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Capillary size exclusion chromatography with picogram sensitivity for analysis of monoclonal antibodies purified from harvested cell culture fluid

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ABSTRACT

Size exclusion chromatography (SEC) is widely used in the characterization and quality control of therapeutic proteins to detect aggregates. Aggregation is a carefully monitored quality attribute from the earliest stages of clinical development owing to the possibility of eliciting an immunogenic response in the patient. During early stage molecule assessment for cell culture production, small-scale screening experiments are performed to permit rapid turn-around of results so as to not delay timelines. We report the development of a capillary SEC methodology for preliminary molecule assessment to support the evaluation of therapeutic candidates at an early stage of development. By making several key modifications to a commercially available liquid chromatography system, we demonstrate a platform process to perform capillary SEC with excellent precision, picogram sensitivity and good ruggedness. The limit of quantitation was determined to be approximately 15 pg; picogram sensitivity for SEC analysis of monoclonal antibodies had not been achieved prior to this work. In addition, we have developed a method to capture low levels of antibody (1 μg/mL) from harvested cell culture fluid (HCCF) for capillary SEC analysis. Up to 40% recovery efficiency was achieved using this micro-recovery method on 3 mL HCCF samples. Using early stage cell culture transient transfection samples, which typically have much lower titers than stable cell line samples, we demonstrate a consistent method for analyzing aggregates in low protein concentration HCCF samples for molecule assessment and early stage candidate screening.

1. Introduction

Monoclonal antibodies (mAbs) are a very important class of therapeutic proteins in the biopharmaceutical industry, and have been developed to treat a variety of indications to fulfill significant unmet medical needs [1]. Monoclonal antibodies are generally target-specific and well tolerated with a relatively long half-life, contributing to the success of the molecule class for drug development [2].

Recombinant monoclonal antibodies are typically produced in mammalian cell lines under defined cell culture conditions. In order to develop the most productive cell culture processes for biopharmaceutical manufacture, many factors are investigated such as molecule sequence, expression host and vector, transfection method, culture media components and process parameters [3,4]. The selected conditions would have optimal production levels of product with the desired profile of product quality attributes [5,6]. Molecules that are difficult to express or have undesirable quality attributes can become resource sinks and delay timelines if not removed from the development process early [7]. Small-scale cell culture studies performed early in the development process can potentially uncover problematic cell culture conditions or poorly expressing molecules, which can be re-engineered prior to scale-up.

Two attributes that are typically deemed the most critical in selecting stable clones and cell culture conditions are the titer and the aggregation level of the protein. Production titers directly correlate to the cost of the process and are desired to be as high as possible without affecting other quality attributes of the product [8]. Aggregates are a carefully monitored product impurity from the earliest stages of clinical development, as aggregates may pose a safety risk to the patient due to the potential for immunogenic

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response [9]. Aggregation has been documented as a critical quality attribute and should be carefully controlled [10].

Antibody products are characterized by physicochemical, immunochemical, and biological methods. Many of the recommended protein characterization assays are based on liquid chromatography methods, such as size exclusion chromatography (SEC) for size heterogeneity [11]. Typical analytical columns for mAb analysis range from 1 mm to 7.8 mm in diameter and require >5 μg of mAb for analysis. However, microwell plate cell culture studies often yield less than 5 μg mAb. Thus, these small-scale cell culture experiments do not yield enough material for conventional scale analysis.

Capillary SEC has been demonstrated using proteins and peptides with molecular weights of less than 70 kDa using highly customized equipment [12,13]. We report herein the development of a capillary SEC methodology with picogram detection limits for the characterization of monoclonal antibodies (~150 kDa) purified from cell culture fluid. In part, we developed a micro-recovery method to extract low abundance antibodies from cell culture fluid obtained from microwell plate molecule assessment studies. Importantly, we made several key modifications to a commercially available liquid chromatography system to reduce the system dead volume, which is critical to reduce band broadening and enable capillary SEC separation. The results herein demonstrate the precision, ruggedness and applicability of this micro SEC method, which can be used to recover and analyze low abundance antibodies in biological matrices.

2. Experimental

2.1. Instrumentation

Chromatographic experiments were performed on a Dionex (Sunnyvale, CA) Ultimate 3000 Nano/Cap/Micro biocompatible liquid chromatography system. Components of the system included dual ternary low-pressure gradient pumps, an autosampler with sample temperature control capability, and a four channel UV–vis detector. Instrument control, data acquisition and compilation of results were performed using Dionex Chromeleon software, version 6.8.

For the capillary chromatography, the chromatography system was equipped with a Dionex FLM-3000 flow manager, configured with a flow splitter cartridge (1:1000 split ratio). The capillary columns were connected directly to a four-port Valco injector (Houston, TX) containing a 20 nL internal loop. The detector contained a Dionex 45 nL, 1 cm path length flow cell. Further operational parameters of the capillary chromatograph were optimized and are discussed in Section 3.

An Eppendorf (Hauppauge, NY) micro-centrifuge capable of 14,000 × *g* was used to perform all of the centrifugation steps. A Fisher Scientific (Pittsburgh, PA) vortex mixer, equipped with a micro-well plate foam rack, was used for the mixing of the extraction beads with the harvested cell culture fluid (HCCF) samples.

2.2. Columns, chemicals and equipment

Capillary SEC columns (300 μm × 300 mm,) containing Tosoh Biosciences (Montgomery, PA) TSKgel Super SW3000 stationary phase (4 μm particle size, 250 Å mean pore size) were obtained from Dionex Benelux (Amsterdam, The Netherlands). The capillary columns were fitted with a fused silica capillary outlet (20 μm ID) for connecting the column outlet to the detector. Conventional scale SEC columns containing Tosoh Biosciences TSKgel Super SW3000 stationary phase of dimensions 1 × 300 mm and 4.6 × 300 mm were obtained from Tosoh Biosciences.

Table 1
Summary of mobile phases and buffers used in this work.

Buffer	Composition
SEC mobile phase	200 mM potassium phosphate, 250 mM potassium chloride, pH 6.2
Low salt wash – Protein A	25 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 7.1
High salt wash – Protein A	0.4 M potassium phosphate, pH 7.0
Elution – Protein A	100 mM acetic acid, pH 2.9
Neutralizing – Protein A	1 M HEPES, pH 7.3

Sodium hydroxide, potassium chloride, ethylenediaminetetraacetic acid (EDTA), and acetic acid were obtained from Mallinckrodt (Phillipsburg, NJ). Trizma HCl, Trizma base, potassium phosphate (mono- and dibasic), and glycine were Sigma brand reagents (St. Louis, MO). 4-(2-hydroxyethyl)-1-Piperazineethanesulfonic acid (HEPES) was obtained from Cellgro (Manassas, VA). All other common materials were obtained from Fisher Scientific (Waltham, MA) unless otherwise indicated.

Consumables used for the antibody extraction include: Millipore (Billerca, MA) ProSep vA Protein A resin, Pierce (Rockford, IL) micro-spin columns to retain the Protein A resin, Becton Dickenson (Franklin Lakes, NJ) 12-well polypropylene culture plates, 3 M (St. Paul, MN) microplate sealing tape, 2 mL microcentrifuge tubes obtained from E&K Scientific Products (Santa Clara, CA), Pall (Port Washington, NY) Nanosep 30 kDa MWCO centrifugal devices, and Waters Corporation (Milford, MA) maximum recovery HPLC vials.

Monoclonal antibodies (mAbs) and cell culture samples used in this work were produced in-house at Genentech (South San Francisco, CA).

2.3. Mobile phase and buffer preparation

Appropriate amounts of buffer components were dissolved in de-ionized water equal to 90% of the final target volume of the solution. Once dissolved, the solution was titrated to the appropriate pH by the addition of 10 N sodium hydroxide, as necessary. Once titrated, de-ionized water was added to bring the solutions to the required volume. The buffers were then individually filtered through a fresh 0.2 μm nylon filter prior to use. Mobile phases and buffers prepared for this work are listed in Table 1.

2.4. Protein A affinity antibody extraction

To recover the mAbs from harvested cell culture fluid (HCCF), which is cell culture fluid separated from the cells, frozen HCCF samples were thawed at room temperature and mixed briefly. Samples (3 mL HCCF containing antibody) were transferred to each well of a 12-well culture plate. ProSep vA Protein A resin (40 μL slurry, supplied in 0.1 M acetate buffer, 1% benzyl alcohol, pH 5.2) was added to each well. The plate was then sealed with a microwell plate tape sheet and was agitated on a vortex mixer for 25 min. A foam microwell plate adapter secured the plate on the vortex mixer. The vortexer was set at an appropriate speed (~850 rpm) to ensure that a vortex was established in each well, which avoided any settling of the resin and ensured good mixing.

After this binding step, the sealing tape was removed and approximately 600 μL of sample with Prosep vA resin was transferred to a microspin column, which was placed in a 2 mL microcentrifuge tube. The microspin columns were centrifuged for 10 s at 1000 × *g*. Flow through was discarded while the Protein A resin was retained in the spin column. This transfer step was repeated until the total volume present in each well of the 12-well plate, including resin and HCCF, was transferred from the 12-well plate to the spin columns.

The resin was then washed by adding 500 μL of low salt wash buffer to the resin, centrifuging the column for 10 s at 1000 × *g*, and discarding the flow through. This low salt wash step was repeated.

After the low salt wash step, the resin was washed by adding 500 μL of high salt wash buffer to the resin, centrifuging the column for 10 s at 1000 × *g*, and discarding the flow through. This high salt wash step was repeated. After the high salt wash step, the resin was washed further by following the low salt wash step described above.

To elute the protein from the resin, 300 μL of elution buffer was added to the spin column. The column was allowed to incubate for 5 min at room temperature. After incubation, the column was centrifuged for 10 s at 1000 × *g* in a new 2 mL micro-centrifuge tube containing 60 μL of neutralization buffer. After this recovery step, 100 μL of elution buffer was added to the resin and the column was centrifuged for 10 s at 1000 × *g* into the same collection tube.

To concentrate the samples for capillary SEC analysis, eluted samples were added to Nanosep centrifugal spin units (MWCO 30 kDa), then centrifuged for 25 min at 14,000 × *g*. To recover the protein, 5 μL of SEC mobile phase was added directly to the Nanosep filter. The filter unit was gently agitated on a vortex mixer for 30 s. The sample was then recovered from the membrane filter using a micropipette and transferred to a maximum recovery HPLC vial for subsequent capillary SEC analysis. This centrifugation step was designed to remove all neutralized elution buffer, so that the total sample volume in the HPLC vial would be approximately 5 μL in SEC mobile phase.

2.5. Chromatographic procedures

Monoclonal antibody samples were kept at a temperature of 5 °C ± 3 °C in the auto-sampler. The columns were kept at ambient temperature. The capillary column effluent was monitored at 214 nm. For other column scales, the column effluent was monitored at 280 nm. Monitoring at 214 nm for capillary SEC, as opposed to 280 nm, increased signal-to-noise and resulted in better assay consistency. Flow rates and injection volumes were approximately scaled linearly according to the nominal column cross-sectional area. Injection volumes for 1 mm and 4.6 mm ID columns were 1 μL and 20 μL, respectively. For 1 mm × 300 mm columns, the flow rate was set to 16 μL min^{-1}. For 4.6 mm × 300 mm columns, the flow rate was set to 250 μL min^{-1}. For the 300 μm ID columns, the flow rate through the column was set at 1 μL min^{-1}. Injection volumes of 20 nL were used throughout the studies for the capillary SEC column.

A sample matrix injection as a blank was performed with each sequence prior to sample injection. Furthermore, bracketing control samples of mAb reference material were run with each sequence to monitor system performance. After the installation of a new column, conditioning runs were performed until consistent profiles were achieved. Each chromatogram was carefully integrated to ensure that only peaks not present in the associated blank were considered to be protein. The size exclusion profiles were divided into two distinct components: the main peak (monomer) and the high molecular weight species (HMWS), which represent aggregates. A typical chromatogram (mAb1 reference standard) is shown in Fig. 1. Low molecular weight species (LMWS), if present, were included with the main peak. For HMWS and main peak, the percent peak area was calculated per Eq. (1).

$$\% \text{ Component} = \frac{\text{Component peak area}}{\text{Total peak area}} \times 100 \quad (1)$$

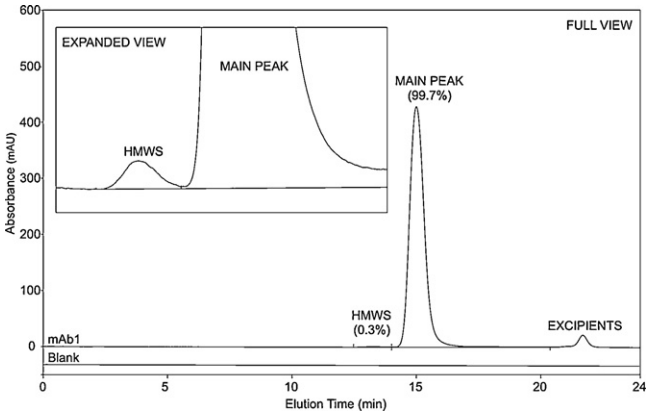


Fig. 1. Typical profile obtained using capillary SEC for a monoclonal antibody (mAb1). Main peak and high molecular weight species (HMWS) are denoted. A protein amount of 20 ng (20 nL) was injected onto the column, using a mobile phase flow rate of 1 μL min^{-1}. Column effluent was monitored at 214 nm. A representative integration is shown, and relative peak area percentages for main peak and HMWS are in parentheses.

3. Results and discussion

3.1. Chromatograph modifications

In this work, capillary SEC was developed to analyze low amounts of monoclonal antibody in HCCF from transiently transfected cells. To achieve this, several challenges were addressed that were specific to SEC; since SEC separation is primarily based on size, resolution is not readily improved through the use of mobile phase modifiers. Several modifications were made to the commercially available liquid chromatograph to perform the capillary SEC analysis. First, the factory fitted six-port valve was replaced by a four-port valve with a 20 nL internal loop in order to minimize the amount of sample needed for analysis. The bore size on the valve was 150 μm as opposed to the factory-installed valve, which had a bore size of 250 μm in diameter. Second, a fused silica sample transfer line with a 50 μm ID was installed in the auto-sampler to minimize the needle volume (∼0.55 μL). The tubing hold up volume of the injector was thus minimized, and a low draw speed (20 nL/s) was used to accurately draw nanoliter volumes without incurring prohibitively long injection cycle durations. Finally, the fused silica connecting capillary from the column outlet to the detector had a nominal inner diameter of 20 μm, as opposed to a more typical inner diameter of 50 μm, thus minimizing post column band spreading as much as practically possible.

Several detector cells and flow splitter cartridges were tested; a 45 nL flow cell was found to produce a more stable baseline than a 3 nL flow cell, and a 1:1000 split ratio cartridge was found to maintain pump pressure with minimal fluctuations at low flow rates more consistently than a 1:100 split ratio cartridge. To improve sensitivity, proteins were detected at a wavelength of 214 nm, the wavelength at which carbonyl groups absorb, as opposed to the typical wavelength of 280 nm. Monitoring at 214 nm as opposed to 280 nm increased peak height by 13-fold (data not shown).

To further reduce the amount of sample necessary for analysis, a user-defined program was implemented for sample uptake. Rather than filling the fused silica sample transfer line completely with sample, a transport liquid (SEC mobile phase) was used to partially fill the sample needle (Fig. 2). Air pockets of 50 nL were programmed to occupy the sample transfer line both before and after the sample plug to avoid sample contact and dilution with the transport liquid. A user defined program with 450 nL transport liquid uptake and 300 nL sample uptake was used throughout the study. The reduction of sample usage was necessary to be

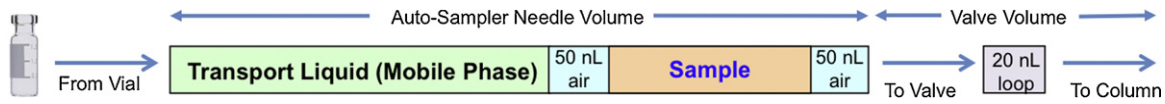


Fig. 2. User-defined sample uptake method for reducing sample usage. Schematic of the sample transport from sample vial to column is shown. Transport liquid, air, and sample uptake volumes are represented. Air pockets prevent mixing of adjacent liquid volumes.

able to perform multiple injections from the same vial; multiple injections are often required during sample analysis to verify chromatographic results.

3.2. Performance of capillary SEC

After optimizing the commercially available liquid chromatography system for capillary SEC by modifying valve configuration, sample needle diameter, sample uptake volume, column tubing dimensions and detector cell size, studies were performed to demonstrate the precision, LOQ, and ruggedness of the capillary SEC method. In addition, comparability to conventional scale SEC and the ability of the capillary SEC method to analyze different antibodies in a single sequence were assessed.

3.2.1. Precision

The precision of an analytical procedure expresses the level of agreement between a series of measurements obtained from multiple analyses of the homogeneous sample under the prescribed conditions, and is often expressed as relative standard deviation (RSD). To calculate injection repeatability, a single sample preparation of mAb was analyzed using six replicate injections. The repeatability of the user-defined program was demonstrated, with a RSD value of 0.04% for main peak relative area for mAb2 (Table 2). The overlay for six sequential replicate injections from a single sample vial demonstrated excellent reproducibility of the elution profile (Fig. 3). Thus, we demonstrate a capillary SEC method with low sample usage and good precision after employing modifications to a commercially available chromatography system.

3.2.2. Limit of quantitation

The limit of quantitation refers to the lowest amount of an analyte in a sample that can be quantitatively determined with suitable precision and accuracy [14]. To determine the limit of quantitation of the method, triplicate injections of various sample concentrations (0.1–1.0 mg/mL) of a mAb with a low level of HMWS (0.3%) were analyzed at 214 nm. The minimum mAb concentration that was above the limit of quantitation was 0.25 mg/mL; concentrations below 0.25 mg/mL produced inconsistent results due to the difficulty of integration at very low injection amounts. This limit translates to a total protein load of 5 ng, with method sensitivity of approximately 15 pg (amount of HMWS in the sample). Picogram sensitivity for a size-exclusion chromatography method that analyzes monoclonal antibodies had not been demonstrated prior to this work.

Table 2
Injection repeatability of the user-defined sample uptake program for mAb2. Six replicate injections were made sequentially from a single vial (preparation) of the sample.

Injection #	Relative peak area (%)		Total peak area (mA U × min)
	HMWS	Main peak	HMWS + main peak
1	1.9	98.1	184.7
2	2.0	98.0	184.8
3	2.0	98.1	184.4
4	2.0	98.0	183.7
5	2.0	98.0	183.4
6	2.0	98.0	184.0
Mean	2.0	98.0	184.1
SD	0.04	0.04	0.58
RSD (%)	1.87	0.04	0.31

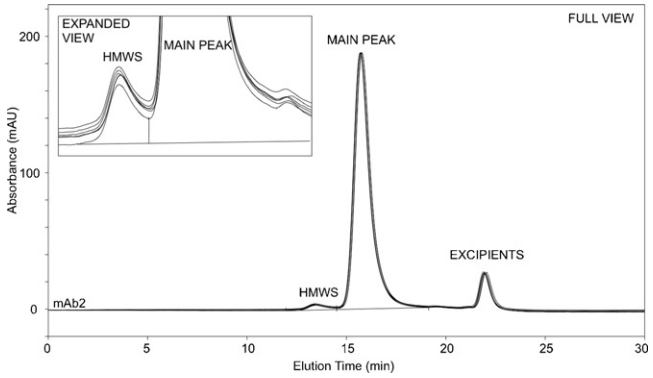


Fig. 3. Overlay of six sequential replicate injections of mAb2 from a single sample vial demonstrating the reproducibility of the elution profile. Conditions as per Fig. 1.

3.2.3. Ruggedness

Ruggedness of an analytical method is defined as the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, and different days [15]. Without deliberately changing the method parameters, the method is thus performed under different test conditions, and their influences can be evaluated. The capillary SEC method demonstrated remarkable ruggedness; for a total of 37 data points (control sample injections of mAb3) from at least three column lots, three analysts and approximately 4 months of analysis, the 3SD (3 standard deviations of the mean) of relative main peak area was 0.5% (Fig. 4). These 37 control sample injections of mAb3 were used to monitor the performance of the method during the analysis of approximately 600 actual sample injections amongst the

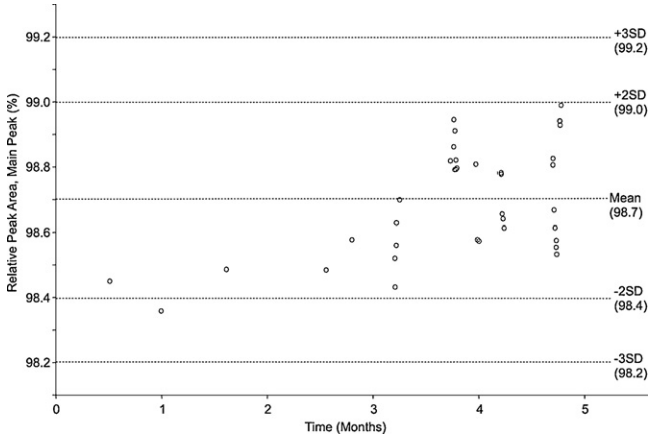
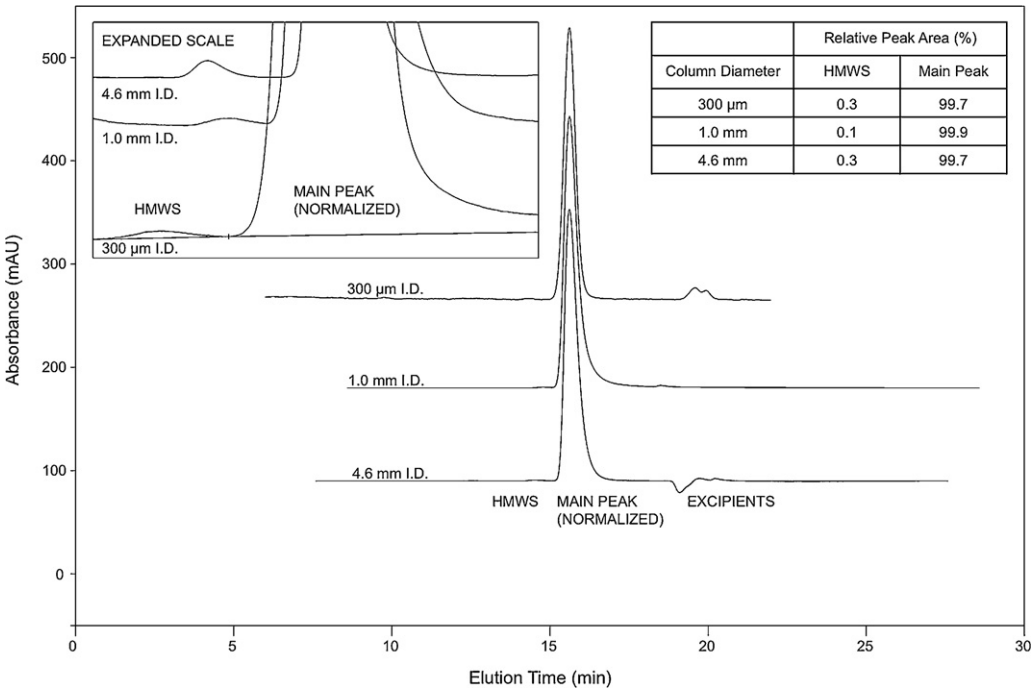


Fig. 4. Trending data for capillary SEC using mAb3 reference standard. Relative main peak areas for mAb3 were tracked for approximately 4 months and graphically represented. Data was obtained by three analysts using three different columns. Mean and standard deviations (SD) are represented by horizontal dashed lines.



	Relative Peak Area (%)	
Column Diameter	HMWS	Main Peak
300 µm	0.3	99.7
1.0 mm	0.1	99.9
4.6 mm	0.3	99.7

Fig. 5. Capillary SEC comparability to conventional scale SEC. Elution profiles obtained with columns of various dimensions for mAb1 are shown. Results from the 300 µm ID column were obtained from the capillary chromatography system, while data for the remaining columns were obtained on a conventional scale chromatography system. Integration results are tabulated.

three column lots. Thus, the method is robust to changes in analysts, columns and days of analysis, which demonstrates the ruggedness of the method.

For a single capillary SEC column, consistency and long column life were demonstrated. In a study to evaluate column life, of 111 mAb3 control injections performed amongst a total of 571 mAb sample injections onto a single column, the mean and RSD of relative main peak area for the control injections were 98.8% and 0.1%, respectively. The column performed consistently at both early injections and at later injections. Column failure was typically indicated by loss of resolution as opposed to column blockage. Column or capillary blockage was not observed during normal usage as described in this paper.

3.2.4. Comparison to conventional scale SEC

Capillary SEC was compared to more conventional scale SEC columns, with column diameters of 1.0 mm and 4.6 mm (Fig. 5). Flow rates and injection amounts were approximately scaled linearly according to cross-sectional area; superficial linear velocity of the capillary column was approximately 1.4 cm min^{-1}. As shown in Fig. 5, the main peak width and relative peak areas were comparable between capillary SEC and conventional scale SEC for mAb1. This result is notable considering the potential for capillary scale SEC to degrade by instrumental band broadening. The modifications to the capillary chromatograph take this into account; thus, significant band broadening was not observed for capillary SEC compared to conventional SEC. In addition, comparing across column scales at different detection wavelengths (214 nm for capillary SEC and 280 nm for conventional SEC) resulted in comparable main peak and HMWS relative areas.

3.2.5. Multiple antibodies in sequence

To demonstrate the applicability of capillary SEC to multiple products, six different antibodies were injected back-to-back in a single sequence (Fig. 6). The different antibody samples contained different levels of high molecular weight species. The capillary SEC method was able to detect the different HMWS amounts for the different samples. The relative peak areas for HMWS and main peak were comparable to conventional scale SEC (data not shown). The formulations for the different mAbs contain different excipients, which may explain the slight differences in elution times for the excipient peaks. The ability to analyze different antibodies in a single sequence demonstrates the utility and throughput of the method.

3.3. Performance of antibody recovery/capillary SEC method

A micro-scale antibody recovery method was developed to recover antibody from low titer HCCF samples using Protein A resin to capture the antibodies. The recovery development was particularly challenging due to the low amount of antibody in the sample and the relatively large sample volume in respect to analytical instrumentation working volumes. Initial recovery methods utilized Protein A HPLC columns, but the low concentrations of antibody in the cell culture samples resulted in low recoveries. Pipette tips packed with Protein A resin were also explored; however, these tips had limited volume capacities, which were not suitable for the amounts of cell culture fluid per sample. Thus, we developed a method that allowed for batch binding in a well-plate using suspended Protein A resin, and utilized micro-spin columns for antibody recovery.

3.3.1. Recovery of antibody using a micro-scale Protein A method

To determine the recovery efficiency of the micro-scale antibody recovery method, different amounts of antibody were spiked in 3 mL blank HCCF (HCCF without mAb present) and recovered using the Protein A batch binding method (Table 3). The recovery efficiency was determined by calculating the amount of antibody recovered at the end of the sample preparation procedure, as determined by measuring protein concentration using a UV-vis spectrophotometer, then dividing by the total amount of antibody spiked into the blank HCCF. The percent recovery of the

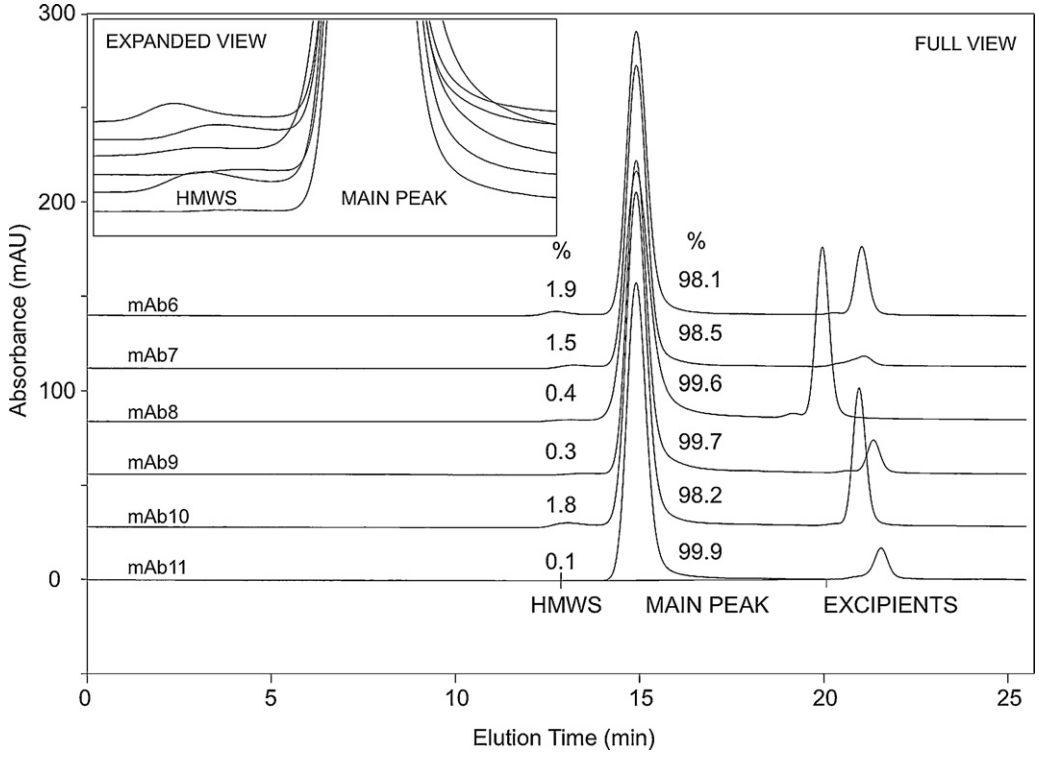


Fig. 6. Capillary SEC with multiple antibodies injected sequentially. Elution profiles obtained for six different antibodies (mAb6, mAb7, mAb8, mAb9, mAb10, and mAb11) with varying HMWS content are shown. Integration results are indicated as relative peak areas. Conditions as per Fig. 1.

extraction process increased as antibody concentration increased, with up to ~40% recovery with a starting concentration of 0.01 mg/mL antibody in HCCF. Fixed losses of protein to the disposable consumables, such as tubes and filters, account for loss in recovery efficiency. Finally, by comparing capillary SEC results of mAb1 and mAb3 before spiking into blank HCCF and after Protein A recovery, there was no indication of speciation or sample bias due to the micro-scale recovery method.

3.3.2. Precision and robustness of antibody recovery/capillary SEC method

Capillary SEC is precise and robust for antibody reference standards in mobile phase, as demonstrated in Section 3.2. Here, we set out to determine if we could analyze cell culture samples (3 mL) containing low amounts of antibody (~3 μg) with suitable precision and robustness for molecule assessment studies. Unlike reference standard in formulation buffer or mobile phase, HCCF samples cannot be directly injected onto an SEC column due to matrix incompatibility, particularly the presence of various host cell proteins. Antibodies must first be extracted from the HCCF and transferred to a compatible sample matrix, such as mobile phase.

To determine the precision of this micro-scale antibody recovery/capillary SEC method, antibody was spiked into 3 mL blank HCCF and then recovered and analyzed. The antibody recovery/capillary SEC method was consistent; for a total of 25 data points (spiked control sample recovered from HCCF) from 2 different columns, over approximately 2 months of analysis, the mean relative main peak area was 99.1% with an RSD of 0.3% (Fig. 7). Thus, the antibody recovery/capillary SEC method demonstrated consistency with blank HCCF spiked with antibody standard. Compared to control injections of mAb reference standard in mobile phase without Protein A recovery, which had a 3SD (3 standard deviations of the mean) of 0.5%, the antibody recovery/capillary SEC method demonstrated only a slightly higher standard deviation, with a 3SD of 1.0%.

Table 3
Recovery efficiency using the micro-scale Protein A recovery method as a function of starting antibody concentration in 3 mL harvested cell culture fluid (HCCF).

Antibody concentration (mg/mL)	Recovery efficiency (%)	Standard deviation (%)
0.001	13.9	0.3
0.003	21.7	1.4
0.007	28.9	4.2
0.014	39.4	3.5

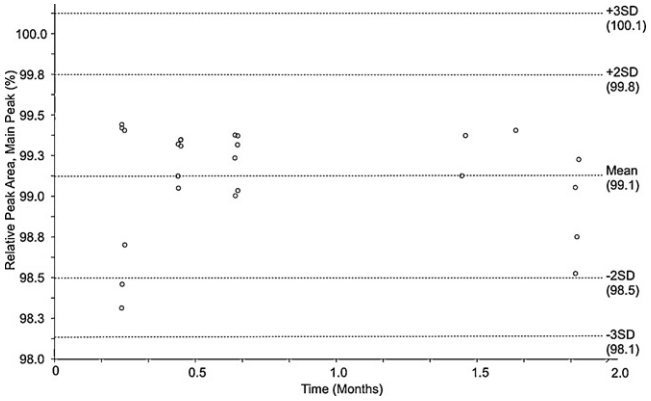


Fig. 7. Trending data for Protein A recovery followed by capillary SEC. Relative main peak areas for mAb3 reference standard spiked into blank HCCF were tracked for approximately 2 months and graphically represented. Data was obtained using two different columns. Mean and standard deviations (SD) are represented by horizontal lines.

Table 4
Several mAbs are used to demonstrate the reproducibility of the Protein A/capillary SEC method. mAbs were recovered from transiently transfected cell culture using the batch extraction Protein A method.

	Percent peak area	
	HMWS	Main peak
mAb3 week #		
1	1.2	98.8
2	1.2	98.8
3	1.1	98.9
Mean	1.2	98.8
Standard deviation	0.05	0.05
mAb4 week #		
1	0.7	99.3
2	1.1	98.9
3	0.6	99.4
Mean	0.8	99.2
Standard deviation	0.24	0.24
mAb5 week #		
1	0.6	99.4
2	0.6	99.4
3	0.6	99.4
Mean	0.6	99.4
Standard deviation	0.03	0.03

3.3.3. Week-to-week consistency of transiently transfected cell culture analysis

Micro-scale antibody recovery/capillary SEC demonstrated good precision for antibodies spiked into blank HCCF, as demonstrated in the previous section. Here, we set out to determine the consistency of the method using transiently transfected cell culture samples. Monoclonal antibodies were produced in transiently transfected Chinese hamster ovary (CHO) cells for subsequent capillary SEC analysis. Transiently transfected cell culture samples (3 mL) ranged in concentration from ~500 ng/mL to 3000 ng/mL. To determine the week-to-week consistency of the method using transiently transfected cell culture samples, HCCF aliquots from pooled samples were analyzed once a week over 3 weeks for Protein A recovery and capillary SEC analysis. Capillary SEC results for three different antibodies with titers above 1000 ng/mL are tabulated in Table 4. The results demonstrate good week-to-week reproducibility of the antibody recovery/capillary SEC method for samples with titers greater than 1000 ng/mL. Samples with titers below 1000 ng/mL gave more variable results due to the contribution of baseline noise.

4. Conclusion

Low abundance antibodies in various biological sample matrices are being generated for research and development purposes. In this study, a capillary SEC methodology was developed in conjunction with micro Protein A recovery in order to characterize these low abundance antibodies from HCCF. The testing of this method with different mAbs, different columns and different analysts demonstrates a highly robust method with results consistent across column scales. The capillary SEC method compares very favorably to conventional scale SEC.

The application of capillary SEC to analyze very small amounts of mAbs is significant because product quality information was previously not attainable at these scales. This method reduces potential setbacks due to poor product quality by investigating protein aggregation early in development. By screening out protein candidates that have poor product quality, time and resources that would have been spent on problematic molecules are conserved. To date, over 300 cell culture samples have been analyzed using the methodology described herein. Ultimately, early molecule assessment can result in faster delivery of protein therapeutics to the clinic.

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