

Antibody Analysis using microfluidic technology in high throughput Quality by Design Experiments

Abstract

Current initiatives in Process Analytical Technology (PAT) and Quality by Design (QbD) are driving the need to identify and thoroughly understand the relation between Critical Process Parameters (CPP) and Critical Quality

Attributes (CQAs)¹. Design of Experiment (DOE) studies to test the effect of cell culture changes on post translational modifications of monoclonal antibodies (MAb) or to monitor its purification process, produces large number of samples that can greatly exceed the capacity of modern analytical laboratories. With increased samples comes the demand for a high throughput analytical platform with superior precision, automation, and ease of use.

Here we describe the use of the LabChip® microfluidics platform to analyze antibodies, demonstrating the assay's precision in determining sample purity and quantification, its linear dynamic range, and capability to resolve low level impurities. The antibodies are analyzed under reducing and non-reducing conditions on a reusable microfluidic chip in an automated high throughput mode with analysis time of 41 seconds per sample.



Figure 1. LabChip GXII Touch System.

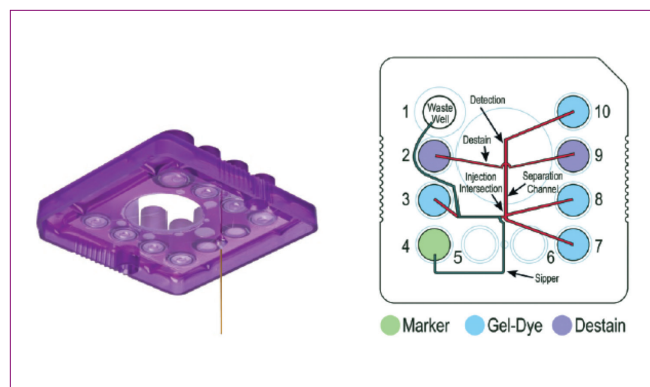


Figure 2. Image of the sipper LabChip GXII Touch protein chip (L) and top down schematic diagram (R) of the microfluidic channel layout.

Introduction

Monoclonal antibody products are inherently heterogeneous because of post-translation modification that often occurs during the fermentation process. Therefore, thorough characterization of MAb products is required in order to assess their CQAs. In early stage process development, DOE or Factorial Experimental Design (FED), experiments are performed to understand the relationships between the process parameters and product quality in order to define a process design space around the variability in the process parameters. The increased number of variables to be examined in a DOE study of cell culture conditions or purification process produces large number of samples that need to be processed. Current method of monitoring protein product quality includes SDS-PAGE and HPLC. SDS-PAGE is both labor intensive and difficult to automate and has been supplanted by CE-based separation in modern analytical laboratories. CE and HPLC analysis require separation time of 15-60 minutes per sample, which can limit DOE studies.

In the past several years, microfluidic-based assays for sizing, quantification, and purity assessment of proteins are finding wide use because they address the limitations of SDS-PAGE, HPLC, and CE methods ^{2,3}. Chen et. al.² studied the use of the LabChip technology for screening MAb product quality attributes. They analyzed MAbs from crude CHO cell culture supernatants and purified samples under reducing and non-reducing conditions. They showed similar resolution and sensitivity as conventional CE-SDS on a 20 cm capillary but a time scale of approximately 70 times faster (41 sec versus 50 min per sample).

The LabChip GXII Touch platform provides an automated platform for rapid analysis of proteins and nucleic acids. Sampling directly from a 24-, 96- or 384-well plate, the LabChip GXII Touch offers ease of use and walk away time. Each individual sample can be analyzed in 41 seconds or less, enabling a 96-well plate to be analyzed in slightly longer than 1 hour (Figure 1).

The protein sizing on the microfluidic chip (Figure 2) is achieved by integration of the main features of one-dimensional SDS-PAGE: these include separation, staining, destaining and detection steps. Denatured proteins are brought onto the chip directly from a microtiter plate through a capillary sipper. The samples are then electrokinetically loaded and injected into the 14mm long separation channel that contains a low viscosity matrix of entangled polymer solution. The protein-SDS complex and free SDS micelles in the sample plug are immediately stained by dye present in the sieving matrix. At the end of the separation channel, the sample is diluted to reduce the SDS concentration below its critical micelle concentration in order to reduce the background fluorescence so that protein-SDS-dye complexes can be detected.

The integrated microchip-based assay was used to separate and quantify monoclonal antibodies and their fragments with minimal sample preparation artifacts, including crude samples. Illustrations of the reproducibility, resolution, linearity, quantification, and sensitivity are described in this application note.

Experimental Results and Discussion

All experiments were performed in accordance to the user's guide, using the HT Antibody Analysis 200 assay on the LabChip GXII system (PerkinElmer).

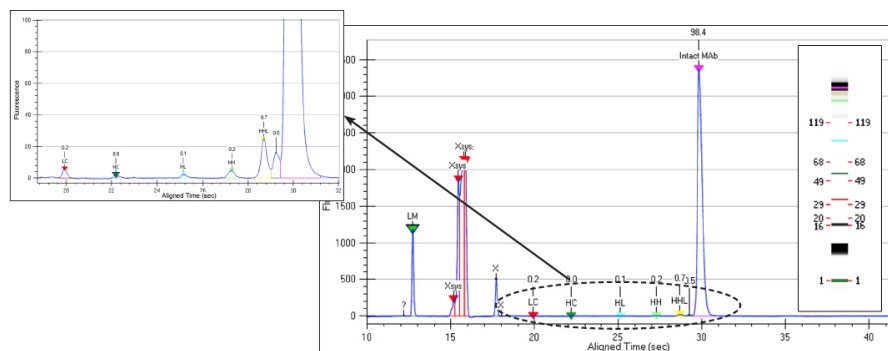


Figure 3. Non-reduced MAb electropherogram indicating the % purity of the fragments and intact MAb. The peak marked X at ~ 17.5 seconds is lysozyme spiked into the antibody and used as an internal standard. LM is the lower marker and Xsys are the system peaks.

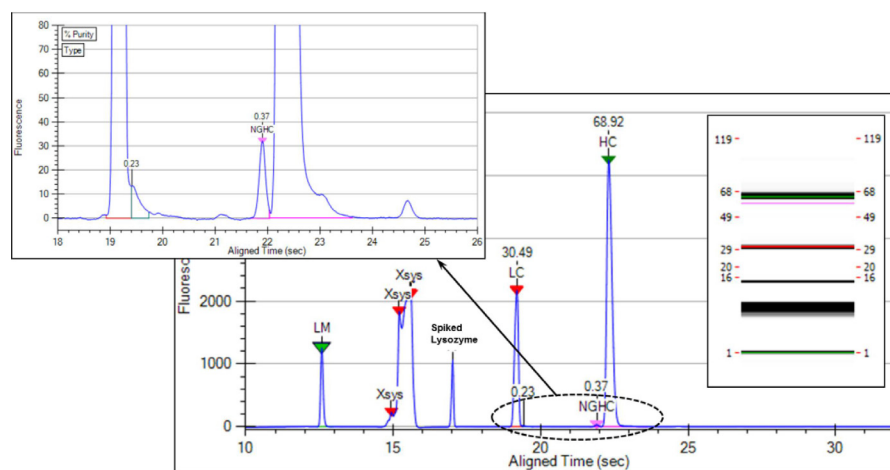


Figure 4. Reduced MAb electropherogram

Electropherogram Analysis

Figure 3 shows an example of an electropherogram for a non-reduced MAb at a concentration of 1 mg/mL. The inset plot shows that the low level fragments, such as the light chain, heavy chain, heavy-light chain, heavy-heavy chain, heavy-heavy-light chain, and intact MAb are all well resolved. The electropherogram for the reduced MAb is shown in Figure 4, illustrating sufficient resolution of the non-glycosylated heavy chain (NGHC) from the heavy chain for accurate quantification. Using the HT Protein Express Assay, Chen et. al.² quantified the NGHC in crude samples in order to measure the relative level of the high mannose. They concluded that the assay could provide valuable feedback for early stage cell culture screening.

Detection Linearity

Linearity of assay detection was tested by running dilutions of a MAb under non-reducing conditions over the range of 8– 2000 µg/mL. Eight replicates were run at each concentration. The linearity plot of integrated time corrected area versus expected concentration with an r^2 value of 0.9997 (Figure 5). The linear

dynamic range of 3 logs indicates the potential for the assay to be used for titer determination.

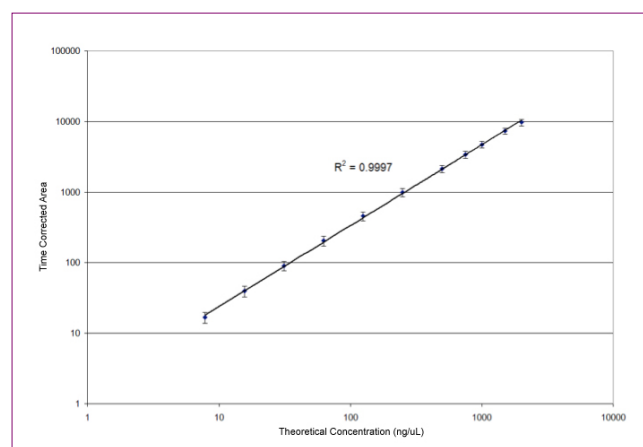


Figure 5. Linear dynamic range: time corrected area versus theoretical MAb concentration over the range 8 to 2000 µg/m

Assay Precision

The inter-assay precision was determined by calculating the relative standard deviation (RSD) of the percent purity of the intact MAb, light chain, heavy chain, and NGHC. Three different operators did eight sample preparations for each MAb under reducing and non-reducing conditions. Each operator then ran the samples on separate chips and instruments. Table 1 summarizes the combined results from all three operators (total of 24 sample preparations for each MAb). The RSD of the intact MAb was <0.5%. The heavy chain was <2%, while the light chain and NGHC were <4%.

Assay Quantification

In order to determine sample concentration of non-reduced samples, a standard curve of normalized corrected area versus expected concentration was created using a MAb across the concentration range 8 – 2000 µg/mL. Lysozyme was used as an internal standard and spiked into the sample buffer at 10 µg/mL. The time corrected area of the antibody peak was normalized to the time corrected area of the lysozyme peak for each sample. The standard curve was then used to calculate the concentration of three different MAbs. Each MAb was diluted to an expected concentration of 1000 µg/mL. As in the assay precision determination, three operators did eight sample preparations and ran the samples on separate chips and instruments. Table 2a shows the average calculated concentration and RSD for MAb3 for each operator. Table 2b shows the combined values for all three operators for each MAb. The RSD for the quantification was <2% for MAb1 and MAb3 and <5% for MAb2.

	% Intact MAb		% LC		% HC		% NGHC	
	Average	RSD (%)	Average	RSD (%)	Average	RSD (%)	Average	RSD
MAb1	98.13	0.18	29.38	3.61	69.69	1.67	69.69	1.32
MAb2	95.48	0.51	29.04	3.39	69.15	1.43	69.15	3.72
MAb3	98.53	0.32	29.59	3.27	69.70	1.46	69.70	4.04

Table 1. Inter-assay precision for non-reduced and reduced MAb samples.

MAb3	Concentration (ng/µL)	RSD (%)
Operator 1	1046	1.91
Operator 2	1052	2.19
Operator 3	1036	1.76

Table 2a. MAb3 calculated concentration by operator.

	Concentration (ng/µL)	RSD (%)
MAb1	1039	1.68
MAb2	1007	5.39
MAb3	1046	1.92

Table 2b. Overall calculated concentration.

Low-Level Impurity Detection

In order to demonstrate the detection of low-level impurities lysozyme was spiked into the sample at 1% of the total protein concentration. The MAb was run under reducing conditions at 1mg/mL. Figure 6 illustrates the resolution of the lysozyme peak with a signal to noise ratio of 9:1.

The ProfilerPro Glycan Profiling Assay provides a faster more efficient means to assess the main glycoforms of IgG and related proteins. PerkinElmer offers a complete screening kit consisting of sample prep plates and microfluidic assay components to enable the rapid digestion, dyeing and identification of main glycans (N-Glycans) in the Fc region of recombinant monoclonal antibodies (MAb).

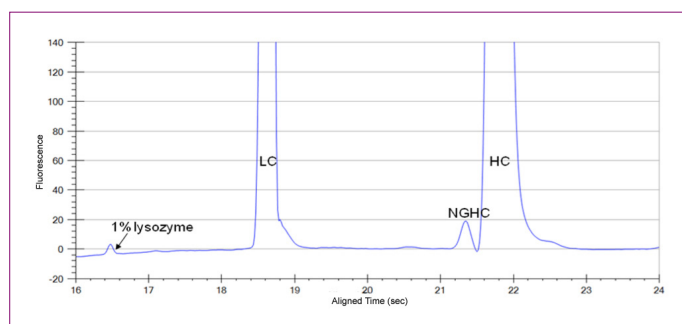


Figure 6. Detection of lysozyme spiked in at 1% of the total protein concentration.

High Throughput Screening of Neutral Glycans

The ProfilerPro Glycan Profiling Screening Assay is 60-70% faster, and 10-60% less expensive than competing technologies. Profiling of 96 samples can be performed in as little as half a day (Figure7).

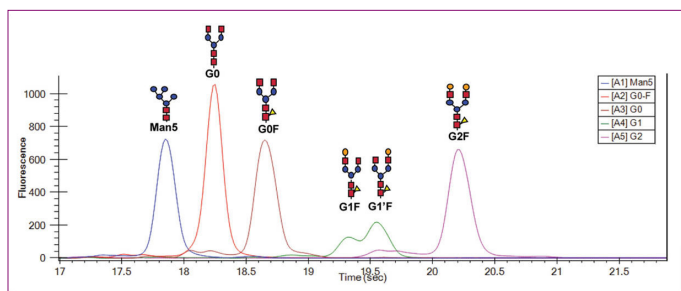


Figure 7. Overlay electropherogram of the five main glycan standards.

Charge Variant Assay for clone selection to formulation

Another assay on the LabChip system is used to assess the charge heterogeneity on the biomolecules (specifically antibodies) via capillary zone electrophoresis. One of the major factors of heterogeneity in biomolecules is stress induced variability. The heterogeneity often has an impact on the pharmacokinetic profiles. In this method, antibodies in the pI range of 7.5 to 9 are fluorescently labeled in a ten minute reaction while conserving the overall charge. The labeled samples are then introduced into the microchip through a sipper, using vacuum. The sample in the chip is electrokinetically injected and separated in a separation channel. The method achieves adequate separation of the main peak, the acidic and basic peaks in less than 60 seconds. A typical profile of a monoclonal antibody is shown in Figure 8.

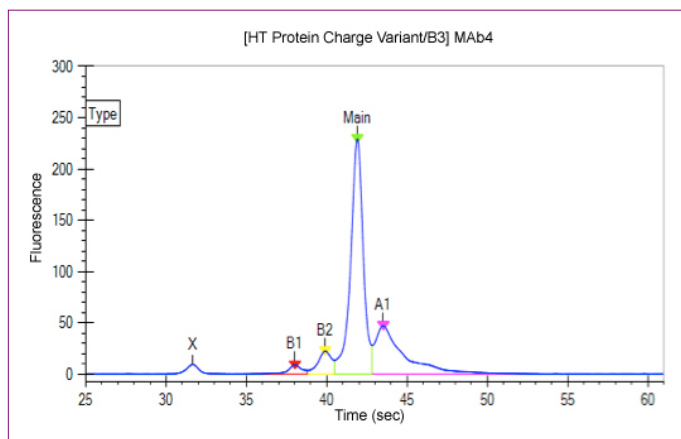


Figure 8. The figure shows a typical mAb profile generated in about 60 seconds on the LabChip. Basic region is on the left of the main peak (indicated by B1 and B2) and the acidic region on the right (indicated by A1). The separation of the antibody depends on the pI of the antibody molecule (and pH of the separation buffer).

Summary

The HT Antibody Analysis 200 assay on the LabChip microfluidics platform can be used as a high throughput automated alternative to SDS-PAGE and conventional CE- SDS for screening antibody product quality attributes including quantification, fragmentation, and purity analysis. It offers a quick time to result (41 seconds per sample) with < 1% precision. Good linearity was achieved for the assay over the concentration range 8 – 2000 µg/mL as well as the ability to detect low level impurities with a signal to noise ratio of 9:1.

References

1. Rathore A. S. and Winkle H., Quality by design for biopharmaceuticals, Nature Biotechnology 2009, 27, 26-34.
2. Chen X. et. al. Microchip assays for screening monoclonal antibody product quality. Electrophoresis 2008, 29, 4993-5002.
3. Swalley S. E. et. al., Screening Factors Effecting a Response in soluble protein expression: formalized approach using design of experiments. Analytical Biochemistry, 2006, 351, 122-127.
4. Bousse L. et. al., Protein sizing on a microchip Anal. Chem. 2001, 73, 1207-1212.