High Speed Monolithic Assays for IgM Quantitation in Cell Culture Production and Purification Process Monitoring

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Introduction

A number of IgM monoclonal antibodies are currently in development for treatment of autoimmune disease, infectious disease, and cancer. Growing interest in these molecules has created a need for an accurate, rapid, simple analytical method to measure IgM levels in cell culture supernatants, and to document the distribution of IgM and protein contaminants in chromatography fractions. High performance protein A columns are used for this application with IgG monoclonals, but IgMs are easily denatured by the harsh conditions required for elution of most affinity ligands. However, IgM monoclonals often exhibit strong retention on either cation exchangers, or anion exchangers, or both, making ion exchange chromatography a potential candidate for this application.

The large size of IgMs makes them a major challenge to particle-based chromatography media. Pentameric IgM has a mass of about 0.96 Md, and hexameric IgM about 1.15 Md. Their diffusion constants are about 2.5 x10^{-7} cm^2/sec, about twice as slow as IgG. Since particle-based chromatography media mostly rely on diffusion for mass transport, both resolution and capacity are impaired, and increasingly so at higher flow rates.

Monolithic ion exchangers are characterized by an interconnected system of channels with diameters ranging 0.5 to 2.0 microns. This pore architecture supports convective flow, which conserves high resolution at high flow rates.[1] The lack of a void volume removes the major source of dispersion in chromatographic systems. This contributes to sharper peaks, which improves both resolution and sensitivity. Capacity is also conserved at high flow rates. This permits use of a microcolumn format that minimizes assay time and buffer consumption. This combination of features should make monoliths effective analytical tools for IgM.

Materials and methods

All chromatography was conducted on an AKTA Explorer 100 (GE Healthcare). Mouse IgM cell culture supernatant and hydroxypatite chromatography fractions were chromatographed on 76 μL analytical CIM® QA (anion exchange) and SO_3 (cation exchange) monoliths (4.4 x 5.0 mm, BIA Separations) run at 2 mL/min (789 cm/hr). Ceramic hydroxyapatite CHT™ type II 40 μm (Bio-Rad Laboratories) was packed into MediaScout® minichrom columns (ATOLL). See text and Figure legends for details.

Results and Discussion

Anion and cation exchange monoliths were both evaluated for this application. The IgM eluted in a cluster of contaminants on the cation exchanger so it was not considered further. Cation exchange has proven useful with other IgMs but anion exchange was better suited to this particular monoclonal. Figure 1 illustrates

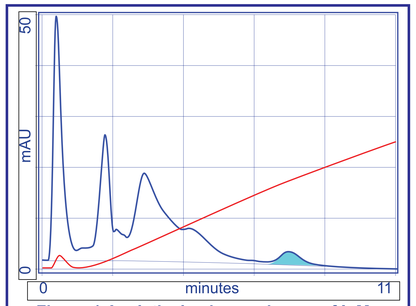

Figure 1.Analytical anion exchange of IgM cell cupernatant. Equilibrate 10 mM sodium phosphate pH 7.0. Inject 100 µL sample. Wash 1 minute 10 mM sodium phosphate. Elute 10 minute (20 mL) linear gradient to 250 mM sodium phosphate. IgM highlighted in blue.

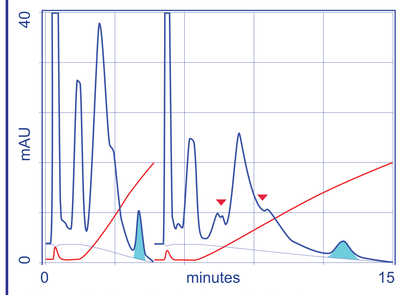

Figure 2. The effect of gradient volume on resolution and sensitivity. Same buffers as Figure 1 but both profiles include a longer pre-column wash, and the first profile shows a 5 minute (10 mL) linear gradient. Red triangles highlight better discrimination in the longer gradient.

Figure 3. Anion exchange analysis of hydroxyapatite chromatography fractions (above right). Same conditions as Figure 1. Sample A: cell culture supernatant, B: flow-through from sample load, C: Pre-elution wash, D: IgM elution peak, E: post-elution cleaning. Results confirm that all of the IgM bound to the hydroxyapatite column, that it remained bound during the wash, and that it was fully eluted in the gradient. Additional chromatograms from this series were used to determine which fractions to pool, based on the proportion of IgM to contaminating proteins. The buffer conditions used for this evaluation are suitable for anion and cation exchange screening of most IgMs.

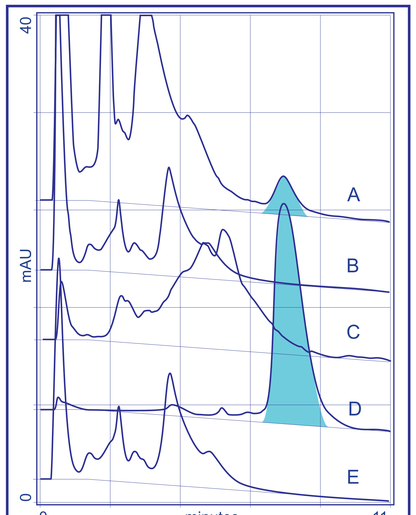

Figure 3. Analytical anion exchange analysis of hydroxyapatite chromatography fractions See legend below left.

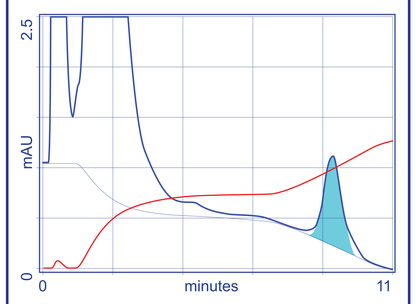

Figure 4. Analytical anion exchange gradient for analyzing cell culture supernatant. Equilibrate 10 mM sodium phosphate pH 7.0. Inject 100 µL dilute IgM cell supernatant. Wash 1 minute 10 mM sodium phosphate. Wash 5 minutes 100 mM sodium phosphate. Elute, 3 minute linear gradient to 250 mM phosphate. Wash 2 minutes 250 mM phosphate. This approach is useful when it is necessary to isolate the product peak as much as possible, but it needs to be customized to each monoclonal. Gradient volume could have been reduced with the combined benefits of higher sensitivity, reduced analysis time, and reduced buffer consumption.

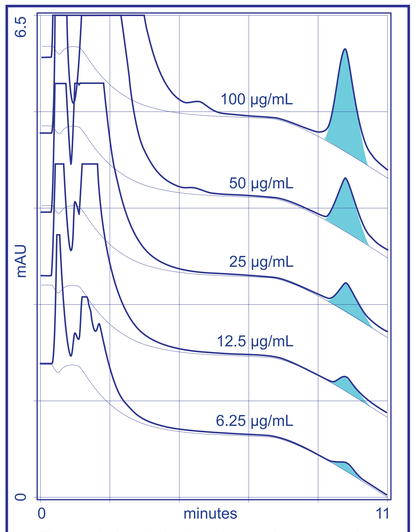

Figure 5. Analytical anion exchange analysis of IgM cell culture supernatant dilutions. Same conditions as Figure 4. Note that the baseline follows an inverse trace of the conductivity profile. This is from differential UV absorbance by the low and high salt buffers. Reducing the gradient volume would increase sensitivity, permit detection with less sensitivity, and proportionately reduce the baseline effect.

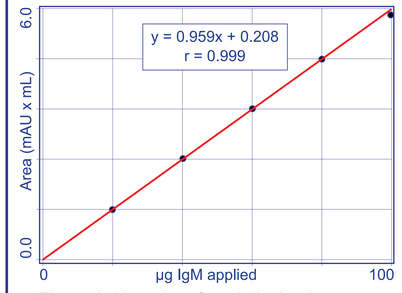

Figure 6. Linearity of analytical anion exchange assay. Peak area data from Figure 5. The linearity and intercept suggest the feasibility of single-point calibration. This would add to the utility of the assay for quick convenient quantitation of IgM in cell culture supernatants during cell line development and for monitoring cell culture production.

fractionation of cell supernatant. The IgM eluted in a distinct peak, well isolated from contaminants. Figure 2 illustrates the effect of gradient volume on resolution and sensitivity. Sensitivity is higher in the shorter gradient, but adequate in the longer gradient, and the longer gradient discriminates more proteins. The longer gradient also highlights the low dispersion of monoliths: separation performance and detectability are maintained in a 262 column volume gradient, despite a bed height of only 5 mm. Figure 3 illustrates the application of this method to a series of hydroxyapatite chromatography fractions. Results quickly confirmed that the IgM was present in the fractions where it was expected, and absent from other fractions. The ability to perform this assay with the same instrument and buffers used for process development allows developers to obtain immediate feedback, without the delay of sending samples for analysis by PAGE or ELISA.

Figure 4 illustrates a modified anion exchange gradient configuration for monitoring the amount of IgM expressed in cell culture supernatants. A wash step was introduced to better remove contaminants from the region of IgM elution to allow more accurate quantitiation. Figure 5 illustrates results from a 2-fold dilution series from cell culture supernatant. The IgM concentration was estimated by ELISA to be about 100 μg/mL. IgM peak areas were integrated and plotted in Figure 6. The linear range of the assay easily covers the production ranges necessary to accommodate developmental and manufacturing cell cultures.

Conclusions

Experimental results demonstrate the utility of monolithic assays for monitoring product and contaminant distribution in chromatography fractions. Data documenting that the purification process is operating within specifications can be obtained much more rapidly than by PAGE or immunological assays. Such data may also be valuable for purification process characterization during early development, and later for process validation. The sensitivity, linearity, accuracy, and speed of the system are also well suited for IgM quantitation of cell cuture supernatants.

Literature cited

1. Strancar et al, 2002, *Adv. Biochem. Eng. Biotechnol.*, **76** *50*

Acknowledgements

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