that as you increase the flow rate there is little effect on the amount of IgG that binds to the column. The increase in total area (Table 1) is due to the use of the same data collection rate of the detector.

FIGURE 3. Example of effect of flow rate on column backpressure.

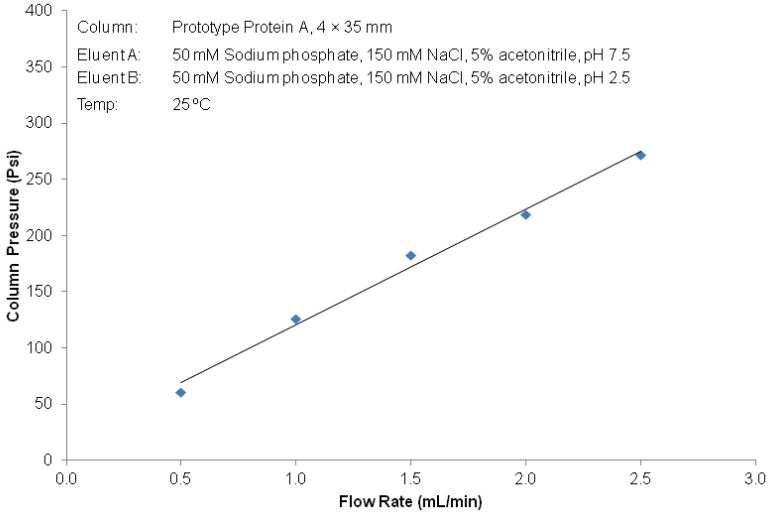


FIGURE 4. Effect of flow rate on IgG binding.

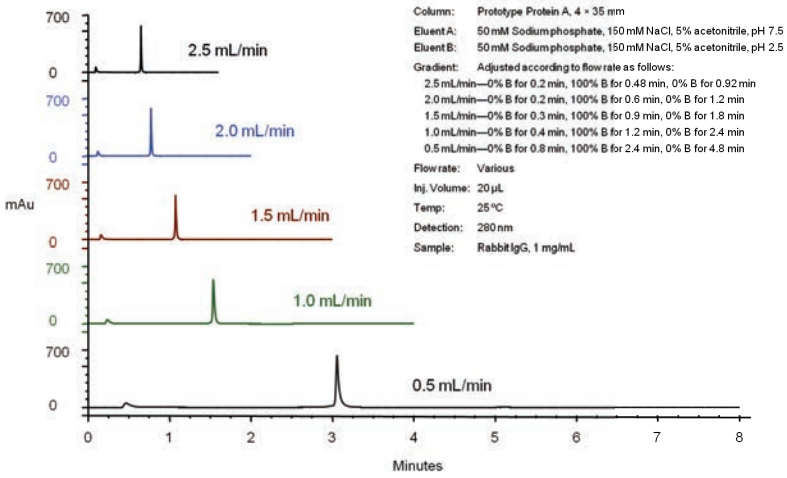


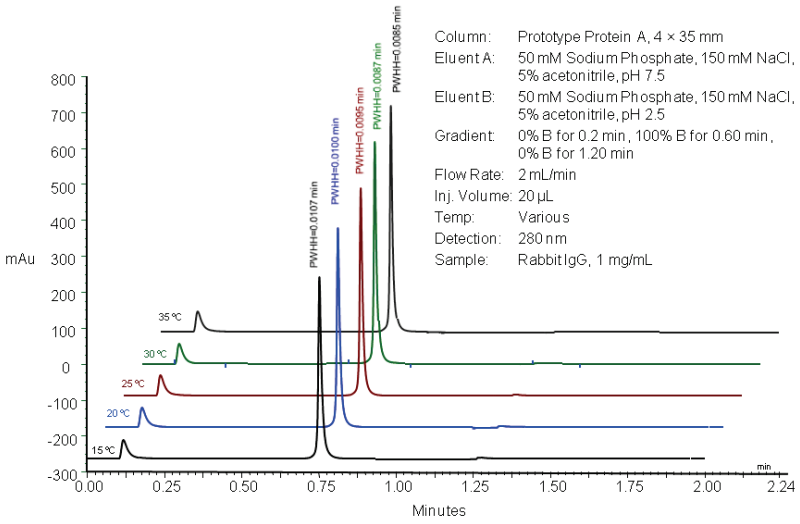
Table1. Effect of flow rate on IgG peak area.

Flow Rate (mL/min)	Total Area (mAu/min)	Unbound Area (mAu/min)	Unbound Relative Area (%)	IgG Area (mAU/min)	IgG Relative Area (%)
2.5	7.381	1.196	16.20	6.185	83.80
2.0	8.826	1.419	16.08	7.407	83.92
1.5	12.111	2.068	17.08	10.043	82.92
1.0	17.895	2.937	16.41	14.958	83.59
0.5	34.219	5.582	16.31	28.637	83.69

Influence of Temperature on Binding Efficiency

It is recommended that the prototype Protein A column be used at temperatures no higher than 35 ˚C. To ensure data consistency and to prolong the column lifetime, it is recommended that a column oven be used to control the temperature to 25 ˚C. Figure 5 shows how rabbit IgG binding is minimally affected by temperature. Proteins with different binding association may be affected differently.

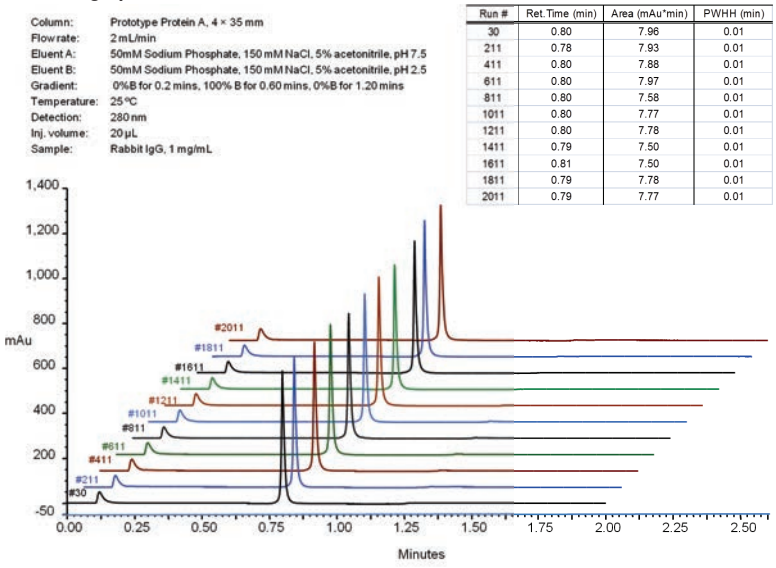
FIGURE 5. Temperature effect on binding efficiency.



Column Ruggedness

The prototype Protein A column has been tested continuously for 2,000 cycles. Every hundred cycles, a set of calibration standards (from 0.01 mg/mL to 5 mg/mL) was analyzed. As shown in Figure 6, the retention time, peak area, and peak width of IgG remain unchanged. In the upper range, there is no loss of binding capacity and in the lower range sensitivity is maintained.

FIGURE 6. Chromatograms of rabbit IgG analyzed on the prototype Protein A column. 20 µg rabbit IgG was injected every 100 cycles in the course of 2,000 runs. Retention time, peak area, and peak width of IgG from each chromatographic run are listed on the inserted table.

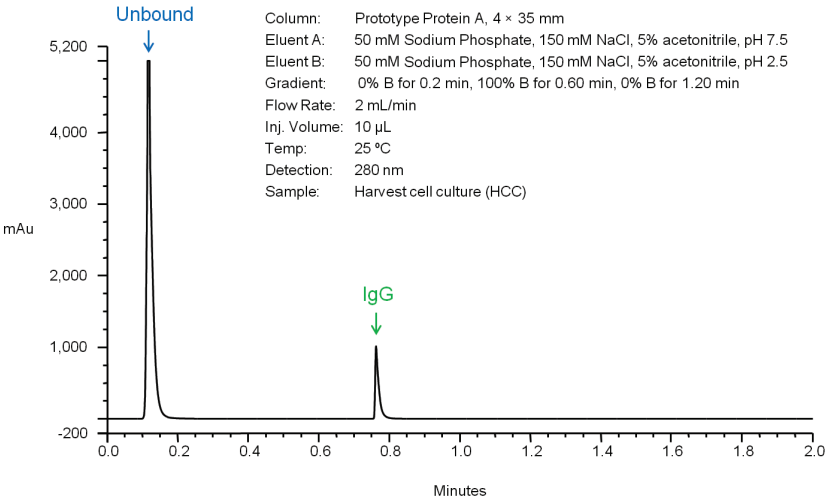


Run #	Ret.Time (min)	Area (mAu*min)	PWHH (min)
30	0.80	7.96	0.01
211	0.78	7.93	0.01
411	0.80	7.88	0.01
611	0.80	7.97	0.01
811	0.80	7.58	0.01
1011	0.80	7.77	0.01
1211	0.80	7.78	0.01
1411	0.79	7.50	0.01
1611	0.81	7.50	0.01
1811	0.79	7.78	0.01
2011	0.79	7.77	0.01

Determination of MAb titer of harvest cell culture

The prototype Protein A column is used to measure the MAb titer from harvest cell culture (HCC). In the chromatogram below, 10 µL of HCC sample was injected onto prototype Protein A column. A large peak elutes in the initial (binding) portion of the method and represents unbound material. The MAb was released using a low pH wash at pH 2.5. The MAb titer is determined to be about 2 mg/mL using a calibration curve previously generated (such as the example in Figure 2) and the integrated IgG peak area (Figure 7).

FIGURE 7. Analysis of HCC on the prototype Protein A (4 × 35 mm) column.



Conclusions

- The prototype Protein A column has a dynamic loading capacity of at least 100 µg. It is capable of quantifying MAbs in the range of 0.01 mg/mL to 5 mg/mL.
- The prototype Protein A column has a fast cycle time. At 2 mL/min, a complete titer analysis takes 2 min.
- The prototype Protein A column has been successfully tested through 2,000 cycles without loss of binding capacity.

References

1. Hober, S.; Nord, K.; Linhult, M. Protein A Chromatography for Antibody Purification. *J. Chromatogr. B*, **2007**, *848*, 40–47.

Acknowledgements

We would like to thank Dr. Wim Decrop at Thermo Fisher Scientific for internal testing.

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