Analysis of NIST Monoclonal Antibody RM 8671 with Novilytic's Proteometer-CV Kit

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ABSTRACT

Novilytic's Proteometer-CV Kit enables rapid analysis of titer and charge variant composition of biologics containing a human Fc region, including human, humanized, or chimeric monoclonal antibodies (mAbs), bispecific antibodies (bsAb), antibody-drug conjugates (ADCs), and Fc-fusion proteins. Charge variant profile and titer are critical quality attributes analyzed throughout process development of therapeutic antibodies. Both quality attributes are determined without prior purification in 25 minutes. This application note details the analysis of NIST monoclonal antibody (NmAb) formulated in clarified fermentation broth (CFB) using the Proteometer-CV Kit. The kit's effectiveness is demonstrated by evaluating key analytical performance metrics, specifically repeatability, linearity, and robustness.

INTRODUCTION

Determining the charge variant profiles is an essential step in the development of therapeutic antibodies. It is essential to understand these profiles and closely monitor any changes throughout the life cycle of the antibody to gain insights into chemical and structural variations[1]. These variations may lead to loss of therapeutic potency, increased toxicity, or decreased stability, all of which are undesireable. Therefore, they must be removed from the final product through purification, a process that can lower yields and increase costs for the antibody manufacturer[1]. The ability to determine charge variant profiles without relying on Protein A purification is of great utility. By eliminating the Protein A purification step, which involves denaturation of the molecule, the charge variant profile better represents the true state of t e antibody as it exists in the fermentor.

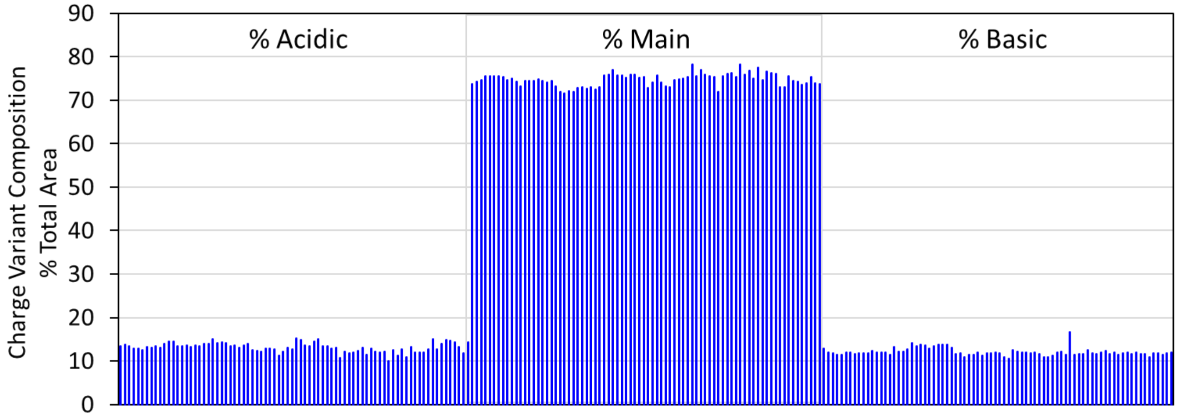
Traditionally, charge variant analysis of an antibody is a two-step process that involves Protein A purification of each sample to eliminate interference from CFB components, followed by chromatography or electrophoresis to fractionate the variants. The Proteometer-CV assay achieves the same objectives by combining separation, affinity selection, and fluorescence labeling of the antibody in a single analysis. The separation step is followed by specific coding of the biologic analyte--any protein containing the Fc domain of human IgG--using a fluorescently labeled affinity selector. Novilytic's Proteometer-CV Kit enables the analysis of biologics containing the Fc domain of human IgG such as mAbs, bsAbs, ADCs and Fc fusion proteins. NIST Monoclonal Antibody Reference Material, SRM 8671 (NmAb) was utilized to develop and qualify the Proteometer-CV method. This reference material is a highly characterized mAb available for purchase and is commonly used as a standard in analytical techniques[2].

RESULTS

NmAb was formulated at a concentration of 1 mg/mL in CFB and injected in varying amounts into an HPLC system equipped with a fluorescence detector. The Proteometer-CV Kit was separate days used for the system setup with the quaternary pump method, using the suggested gradient of 7-14% mobile phase B[3]. Ten injections of NmAb (8 µg each) were used for repeatability testing. Triplicate injections of 8, 12, 16, 20, and 24 µg were used for linearity testing. To test robustness, Proteometer-CV Reactors from three different lots were used. Two different analysts conducted a repeatability test and a linearity test on three seperatedays for each reactor. Charge heterogeneity, defined as percent acidic, main, and basic variants, was calculated by measuring peak areas.

Repeatability

Data from all injections for repeatability tests (*n* = 90) were plotted against the injection number, as shown in Figure 1. The resultsdemonstrate excellent repeatability and robustness of charge variant analysis using the Proteometer-CV with NmAb as the analyte.



(*n* = 90)	% Acidic	% Main	% Basic
Average	13.13	74.72	12.08
Std Dev	1.08	1.45	0.90
% CV	8.19	1.94	7.46

Figure 1. Repeatability and robustness of NmAb charge variant analysis with the Proteometer-CV

Linearity

Overlaid chromatograms across all injection amounts for NmAb linearity analysis using the Proteometer-CV are shown in Figure 2. No signal was observed for the CFB control injection. Figure 2 (inset) also shows the linear regression of the total peak area as a function of mAb injection amount (*n* = 3). Nine similar linearity analyses were performed (three per Proteometer-CV Reactor for three different reactor lots). Linear regressions of these data sets resulted in R^2 of 0.995 or greater in all nine data sets where R^2>0.990 is generally acceptable for impurity analysis methods such as the Proteometer-CV[4].

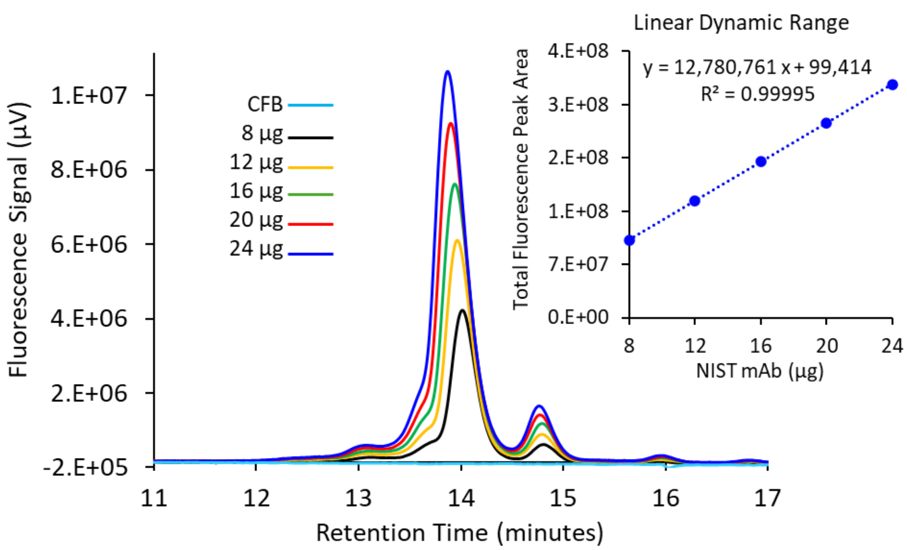
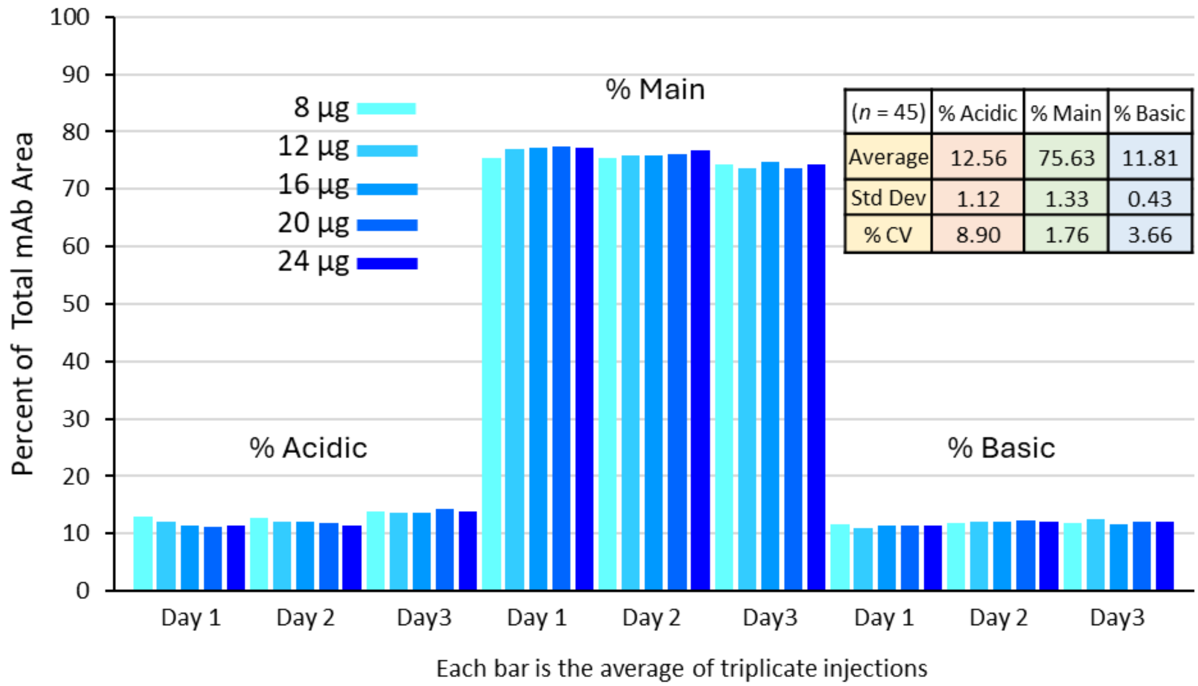


Figure 2. Linear response of NIST mAb in CFB for sample loads of 8-24 µg. The inset on the right shows linear regression of average total peak area (*n*=3) versus NIST mAb load (µg).

Robustness and Charge Variance Repeatability

For the reactor used in the analysis shown in Figure 2, the average charge variant distribution was calculated for each injection amount. Figure 3 shows a graphical representation of the data obtained from three analysis runs by two operators on two LC systems. Over the total number of injections (*n*=45), the average charge heterogeneity was 12.56% acidic variants, 75.63% main component, and 11.81% basic variants. The greatest coefficient of variance observed among the components was 8.90% for the acidic variants (Figure 3 inset).

Similar analyses were performed with two additional reactor lots to compare the variability between lots. Comparable charge heterogeneity data was obtained from the three different Proteometer-CV Reactors tested, with the highest variability observed for the acidic variants, which had a coefficient of variance of 9.60%.



(n = 45)	% Acidic	% Main	% Basic
Average	12.56	75.63	11.81
Std Dev	1.12	1.33	0.43
% CV	8.90	1.76	3.66

Figure 3. Reproducibility of charge variant composition of NmAb in CFB for various injection amounts.

The relationship between charge variant composition and sample load was examined for the data shown in figure 3. The trendlines of the sample µg load versus the percent acid, main, and basis peak areas each have slopes within the range 0 +/- 0.02, indicating that the sample load does not affect the charge variant composition reported by Proteometer-CV. Additionally, Analysis of Variance (ANOVA) of % acidic, % basic, and % main for sample loads over 8-24 µg of NmAb yields p-values of 0.3303, 0.5322, and 0.8595 respectively. The value $p \geq 0.05$ (5% significance level) indicates that there is no statistically significant difference in the values obtained for each class of charge variants over the tested sample loads. This indicates that the charge variant composition results provided by the Proteometer-CV Kit through direct analysis of NISTmAb in CFB are highly reproducible and independent of sample loads between 8 µg and 24 µg.

CONCLUSION

These results demonstrate the exceptional repeatability, linearity, and robustness of the Novilytic Proteometer-CV Kit. The charge variant composition values obtained by the Proteometer-CV for CFB samples containing NIST mAb, ranging from 8 to 24 µg, will not be significantly different. Additionally, results across multiple days and various Proteometer-CV Reactor lots are comparable. Therefore, the Proteometer-CV can be used to obtain two critical quality attributes of an antibody: (1) charge variant composition and (2) titer. By eliminating the need for Protein A purification prior to charge-variant analysis, the Proteometer-CV methodology saves both time and labor, while providing results that are most representative of the antibody in the production environment.

REFERENCES

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